THE INTRICATE INNATE IMMUNE-CANCER CELL RELATIONSHIP IN THE CONTEXT OF TUMOR ANGIOGENESIS, IMMUNITY AND MICROBIOTA: THE ANGIOGENIC SWITCH IN THE TUMOR MICROENVIRONMENT AS A KEY TARGET FOR IMMUNOTHERAPIES

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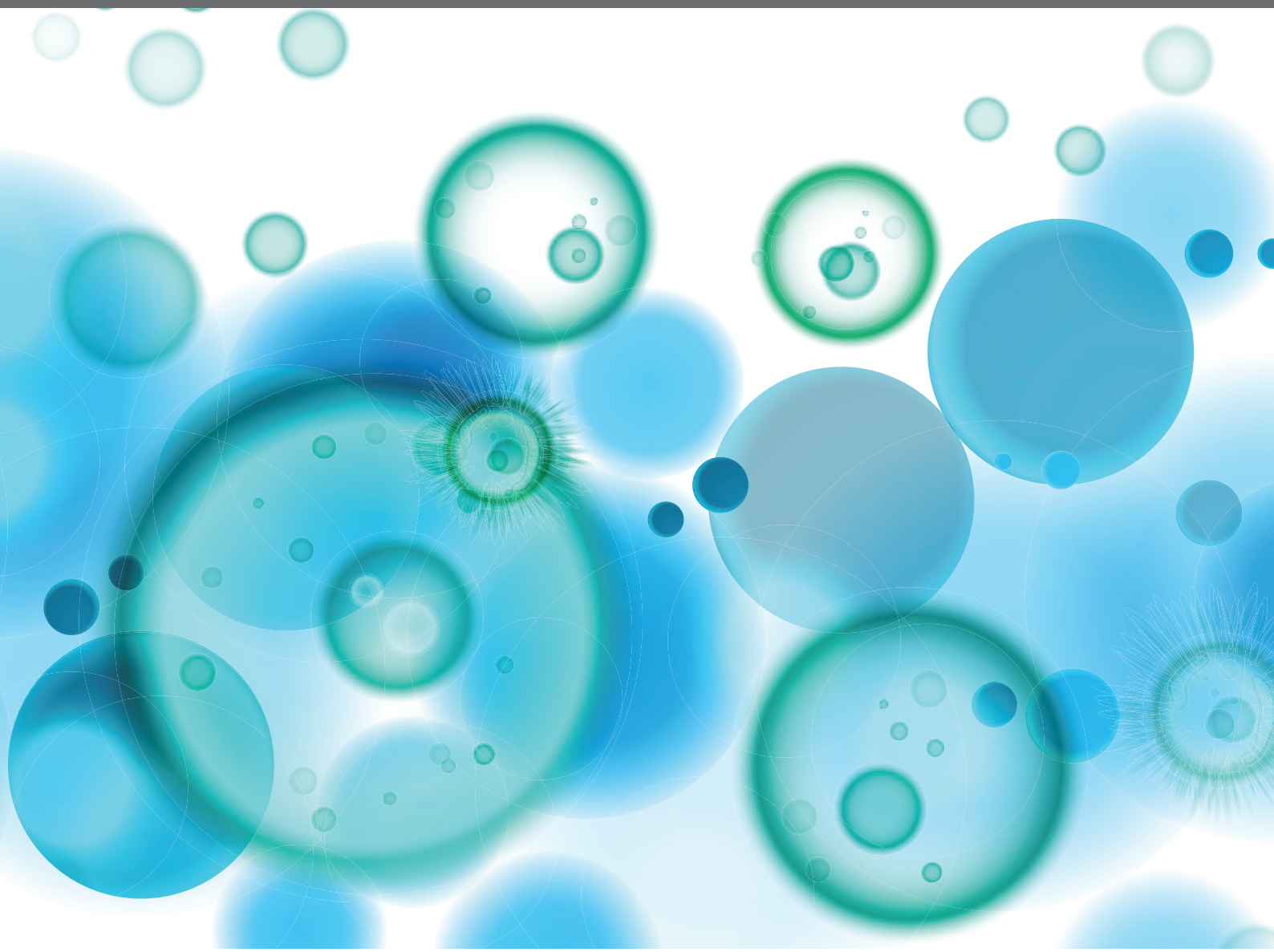
PUBLISHED IN: Frontiers in Immunology and Frontiers in Oncology



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ISSN 1664-8714
ISBN 978-2-83250-551-9
DOI 10.3389/978-2-83250-551-9

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Citation: Mortara, L., Benest, A. V., Chouaib, S., Derosa, L., Ribatti, D., eds. (2022). The Intricate Innate Immune-Cancer Cell Relationship in the Context of Tumor Angiogenesis, Immunity and Microbiota: The Angiogenic Switch in the Tumor Microenvironment as a Key Target for Immunotherapies.
Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83250-551-9

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frontiers | Frontiers in Immunology

TYPE Editorial
PUBLISHED 06 October 2022
DOI 10.3389/fimmu.2022.1045074

OPEN ACCESS

EDITED AND REVIEWED BY
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SPECIALTY SECTION
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

RECEIVED 15 September 2022
ACCEPTED 22 September 2022
PUBLISHED 06 October 2022

CITATION
Mortara L, Benest AV, Derosa L, Chouaib S and Ribatti D (2022) Editorial: The intricate innate immune-cancer cell relationship in the context of tumor angiogenesis, immunity and microbiota: The angiogenic switch in the tumor microenvironment as a key target for immunotherapies. *Front. Immunol.* 13:1045074. doi: 10.3389/fimmu.2022.1045074

Editorial: The intricate innate immune-cancer cell relationship in the context of tumor angiogenesis, immunity and microbiota: The angiogenic switch in the tumor microenvironment as a key target for immunotherapies

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KEYWORDS

Cancer, tumor microenvironment, tumor angiogenesis, Immunity, microbiota, immune checkpoint inhibitors, immunotherapies

Editorial on the Research Topic
The intricate innate immune-cancer cell relationship in the context of tumor angiogenesis, immunity and microbiota: The angiogenic switch in the tumor microenvironment as a key target for immunotherapies

The tumor microenvironment (TME) represents a complex multicellular network which comprises host-derived stromal, immune and endothelial cells with potential dual role in tumor development and dissemination. For example, immune cells have the ability to recognize and orchestrate anti-tumor responses leading to cancer cell death, however in the meantime they can become exhausted whereas innate immune cells can acquire pro-tumorigenic and/or pro-angiogenic activities.

This Research Topic was designed to dissect various aspects of interactions that tumor cells must set up with the TME to trigger tumor blood vessel proliferation, to tamper host anti-tumor

responses and to modulate microbiota, and to investigate feasibility to target these pathways to improve immunotherapies.

Here, Genova et al. examines the impact that the TME can have on immune checkpoint inhibitors (ICIs) in non-small cell lung cancer (NSCLC). They discuss on the pro-angiogenic and immunosuppressive role of the TME exerted by many distinct cells as well as on multiple clinical studies focusing on alternative immune checkpoint receptors that could lead to exhausted T and natural killer (NK) cells and resistance to ICIs. Importantly, they provide an update on novel predictors of response from currently available ICI and novel therapeutic targets. In fact, there are many promising preclinical and trials data in NSCLC, where in parallel with classical ICIs targeting PD-1/PD-L1, new target molecules could be used, such as: LAG-3 and TIM-3.

In turn, the review by Baci et al. takes under consideration the role of tumor immune microenvironment in NSCLC and the interactions between tumor cells and immune infiltrate with the aim to define new targetable drivers of immunotherapy. In particular, they pinpoint the effects exerted by neutrophils, myeloid-derived suppressor cells (MDSCs), NK cells, NKT cells, dendritic cells (DCs), Treg cells and mast cells on the orchestration of primary resistance to ICIs. This review also includes the discussion about the relevance of combination of anti-angiogenic therapies with ICIs.

Concerning anti-angiogenesis therapy, Solimando et al. in their mini-review, examine this phenomenon in metastatic castration-resistant prostate cancer (mCRPC). Targeting angiogenesis has failed to impact overall survival in patients with mCRPC despite promising preclinical and early clinical data. Narrowing the gap between the bench and bedside appears critical for developing novel therapeutic strategies. Several other compounds with known anti-angiogenic properties, including metformin or curcumin, are currently investigated. Angiogenesis-targeting strategies include biomarker-guided treatment stratification as well as combinatorial approaches. Beyond established angiogenesis inhibitors, therapies aiming at prostate specific membrane antigen (PSMA) have a substantial anti-angiogenic effect, due to PSMA´s expression in tumor vasculature.

Understanding the interactions between all the constituents of the TME remains a challenging task. Currently most patients still do not benefit from cancer immunotherapies notably because of the hostility imposed by the hypoxic microenvironment inducing immune suppression and tumor plasticity and resistance. Khouzam et al. review the mechanisms by which hypoxic stress impacts immune cell functions and how that could translate to predicting response to immunotherapy. Of particular interest is the discussion relating to how multi modal diagnostic techniques are being aligned with *in silico* approaches. Along the same line of research, Janji and Chouaib summarize the contribution of hypoxic stress to tumor progression, and its impact upon conventional anti-tumor therapies. However, although increasing evidence, the acceptance that targeting hypoxia in combination with immunotherapy might offer further clinical benefit is less well established. HIF1a signaling is a known modulator of multiple inflammatory cytokines and checkpoint expressions and therefore offers new avenues to explore as immunotherapy becomes a standard treatment.

Interestingly, Wang et al. in their review investigate the relevance of the TME in the hepatocellular carcinoma, a cancer with high worldwide incidence and with serious therapeutic implications. They illustrate the possibility of targeting the TME using immunomodulatory therapy (ICIs, new immune checkpoints, combination of ICIs with multiple kinase inhibitors), or oncolytic viruses or anti-angiogenesis therapies.

Taken together, the TME is not simply pro-angiogenic or pro/anti-inflammatory, rather is a dynamic milieu of complex interactions and cellular consequences. Of note, Xu et al. highlight the practical application of this in their report; the particularly rare splenic angiosarcoma is treatable with anti-PD-L1 antibody and tyrosine kinase receptor inhibitors. Whereas this is a case report, and full clinical trials will need to be registered and completed it offers promise to an otherwise poor prognosis, indeed at 3 months no metastatic colonization was observed. Of particular interest is the use of computed tomography (CT) and magnetic resonance imaging (MRI) to assess the efficacy of combination treatment.

Instead, Etxebeste-Mitxeltorena et al. in their review, analyze the role of adoptive cellular immunotherapy using chimeric antigen receptor (CAR)-modified T cells and NK cells in cancer. Whereas CAR-T cells induce outstanding responses in a subset of hematological malignancies, responses are much more deficient in solid tumors. Authors describe plasticity of immune cells and how these cells change their activity and phenotype depending on the stimuli they receive from molecules secreted in the TME. For example, this phenomenon could affect tumor cell phagocytosis by macrophages, which is required to remove dying tumor cells after the attack of NK cells or CAR-T cells, and it can be avoided in the TME.

Concerning ICIs resistance in solid tumors, the review by Roberto et al. analyzes how microbiota is affected by intestinal microenvironment and how microenvironment alterations may influence the response to ICIs. They showed how diet is emerging as a fundamental determinant of microbiota's community structure and function and describe the role of certain dietary factors, as well as the use of probiotics, prebiotics, postbiotics, and antibiotics in modifying the human microbiota. Finally, they shed new light on the possibility of administering fecal microbiota transplantation to modulate the gut microbiota in cancer treatment.

Within the frame of this Research Topic, the article of Qing et al. probed the Cancer Genome Atlas (TCGA) and the GEO repository for gene signatures relating to angiogenesis and immune cells infiltration and combined the transcriptomic data with prognostic data to predict therapeutic responses. The resultant data were used to generate a prognostic nomogram, allowing clinicians to match tumor characteristics with potential personalized therapeutic opportunities.

On the other hand, Zhang et al. in their work systematically collected and evaluated the infiltration pattern of 65 immune cells.

They constructed the immune cell pair (ICP) score based on the cell pair algorithm across 12 independent cancer types. The ICP score showed reliability and efficacy in predicting the survival of patients with gliomas, in pan-cancer samples, and six independent cancer types. Moreover, the ICP score was correlated with the genomic alteration features in gliomas, exhibited a remarkable association with multiple immunomodulators that could potentially mediate immune escape, and predicted immunotherapeutic responses with a high sensitivity.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

frontiers in Immunology

REVIEW
published: 10 August 2021
doi: 10.3389/fimmu.2021.717850

Tumor Secretome to Adoptive Cellular Immunotherapy: Reduce Me Before I Make You My Partner

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Adoptive cellular immunotherapy using chimeric antigen receptor (CAR)-modified T cells and Natural Killer (NK) cells are common immune cell sources administered to treat cancer patients. In detail, whereas CAR-T cells induce outstanding responses in a subset of hematological malignancies, responses are much more deficient in solid tumors. Moreover, NK cells have not shown remarkable results up to date. In general, immune cells present high plasticity to change their activity and phenotype depending on the stimuli they receive from molecules secreted in the tumor microenvironment (TME). Consequently, immune cells will also secrete molecules that will shape the activities of other neighboring immune and tumor cells. Specifically, NK cells can polarize to activities as diverse as angiogenic ones instead of their killer activity. In addition, tumor cell phagocytosis by macrophages, which is required to remove dying tumor cells after the attack of NK cells or CAR-T cells, can be avoided in the TME. In addition, chemotherapy or radiotherapy treatments can induce senescence in tumor cells modifying their secretome to a known as "senescence-associated secretory phenotype" (SASP) that will also impact the immune response. Whereas the SASP initially attracts immune cells to eliminate senescent tumor cells, at high numbers of senescent cells, the SASP becomes detrimental, impacting negatively in the immune response. Last, CAR-T cells are an attractive option to overcome these events. Here, we review how molecules secreted in the TME by either tumor cells or even by immune cells impact the anti-tumor activity of surrounding immune cells.

Keywords: tumor secretome, SASP, senescence, immunotherapy, macrophages, CAR-T cells, NK cells, T cells

OPEN ACCESS

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Specialty section:
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

Received: *31 May 2021*
Accepted: *26 July 2021*
Published: *10 August 2021*

Citation:
Etxebeste-Mitxeltorena M, del Rincón-Loza I and Martín-Antonio B (2021) Tumor Secretome to Adoptive Cellular Immunotherapy: Reduce Me Before I Make You My Partner. Front. Immunol. 12:717850. doi: 10.3389/fimmu.2021.717850

INTRODUCTION

Today, it is widely recognized that chronic inflammation is a driver of cancer (1), being estimated that 15-20% of cancers are inflammation-related (2). This association has been observed in different contexts, such as persistent *Helicobacter pylori* infection or autoimmune diseases like inflammatory bowel disease that increase the risk of developing gastric cancer (3) or colorectal cancer (4), respectively. Numerous studies have found associations of inflammatory markers with a higher risk of developing cancer. For instance, 15% of patients with cardiovascular disease, after a median follow-up of 8.3 years, developed different types of cancer whose incidence was associated with high C-reactive protein (CRP) levels (5). In addition, IL6 levels are also associated with an increased risk

of developing different types of cancer (6). Moreover, IL1β inhibition reduced CRP and IL6 levels and the incidence of developing lung cancer in patients with atherosclerosis who had a myocardial infarction (7).

Both immune and tumor cells promote this pro-inflammatory microenvironment. Expressly, tumor cells release a secretome that displays an altered composition compared to the normal tissue from which they are derived (8). This secretome contains cytokines, chemokines, hormones, metabolites, and growth factors involved in cell-cell communication, angiogenesis, hypoxia, metastasis, extracellular matrix remodeling, and drug resistance (8, 9), where tumor cells employ it as a mechanism of immune evasion (10–12). On the other side, the different subsets of immune cells will also release immunosuppressive and inflammatory factors that will shape the tumor microenvironment (TME), promoting or inhibiting cancer progression (13).

The anti-tumor activity of immune cells infiltrating tumors led to the development of adoptive cellular immunotherapy administering natural killer (NK) cells, T cells, or genetically modified chimeric antigen receptor (CAR)-T cells in cancer patients (14–17). Clinical results administering different immune cells have been reviewed by others (**Table 1**). However, despite promising results in these studies for some malignancies (26), immune cells do not persist long for other malignancies, and patients end up relapsing (27). Once immune cells achieve the tumor, they will have to face tumor cells and their secretome that may polarize their anti-tumor activity to a pro-tumoral one, increasing angiogenesis and enhancing tumor growth (28). Moreover, after chemotherapy treatment, tumor cells can reach a senescent state, known as therapy-induced senescence (TIS), that shapes the tumor secretome to a variety of pro-inflammatory and angiogenic proteins known as "senescence-associated secretory phenotype" (SASP). The SASP may enhance the immune response at initial stages and contribute to a favorable environment for tumor growth at late stages (29). For example, senescent fibroblasts, much more than pre-senescent fibroblasts, secrete VEGF that causes premalignant and malignant epithelial cells to form tumors, suggesting that although cellular senescence suppresses tumorigenesis early in life, it may also promote cancer (30).

Here, we review how the tumor secretome can shape the immune response achieving a state when immune cells no longer recognize tumor cells and instead, they secrete proteins that breed the TME. We will specifically focus on the impact on T cells, CAR-T cells, and NK cells, which are currently used in adoptive cellular immunotherapy (14–17, 31), and macrophages due to their relevant role in removing dying/senescent tumor cells after cancer treatment (32). The impact of these molecules is summarized in **Table 2**. Moreover, we will review the effect of the tumor secretome in the immune response when tumor cells become senescent due to chemotherapy treatments.

TABLE 1 | Reviews indicating clinical results with different types of immune cells administered in immunotherapy studies in cancer patients.

Types of immune cell administered	Reference
NK cells	(18, 19)
CAR-T cells	(20–22)
TILs:	(23–25)

NK, natural killer; CAR, chimeric antigen receptor; TILs, tumor infiltrating lymphocytes.

IMPACT OF TUMOR SECRETOME IN THE ANTI-TUMOR ACTIVITY OF IMMUNE CELLS

T Cells

Tumor cells with stromal cells, endothelial cells, fibroblasts, and immune cells create a suitable TME that favors tumor progression (79–81). The ability of T cells to infiltrate this TME has led to the development of adoptive cellular immunotherapy to treat cancer patients with tumor-infiltrating lymphocytes (TILs) or CAR-T cells (14, 15, 31). Interestingly, the TME can shape the anti-tumor activity of T cells depending on a variety of secreted molecules. We detail here the impact of some of these released factors.

TGF-β, a highly recognized immunosuppressive cytokine secreted by tumor cells (33), suppresses IFN-γ production by Th1 and effector CD8 T cells, inducing the differentiation of CD4 T cells to both regulatory (T-reg) cells and Th17 cells. T-reg cells that also release TFG-β and IL10 will further suppress the activation of CD8 T cells, promoting tumor cell growth (34, 35). IL10 production by tumor cells down-regulates HLA-I and HLA-II on tumor cells and HLA-II on antigen-presenting cells (APCs), inhibiting antigen presentation becoming an escape mechanism from immune surveillance (42, 82–84). On the other side, cancer models have shown that IL10 also induces intratumoral antigen presentation with infiltration and activation of tumor-specific cytotoxic CD8 T cells expressing IFNγ and granzymes (43) (**Figure 1**).

A wide field of research in cancer immunotherapy consists of inhibiting immune-checkpoint receptors on immune cells and their ligands in tumor cells. The interaction of these receptors/ligands modulates the activity of immune cells to limit the development of auto-immunity and create immunotolerant T cells. Therefore, the inhibition of these interactions with monoclonal antibodies increases their anti-tumor activity. The most common immune checkpoints include cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed death 1 (PD-1), T cell immunoglobulin and mucin-3 (TIM-3), B and T lymphocyte attenuator (BTLA), lymphocyte activation gene 3 (LAG3), adenosine 2A receptor (A2AR) and T cell immunoglobulin and ITIM domain (TIGIT) (85–91). Secreted molecules by tumor cells impact the expression of immune-checkpoint receptors on immune cells. For instance, release of soluble HLA-G by tumor cells up-regulates CTLA-4, PD-1, TIM-3, and CD95 on CD8 T cells impacting their anti-tumor activity (44). On the other hand, cytokines released by activated immune cells can up-regulate ligands of immune-checkpoints in tumor cells. Thus, IFNγ release by activated T cells induces PD-L1 up-regulation in tumor cells (45) (**Figure 2**).

TABLE 2 | Impact of secreted factors in the tumor microenvironment (TME) over the different immune cell populations and description of receptors acting as eat me or don't eat me signals for phagocytic activity of macrophages.

Factor	Type of cell	Effect	Reference
TGFβ	CD8	Suppresses IFN-γ production	(33)
	Th1	Suppresses IFN-γ production and induces differentiation to T-reg and Th17 cells.	(34, 35)
	PB-NK	Converts cytotoxic $CD56^{dim}$ and $CD56^{bright}$ PB-NK cells into dNK-like cells.	(36, 37)
		Added to IL15 and IL18 the effects are enhanced.	(38)
	PB-NK	Down-regulates NKP30, NKG2D and DAP10 and, consequently, NKG2D.	(39, 40)
	PB-NK	At low doses up-regulates CXCR4 and CXCR3. At high doses, down-regulates NKp30, limiting NK killer activity.	(41)
	PB-NK	In combination with hypoxia and 5-aza-2′-deoxycytidine polarizes PB-NK cells to dNK-like cells.	(37)
IL10	APCs	Down-regulates HLA-II on APCs inhibiting antigen presentation.	(42)
	CD8	Induces intratumoral antigen presentation with infiltration and activation of CD8 T cells expressing IFNγ and granzymes.	(43)
HLA-G	CD8	Up-regulates CTLA-4, PD-1, TIM-3, and CD95.	(44)
IFNγ	Tumor cells	PD-L1 up-regulation.	(45)
FGL1	CD8	LAG-3 up-regulation with T cell inhibition.	(46)
Gal-9	Th1	Loss of IFNg producing cells and suppression of Th1 autoimmunity.	(47)
Nectin-3	T cells and monocytes	Promote lymphocyte transmigration through interaction with Nectin-2 on endothelial cells.	(48)
Nectin-2	T cell	T cell homing migration to the spleen through TIGIT interaction.	(49)
	PB-NK	Binds to TIGIT inhibiting NK cell cytotoxicity.	(50)
PVR	PB-NK	Binds to TIGIT inhibiting NK cell cytotoxicity.	(50)
PGE2	CD8	Suppression of activity.	(51)
	CD4	Suppression of Th1 activity and promotion of Th2, Th17 and T-reg.	(51)
	PB-NK	In thyroid cancer and melanoma inhibits NKG2D, NKp44, NKp30, and TRAIL suppressing NK cell cytotoxicity.	(10, 52)
	PB-NK	In melanoma down-regulates NKp44 and NKp30 leading to NK cell inhibition.	(53)
	Macrophages	Reduction of CCL5 production.	(54)
IDO	CART-19	Inhibition of CART cell activity.	(55)
Lactic acid	CD8	Suppresses nutrient uptake leading to impaired activation.	(56)
	NK	Suppresses nutrient uptake leading to impaired activation.	(56)
Glycodelin-A	$CD56^{bright}$ PB-NK	Polarizes $CD56^{bright}$ into dNK-like cells.	(57)
HLA-G	PB-NK	Induction of senescence with SASP secretion promoting vascular remodeling and angiogenesis.	(58)
Hypoxia	T cells	Favors a glycolytic metabolism and increased lactate production, dampening T effector functions.	(59)
	PB-NK	Avoids the ability to upregulate NKp46, NKp30, NKp44, and NKG2D in response to activating cytokines.	(60)
	PB-NK	Degrades NK cell granzyme B by autophagy.	(61)
	PB-NK	Reduced ability to release IFNγ, TNFα, GM-CSF, CCL3, and CCL5, and preservation of immature $CD56^{bright}$ NK cells expressing CCR7 and CXCR4, resembling dNK-like cells.	(62)
	Macrophages	Activates granulin expression in macrophages through VEGF, conferring increased angiogenic potential.	(63)
	Macrophages	In pancreatic cancer promotes release of exosomes containing miR-301a-3p that induce M2 polarization.	(64)
	Macrophages	Induces CXCL12 and CXCR4 expression, which modulate the migration of monocyte-derived macrophages, and TAMs.	(65)
IL6	Macrophage	Induces M2 polarization in colorectal cancer models.	(66)
OSM	Macrophage	M2 polarization *via* mTOR signaling complex 2-Akt1.	(67)
CCL2	Macrophage	Recruitment of M1 to polarize them to metastasis-associated macrophages.	(68)
IL34	Macrophage	Increase recruitment of M2 macrophages in osteosarcoma.	(69)
VEGF-A	Macrophage	With IL10 and IL4 secreted by tumor cells and macrophages, respectively, induced M2 polarization.	(70)
Versican	Macrophage	Activates macrophages to release TNFα enhancing growth of tumor cells.	(71)
MIF	Macrophage	Recruitment of macrophages through TGFβ secretion by Kupffer cells that creates a fibrotic microenvironment.	(72)
ST2	Macrophage	M1 macrophage polarization in models of lung cancer.	(73)
miR-21	Macrophage	Polarization of monocytes to M2 macrophages, secretion of IL6, IL8, CCL2, and CCL5.	(74)
CD47	Macrophage	In tumor cells is a don't eat me signal for macrophages.	(75)
PD-1	Macrophage	Don't eat signal in macrophages.	(76)
β2M subunit (HLA-I)	Macrophage	In tumor cells is a don't eat me signal for macrophages through interaction with LILRB1.	(77)
CD24	Macrophage	In tumor cells is a don't eat me signal for macrophages.	(78)

PB-NK, peripheral blood NK cells; dNK, decidual NK cells; T-reg, regulatory T cell; APCs, antigen presenting cells; IFN-γ, interferon-γ; TGFβ, transforming growth factorβ; FGL1, fibrinogen-like 1; GAL-9, galectin-9; IL, interleukin; HLA, human leukocyte antigen; miR, microRNA; OSM, oncostatin-M; VEGF, vascular endothelial growth factor; MIF, macrophages migration inhibitory factor; ST2, suppression of tumorigenicity 2.

HLA-II over-expression by tumor cells (92) and fibrinogen-like 1 (FGL1), a protein secreted by liver cells and tumor cells (46), are ligands of LAG-3, and their secretion impact the expression of LAG-3 in T cells, promoting an immunosuppressive function. TIM-3 is expressed on Th1 cells, and its interaction with its ligand Galectin-9 (Gal-9) on tumor cells inhibits Th1 cell responses (47) (**Figure 2**). Both overexpression of Gal-9 on gastric cancer cells and expression of TIM-3 on immune cells correlates negatively with poor outcomes in cancer patients (93) and lead to an increase in granulocytic myeloid-derived suppressor cells that inhibit immune responses impacting tumor growth (94).

TIGIT ligands include CD155 (PVR), and the Nectin family (95, 96) (**Figure 2**), which are over-expressed in many human malignancies (97). Specifically, soluble PVR is a valuable

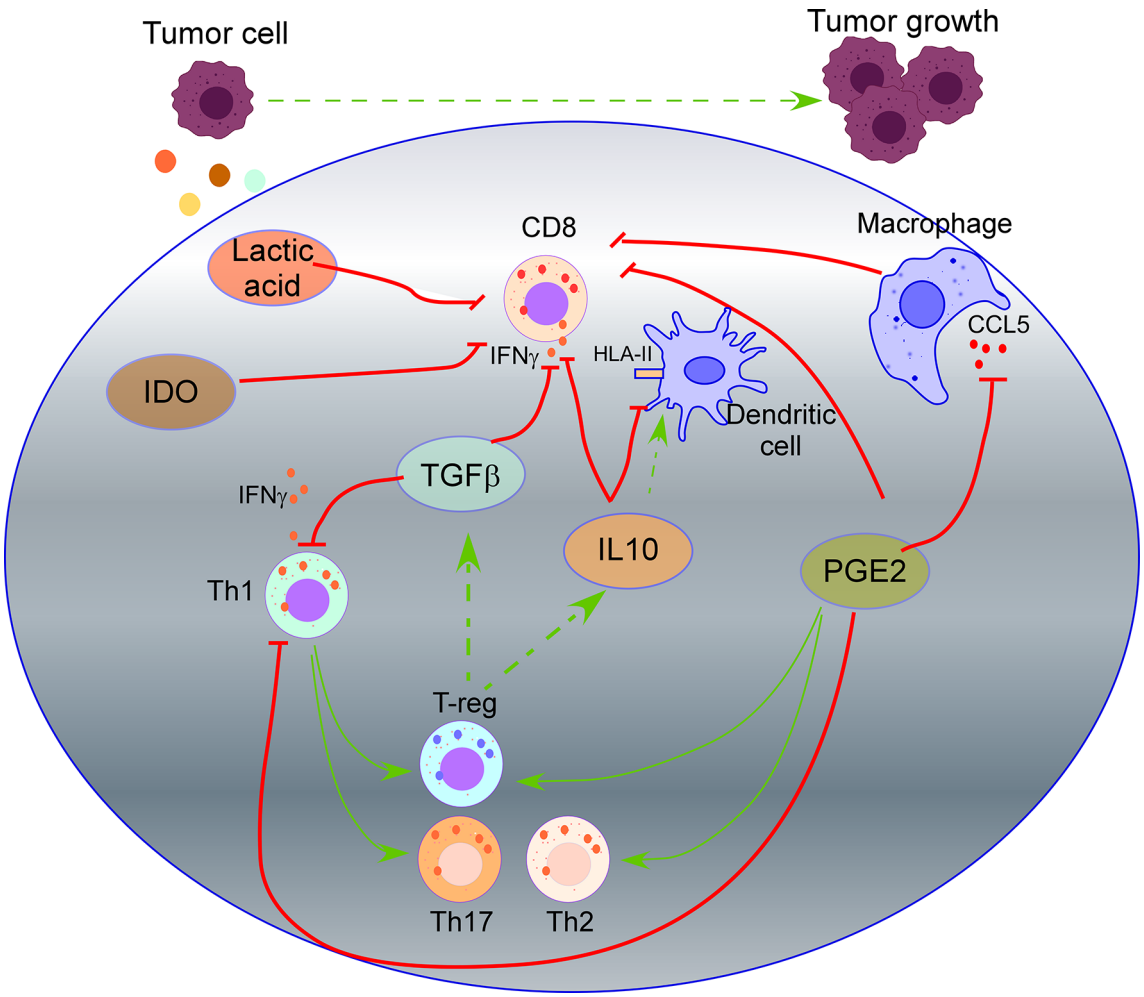

FIGURE 1 | Impact of tumor secretome in T cell activity. TGF-β secreted by tumor cells suppresses IFN-γ production by Th1 and effector CD8 T cells, inducing the differentiation of CD4 T cells to regulatory (T-reg) cells and Th17 cells. T-reg cells also release TFG-β and IL10 that will suppress the activation of CD8 T cells. IL10 secreted by tumor cells down-regulates HLA-II on dendritic cells, inhibiting antigen presentation. Prostaglandin 2 (PGE2) secreted by tumor cells suppresses the functions of CD8 T cells and Th1 cells, and promotes Th2, Th17, and T-reg cell response. PGE2 reduces CCL5 production by macrophages, which is required for T cell proliferation. Secretion of Indoleamine 2,3 dioxygenase (IDO) by tumor cells produces metabolites that inhibit T cell activity. Lactic acid produced by tumor cells suppresses nutrient uptake by CD8 T cells.

biomarker for cancer development, where higher soluble PVR levels are detected in lung, gastrointestinal, breast, and gynecologic cancers compared to healthy donors, being even higher at advanced stages of the disease (98). Of interest, Nectins promote the transendothelial migration of cells and associate with poor prognosis and advanced disease stages in different types of cancer (99). Soluble Nectin-4 released by cancer cells interacts with integrin-β4 on endothelial cells, promoting angiogenesis (100). Of interest, Nectins also mediate transendothelial migration of immune cells (48). For instance, Nectin-2 promotes endothelial cell migration, endothelial tube formation, and T cell homing migration to the spleen, promoting an angiogenic function (49); Nectin-3 expressed by T cells and monocytes binds to endothelial cells through Nectin-2 promoting the transmigration of immune cells (48). This angiogenic function of soluble Nectins released by tumor cells suggests an essential role of the tumor secretome polarizing the cytotoxic activity of T cells to an angiogenic one.

Prostaglandin 2 (PGE2) is a crucial mediator of immunopathology in chronic infections and cancer. PGE2 secreted by tumor cells suppresses the effector functions of CD8 T cells and Th1 cells, promotes Th2, Th17, and T-reg cell response, and inhibits the attraction of immune cells (51). Moreover, PGE2 reduces CCL5 production by macrophages (54), which is required for IL2, IFN-γ production, and T cell proliferation (101). Recent studies revealed that COX2/mPGES1/PGE2 pathway in tumor cells up-regulates PD-L1 in tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs), which is followed by T cell elimination (102).

In addition, the tumor secretome impacts the metabolic activity of T cells through the competitive removal of essential

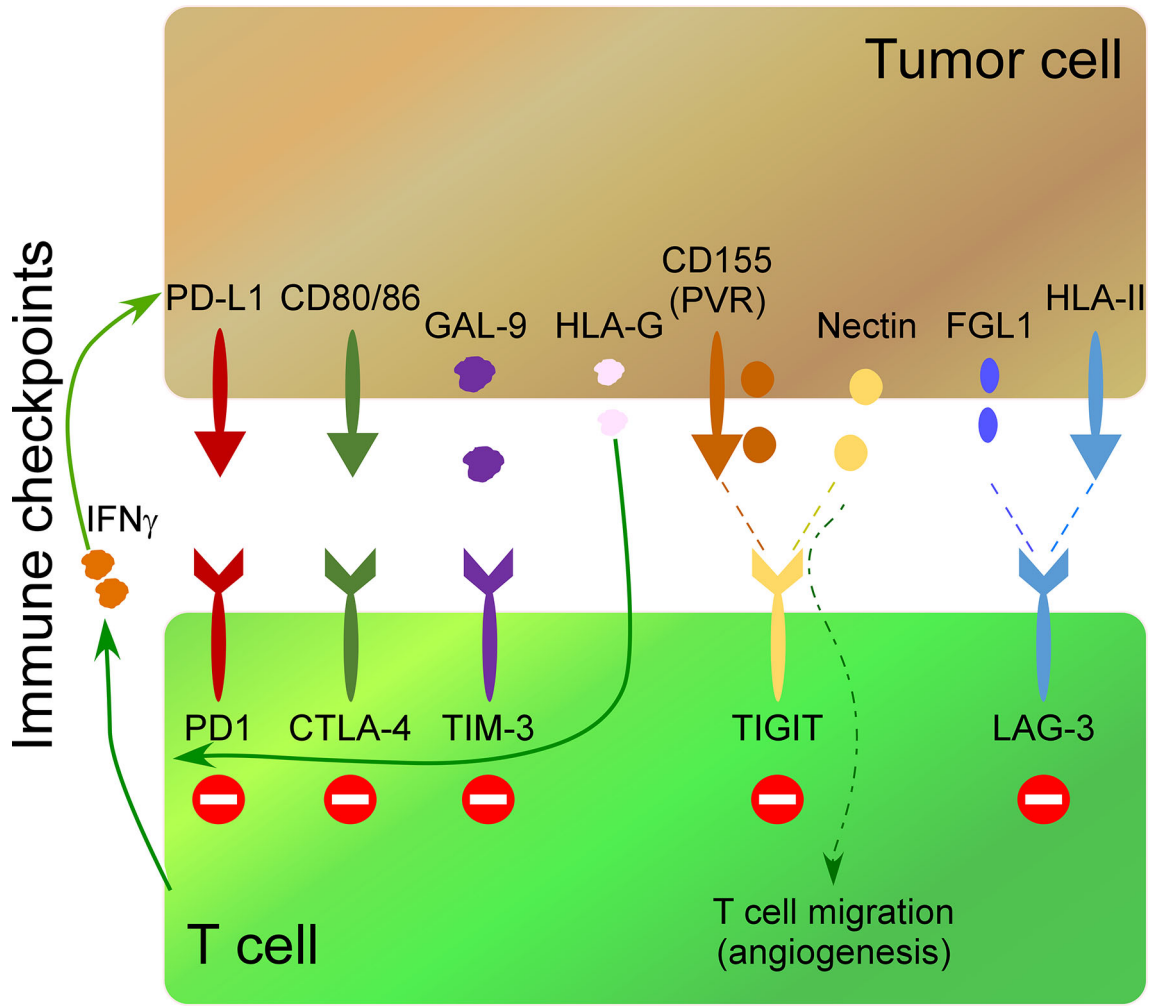

FIGURE 2 | Impact of some secreted molecules in the TME on the expression of immunocheckpoints in T cells. The most common immune checkpoints on T cells include programmed death 1 (PD-1), cytotoxic T lymphocyte antigen 4 (CTLA-4), T cell immunoglobulin and mucin-3 (TIM-3), T cell immunoglobulin and ITIM domain (TIGIT) and lymphocyte activation gene 3 (LAG3), which interact with their ligands on tumor cells. IFNγ release by activated T cells induces PD-L1 up-regulation in tumor cells. TIM-3 interaction on Th1 cells with Galectin-9 (Gal-9) on tumor cells inhibits Th1 cell responses. Soluble HLA-G released by tumor cells up-regulates PD-1, CTLA-4, and TIM-3, on T cells. CD155 (PVR), and the Nectin family are ligands of TIGIT. Soluble PVR is released by tumor cells. Soluble Nectins released by cancer cells mediate transendothelial migration of immune cells promoting angiogenesis. HLA-II over-expression by tumor cells and fibrinogen-like 1 (FGL1) secreted by tumor cells impact the expression of LAG-3 in T cells.

nutrients for T lymphocytes. In this sense, secretion of "Indoleamine 2,3 dioxygenase" (IDO), which catalyzes tryptophan degradation, produces metabolites that inhibit T cell activity. In a murine lymphoma model with CAR-T cells targeting CD19, over-expression of IDO depleted the anti-tumor activity of CAR-T cells and inhibited the cytokine-dependent expansion of CAR-T cells, cytokine secretion, and increased their apoptosis (55) (**Figure 1**). Production of lactic acid by tumor cells also inhibits the activity of CD8 T cells and NK cells. In detail, most tumors rely on glycolytic metabolism to sustain rapid cell growth through the enzyme lactate dehydrogenase-A that produces lactic acid. CD8 T cells and NK cells undergo a similar metabolic switch activating a glycolytic metabolism when they evolve from a naive to an activated state. However, highly glycolytic tumor cells are superior competitors for glucose and amino acids than CD8 T cells and NK cells. In addition, lactic acid production further suppresses nutrient uptake by CD8 T cells and NK cells, dampening their metabolic programs, leading to impaired activation of CD8 T cells and NK cells with the subsequent overcoming of immune surveillance by tumor cells (56).

CAR-T Cells, a Strategy to Inhibit the Immunosuppressive TME and the Impact of Tumor Secretome

Adoptive cellular immunotherapy administering CAR-T cells has achieved outstanding and permanent responses in pediatric-B cell hematological malignancies with persistence of CAR-T cells over the years (26). However, in other hematological

malignancies (15, 27) and solid tumors, results have been more inferior due to a short persistence of CAR-T cells and the barriers that CAR-T cells have to face in the TME, such as the impact of the tumor secretome. Fourth-generation CAR-T cells, termed armored or TRUCK CARs, are equipped with different features that can remodel the TME to enhance the activity of CAR-T cells.

Thus, a variety of armored CAR-T cells that secrete different cytokines have been developed. For instance, CART-19 cells that secrete IL12 show increased cytotoxicity and resistance to T-reg cell-mediated inhibition, better engraftment, and enhanced anti-tumor activity in models of B-cell malignancies (103) and ovarian cancer (104). Of note, severe adverse events were observed in a clinical trial with TILs secreting IL12 (105). Therefore, decreasing the amount of cytokines released by CART cells, in this case, IL12, could be modulated *via* different gene-expression cassettes, such as promoters in the CAR with inducible nuclear factor of activated T cells (NFAT) binding motifs (106). IL15 enhances the differentiation, homeostasis, and survival of T cells and NK cells. CART-19 cells secreting IL15 demonstrated increased expansion and efficacy, with decreased apoptosis and PD-1 expression, in models of Burkitt lymphoma (107). CAR-T cells secreting IL18 have caused increased M1-polarization in macrophages of the TME, depletion of M2-macrophages and T-reg cells (108), and recruitment of endogenous T cells (109). Nevertheless, as IL18 is pro-inflammatory, it has pathogenic roles in autoimmune diseases (110) and might also promote tumor progression, angiogenesis, immune escape, and metastasis (111). CAR-T cells secreting IL7 and CCL19 have also improved cell infiltration of dendritic cells (DCs) and survival of CAR-T cells (112). In addition, inhibition of TGFβ is achieved by co-expression in the CAR of a dominant-negative receptor for TGFβ that blocks TGFβ signaling, increasing proliferation and persistence of CAR-T cells in models of prostate cancer (113).

Armored CAR-T cells also avoid the negative impact of immune checkpoints. Thus, in lymphoma, the TME is marked by exacerbated lymphoid stroma activation and increased recruitment of follicular helper T cells, resulting from the disruption of the inhibitory checkpoint HVEM/BTLA. Secretion of HVEM by CAR-T cells binds BTLA avoiding this event (114). In addition, CAR-T cells that secrete anti-PD-L1 antibodies prevent T cell exhaustion and recruit NK cells to the tumors (115).

Furthermore, hypoxia is found in the TME and contributes to the rapid growth of tumor cells. Under hypoxia, glucose is fermented to lactate. The hypoxic TME also favors a glycolytic metabolism and increased lactate production, dampening T and NK cell effector functions and survival (59). Thus, armored CAR-T cells that secrete catalase (CAT-CAR) overcome hypoxia and reactive oxygen species (ROS) present in the TME (116). Another option to overcome these obstacles is to modify the CAR to express anti-oxidant factors such as N-acetylcysteine (NAC) that reduces DNA damage in CAR-T cells lowering activation induced-cell death in CAR-T cells (117).

Decidual-Like NK Cells: An NK Cell Population Poorly Studied in Immunotherapy

The well-recognized anti-tumor activity of NK cells has led to many clinical studies administering either NK cells or CAR-modified NK cells, although results to date have shown mainly safety but not a high efficacy (18). These findings suggest the need to optimize NK cell anti-tumor efficacy. Here, we present studies that indicate that when NK cells arrive at the TME, events might happen that modify their killer activity.

In this regard, there are two main populations of NK cells in peripheral blood, the mature and cytotoxic NK with $CD56^{low}CD16^{high}$ expression, which constitutes 90% of NK cells, and the immature and immunoregulatory NK cells characterized by $CD56^{high}CD16^{low/neg}CD25^{+}$ expression, which comprise approximately 10% of peripheral blood (PB)-NK (18, 19). A third transient population, known as decidual NK (dNK) cells, present at the fetal-maternal interface during the first months of pregnancy, representing 70% of immune cells in the decidua. dNK cells are also known as uterine NK (uNK) cells, as classically, uNK cells were detected by Dolichos biflorus agglutinin (DBA) lectin staining, where DBA+ cells were defined as dNK cells. Decidualization is triggered during blastocyst implantation and the menstrual cycle, characterized by a marked increase in dNK cells. dNK or uNK cells are a dynamic population, and their origin is not clear. A recent model proposed that there is a first wave of proliferation of tissue-resident NK cells in the pregnant uterus at the onset of the decidualization process. Then, a second wave involves the recruitment of conventional PB-NK cells during the placentation process (118, 119).

dNK cells are immune-tolerant and characterized by $CD56^{bright}CD16^{-}CD9^{+}CD49a^{+}$ and $Eomes^{+}$ expression (120, 121). They are angiogenic, regulate trophoblast invasion and vascular growth during the placental developmental process and cooperate with other cells to serve as constructive elements during early pregnancy. dNK cells produce large amounts of proangiogenic factors, including VEGF, PlGF, CXCL8, IL-10, and angiogenin, critical for decidual vascularization and spiral artery formation (122). dNK cells also express chemokine receptors, including CXCR3, CXCR4, CCR1, CCR9, and the integrin ITGA3 (120), and through the interaction of HLA-G on fetal trophoblast cells with ILT2 and KIR2DL4, they secrete other growth-promoting factors, including pleiotrophin and osteoglycin (121). Moreover, interaction of soluble HLA-G with KIR2DL4 induces a pro-inflammatory response in dNK cells, activating their senescence with SASP secretion that promotes vascular remodeling and angiogenesis in early pregnancy (58).

This "nurturing" role of dNK cells during early pregnancy presents many homologies to NK cells infiltrated in different types of tumors. Thus, a subset of NK cells in non-small cell lung cancer, squamous cell carcinoma, or colorectal cancer turns into dNK-like cells inducing human umbilical vein endothelial cell migration and formation of capillary-like structures (36, 123–125). Various studies have tried to determine different factors during early pregnancy that might be responsible for this polarization of PB-NK cells into dNK-like cells. Results suggest that this polarization seems more specific for CD56bright than for CD56dim NK cells. Of interest, NK cells administered in immunotherapy treatments undergo an *in vitro* expansion that

turns them into CD56^{bright} NK cells (17). Many of the factors responsible for this NK polarization are present in both the decidua and the TME, suggesting that these events occurring in the TME might impact the growth of tumor cells. In the next section, we detail the effect of secreted factors in the TME over the phenotype and polarization of NK cells.

Impact of the Tumor Secretome in the PB-NK Cell Activity and Their Transition of Killer NK to dNK-Like Cells

Glycodelin-A is secreted in large amounts in the decidua and by tumor cells in malignancies, such as Non-Small Cell Lung Cancer (126), mesothelioma (127), ovarian cancer (128), and endometrial cancer (129). Glycodelin-A converts immunoregulatory CD56^{bright} PB-NK cells into dNK-like cells, an effect that does not occur for mature CD56^{low} PB-NK cells. This mechanism occurs through binding of Glycodelin-A to sialylated glycans on CD56^{bright} NK cells and causes enhanced expression of CD9, CD49a, and production of VEGF and IGFBP-1 that regulate endothelial cell angiogenesis and trophoblast invasion (57).

Soluble HLA-G is associated with bad prognosis in different tumors (130–134). Of interest, soluble HLA-G mediates polarization of PB-NK cells to dNK-like cells, with a senescent phenotype, secretion of growth factors, and reduced killer activity (58), thus, emerging as an essential target that can polarize the activity of NK cells.

TGFβ secretion can be beneficial at early stages and detrimental at late-stage tumor development by remodeling the TME to favor tumor growth (130, 135). TGFβ converts both cytotoxic CD56^{dim} and CD56^{bright} PB-NK cells into dNK-like cells (36, 37) (**Figure 3**). Moreover, IL15 and IL18 added to TGFβ enhance the impact on the polarization of PB-NK cells toward a dNK cell phenotype with increased expression of CD9, CD49a, ITGA3, and CXCR4 (38). Of interest, as previously mentioned, IL15 and IL18 are beneficial for CAR-T cells (107–109), suggesting the negative role of these cytokines when TGFβ is added. Additional effects of TGFβ over NK cells include down-regulation of NKP30, NKG2D (39), and DAP10 and, consequently, NKG2D (40) inhibiting NK cell function (**Figure 3**). Of interest, this dual role of TGFβ in the TME is observed when at low doses facilitates NK cell recruitment to the tumor by up-regulating CXCR4 and CXCR3, markers of dNK; and at high doses, down-regulates NKp30, limiting NK killer activity (41).

PGE2 secretion in thyroid cancer and melanoma inhibits the expression of NKG2D, NKp44, NKp30, and TRAIL on PB-NK cells and their functional maturation leading to suppressed NK cell cytotoxicity (10, 52) (**Figure 3**). PGE2 release by cancer-associated fibroblasts in melanoma down-regulates NKp44 and NKp30 leading to NK cell inhibition (53). Soluble PVR and Nectin-2 released by tumor cells bind to TIGIT on NK cells inhibiting NK cell cytotoxicity (50).

Hypoxia is another factor present in both the decidua and the TME. Hypoxia in the TME avoids the ability of NK cells to upregulate NKp46, NKp30, NKp44, and NKG2D in response to activating cytokines (60) and degrades NK cell granzyme B by autophagy (61), impairing the ability to kill and promoting immune evasion (**Figure 3**). Moreover, exposure to a combination of hypoxia, TGFβ, and 5-aza-2′-deoxycytidine, results in the polarization of PB-NK cells to dNK-like cells. These changes are more pronounced when all the factors are together and lead to the expression of CD9, CD49a, chemokine receptors, and VEGF secretion that leads to dNK-like cells with capacity to promote invasion of trophoblast cell lines and reduced cytotoxicity. Significantly, these parameters are reversed after returning to normal conditions, indicating the plasticity of immune cells (37). Exposure of PB-NK cells to hypoxia also causes reduced NK cell ability to release IFNγ, TNFα, GM-CSF, CCL3, and CCL5, and preservation of immature CD56^{bright} NK cells expressing CCR7 and CXCR4, resembling dNK-like cells (62).

The impact of these tumor secreted factors occur mainly on CD56^{bright} PB-NK cells, and NK cells used in immunotherapy undergo an *in vitro* expansion that turn them into CD56^{bright} NK cells (17). These events suggest that in cases that NK cells do not achieve complete removal of tumor cells they might have polarized into dNK-like cells. Therefore, monitoring these changes in immunotherapy NK cell studies will provide relevant information to improve the clinical outcome of patients.

Role of Macrophages in Immune Surveillance

Macrophages are innate immune cells with high plasticity which traditionally, have been classified as two extremes being either pro-inflammatory (M1: activated) or anti-inflammatory (M2: alternatively activated). M1 inhibits cell proliferation and causes tissue damage, while M2 promotes cell proliferation and tissue repair. M1 and M2 enable Th1, and Th2 responses, respectively, and consequently, Th1 and Th2 cytokines regulate their activity. Thus, M1 responds to IFN-γ, TNF-α, and TLR4 activation, and M2 to IL-4 and IL-13 (136). However, macrophages present high plasticity and convert to a wide variety of subpopulations depending on the stimuli they receive from the TME (63, 137). Macrophages represent the largest population of all infiltrating leukocytes in the tumor (138), where tumor-associated macrophages (TAMs), which present an M2-like phenotype, are considered highly responsible for tumor progression, and many studies have focused on trying to polarize M2-like macrophages to M1 (139). However, M2 are the macrophages with the highest phagocytic activity against apoptotic tumor cells (140), suggesting that removing this activity might also be detrimental. Therefore, efforts should be directed to preserve M1 macrophage activity while also enhancing the phagocytic activity of M2 macrophages. Here, we will pay special attention to the phagocytic function of M2 macrophages to remove tumor cells and how secreted molecules in the TME can polarize macrophages to an M2-like or M1 phenotype.

Phagocytosis of tumor cells by macrophages is performed after recognizing "eat me" or don't eat me" signals that will or will not trigger phagocytosis. "Eat me" and "don't eat me" signals act as ligands for phagocytic receptors that will or will not trigger the engulfment of the target. Different studies have shown the beneficial impact in tumor regression of inhibiting these "don't eat me" signals. For instance, CD47 expression in small-cell lung cancer cells engages SIRPα on macrophages inhibiting their phagocytic activity, which is recovered with an anti-CD47 (75).

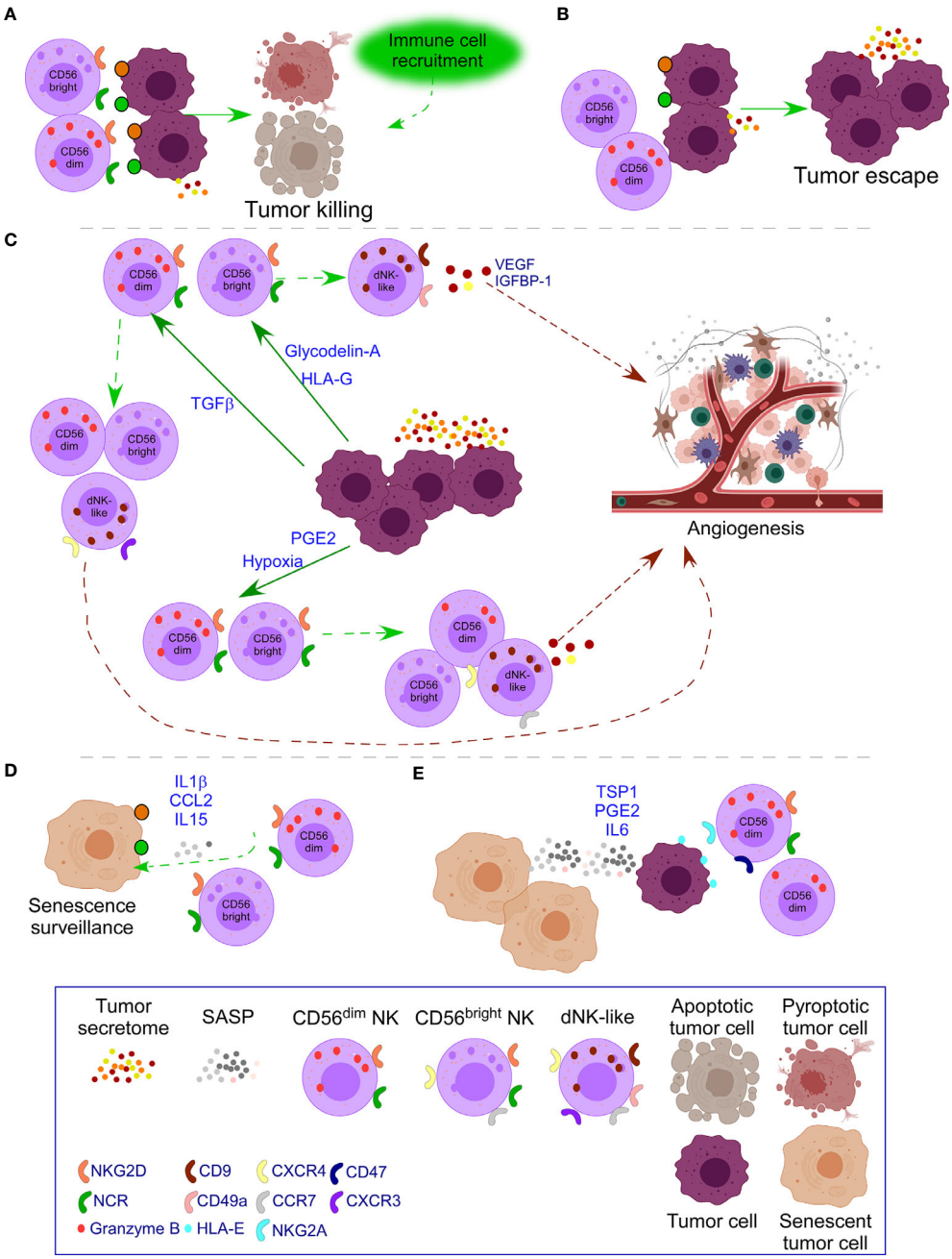

FIGURE 3 | Impact of tumor secretome in NK cell activity. **(A)** In healthy conditions, NK cells recognize transformed cells through ligands of NKG2D and the family of NCR receptors (NKp30, NKp44, NKp46) which are over-expressed in transformed cells. Pro-inflammatory forms of cell death attract additional immune cells to cooperate in the killing. **(B)** In some cases, tumor cells down-regulate ligands for NK cell receptors or the tumor microenvironment (TME) causes down-regulation of activating NK cell receptors leading to tumor escape with additional secretion of tumor secretome. **(C)** When tumor escape occurs, increased tumor secretome leads to additional changes in NK cells. Specifically, release of Glycodelin-A and HLA-G converts immunoregulatory $CD56^{bright}$ PB-NK cells into dNK-like cells. TGFβ converts both cytotoxic $CD56^{dim}$ and $CD56^{bright}$ NK cells into dNK-like cells; and down-regulates NK cell activating receptors limiting NK killer activity. PGE2 and hypoxia inhibit the expression of NK cell activating receptors and their functional maturation leading to suppressed NK cell cytotoxicity. Moreover, hypoxia, preserves immature $CD56^{bright}$ NK cells with expression of receptors of dNK cells, resembling to dNK-like cells. In all cases, dNK-like cells will activate angiogenesis processes. **(D)** Emergence of senescent tumor cells leads to SASP secretion that attracts NK cells to mediate their clearance. **(E)** When the number of senescent cells increases, the SASP also does, leading to inhibition of NK cell activity, through mechanisms, such as the interaction of HLA-E with the inhibitory receptor NKG2A in NK cells and binding of TSP1 with CD47 that inhibit NK cell activity. PGE2 and IL6 in the SASP also down-regulate NK cell activating receptors. Moreover, therapy-induced senescence in established tumors down-regulates NK cell activating receptors on mature NK cells and their ligands on tumor cells.

Moreover, inhibition of CD47 in tumor cells promoted their phagocytosis and the anti-tumor activity of CD8 T cells while inhibiting T-reg cells (141). Blocking PD-1 expressed in TAMs or M2-like macrophages increases macrophage phagocytosis and reduces tumor growth (76) (**Figure 4**).

Furthermore, anti-PD-L1 treatment reverses the immunosuppressive status of the TME and enhances specific T cell anti-tumor effects in murine models of cancer (142). Interaction of β2M subunit of HLA-I in tumor cells with LILRB1 on macrophages protects tumor cells from phagocytosis by TAMs, and disruption of this interaction potentiates phagocytosis of tumor cells (77). In ovarian cancer and triple-negative breast cancer, tumor cells evade clearance by macrophages through over-expression of CD24 that interacts with Siglec-10 in TAMs, and its blockade augments the phagocytosis of CD24-expressing tumors leading to a reduction of tumor growth (78). Dectin-2, a C-type lectin receptor in macrophages resident in the liver (Kupffer cells), promotes phagocytosis of cancer cells, avoiding liver metastasis (143) (**Figure 4**).

Phagocytosis requires an intimate contact of the macrophage and the target, where the glycocalyx, a layer that surrounds the plasma membrane containing glycolipids, glycoproteins, and surface-associated glycosaminoglycans, acts as a barrier for these contacts. The size and charge of this glycocalyx can be modified and modulated by enzymes or other molecules present on the TME to promote phagocytosis (144). Of interest, we described that NK cells release histones that bind to and degrade the syndecans on the glycocalyx of multiple myeloma cells (145), suggesting that by doing this, NK cells might also promote the phagocytosis of tumor cells by macrophages, an event observed during fungal infection (146).

Molecules secreted in the TME will also impact promoting an anti-inflammatory or pro-inflammatory environment that will

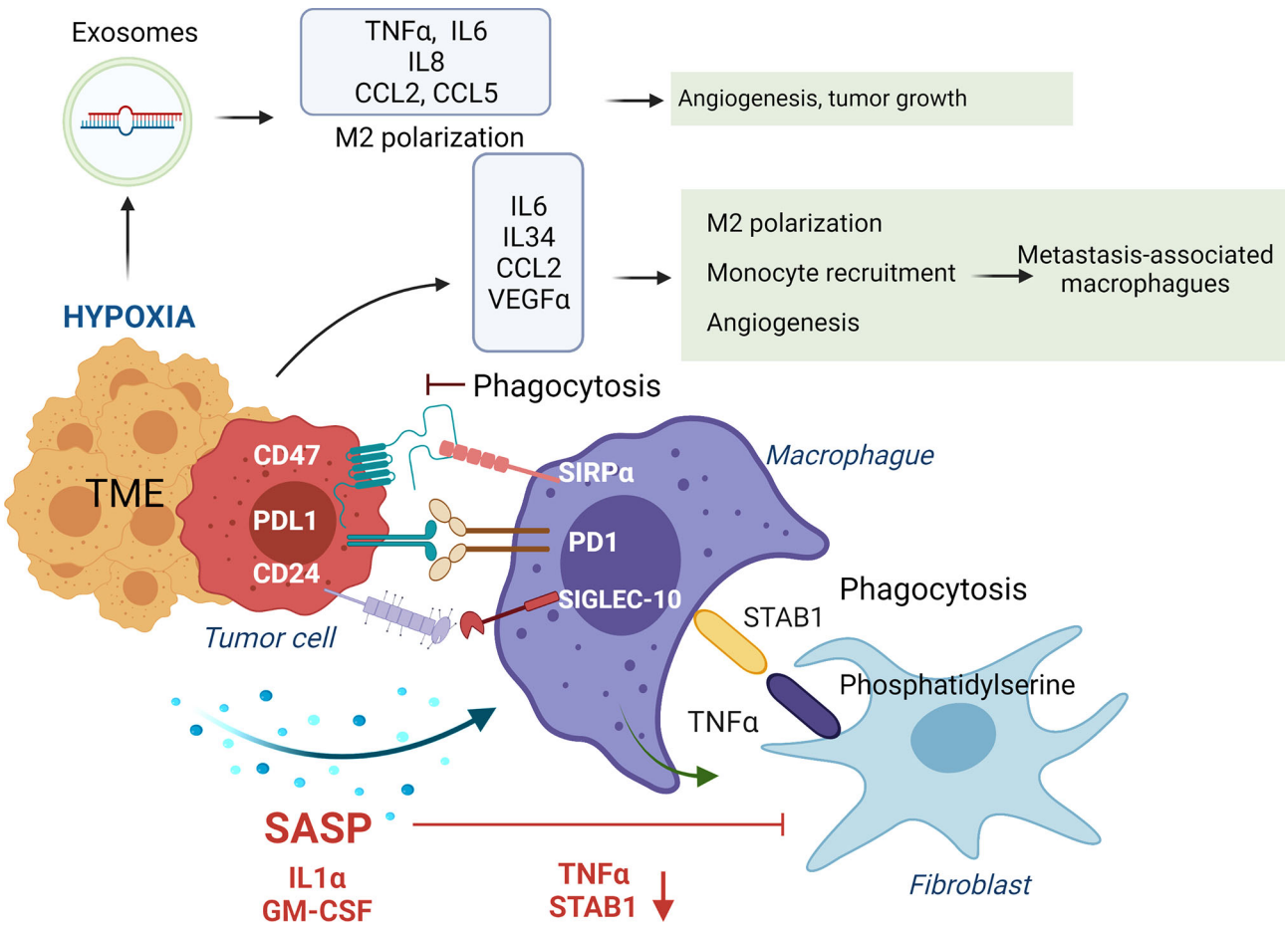

FIGURE 4 | Impact of tumor secretome in the phagocytic activity of macrophages. In healthy conditions macrophages phagocyte transformed cells and senescent fibroblasts to maintain tissue homeostasis. Normally, macrophages, through release of TNFα, induce apoptosis in senescent fibroblasts, leading to expression of phosphatidylserine in their surface, which is recognized by STAB1 on macrophages to promote their phagocytosis. In advanced stages of senescence, phagocytic activity of macrophages is inhibited by over-expression of ligands of immune-checkpoints (CD47, PDL-1 and CD27) that interact with their receptors on macrophages (SIRPα, PD1 and SIGLEC-10). Moreover, SASP factors, including IL1α and GM-CSF, down-regulate STAB1 and TNFα expression, avoiding the phagocytosis of senescent fibroblasts by macrophages. In addition, IL6, IL34, CCL2 and VEGFa secretion in the TME, induce M2 macrophage polarization and recruitment of inflammatory monocytes that polarize to metastasis-associated macrophages that in summary promote tumor growth. Hypoxia in established tumors also promotes the release of exosomes containing the miRNAs miR-301a-3p and miR-21 that promote M2 polarization, and TNFα, IL6, IL8, CCL2 and CCL5 secretion impacting in higher angiogenesis, and tumor growth.

polarize macrophages into TAMs/M2-like or M1 phenotypes. For instance, IL6 secretion in the TME induces M2 macrophage polarization in colorectal cancer models (66). The release of oncostatin M in the TME is involved in M2 polarization *via* mTOR signaling complex 2-Akt1 (67). At breast cancer, the release of CCL2 by tumor cells recruits inflammatory monocytes that polarize to metastasis-associated macrophages, which secrete CCL3, promoting lung metastasis (68). IL34 secretion by tumor cells binds to CSF1R in macrophages and polarizes them to M1 and M2 (147). Also, IL34 contributes to osteosarcoma growth by increasing the neo-angiogenesis and recruitment of M2 macrophages (69). In a skin carcinogenesis model, VEGF-A expression on tumor cells with IL10 and IL4 secreted by tumor cells and macrophages, respectively, induced M2 polarization that promoted tumor growth (70). Release of the proteglycan versican by lung carcinoma cells activates macrophages to release TNFα enhancing growth of tumor cells (71) (**Figure 4**).

Tumor hypoxia, a feature of the TME, promotes ID4 expression in cancer cells which, through VEGF, activates increased expression of granulin in macrophages, conferring increased angiogenic potential (63). In pancreatic cancer cells, the presence of hypoxia promotes the release of exosomes containing the miRNA miR-301a-3p that binds to TLR macrophages receptors, promoting M2 polarization, TNFα, and IL6 production, creating a pro-metastatic environment (64). Hypoxia induces CXCL12 and CXCR4 expression, which modulate the migration of monocytes, monocyte-derived macrophages, and TAMs (65) (**Figure 4**). Of interest, when hypoxia is absent in tumor cells, TAMs can enhance tumor hypoxia and glycolysis (148), being both features that promote tumor aggressiveness (149).

Exosomes released in liver tumors bind to macrophages through exosome integrins and prepare the pre-metastatic niche (150). In pancreatic ductal adenocarcinomas, tumor-derived exosomes with macrophage migration inhibitory factor are taken by Kupffer cells causing TGFβ secretion. Consequently, a fibrotic microenvironment emerges that recruits macrophages, creating a liver pre-metastatic niche (72). Of interest, the release of ST2 in Rab37 exosomes skewed M1 macrophage polarization leading to reduced tumor growth in models of lung cancer. Moreover, lung cancer patients with low Rab37, low soluble ST2, and low M1/M2 ratio presented worse overall survival (73). SNAIL, a transcription factor expressed during epithelial-mesenchymal transition, activates the production of tumor-derived exosomes containing miR-21 that will be phagocyted by monocytes leading to M2 macrophages, secretion of IL6, IL8, CCL2, and CCL5 impacting in higher angiogenesis, and tumor growth (74) (**Figure 4**).

ACQUISITION OF THERAPY INDUCED-SENESCENCE (TIS) AFTER CHEMOTHERAPY AND ITS IMPACT ON IMMUNE CELLS

Studies have demonstrated that chemotherapy treatment can lead to acquired resistance and the emergence of more aggressive tumor cells. In this regard, the tumor secretome is shaped by chemotherapy treatment that will impact the immune response and increase tumor aggressiveness. For instance, in breast cancer, IL6 release after treatment converts differentiated tumor cells to cancer stem cells through the IL6-JAK1-STAT3 pathway (151). In non-small cell lung cancer, cisplatin induces IL6 secretion that increases tumor progression and resistance to treatment through up-regulation of anti-apoptotic proteins and DNA repair associated genes (152). Paclitaxel enhances IRE1 RNase activity that leads to the production of IL6, IL8, CXCL1, GM-CSF, and TGFβ2 in breast cancer cells contributing to the expansion of tumor-initiating cells (153). Doxycycline treatment in squamous cell carcinoma leads to TGFβ secretion that activates the TGF-β/SMAD pathway increasing tumorigenic potential (154). Treatment with kinase inhibitors causes secretion of positive mediators of the AKT pathway, including IGF1, EGF, ANGPTL7, and PDGFD, accelerating the expansion and dissemination of drug-resistant clones (155). Docetaxel induces secretion of extracellular vesicle-encapsulated miRNAs, including miR-9-5p, miR-195-5p, and miR-203a-3p, which down-regulate the transcription factor ONECUT2, leading to up-regulation of stemness-associated genes, that stimulate cancer stem-like cells and resistance to therapy in breast cancer (156).

In addition, chemotherapy and radiotherapy treatments trigger a premature state of senescence in tumor cells termed "therapy-induced senescence" (TIS) that will shape the tumor secretome (29, 157). TIS can reactivate the cell cycle and bring on cancer daughter cells that survive therapy more transformed than the original population (158, 159). This secretome is unique because it is induced by senescence, being termed senescence-associated secretory phenotype (SASP). SASP includes various cytokines, chemokines, growth factors, and matrix metalloproteinases, such as IL1α, IL1β, IL6, IL8, CXCL1, CCL2, VEGF, and CXCR2 (29, 160, 161), that interfere with the paracrine activity of senescent cells. Of interest, SASP released by tumor cells after TIS induces transmission of senescence to non-senescent neighboring cells (162, 163). The SASP can foster an immunosuppressive environment favoring metastasis (160), and on the other side, attracts immune cells including macrophages, neutrophils, and NK cells to remove senescent cells, a process known as "senescence surveillance" (164–167).

Moreover, cancer is associated with aging. A physiological consequence of aging is the development of immunosenescence due to a functional degradation of the thymus, resulting in decreased functional naïve CD4 and CD8 T cells and a peripheral oligo-clonal expansion of memory T cells. These events provide a contracted T cell antigen receptor (TCR)-repertoire diversity with secretion of SASP (29, 168). Immunosenescence associated with aging also occurs due to exposure to virus infections or chronic inflammation (169); and additional factors such as nutrition, sex, genetics, previous diseases, or tumors (170, 171). Therefore, the immune cells of elderly cancer patients will probably be already senescent; and moreover, SASP secretion by senescent tumor cells after chemotherapy will accelerate this immunosenescence process

(171). Here, we will mention some SASP factors released by tumor cells that impact the anti-tumor immune response.

Impact of the SASP in T Cells and Immunosenescent T Cells

Studies have shown a significant accumulation of senescent T cells in certain types of cancer patients (172), and that tumor SASP induces T cell senescence leading to suppression of responses of naïve/effector T cells (173), suggesting that this might be a strategy used by malignant cells to evade immune surveillance. Transformed senescent T cells are in cell cycle arrest and develop significant phenotypic alterations, such as down-regulation or loss of CD27 and CD28.Through SASP factors including pro-inflammatory cytokines or inhibitory molecules like IL10 or TGFβ, senescent T cells will amplify the immunosenescence process. Moreover, the development of exhaustion with high expression of immune checkpoints, such as TIM-3 and other co-inhibitory receptors as CD57 or KLRG-1, will promote replicative senescence of T cells (174).

TGFβ1 and TGFβ3 are early SASP factors that regulate thymic T cell homeostasis, inhibit cytotoxic T cell proliferation, and promote T-reg generation (175). Tumor senescent cells up-regulate NOTCH1 and drive a TGFβ-rich secretome that suppresses the release of a pro-inflammatory SASP and contributes to the transmission of senescence through cell-cell interaction *via* NOTCH-JAG1 pathway. Of interest, NOTCH1 inhibition recovers the secretion of pro-inflammatory cytokines, promoting lymphocyte recruitment and senescence surveillance (176). Senescent cells, after genotoxic stress, secrete IL6 and IL8 that promote epithelial-mesenchymal transition, increasing tumor cells' invasiveness. Moreover, IL6 recruits myeloid cells that inhibit T cell responses (177).

MAPK signaling is a relevant pathway that controls T cell senescence (178) through activation of p53, p21, and p16 (179). Recent research demonstrated that tumor-derived T-reg cells exhibit an accelerated glucose uptake, competing with effector T cells for glucose through TLR8 signaling, leading to MAPK activation, which induces T cell senescence (180). Another study showed that T-reg cells, through p38, ERK1/2 signaling, p16, p21, and p53 induce senescence in responder naïve and effector T cells. This event is reverted by the block of TLR8 signaling and or by specific ERK1/2 and p38 inhibition (181). Moreover, the p53 isoforms Δ133p53 and p53β regulate proliferation and senescence in human T lymphocytes. Thus, decreased Δ133p53 and increased p53β expression in healthy individuals and lung cancer patients associated with age-dependent accumulation of senescent CD8 T cells (182).

The hypoxic TME leads to the accumulation of adenosine and tumor-derived cAMP. This cAMP is a SASP factor that induces T cell senescence in naïve/effector T cells. Of interest, activation of TLR8 signaling in tumor cells reverses this event resulting in enhanced anti-tumor immunity (183). Moreover, the accumulation of adenosine in the TME also inhibits the anti-tumor activity of T cells through the adenosine receptor A2AR, which in healthy conditions regulates immune cells protecting from inflammatory damage (184).

CAR-T Cells

Whereas the immunosenescence process has been widely studied in T cells, there is a lack of information related to CAR-T cell senescence. It could be exciting to delve into the mechanisms of senescence of this type of cells to find pathways to inhibit senescence without impacting their anti-tumor activity. Specifically, CAR-T cells undergo a significant *in vitro* expansion (185) to obtain enough CAR-T cells to treat the patients. This expansion might impact the development of senescence due to continuous *in vitro* proliferation. Moreover, the transfer of senescence from tumor cells in the TME mediated by cell-cell contact or through factors present in the SASP will impact CAR-T cell activity. CAR-T cells can be engineered to avoid these events. Thus, recently, CAR-T cells have been used as senolytic agents in lung adenocarcinoma to remove chemically induced senescent cells by targeting the urokinase-type plasminogen activator receptor (186).

A tempting option that could be tested is to reverse early-stage senescent CAR-T cells by blocking critical mediators of this process, such as proteins involved in the DDR, p38, p53, p21, or ATM (187). However, these changes could also decrease T cell functionality by impacting other relevant functions. For instance, p38 is involved in the induction of senescence and IFNγ and TNFα secretion (188), and its inhibition have diminished these cytokines in different inflammation or virus infection models (189, 190). Moreover, blockage of DDR and p53 involves a risk of DNA damage on T cells that might induce malignancy (191).

SASP Impact in NK Cells and Senescence Surveillance

NK cells have an essential role in the senescence surveillance of tumor cells. Senescence surveillance is initiated by the SASP that activates immune cells to clear senescent cells preventing tumor initiation (167), where both macrophages and NK cells have an important task (32, 192, 193). Proteins present in the SASP, such as CCL2, attract PB-NK cells to remove senescent cells through NKG2D (194). Of interest, this role of PB-NK cells removing senescent cells is also observed by decidual uterine NK cells to control embryo implantation. Specifically, dNK cells after being activated by IL15, present in the SASP, target and clear decidual cells that became senescent in an IL8 dependent manner. This mechanism of NK cells is mediated through granule exocytosis and involvement of NKG2D (195).

SASP secretion by senescent tumor cells up-regulates HLA-E, the ligand of the inhibitory NKG2A NK receptor (196), and cleave NKG2D ligands inhibiting NK cell activity (197).

Soluble Thrombospondin-1 (TSP1), released in the SASP, is involved in Ras-induced senescence (198). Moreover, TSP1 released by tumor cells binds CD47 on NK cells inhibiting its activity (199). CD47 is described as a relevant modulator of NK cell function in virus infection (200). Of interest, after TIS, binding of soluble TSP1 to CD47 causes emergence of tumor-resistant cells and metastasis in triple-negative breast cancer (201), and inhibits anti-melanoma NK cell activity with reduced granzyme B and IFNγ production (202) (**Figure 3**).

IL1β is another crucial molecule present in the SASP with a relevant pro-tumor activity (203). In detail, IL1 signaling controls the SASP production (204), and transmission of IL1β

to neighboring cells induces cell senescence (205, 206). A dual role for IL1β is observed in NK cell activity. For example, IL1β is required by $CD56^{bright}$ NK cells to produce IFNγ (207) to activate pyroptosis, necessary for the anti-microbial (208) and anti-tumor (145) activity of NK cells. In addition, IL1β released by M1 macrophages increases NK cell cytotoxicity up-regulating NKp44 and NKG2D and triggering IFNγ production by NK cells. Of interest, these IL1β-primed NK cells can reverse M2 macrophage polarization (209). On the other side, a negative impact of IL1β has been described over NK activity. Thus, tumor-derived IL1β induces accumulation of MDSCs that impair NK cell development and functions (210). Moreover, a higher secretion of IL1β in endometrial cancer patients compared to healthy tissues correlates with infiltrating $CD56^{bright}$ NK cells in the tumor with exhausted phenotype, indicated by TIGIT and TIM3 expression (211).

IL6 and IL8, present in the SASP, favor the acquisition of migration/invasion and stem-like features, increasing tumor aggressiveness in breast cancer cells (212, 213). Moreover, IL6 also inhibits NK cytotoxic activity by down-regulating perforin and Granzyme B (214). In esophageal squamous cell carcinoma, tumor cells activate the STAT3 pathway on NK cells through IL6 and IL8, leading to down-regulation of NKp30 and NKG2D on NK cells and tumor progression (215) (**Figure 3**). In addition, increased levels of IL6 in the peritoneal fluid of endometriosis patients reduced the cytolytic activity of NK cells with down-regulation of granzyme B and perforin (216). IL8 activates and recruits immune cells (217) but also has tumor-promoting functions (218). IL8 is produced by CD56 bright NK cells (219), and stimulation with IL18 and IL12 induces higher IL8 production by NK cells (220).

PGE2 secretion, present in the tumor secretome, inhibits NK cell activity (10, 52, 53). Moreover, PGE2 is also present in the SASP at early tumorigenesis stages, secreted by COX-2, a critical regulator of the SASP, and promotes senescence surveillance (221) (**Figure 3**).

Senescent cells show high ROS levels and lactate production that induce and maintain cell senescence (222, 223). ROS can present contradictory effects on the activity of NK cells. Specifically, lactate production by metastatic colorectal cancer cells induces mitochondrial stress, increased ROS, and apoptosis in NK cells (224). On the other side, ROS is required for the anti-tumor activity of NK cells (225). Moreover, TIS up-regulates NKG2D ligands (MICA, MICB, and PVR) in an oxidant-dependent manner, resulting in enhanced NK cell activity against myeloma cells (226). This up-regulation of NKG2D ligands upon oxidative stress was also observed in colon carcinoma cells, leading to improved NK cell killing (227). However, in established tumors, ROS down-regulates NKp46 and NKG2D on mature $CD56^{dim}$ NK cells inducing suppression of NK activity against melanoma (228) and acute myeloid leukemia cells (229). Of interest, we previously observed that cord blood-derived NK cells reduce ROS levels in multiple myeloma cells (230). This negative role of ROS in tumors has led to antioxidant treatments in cellular immunotherapy studies. For instance, as previously mentioned, in solid tumors, CAR-T cells modified to express the enzyme catalase presented an anti-oxidant capacity to protect bystander T cells and NK cells (116).

All these studies suggest the beneficial and detrimental role of the SASP at early and late stages of tumorigenesis, respectively. As high levels of SASP inhibit NK cell activity, a strategy to treat advanced cancer patients with cellular immunotherapy, could be to administer senescence inhibitors to decrease the number of senescent cells. Once reduced levels of SASP are achieved, immune cells could be administered, that would be attracted to remove the remaining senescent tumor cells.

Macrophages

Macrophages are attracted and stimulated by SASP factors including MCP-1, MIP-1α, and GM-CSF to remove senescent cells (231). Macrophages are also affected by age-related immunosenescence and the consequences of inflammaging, a chronic inflammation occurring with aging, leading to macrophage dysfunction. Increased levels of A20, a suppressor of the NFκB and MAPK signaling, mediated this dysfunction, leading to poor NFκB and MAPK activation following TLR stimulation (232).

There is a disparity in the impact of TIS and the SASP in macrophage polarization and their phagocytic activity. Thus, in a model of skin aging, macrophage activity is inhibited when there are a high number of senescent cells (233). Specifically, through TNFα release, macrophages induce apoptosis in senescent fibroblasts, leading to the expression of phosphatidylserine on their surface. Phosphatidylserine is recognized by the STAB1 receptor on macrophages to promote their phagocytosis. However, SASP factors, including IL1α and GM-CSF, down-regulate STAB1 and TNFα expression, avoiding the killing and phagocytosis of macrophages, with no impact observed in the macrophage polarization (233).

In a model of thyroid cancer, monocytes exposed to conditioned media from senescent thyrocytes and thyroid tumor cells, undergo M2-like polarization displaying tumor-promoting. These events were related to the production of PGE2 (234). In liver fibrosis and cirrhosis, hepatic stellate cells made senescent by carbon tetrachloride treatment produce cytokines that recruit M1 macrophages, promoting a tumor-suppressive environment. However, in the absence of p53, a promoter of senescence, the released secretome induces M2 polarization, enhancing premalignant cells' proliferation (235). In a model of pancreatic cancer with oncogene-induced senescence, the SASP factor CXCL1 activates CXCR2 that leads to recruitment of M1 macrophages, inhibiting carcinogenesis. However, oncogene-induced senescence and SASP are bypassed at late stages, and M2 macrophages are recruited to enhance the proliferation of the transformed pancreatic cancer cells (236).

IMPACT OF THE TYPE OF CELL DEATH ACTIVATED IN THE TUMOR SECRETOME

Finally, we call the reader's attention to the type of cell death activated in tumor cells after the attack of immune cells in adoptive cellular immunotherapy. Inflammatory forms of cell

death include pyroptosis, which activates the NLRP3 inflammasome, leading to IL1β production (237). As previously mentioned, IL1 signaling controls the SASP production (204). Of interest, CAR-T cells and NK cells used in adoptive cellular immunotherapy activate pyroptosis when they encounter the tumor cell (145, 238). These events suggest that the consequences of this IL1β release should be considered. Expressly, inflammasome activation and pyroptosis execution represent a double edge-sword in cancer immunotherapy, as on one side, pyroptosis executes cell death. On the other side, pyroptosis and IL1β production activate multiple signaling pathways and inflammatory mediators that promote tumor growth and metastasis in cancer models (239, 240), triggering TAMs to boost tumor angiogenesis (241). Moreover, the role of pyroptosis is highly relevant to attracting other immune cells through IL1β and IL18 secretion. These events are observed in microbial infections, where pyroptosis attract immune cells to kill the previously trapped pathogen and remove the infected cell (208, 242). In adoptive cellular immunotherapy, removing dead tumor cells after being killed by immune cells is required, suggesting an advantage of pyroptosis in this context.

CONCLUSIONS

To conclude, adoptive cellular immunotherapy has emerged as a promising treatment to treat cancer patients in the last years. However, results still need to be improved in a variety of malignancies. Immune cells present a high capacity of plasticity when they receive stimuli from secreted molecules in the TME. Thus, if immune cells do not remove tumor cells, tumor secretome could modify their killer activity to an angiogenic or immunosuppressive one. A highly relevant aspect that needs to be considered to avoid these events is an efficient removal by macrophages of dying/dead tumor cells after the attack of immune cells, such as NK cells or CAR-T cells. Of interest, NK cells present additional functions to their classic killer activity that might help in this tumor cell surveillance. Inflammatory forms of cell death activated by *in vitro* expanded immune cells might also impact these processes. In summary, to achieve complete and permanent responses in cancer patients treated with adoptive cellular immunotherapy, all these aspects together need to be considered and count on the activity of the whole immune response and not just one immune cell population.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

FUNDING

This research was funded by Fondos Feder with a grant of the Institute of Health Carlos III, grant number PI20/00991.

ACKNOWLEDGMENTS

Some sections of the Figures were made with Biorender.

REFERENCES

1. Grivennikov SI, Greten FR, Karin M. Immunity, Inflammation, and Cancer. *Cell* (2010) 140:883–99. doi: 10.1016/j.cell.2010.01.025
2. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-Related Inflammation. *Nature* (2008) 454:436–44. doi: 10.1038/nature07205
3. Kumar S, Metz DC, Ellenberg S, Kaplan DE, Goldberg DS. Risk Factors and Incidence of Gastric Cancer After Detection of Helicobacter Pylori Infection: A Large Cohort Study. *Gastroenterology* (2020) 158:527–36.e7. doi: 10.1053/j.gastro.2019.10.019
4. Waldner MJ, Neurath MF. Colitis-Associated Cancer: The Role of T Cells in Tumor Development. *Semin Immunopathol* (2009) 31:249–56. doi: 10.1007/s00281-009-0161-8
5. van't Klooster CC, Ridker PM, Hjortnaes J, van der Graaf Y, Asselbergs FW, Westerink J, et al. On Behalf of the UCC-SMART Study Group. The Relation Between Systemic Inflammation and Incident Cancer in Patients With Stable Cardiovascular Disease: A Cohort Study. *Eur Heart J* (2019) 40:3901–9. doi: 10.1093/eurheartj/ehz587
6. Heikkilä K, Harris R, Lowe G, Rumley A, Yarnell J, Gallacher J, et al. Associations of Circulating C-Reactive Protein and Interleukin-6 With Cancer Risk: Findings From Two Prospective Cohorts and a Meta-Analysis. *Cancer Causes Control* (2009) 20:15–26. doi: 10.1007/s10552-008-9212-z
7. Ridker PM, MacFadyen JG, Thuren T, Everett BM, Libby P, Glynn RJ. CANTOS Trial Group. Effect of Interleukin-1β Inhibition With Canakinumab on Incident Lung Cancer in Patients With Atherosclerosis: Exploratory Results From a Randomised, Double-Blind, Placebo-Controlled Trial. *Lancet* (2017) 390:1833–42. doi: 10.1016/S0140-6736(17)32247-X
8. da Cunha BR, Domingos C, Stefanini ACB, Henrique T, Polachini GM, Castelo-Branco P, et al. Cellular Interactions in the Tumor Microenvironment: The Role of Secretome. *J Cancer* (2019) 10:4574–87. doi: 10.7150/jca.21780
9. Mukherjee P, Mani S. Methodologies to Decipher the Cell Secretome. *Biochim Biophys Acta* (2013) 1834:2226–32. doi: 10.1016/j.bbapap.2013.01.022
10. Park A, Lee Y, Kim MS, Kang YJ, Park Y-J, Jung H, et al. Prostaglandin E2 Secreted by Thyroid Cancer Cells Contributes to Immune Escape Through the Suppression of Natural Killer (NK) Cell Cytotoxicity and NK Cell Differentiation. *Front Immunol* (2018) 9:1859. doi: 10.3389/fimmu.2018.01859
11. Teng MWL, Galon J, Fridman W-H, Smyth MJ. From Mice to Humans: Developments in Cancer Immunoediting. *J Clin Invest* (2015) 125:3338–46. doi: 10.1172/JCI80004
12. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. *Cell* (2011) 144:646–74. doi: 10.1016/j.cell.2011.02.013
13. Bui JD, Schreiber RD. Cancer Immunosurveillance, Immunoediting and Inflammation: Independent or Interdependent Processes? *Curr Opin Immunol* (2007) 19:203–8. doi: 10.1016/j.coi.2007.02.001
14. Perez-Amill L, Suñe G, Antoñana-Vildosola A, Castella M, Najjar A, Bonet J, et al. Preclinical Development of a Humanized Chimeric Antigen Receptor Against B Cell Maturation Antigen for Multiple Myeloma. *Haematologica* (2021) 106:173–84. doi: 10.3324/haematol.2019.228577
15. Zhao W-H, Liu J, Wang B-Y, Chen Y-X, Cao X-M, Yang Y, et al. A Phase 1, Open-Label Study of LCAR-B38M, a Chimeric Antigen Receptor T Cell Therapy Directed Against B Cell Maturation Antigen, in Patients With Relapsed or Refractory Multiple Myeloma. *J Hematol Oncol* (2018) 11:141. doi: 10.1186/s13045-018-0681-6

16. Liu E, Marin D, Banerjee P, Macapinlac HA, Thompson P, Basar R, et al. Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors. *N Engl J Med* (2020) 382:545–53. doi: 10.1056/NEJMoa1910607
17. Shah N, Martin-Antonio B, Yang H, Ku S, Lee DA, Cooper LJN, et al. Antigen Presenting Cell-Mediated Expansion of Human Umbilical Cord Blood Yields Log-Scale Expansion of Natural Killer Cells With Anti-Myeloma Activity. *PloS One* (2013) 8:e76781. doi: 10.1371/journal.pone.0076781
18. Bachiller M, Battram AM, Perez-Amill L, Martín-Antonio B. Natural Killer Cells in Immunotherapy: Are We Nearly There? *Cancers (Basel)* (2020) 12 (11):3139. doi: 10.3390/cancers12113139
19. Martín-Antonio B, Suñe G, Perez-Amill L, Castella M, Urbano-Ispizua A. Natural Killer Cells: Angels and Devils for Immunotherapy. *Int J Mol Sci* (2017) 18(9):1868. doi: 10.3390/ijms18091868
20. Perez-Amill L, Marzal B, Urbano-Ispizua A, Juan M, Martín-Antonio B. CAR-T Cell Therapy: A Door is Open to Find Innumerable Possibilities of Treatments for Cancer Patients. *Turk J Haematol* (2018) 35:217–28. doi: 10.4274/tjh.2018.0196
21. Marofi F, Rahman HS, Achmad MH, Sergeevna KN, Suksatan W, Abdelbasset WK, et al. A Deep Insight Into CAR-T Cell Therapy in non-Hodgkin Lymphoma: Application, Opportunities, and Future Directions. *Front Immunol* (2021) 12:681984. doi: 10.3389/fimmu.2021.681984
22. Martino M, Alati C, Canale FA, Musuraca G, Martinelli G. Cerchione C. A Review of Clinical Outcomes of CAR T-Cell Therapies for B-Acute Lymphoblastic Leukemia. *Int J Mol Sci* (2021) 22:2150. doi: 10.3390/ijms22042150
23. Dafni U, Michielin O, Lluesma SM, Tsourti Z, Polydoropoulou V, Karlis D, et al. Efficacy of Adoptive Therapy With Tumor-Infiltrating Lymphocytes and Recombinant Interleukin-2 in Advanced Cutaneous Melanoma: A Systematic Review and Meta-Analysis. *Ann Oncol* (2019) 30:1902–13. doi: 10.1093/annonc/mdz398
24. Moreno V, Hernandez T, de Miguel M, Doger B, Calvo E. Adoptive Cell Therapy for Solid Tumors: Chimeric Antigen Receptor T Cells and Beyond. *Curr Opin Pharmacol* (2021) 59:70–84. doi: 10.1016/j.coph.2021.05.004
25. Lin B, Du L, Li H, Zhu X, Cui L, Li X. Tumor-Infiltrating Lymphocytes: Warriors Fight Against Tumors Powerfully. *Biomed Pharmacother* (2020) 132:110873. doi: 10.1016/j.biopha.2020.110873
26. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in Children and Young Adults With B-Cell Lymphoblastic Leukemia. *N Engl J Med* (2018) 378:439–48. doi: 10.1056/NEJMoa1709866
27. Raje N, Berdeja J, Lin Y, Siegel D, Jagannath S, Madduri D, et al. Anti-BCMA CAR T-Cell Therapy Bb2121 in Relapsed or Refractory Multiple Myeloma. *N Engl J Med* (2019) 380:1726–37. doi: 10.1056/NEJMoa1817226
28. Bruno A, Pagani A, Pulze L, Albini A, Dallaglio K, Noonan DM, et al. Orchestration of Angiogenesis by Immune Cells. *Front Oncol* (2014) 4:131. doi: 10.3389/fonc.2014.00131
29. Battram AM, Bachiller M, Martín-Antonio B. Senescence in the Development and Response to Cancer With Immunotherapy: A Double-Edged Sword. *Int J Mol Sci* (2020) 21:4346. doi: 10.3390/ijms21124346
30. Krtolica A, Parrinello S, Lockett S, Desprez PY, Campisi J. Senescent Fibroblasts Promote Epithelial Cell Growth and Tumorigenesis: A Link Between Cancer and Aging. *Proc Natl Acad Sci USA* (2001) 98:12072–7. doi: 10.1073/pnas.211053698
31. Wolf B, Zimmermann S, Arber C, Irving M, Trueb L, Coukos G. Safety and Tolerability of Adoptive Cell Therapy in Cancer. *Drug Saf* (2019) 42:315–34. doi: 10.1007/s40264-018-0779-3
32. Kale A, Sharma A, Stolzing A, Desprez P-Y, Campisi J. Role of Immune Cells in the Removal of Deleterious Senescent Cells. *Immun Ageing* (2020) 17:16. doi: 10.1186/s12979-020-00187-9
33. Massagué J. Tgfbeta in Cancer. *Cell* (2008) 134:215–30. doi: 10.1016/j.cell.2008.07.001
34. Sun X, Cui Y, Feng H, Liu H, Liu X. TGF-β Signaling Controls Foxp3 Methylation and T Reg Cell Differentiation by Modulating Uhrf1 Activity. *J Exp Med* (2019) 216:2819–37. doi: 10.1084/jem.20190550
35. Chen W, Jin W, Hardegen N, Lei K-J, Li L, Marinos N, et al. Conversion of Peripheral CD4+CD25- Naive T Cells to CD4+CD25+ Regulatory T Cells by TGF-Beta Induction of Transcription Factor Foxp3. *J Exp Med* (2003) 198:1875–86. doi: 10.1084/jem.20030152
36. Bruno A, Focaccetti C, Pagani A, Imperatori AS, Spagnoletti M, Rotolo N, et al. The Proangiogenic Phenotype of Natural Killer Cells in Patients With non-Small Cell Lung Cancer. *Neoplasia* (2013) 15:133–42. doi: 10.1593/neo.121758
37. Cerdeira AS, Rajakumar A, Royle CM, Lo A, Husain Z, Thadhani RI, et al. Conversion of Peripheral Blood NK Cells to a Decidual NK-Like Phenotype by a Cocktail of Defined Factors. *J Immunol* (2013) 190:3939–48. doi: 10.4049/jimmunol.1202582
38. Siewiera J, Gouilly J, Hocine H-R, Cartron G, Levy C, Al-Daccak R, et al. Natural Cytotoxicity Receptor Splice Variants Orchestrate the Distinct Functions of Human Natural Killer Cell Subtypes. *Nat Commun* (2015) 6:10183. doi: 10.1038/ncomms10183
39. Castriconi R, Cantoni C, Della Chiesa M, Vitale M, Marcenaro E, Conte R, et al. Transforming Growth Factor Beta 1 Inhibits Expression of Nkp30 and NKG2D Receptors: Consequences for the NK-Mediated Killing of Dendritic Cells. *Proc Natl Acad Sci USA* (2003) 100:4120–5. doi: 10.1073/pnas.0730640100
40. Park YP, Choi S-C, Kiesler P, Gil-Krzewska A, Borrego F, Weck J, et al. Complex Regulation of Human NKG2D-DAP10 Cell Surface Expression: Opposing Roles of the Γc Cytokines and TGF-β1. *Blood* (2011) 118:3019–27. doi: 10.1182/blood-2011-04-346825
41. Castriconi R, Dondero A, Bellora F, Moretta L, Castellano A, Locatelli F, et al. Neuroblastoma-Derived TGF-β1 Modulates the Chemokine Receptor Repertoire of Human Resting NK Cells. *J Immunol* (2013) 190:5321–8. doi: 10.4049/jimmunol.1202693
42. Steinbrink K, Graulich E, Kubsch S, Knop J, Enk AH. CD4(+) and CD8(+) Anergic T Cells Induced by Interleukin-10-Treated Human Dendritic Cells Display Antigen-Specific Suppressor Activity. *Blood* (2002) 99:2468–76. doi: 10.1182/blood.v99.7.2468
43. Mumm JB, Emmerich J, Zhang X, Chan I, Wu L, Mauze S, et al. IL-10 Elicits Ifnγ-Dependent Tumor Immune Surveillance. *Cancer Cell* (2011) 20:781–96. doi: 10.1016/j.ccr.2011.11.003
44. Schwich E, Hò G-GT, LeMaoult J, Bade-Döding C, Carosella ED, Horn PA, et al. Soluble HLA-G and HLA-G Bearing Extracellular Vesicles Affect ILT-2 Positive and ILT-2 Negative CD8 T Cells Complementary. *Front Immunol* (2020) 11:2046. doi: 10.3389/fimmu.2020.02046
45. Chen J, Feng Y, Lu L, Wang H, Dai L, Li Y, et al. Interferon-Γ-Induced PD-L1 Surface Expression on Human Oral Squamous Carcinoma *via* PKD2 Signal Pathway. *Immunobiology* (2012) 217:385–93. doi: 10.1016/j.imbio.2011.10.016
46. Wang J, Sanmamed MF, Datar I, Su TT, Ji L, Sun J, et al. Fibrinogen-Like Protein 1 Is a Major Immune Inhibitory Ligand of LAG-3. *Cell* (2019) 176:334–47.e12. doi: 10.1016/j.cell.2018.11.010
47. Zhu C, Anderson AC, Schubart A, Xiong H, Imitola J, Khoury SJ, et al. The Tim-3 Ligand Galectin-9 Negatively Regulates T Helper Type 1 Immunity. *Nat Immunol* (2005) 6:1245–52. doi: 10.1038/ni1271
48. Devilard E, Xerri L, Dubreuil P, Lopez M, Reymond N. Nectin-3 (CD113) Interacts With Nectin-2 (CD112) to Promote Lymphocyte Transendothelial Migration. *PloS One* (2013) 8:e77424. doi: 10.1371/journal.pone.0077424
49. Russo E, Runge P, Jahromi NH, Naboth H, Landtwing A, Montecchi R, et al. CD112 Regulates Angiogenesis and T Cell Entry Into the Spleen. *Cells* (2021) 10(1):169. doi: 10.3390/cells10010169
50. Stanietsky N, Simic H, Arapovic J, Toporik A, Levy O, Novik A, et al. The Interaction of TIGIT With PVR and PVRL2 Inhibits Human NK Cell Cytotoxicity. *Proc Natl Acad Sci USA* (2009) 106:17858–63. doi: 10.1073/pnas.0903474106
51. Kalinski P. Regulation of Immune Responses by Prostaglandin E2. *J Immunol* (2012) 188:21–8. doi: 10.4049/jimmunol.1101029
52. Pietra G, Manzini C, Rivara S, Vitale M, Cantoni C, Petretto A, et al. Melanoma Cells Inhibit Natural Killer Cell Function by Modulating the Expression of Activating Receptors and Cytolytic Activity. *Cancer Res* (2012) 72:1407–15. doi: 10.1158/0008-5472.CAN-11-2544
53. Balsamo M, Scordamaglia F, Pietra G, Manzini C, Cantoni C, Boitano M, et al. Melanoma-Associated Fibroblasts Modulate NK Cell Phenotype and Antitumor Cytotoxicity. *Proc Natl Acad Sci USA* (2009) 106:20847–52. doi: 10.1073/pnas.0906481106
54. Qian X, Zhang J, Liu J. Tumor-Secreted PGE2 Inhibits CCL5 Production in Activated Macrophages Through Camp/PKA Signaling Pathway. *J Biol Chem* (2011) 286:2111–20. doi: 10.1074/jbc.M110.154971
55. Ninomiya S, Narala N, Huye L, Yagyu S, Savoldo B, Dotti G, et al. Tumor Indoleamine 2,3-Dioxygenase (IDO) Inhibits CD19-CAR T Cells and Is

Downregulated by Lymphodepleting Drugs. *Blood* (2015) 125:3905–16. doi: 10.1182/blood-2015-01-621474
56. Brand A, Singer K, Koehl GE, Kolitzus M, Schoenhammer G, Thiel A, et al. LDHA-Associated Lactic Acid Production Blunts Tumor Immunosurveillance by T and NK Cells. *Cell Metab* (2016) 24:657–71. doi: 10.1016/j.cmet.2016.08.011
57. Lee C-L, Vijayan M, Wang X, Lam KKW, Koistinen H, Seppala M, et al. Glycodelin-a Stimulates the Conversion of Human Peripheral Blood CD16-CD56bright NK Cell to a Decidual NK Cell-Like Phenotype. *Hum Reprod* (2019) 34:689–701. doi: 10.1093/humrep/dey378
58. Rajagopalan S, Long EO. Cellular Senescence Induced by CD158d Reprograms Natural Killer Cells to Promote Vascular Remodeling. *Proc Natl Acad Sci USA* (2012) 109:20596–601. doi: 10.1073/pnas.1208248109
59. Schurich A, Magalhaes I, Mattsson J. Metabolic Regulation of CAR T Cell Function by the Hypoxic Microenvironment in Solid Tumors. *Immunotherapy* (2019) 11:335–45. doi: 10.2217/imt-2018-0141
60. Balsamo M, Manzini C, Pietra G, Raggi F, Blengio F, Mingari MC, et al. Hypoxia Downregulates the Expression of Activating Receptors Involved in NK-Cell-Mediated Target Cell Killing Without Affecting ADCC. *Eur J Immunol* (2013) 43:2756–64. doi: 10.1002/eji.201343448
61. Baginska J, Viry E, Berchem G, Poli A, Noman MZ, van Moer K, et al. Granzyme B Degradation by Autophagy Decreases Tumor Cell Susceptibility to Natural Killer-Mediated Lysis Under Hypoxia. *Proc Natl Acad Sci USA* (2013) 110:17450–5. doi: 10.1073/pnas.1304790110
62. Parodi M, Raggi F, Cangelosi D, Manzini C, Balsamo M, Blengio F, et al. Hypoxia Modifies the Transcriptome of Human NK Cells, Modulates Their Immunoregulatory Profile, and Influences NK Cell Subset Migration. *Front Immunol* (2018) 9:2358. doi: 10.3389/fimmu.2018.02358
63. Donzelli S, Milano E, Pruszko M, Sacconi A, Masciarelli S, Iosue I, et al. Expression of ID4 Protein in Breast Cancer Cells Induces Reprogramming of Tumour-Associated Macrophages. *Breast Cancer Res* (2018) 20:1–15. doi: 10.1186/s13058-018-0990-2
64. Wang X, Luo G, Zhang K, Cao J, Huang C, Jiang T, et al. Hypoxic Tumor-Derived Exosomal MiR-301a Mediates M2 Macrophage Polarization *via* PTEN/PI3Kγ to Promote Pancreatic Cancer Metastasis. *Cancer Res* (2018) 78:4586–98. doi: 10.1158/0008-5472.CAN-17-3841
65. Schioppa T, Uranchimeg B, Saccani A, Biswas SK, Doni A, Rapisarda A, et al. Regulation of the Chemokine Receptor CXCR4 by Hypoxia. *J Exp Med* (2003) 198:1391–402. doi: 10.1084/jem.20030267
66. Chen L, Wang S, Wang Y, Zhang W, Ma K, Hu C, et al. IL-6 Influences the Polarization of Macrophages and the Formation and Growth of Colorectal Tumor. *Oncotarget* (2018) 9:17443–54. doi: 10.18632/oncotarget.24734
67. Shrivastava R, Asif M, Singh V, Dubey P, Ahmad Malik S, Lone M-U-D, et al. M2 Polarization of Macrophages by Oncostatin M in Hypoxic Tumor Microenvironment is Mediated by Mtorc2 and Promotes Tumor Growth and Metastasis. *Cytokine* (2019) 118:130–43. doi: 10.1016/j.cyto.2018.03.032
68. Kitamura T, Qian B-Z, Soong D, Cassetta L, Noy R, Sugano G, et al. CCL2-Induced Chemokine Cascade Promotes Breast Cancer Metastasis by Enhancing Retention of Metastasis-Associated Macrophages. *J Exp Med* (2015) 212:1043–59. doi: 10.1084/jem.20141836
69. Ségaliny AI, Mohamadi A, Dizier B, Lokajczyk A, Brion R, Lanel R, et al. Interleukin-34 Promotes Tumor Progression and Metastatic Process in Osteosarcoma Through Induction of Angiogenesis and Macrophage Recruitment. *Int J Cancer* (2015) 137:73–85. doi: 10.1002/ijc.29376
70. Linde N, Lederle W, Depner S, van Rooijen N, Gutschalk CM, Mueller MM. Vascular Endothelial Growth Factor-Induced Skin Carcinogenesis Depends on Recruitment and Alternative Activation of Macrophages. *J Pathol* (2012) 227:17–28. doi: 10.1002/path.3989
71. Kim S, Takahashi H, Lin W-W, Descargues P, Grivennikov S, Kim Y, et al. Carcinoma-Produced Factors Activate Myeloid Cells Through TLR2 to Stimulate Metastasis. *Nature* (2009) 457:102–6. doi: 10.1038/nature07623
72. Costa-Silva B, Aiello NM, Ocean AJ, Singh S, Zhang H, Thakur BK, et al. Pancreatic Cancer Exosomes Initiate Pre-Metastatic Niche Formation in the Liver. *Nat Cell Biol* (2015) 17:816–26. doi: 10.1038/ncb3169
73. Tzeng H-T, Su C-C, Chang C-P, Lai W-W, Su W-C, Wang Y-C. Rab37 in Lung Cancer Mediates Exocytosis of Soluble ST2 and Thus Skews Macrophages Toward Tumor-Suppressing Phenotype. *Int J Cancer* (2018) 143:1753–63. doi: 10.1002/ijc.31569
74. Hsieh C-H, Tai S-K, Yang M-H. Snail-Overexpressing Cancer Cells Promote M2-Like Polarization of Tumor-Associated Macrophages by Delivering Mir-21-Abundant Exosomes. *Neoplasia* (2018) 20:775–88. doi: 10.1016/j.neo.2018.06.004
75. Weiskopf K, Jahchan NS, Schnorr PJ, Cristea S, Ring AM, Maute RL, et al. CD47-Blocking Immunotherapies Stimulate Macrophage-Mediated Destruction of Small-Cell Lung Cancer. *J Clin Invest* (2016) 126:2610–20. doi: 10.1172/JCI81603
76. Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, et al. PD-1 Expression by Tumour-Associated Macrophages Inhibits Phagocytosis and Tumour Immunity. *Nature* (2017) 545:495–9. doi: 10.1038/nature22396
77. Barkal AA, Weiskopf K, Kao KS, Gordon SR, Rosental B, Yiu YY, et al. Engagement of MHC Class I by the Inhibitory Receptor LILRB1 Suppresses Macrophages and Is a Target of Cancer Immunotherapy. *Nat Immunol* (2018) 19:76–84. doi: 10.1038/s41590-017-0004-z
78. Barkal AA, Brewer RE, Markovic M, Kowarsky M, Barkal SA, Zaro BW, et al. CD24 Signalling Through Macrophage Siglec-10 Is a Target for Cancer Immunotherapy. *Nature* (2019) 572:392–6. doi: 10.1038/s41586-019-1456-0
79. Paltridge JL, Belle L, Khew-Goodall Y. The Secretome in Cancer Progression. *Biochim Biophys Acta* (2013) 1834:2233–41. doi: 10.1016/j.bbapap.2013.03.014
80. Hanash S, Schliekelman M. Proteomic Profiling of the Tumor Microenvironment: Recent Insights and the Search for Biomarkers. *Genome Med* (2014) 6:12. doi: 10.1186/gm529
81. Hanahan D, Coussens LM. Accessories to the Crime: Functions of Cells Recruited to the Tumor Microenvironment. *Cancer Cell* (2012) 21:309–22. doi: 10.1016/j.ccr.2012.02.022
82. Mocellin S, Panelli MC, Wang E, Nagorsen D, Marincola FM. The Dual Role of IL-10. *Trends Immunol* (2003) 24:36–43. doi: 10.1016/S1471-4906(02)00009-1
83. Sato T, Terai M, Tamura Y, Alexeev V, Mastrangelo MJ, Selvan SR. Interleukin 10 in the Tumor Microenvironment: A Target for Anticancer Immunotherapy. *Immunol Res* (2011) 51:170–82. doi: 10.1007/s12026-011-8262-6
84. Yue FY, Dummer R, Geertsen R, Hofbauer G, Laine E, Manolio S, et al. Interleukin-10 Is a Growth Factor for Human Melanoma Cells and Down-Regulates HLA Class-I, HLA Class-II and ICAM-1 Molecules. *Int J Cancer* (1997) 71:630–7. doi: 10.1002/(sici)1097-0215(19970516)71:4<630::aid-ijc20>3.0.co;2-e
85. Topalian SL, Drake CG, Pardoll DM. Immune Checkpoint Blockade: A Common Denominator Approach to Cancer Therapy. *Cancer Cell* (2015) 27:450–61. doi: 10.1016/j.ccell.2015.03.001
86. Parry RV, Chemnitz JM, Frauwirth KA, Lanfranco AR, Braunstein I, Kobayashi SV, et al. CTLA-4 and PD-1 Receptors Inhibit T-Cell Activation by Distinct Mechanisms. *Mol Cell Biol* (2005) 25:9543–53. doi: 10.1128/MCB.25.21.9543-9553.2005
87. Sánchez-Fueyo A, Tian J, Picarella D, Domenig C, Zheng XX, Sabatos CA, et al. Tim-3 Inhibits T Helper Type 1-Mediated Auto- and Alloimmune Responses and Promotes Immunological Tolerance. *Nat Immunol* (2003) 4:1093–101. doi: 10.1038/ni987
88. Zhang H, Wang Z, Zhang J, Zhang X, Gui Z, Sun L, et al. The Synergism of B and T Lymphocyte Attenuator (BTLA) and Cytotoxic T Lymphocyte Associated Antigen-4 (CTLA-4) Attenuated Acute T-Cell Mediated Rejection and Prolonged Renal Graft Survival. *Transl Androl Urol* (2020) 9:1990–9. doi: 10.21037/tau-20-728
89. Workman CJ, Wang Y, El Kasmi KC, Pardoll DM, Murray PJ, Drake CG, et al. LAG-3 Regulates Plasmacytoid Dendritic Cell Homeostasis. *J Immunol* (2009) 182:1885–91. doi: 10.4049/jimmunol.0800185
90. Fallarino F, Grohmann U, Vacca C, Bianchi R, Orabona C, Spreca A, et al. T Cell Apoptosis by Tryptophan Catabolism. *Cell Death Differ* (2002) 9:1069–77. doi: 10.1038/sj.cdd.4401073
91. Yu X, Harden K, Gonzalez LC, Francesco M, Chiang E, Irving B, et al. The Surface Protein TIGIT Suppresses T Cell Activation by Promoting the Generation of Mature Immunoregulatory Dendritic Cells. *Nat Immunol* (2009) 10:48–57. doi: 10.1038/ni.1674
92. Yu X, Huang X, Chen X, Liu J, Wu C, Pu Q, et al. Characterization of a Novel Anti-Human Lymphocyte Activation Gene 3 (LAG-3) Antibody for Cancer

Immunotherapy. *MAbs* (2019) 11:1139–48. doi: 10.1080/19420862.2019.1629239
93. Wang Y, Zhao E, Zhang Z, Zhao G, Cao H. Association Between Tim−3 and Gal−9 Expression and Gastric Cancer Prognosis. *Oncol Rep* (2018) 40:2115–26. doi: 10.3892/or.2018.6627
94. Dardalhon V, Anderson AC, Karman J, Apetoh L, Chandwaskar R, Lee DH, et al. Tim-3/Galectin-9 Pathway: Regulation of Th1 Immunity Through Promotion of CD11b+Ly-6G+ Myeloid Cells. *J Immunol* (2010) 185:1383–92. doi: 10.4049/jimmunol.0903275
95. Lozano E, Mena M-P, Díaz T, Martin-Antonio B, León S, Rodríguez-Lobato L-G, et al. Nectin-2 Expression on Malignant Plasma Cells Is Associated With Better Response to TIGIT Blockade in Multiple Myeloma. *Clin Cancer Res* (2020) 26:4688–98. doi: 10.1158/1078-0432.CCR-19-3673
96. Reches A, Ophir Y, Stein N, Kol I, Isaacson B, Charpak Amikam Y, et al. Nectin4 Is a Novel TIGIT Ligand Which Combines Checkpoint Inhibition and Tumor Specificity. *J Immunother Cancer* (2020) 8(1):e000266. doi: 10.1136/jitc-2019-000266
97. Harjunpää H, Guillerey C. TIGIT as an Emerging Immune Checkpoint. *Clin Exp Immunol* (2020) 200:108–19. doi: 10.1111/cei.13407
98. Iguchi-Manaka A, Okumura G, Kojima H, Cho Y, Hirochika R, Bando H, et al. Increased Soluble CD155 in the Serum of Cancer Patients. *PloS One* (2016) 11(4):e0152982. doi: 10.1371/journal.pone.0152982
99. Li M, Qiao D, Pu J, Wang W, Zhu W, Liu H. Elevated Nectin-2 Expression Is Involved in Esophageal Squamous Cell Carcinoma by Promoting Cell Migration and Invasion. *Oncol Lett* (2018) 15:4731–6. doi: 10.3892/ol.2018.7953
100. Siddharth S, Nayak A, Das S, Nayak D, Panda J, Wyatt MD, et al. The Soluble Nectin-4 Ecto-Domain Promotes Breast Cancer Induced Angiogenesis *Via* Endothelial Integrin-β4. *Int J Biochem Cell Biol* (2018) 102:151–60. doi: 10.1016/j.biocel.2018.07.011
101. Dorner BG, Scheffold A, Rolph MS, Huser MB, Kaufmann SHE, Radbruch A, et al. MIP-1alpha, MIP-1beta, RANTES, and ATAC/Lymphotactin Function Together With IFN-Gamma as Type 1 Cytokines. *Proc Natl Acad Sci USA* (2002) 99:6181–6. doi: 10.1073/pnas.092141999
102. Prima V, Kaliberova LN, Kaliberov S, Curiel DT, Kusmartsev S. COX2/Mpges1/PGE2 Pathway Regulates PD-L1 Expression in Tumor-Associated Macrophages and Myeloid-Derived Suppressor Cells. *Proc Natl Acad Sci USA* (2017) 114:1117–22. doi: 10.1073/pnas.1612920114
103. Pegram HJ, Lee JC, Hayman EG, Imperato GH, Tedder TF, Sadelain M, et al. Tumor-Targeted T Cells Modified to Secrete IL-12 Eradicate Systemic Tumors Without Need for Prior Conditioning. *Blood* (2012) 119:4133–41. doi: 10.1182/blood-2011-12-400044
104. Koneru M, Purdon TJ, Spriggs D, Koneru S, Brentjens RJ. IL-12 Secreting Tumor-Targeted Chimeric Antigen Receptor T Cells Eradicate Ovarian Tumors. *vivo. Oncoimmunol* (2015) 4:e994446. doi: 10.4161/2162402X.2014.994446
105. Zhang L, Morgan RA, Beane JD, Zheng Z, Dudley ME, Kassim SH, et al. Tumor-Infiltrating Lymphocytes Genetically Engineered With an Inducible Gene Encoding Interleukin-12 for the Immunotherapy of Metastatic Melanoma. *Clin Cancer Res* (2015) 21:2278–88. doi: 10.1158/1078-0432.CCR-14-2085
106. Zhang L, Kerkar SP, Yu Z, Zheng Z, Yang S, Restifo NP, et al. Improving Adoptive T Cell Therapy by Targeting and Controlling IL-12 Expression to the Tumor Environment. *Mol Ther* (2011) 19:751–9. doi: 10.1038/mt.2010.313
107. Hoyos V, Savoldo B, Quintarelli C, Mahendravada A, Zhang M, Vera J, et al. Engineering CD19-Specific T Lymphocytes With Interleukin-15 and a Suicide Gene to Enhance Their Anti-Lymphoma/Leukemia Effects and Safety. *Leukemia* (2010) 24:1160–70. doi: 10.1038/leu.2010.75
108. Chmielewski M, Abken H. CAR T Cells Releasing IL-18 Convert to T-Bethigh Foxo1low Effectors That Exhibit Augmented Activity Against Advanced Solid Tumors. *Cell Rep* (2017) 21:3205–19. doi: 10.1016/j.celrep.2017.11.063
109. Avanzi MP, Yeku O, Li X, Wijewarnasuriya DP, van Leeuwen DG, Cheung K, et al. Engineered Tumor-Targeted T Cells Mediate Enhanced Anti-Tumor Efficacy Both Directly and Through Activation of the Endogenous Immune System. *Cell Rep* (2018) 23:2130–41. doi: 10.1016/j.celrep.2018.04.051
110. Sedimbi SK, Hägglöf T, Karlsson MCI. IL-18 in Inflammatory and Autoimmune Disease. *Cell Mol Life Sci* (2013) 70:4795–808. doi: 10.1007/s00018-013-1425-y
111. Vidal-Vanaclocha F, Mendoza L, Telleria N, Salado C, Valcárcel M, Gallot N, et al. Clinical and Experimental Approaches to the Pathophysiology of Interleukin-18 in Cancer Progression. *Cancer Metastasis Rev* (2006) 25:417–34. doi: 10.1007/s10555-006-9013-3
112. Adachi K, Kano Y, Nagai T, Okuyama N, Sakoda Y, Tamada K. IL-7 and CCL19 Expression in CAR-T Cells Improves Immune Cell Infiltration and CAR-T Cell Survival in the Tumor. *Nat Biotechnol* (2018) 36:346–51. doi: 10.1038/nbt.4086
113. Kloss CC, Lee J, Zhang A, Chen F, Melenhorst JJ, Lacey SF, et al. Dominant-Negative TGF-β Receptor Enhances PSMA-Targeted Human CAR T Cell Proliferation and Augments Prostate Cancer Eradication. *Mol Ther* (2018) 26:1855–66. doi: 10.1016/j.ymthe.2018.05.003
114. Boice M, Salloum D, Mourcin F, Sanghvi V, Amin R, Oricchio E, et al. Loss of the HVEM Tumor Suppressor in Lymphoma and Restoration by Modified CAR-T Cells. *Cell* (2016) 167:405–18.e13. doi: 10.1016/j.cell.2016.08.032
115. Suarez ER, Chang DK, Sun J, Sui J, Freeman GJ, Signoretti S, et al. Chimeric Antigen Receptor T Cells Secreting Anti-PD-L1 Antibodies More Effectively Regress Renal Cell Carcinoma in a Humanized Mouse Model. *Oncotarget* (2016) 7:34341–55. doi: 10.18632/oncotarget.9114
116. Ligtenberg MA, Mougiakakos D, Mukhopadhyay M, Witt K, Lladser A, Chmielewski M, et al. Coexpressed Catalase Protects Chimeric Antigen Receptor-Redirected T Cells as Well as Bystander Cells From Oxidative Stress-Induced Loss of Antitumor Activity. *J Immunol* (2016) 196:759–66. doi: 10.4049/jimmunol.1401710
117. Scheffel MJ, Scurti G, Simms P, Garrett-Mayer E, Mehrotra S, Nishimura MI, et al. Efficacy of Adoptive T-Cell Therapy is Improved by Treatment With the Antioxidant N-Acetyl Cysteine, Which Limits Activation-Induced T-Cell Death. *Cancer Res* (2016) 76:6006–16. doi: 10.1158/0008-5472.CAN-16-0587
118. Sojka DK, Yang L, Yokoyama WM. Uterine Natural Killer Cells. *Front Immunol* (2019) 10:960. doi: 10.3389/fimmu.2019.00960
119. Huhn O, Zhao X, Esposito L, Moffett A, Colucci F, Sharkey AM. How do Uterine Natural Killer and Innate Lymphoid Cells Contribute to Successful Pregnancy? *Front Immunol* (2021) 12:607669. doi: 10.3389/fimmu.2021.607669
120. Jabrane-Ferrat N. Features of Human Decidual NK Cells in Healthy Pregnancy and During Viral Infection. *Front Immunol* (2019) 10:1397. doi: 10.3389/fimmu.2019.01397
121. Fu B, Zhou Y, Ni X, Tong X, Xu X, Dong Z, et al. Natural Killer Cells Promote Fetal Development Through the Secretion of Growth-Promoting Factors. *Immunity* (2017) 47:1100–13.e6. doi: 10.1016/j.immuni.2017.11.018
122. Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, et al. Decidual NK Cells Regulate Key Developmental Processes at the Human Fetal-Maternal Interface. *Nat Med* (2006) 12:1065–74. doi: 10.1038/nm1452
123. Albini A, Noonan DM. Decidual-Like NK Cell Polarization: From Cancer Killing to Cancer Nurturing. *Cancer Discov* (2021) 11:28–33. doi: 10.1158/2159-8290.CD-20-0796
124. Bassani B, Baci D, Gallazzi M, Poggi A, Bruno A, Mortara L. Natural Killer Cells as Key Players of Tumor Progression and Angiogenesis: Old and Novel Tools to Divert Their Pro-Tumor Activities Into Potent Anti-Tumor Effects. *Cancers (Basel)* (2019) 11(4):461. doi: 10.3390/cancers11040461
125. Bruno A, Bassani B, D'Urso DG, Pitaku I, Cassinotti E, Pelosi G, et al. Angiogenin and the MMP9-TIMP2 Axis are Up-Regulated in Proangiogenic, Decidual NK-Like Cells From Patients With Colorectal Cancer. *FASEB J* (2018) 32:5365–77. doi: 10.1096/fj.201701103R
126. Schneider MA, Granzow M, Warth A, Schnabel PA, Thomas M, Herth FJF, et al. Glycodelin: A New Biomarker With Immunomodulatory Functions in non–Small Cell Lung Cancer. *Clin Cancer Res* (2015) 21:3529–40. doi: 10.1158/1078-0432.CCR-14-2464
127. Schneider MA, Muley T, Kahn NC, Warth A, Thomas M, Herth FJF, et al. Glycodelin Is a Potential Novel Follow-Up Biomarker for Malignant Pleural Mesothelioma. *Oncotarget* (2016) 7:71285–97. doi: 10.18632/oncotarget.12474
128. Scholz C, Heublein S, Lenhard M, Friese K, Mayr D, Jeschke U. Glycodelin a is a Prognostic Marker to Predict Poor Outcome in Advanced Stage Ovarian Cancer Patients. *BMC Res Notes* (2012) 5:551–1. doi: 10.1186/1756-0500-5-551
129. Lenhard M, Heublein S, Kunert-Keil C, Vrekoussis T, Lomba I, Ditsch N, et al. Immunosuppressive Glycodelin a Is an Independent Marker for Poor Prognosis in Endometrial Cancer. *BMC Cancer* (2013) 13:616. doi: 10.1186/1471-2407-13-616

130. Marletta S, Girolami I, Munari E, Pantanowitz L, Bernasconi R, Torresani E, et al. HLA-G Expression in Melanomas. *Int Rev Immunol* (2021) 40(5):330–43. doi: 10.1080/08830185.2020.1869732
131. Lázaro-Sánchez AD, Salces-Ortiz P, Velásquez LI, Orozco-Beltrán D, Díaz-Fernández N, Juárez-Marroquí A. HLA-G as a New Tumor Biomarker: Detection of Soluble Isoforms of HLA-G in the Serum and Saliva of Patients With Colorectal Cancer. *Clin Transl Oncol* (2020) 22:1166–71. doi: 10.1007/s12094-019-02244-2
132. Wlasiuk P, Stec A, Piechnik A, Kaminska W, Dmoszynska A, Ksiazek A, et al. Expression of Soluble HLA-G in Multiple Myeloma Patients and Patients With Renal Failure. *Leuk Res* (2012) 36:881–3. doi: 10.1016/j.leukres.2012.02.015
133. Caocci G, Greco M, Arras M, Cusano R, Orrù S, Martino B, et al. HLA-G Molecules and Clinical Outcome in Chronic Myeloid Leukemia. *Leuk Res* (2017) 61:1–5. doi: 10.1016/j.leukres.2017.08.005
134. Ullah M, Azazzen D, Kaci R, Benabbou N, Pujade Lauraine E, Pocard M, et al. High Expression of HLA-G in Ovarian Carcinomatosis: The Role of Interleukin-1β. *Neoplasia* (2019) 21:331–42. doi: 10.1016/j.neo.2019.01.001
135. Syed V. TGF-β Signaling in Cancer. *J Cell Biochem* (2016) 117:1279–87. doi: 10.1002/jcb.25496
136. Mills CD. M1 and M2 Macrophages: Oracles of Health and Disease. *Crit Rev Immunol* (2012) 32:463–88. doi: 10.1615/critrevimmunol.v32.i6.10
137. Noy R, Pollard JW. Tumor-Associated Macrophages: From Mechanisms to Therapy. *Immunity* (2014) 41:49–61. doi: 10.1016/j.immuni.2014.06.010
138. Gentles AJ, Newman AM, Liu CL, Bratman SV, Feng W, Kim D, et al. The Prognostic Landscape of Genes and Infiltrating Immune Cells Across Human Cancers. *Nat Med* (2015) 21:938–45. doi: 10.1038/nm.3909
139. Genard G, Wera A-C, Huart C, Le Calve B, Penninckx S, Fattaccioli A, et al. Proton Irradiation Orchestrates Macrophage Reprogramming Through Nfκb Signaling. *Cell Death Dis* (2018) 9:728. doi: 10.1038/s41419-018-0757-9
140. Schulz D, Severin Y, Zanotelli VRT, Bodenmiller B. In-Depth Characterization of Monocyte-Derived Macrophages Using a Mass Cytometry-Based Phagocytosis Assay. *Sci Rep* (2019) 9:1925. doi: 10.1038/s41598-018-38127-9
141. Tseng D, Volkmer J-P, Willingham SB, Contreras-Trujillo H, Fathman JW, Fernhoff NB, et al. Anti-CD47 Antibody-Mediated Phagocytosis of Cancer by Macrophages Primes an Effective Antitumor T-Cell Response. *Proc Natl Acad Sci USA* (2013) 110:11103–8. doi: 10.1073/pnas.1305569110
142. Sun N-Y, Chen Y-L, Wu W-Y, Lin H-W, Chiang Y-C, Chang C-F, et al. Blockade of PD-L1 Enhances Cancer Immunotherapy by Regulating Dendritic Cell Maturation and Macrophage Polarization. *Cancers (Basel)* (2019) 11(9):1400. doi: 10.3390/cancers11091400
143. Kimura Y, Inoue A, Hangai S, Saijo S, Negishi H, Nishio J, et al. The Innate Immune Receptor Dectin-2 Mediates the Phagocytosis of Cancer Cells by Kupffer Cells for the Suppression of Liver Metastasis. *Proc Natl Acad Sci USA* (2016) 113:14097–102. doi: 10.1073/pnas.1617903113
144. Imbert PRC, Saric A, Pedram K, Bertozzi CR, Grinstein S, Freeman SA. An Acquired and Endogenous Glycocalyx Forms a Bidirectional "Don't Eat" and "Don't Eat Me" Barrier to Phagocytosis. *Curr Biol* (2021) 31:77–89.e5. doi: 10.1016/j.cub.2020.09.082
145. Martín-Antonio B, Suñe G, Najjar A, Perez-Amill L, Antoñana-Vildosola A, Castella M, et al. Extracellular NK Histones Promote Immune Cell Anti-Tumor Activity by Inducing Cell Clusters Through Binding to CD138 Receptor. *J Immunother Cancer* (2019) 7:259. doi: 10.1186/s40425-019-0739-1
146. Gaforio JJ, Ortega E, Algarra I, Serrano MJ, Alvarez de Cienfuegos G. NK Cells Mediate Increase of Phagocytic Activity But Not of Proinflammatory Cytokine (Interleukin-6 [IL-6], Tumor Necrosis Factor Alpha, and IL-12) Production Elicited in Splenic Macrophages by Tilorone Treatment of Mice During Acute Systemic Candidiasis. *Clin Diagn Lab Immunol* (2002) 9:1282–94. doi: 10.1128/CDLI.9.6.1282-1294.2002
147. Boulakirba S, Pfeifer A, Mhaidly R, Obba S, Goulard M, Schmitt T, et al. IL-34 and CSF-1 Display an Equivalent Macrophage Differentiation Ability But a Different Polarization Potential. *Sci Rep* (2018) 8:256. doi: 10.1038/s41598-017-18433-4
148. Duan Z, Luo Y. Targeting Macrophages in Cancer Immunotherapy. *Signal Transduction Targeted Ther* (2021) 6:1–21. doi: 10.1038/s41392-021-00506-6
149. Jeong H, Kim S, Hong B-J, Lee C-J, Kim Y-E, Bok S, et al. Tumor-Associated Macrophages Enhance Tumor Hypoxia and Aerobic Glycolysis. *Cancer Res* (2019) 79:795–806. doi: 10.1158/0008-5472.CAN-18-2545
150. Najafi M, Hashemi Goradel N, Farhood B, Salehi E, Nashtaei MS, Khanlarkhani N, et al. Macrophage Polarity in Cancer: A Review. *J Cell Biochem* (2019) 120:2756–65. doi: 10.1002/jcb.27646
151. Kim S-Y, Kang JW, Song X, Kim BK, Yoo YD, Kwon YT, et al. Role of the IL-6-JAK1-STAT3-Oct-4 Pathway in the Conversion of non-Stem Cancer Cells Into Cancer Stem-Like Cells. *Cell Signal* (2013) 25:961–9. doi: 10.1016/j.cellsig.2013.01.007
152. Duan S, Tsai Y, Keng P, Chen Y, Lee SO, Chen Y. IL-6 Signaling Contributes to Cisplatin Resistance in Non-Small Cell Lung Cancer *via* the Up-Regulation of Anti-Apoptotic and DNA Repair Associated Molecules. *Oncotarget* (2015) 6:27651–60. doi: 10.18632/oncotarget.4753
153. Logue SE, McGrath EP, Cleary P, Greene S, Mnich K, Almanza A, et al. Inhibition of IRE1 RNAse Activity Modulates the Tumor Cell Secretome and Enhances Response to Chemotherapy. *Nat Commun* (2018) 9:3267. doi: 10.1038/s41467-018-05763-8
154. Brown JA, Yonekubo Y, Hanson N, Sastre-Perona A, Basin A, Rytlewski JA, et al. TGF-β-Induced Quiescence Mediates Chemoresistance of Tumor-Propagating Cells in Squamous Cell Carcinoma. *Cell Stem Cell* (2017) 21:650–64.e8. doi: 10.1016/j.stem.2017.10.001
155. Obenauf AC, Zou Y, Ji AL, Vanharanta S, Shu W, Shi H, et al. Therapy-Induced Tumour Secretomes Promote Resistance and Tumour Progression. *Nature* (2015) 520:368–72. doi: 10.1038/nature14336
156. Shen M, Dong C, Ruan X, Yan W, Cao M, Pizzo D, et al. Chemotherapy-Induced Extracellular Vesicle Mirnas Promote Breast Cancer Stemness by Targeting ONECUT2. *Cancer Res* (2019) 79:3608–21. doi: 10.1158/0008-5472.CAN-18-4055
157. Di Micco R, Fumagalli M, Cicalese A, Piccinin S, Gasparini P, Luise C, et al. Oncogene-Induced Senescence is a DNA Damage Response Triggered by DNA Hyper-Replication. *Nature* (2006) 444:638–42. doi: 10.1038/nature05327
158. Pluquet O, Abbadie C, Coqueret O. Connecting Cancer Relapse With Senescence. *Cancer Lett* (2019) 463:50–8. doi: 10.1016/j.canlet.2019.08.004
159. Childs BG, Durik M, Baker DJ, van Deursen JM. Cellular Senescence in Aging and Age-Related Disease: From Mechanisms to Therapy. *Nat Med* (2015) 21:1424–35. doi: 10.1038/nm.4000
160. Coppé J-P, Patil CK, Rodier F, Sun Y, Muñoz DP, Goldstein J, et al. Senescence-Associated Secretory Phenotypes Reveal Cell-Nonautonomous Functions of Oncogenic RAS and the P53 Tumor Suppressor. *PloS Biol* (2008) 6:2853–68. doi: 10.1371/journal.pbio.0060301
161. Kojima H, Inoue T, Kunimoto H, Nakajima K. IL-6-STAT3 Signaling and Premature Senescence. *JAKSTAT* (2013) 2(4):e25763. doi: 10.4161/jkst.25763
162. Le Duff M, Gouju J, Jonchère B, Guillon J, Toutain B, Boissard A, et al. Regulation of Senescence Escape by the Cdk4-EZH2-AP2M1 Pathway in Response to Chemotherapy. *Cell Death Dis* (2018) 9:199. doi: 10.1038/s41419-017-0209-y
163. Maybruck BT, Pfannenstiel LW, Diaz-Montero M, Gastman BR. Tumor-Derived Exosomes Induce CD8+ T Cell Suppressors. *J Immunother Cancer* (2017) 5:65. doi: 10.1186/s40425-017-0269-7
164. Xue W, Zender L, Miething C, Dickins RA, Hernando E, Krizhanovsky V, et al. Senescence and Tumour Clearance Is Triggered by P53 Restoration in Murine Liver Carcinomas. *Nature* (2007) 445:656–60. doi: 10.1038/nature05529
165. Yi F, Frazzette N, Cruz AC, Klebanoff CA, Siegel RM. Beyond Cell Death: New Functions for TNF Family Cytokines in Autoimmunity and Tumor Immunotherapy. *Trends Mol Med* (2018) 24:642–53. doi: 10.1016/j.molmed.2018.05.004
166. Kang T-W, Yevsa T, Woller N, Hoenicke L, Wuestefeld T, Dauch D, et al. Senescence Surveillance of Pre-Malignant Hepatocytes Limits Liver Cancer Development. *Nature* (2011) 479:547–51. doi: 10.1038/nature10599
167. d'Adda di Fagagna F. Living on a Break: Cellular Senescence as a DNA-Damage Response. *Nat Rev Cancer* (2008) 8:512–22. doi: 10.1038/nrc2440
168. Thomas R, Wang W, Su D-M. Contributions of Age-Related Thymic Involution to Immunosenescence and Inflammaging. *Immun Ageing* (2020) 17:2. doi: 10.1186/s12979-020-0173-8
169. Lian J, Yue Y, Yu W, Zhang Y. Immunosenescence: A Key Player in Cancer Development. *J Hematol Oncol* (2020) 13:151. doi: 10.1186/s13045-020-00986-z
170. Su D-M, Aw D, Palmer DB. Immunosenescence: A Product of the Environment? *Curr Opin Immunol* (2013) 25:498–503. doi: 10.1016/j.coi.2013.05.018

171. Fane M, Weeraratna AT. How the Ageing Microenvironment Influences Tumour Progression. *Nat Rev Cancer* (2020) 20:89–106. doi: 10.1038/s41568-019-0222-9
172. Tsukishiro T, Donnenberg AD, Whiteside TL. Rapid Turnover of the CD8 (+)CD28(-) T-Cell Subset of Effector Cells in the Circulation of Patients With Head and Neck Cancer. *Cancer Immunol Immunother* (2003) 52:599–607. doi: 10.1007/s00262-003-0395-6
173. Ye J, Ma C, Hsueh EC, Eickhoff CS, Zhang Y, Varvares MA, et al. Tumor-Derived Γδ Regulatory T Cells Suppress Innate and Adaptive Immunity Through the Induction of Immunosenescence. *J Immunol* (2013) 190:2403–14. doi: 10.4049/jimmunol.1202369
174. Brenchley JM, Karandikar NJ, Betts MR, Ambrozak DR, Hill BJ, Crotty LE, et al. Expression of CD57 Defines Replicative Senescence and Antigen-Induced Apoptotic Death of CD8+ T Cells. *Blood* (2003) 101:2711–20. doi: 10.1182/blood-2002-07-2103
175. Sanjabi S, Oh SA, Li MO. Regulation of the Immune Response by TGF-β: From Conception to Autoimmunity and Infection. *Cold Spring Harb Perspect Biol* (2017) 9(6):a022236. doi: 10.1101/cshperspect.a022236
176. Hoare M, Ito Y, Kang T-W, Weekes MP, Matheson NJ, Patten DA, et al. NOTCH1 Mediates a Switch Between Two Distinct Secretomes During Senescence. *Nat Cell Biol* (2016) 18:979–92. doi: 10.1038/ncb3397
177. Ruhland MK, Loza AJ, Capietto A-H, Luo X, Knolhoff BL, Flanagan KC, et al. Stromal Senescence Establishes an Immunosuppressive Microenvironment That Drives Tumorigenesis. *Nat Commun* (2016) 7:11762. doi: 10.1038/ncomms11762
178. Iwasa H, Han J, Ishikawa F. Mitogen-Activated Protein Kinase P38 Defines the Common Senescence-Signalling Pathway. *Genes Cells* (2003) 8:131–44. doi: 10.1046/j.1365-2443.2003.00620.x
179. Herbig U, Jobling WA, Chen BPC, Chen DJ, Sedivy JM. Telomere Shortening Triggers Senescence of Human Cells Through a Pathway Involving ATM, P53, and P21(CIP1), But Not P16(INK4a). *Mol Cell* (2004) 14:501–13. doi: 10.1016/s1097-2765(04)00256-4
180. Li L, Liu X, Sanders KL, Edwards JL, Ye J, Si F, et al. TLR8-Mediated Metabolic Control of Human Treg Function: A Mechanistic Target for Cancer Immunotherapy. *Cell Metab* (2019) 29:103–23.e5. doi: 10.1016/j.cmet.2018.09.020
181. Ye J, Huang X, Hsueh EC, Zhang Q, Ma C, Zhang Y, et al. Human Regulatory T Cells Induce T-Lymphocyte Senescence. *Blood* (2012) 120:2021–31. doi: 10.1182/blood-2012-03-416040
182. Mondal AM, Horikawa I, Pine SR, Fujita K, Morgan KM, Vera E, et al. P53 Isoforms Regulate Aging- and Tumor-Associated Replicative Senescence in T Lymphocytes. *J Clin Invest* (2013) 123:5247–57. doi: 10.1172/JCI70355
183. Ye J, Ma C, Hsueh EC, Dou J, Mo W, Liu S, et al. TLR8 Signaling Enhances Tumor Immunity by Preventing Tumor-Induced T-Cell Senescence. *EMBO Mol Med* (2014) 6:1294–311. doi: 10.15252/emmm.201403918
184. Ohta A, Gorelik E, Prasad SJ, Ronchese F, Lukashev D, Wong MKK, et al. A2A Adenosine Receptor Protects Tumors From Antitumor T Cells. *Proc Natl Acad Sci USA* (2006) 103:13132–7. doi: 10.1073/pnas.0605251103
185. Castella M, Caballero-Baños M, Ortiz-Maldonado V, González-Navarro EA, Suñé G, Antoñana-Vidósola A, et al. Point-of-Care CAR T-Cell Production (ARI-0001) Using a Closed Semi-Automatic Bioreactor: Experience From an Academic Phase I Clinical Trial. *Front Immunol* (2020) 11:482. doi: 10.3389/fimmu.2020.00482
186. Amor C, Feucht J, Leibold J, Ho Y-J, Zhu C, Alonso-Curbelo D, et al. Senolytic CAR T Cells Reverse Senescence-Associated Pathologies. *Nature* (2020) 583:127–32. doi: 10.1038/s41586-020-2403-9
187. Beauséjour CM, Krtolica A, Galimi F, Narita M, Lowe SW, Yaswen P, et al. Reversal of Human Cellular Senescence: Roles of the P53 and P16 Pathways. *EMBO J* (2003) 22:4212–22. doi: 10.1093/emboj/cdg417
188. Akbar AN, Henson SM. Are Senescence and Exhaustion Intertwined or Unrelated Processes That Compromise Immunity? *Nat Rev Immunol* (2011) 11:289–95. doi: 10.1038/nri2959
189. Lee M, Kim DW, Khalmuratova R, Shin S-H, Kim Y-M, Han DH, et al. The IFN-Γ-P38, ERK Kinase Axis Exacerbates Neutrophilic Chronic Rhinosinusitis by Inducing the Epithelial-to-Mesenchymal Transition. *Mucosal Immunol* (2019) 12:601–11. doi: 10.1038/s41385-019-0149-1
190. Nayak TK, Mamidi P, Sahoo SS, Kumar PS, Mahish C, Chatterjee S, et al. P38 and JNK Mitogen-Activated Protein Kinases Interact With Chikungunya Virus Non-Structural Protein-2 and Regulate TNF Induction During Viral Infection in Macrophages. *Front Immunol* (2019) 10:786. doi: 10.3389/fimmu.2019.00786
191. Pearson M, Carbone R, Sebastiani C, Cioce M, Fagioli M, Saito S, et al. PML Regulates P53 Acetylation and Premature Senescence Induced by Oncogenic Ras. *Nature* (2000) 406:207–10. doi: 10.1038/35018127
192. Sagiv A, Biran A, Yon M, Simon J, Lowe SW, Krizhanovsky V. Granule Exocytosis Mediates Immune Surveillance of Senescent Cells. *Oncogene* (2013) 32:1971–7. doi: 10.1038/onc.2012.206
193. Krizhanovsky V, Yon M, Dickins RA, Hearn S, Simon J, Miething C, et al. Senescence of Activated Stellate Cells Limits Liver Fibrosis. *Cell* (2008) 134:657–67. doi: 10.1016/j.cell.2008.06.049
194. Iannello A, Thompson TW, Ardolino M, Lowe SW, Raulet DH. P53-Dependent Chemokine Production by Senescent Tumor Cells Supports NKG2D-Dependent Tumor Elimination by Natural Killer Cells. *J Exp Med* (2013) 210:2057–69. doi: 10.1084/jem.20130783
195. Brighton PJ, Maruyama Y, Fishwick K, Vrljicak P, Tewary S, Fujihara R, et al. Clearance of Senescent Decidual Cells by Uterine Natural Killer Cells in Cycling Human Endometrium. *Elife* (2017) 6:e31274. doi: 10.7554/eLife.31274
196. Pereira BI, Devine OP, Vukmanovic-Stejic M, Chambers ES, Subramanian P, Patel N, et al. Senescent Cells Evade Immune Clearance *via* HLA-E-Mediated NK and CD8+ T Cell Inhibition. *Nat Commun* (2019) 10:2387. doi: 10.1038/s41467-019-10335-5
197. Waldhauer I, Goehlsdorf D, Gieseke F, Weinschenk T, Wittenbrink M, Ludwig A, et al. Tumor-Associated MICA Is Shed by ADAM Proteases. *Cancer Res* (2008) 68:6368–76. doi: 10.1158/0008-5472.CAN-07-6768
198. Baek K-H, Bhang D, Zaslavsky A, Wang L-C, Vachani A, Kim CF, et al. Thrombospondin-1 Mediates Oncogenic Ras-Induced Senescence in Premalignant Lung Tumors. *J Clin Invest* (2013) 123:4375–89. doi: 10.1172/JCI67465
199. Kaur S, Bronson SM, Pal-Nath D, Miller TW, Soto-Pantoja DR, Roberts DD. Functions of Thrombospondin-1 in the Tumor Microenvironment. *Int J Mol Sci* (2021) 22(9):4570. doi: 10.3390/ijms22094570
200. Nath PR, Gangaplara A, Pal-Nath D, Mandal A, Maric D, Sipes JM, et al. CD47 Expression in Natural Killer Cells Regulates Homeostasis and Modulates Immune Response to Lymphocytic Choriomeningitis Virus. *Front Immunol* (2018) 9:2985. doi: 10.3389/fimmu.2018.02985
201. Guillon J, Petit C, Moreau M, Toutain B, Henry C, Roché H, et al. Regulation of Senescence Escape by TSP1 and CD47 Following Chemotherapy Treatment. *Cell Death Dis* (2019) 10(3):199. doi: 10.1038/s41419-019-1406-7
202. Nath PR, Pal-Nath D, Mandal A, Cam MC, Schwartz AL, Roberts DD. Natural Killer Cell Recruitment and Activation are Regulated by CD47 Expression in the Tumor Microenvironment. *Cancer Immunol Res* (2019) 7:1547–61. doi: 10.1158/2326-6066.CIR-18-0367
203. Baker KJ, Houston A, Brint E. IL-1 Family Members in Cancer; Two Sides to Every Story. *Front Immunol* (2019) 10:1197. doi: 10.3389/fimmu.2019.01197
204. Man SM, Karki R, Kanneganti T-D. Molecular Mechanisms and Functions of Pyroptosis, Inflammatory Caspases and Inflammasomes in Infectious Diseases. *Immunol Rev* (2017) 277:61–75. doi: 10.1111/imr.12534
205. Acosta JC, Banito A, Wuestefeld T, Georgilis A, Janich P, Morton JP, et al. A Complex Secretory Program Orchestrated by the Inflammasome Controls Paracrine Senescence. *Nat Cell Biol* (2013) 15:978–90. doi: 10.1038/ncb2784
206. Shang D, Hong Y, Xie W, Tu Z, Xu J. Interleukin-1β Drives Cellular Senescence of Rat Astrocytes Induced by Oligomerized Amyloid β Peptide and Oxidative Stress. *Front Neurol* (2020) 11:929. doi: 10.3389/fneur.2020.00929
207. Cooper MA, Fehniger TA, Ponnappan A, Mehta V, Wewers MD, Caligiuri MA. Interleukin-1beta Costimulates Interferon-Gamma Production by Human Natural Killer Cells. *Eur J Immunol* (2001) 31:792–801. doi: 10.1002/1521-4141(200103)31:3<792::aid-immu792>3.0.co;2-u
208. Maltez VI, Tubbs AL, Cook KD, Aachoui Y, Falcone EL, Holland SM, et al. Inflammasomes Coordinate Pyroptosis and Natural Killer Cell Cytotoxicity to Clear Infection by a Ubiquitous Environmental Bacterium. *Immunity* (2015) 43:987–97. doi: 10.1016/j.immuni.2015.10.010
209. Pesant M, Mavilio D. Priming of Human Resting NK Cells by Autologous M1 Macrophages *Via* the Engagement of IL-1β, IFN-β, and IL-15 Pathways. *J Immunol* (2015) 195:2818–28. doi: 10.4049/jimmunol.1500325
210. Elkabets M, Ribeiro VSG, Dinarello CA, Ostrand-Rosenberg S, Di Santo JP, Apte RN, et al. IL-1β Regulates a Novel Myeloid-Derived Suppressor Cell Subset That Impairs NK Cell Development and Function. *Eur J Immunol* (2010) 40:3347–57. doi: 10.1002/eji.201041037

211. Degos C, Heinemann M, Barrou J, Boucherit N, Lambaudie E, Savina A, et al. Endometrial Tumor Microenvironment Alters Human NK Cell Recruitment, and Resident NK Cell Phenotype and Function. *Front Immunol* (2019) 10:877. doi: 10.3389/fimmu.2019.00877
212. Ortiz-Montero P, Londoño-Vallejo A, Vernot J-P. Senescence-Associated IL-6 and IL-8 Cytokines Induce a Self- and Cross-Reinforced Senescence/Inflammatory Milieu Strengthening Tumorigenic Capabilities in the MCF-7 Breast Cancer Cell Line. *Cell Commun Signal* (2017) 15:17. doi: 10.1186/s12964-017-0172-3
213. Barajas-Gómez BA, Rosas-Carrasco O, Morales-Rosales SL, Pedraza Vázquez G, González-Puertos VY, Juárez-Cedillo T, et al. Relationship of Inflammatory Profile of Elderly Patients Serum and Senescence-Associated Secretory Phenotype With Human Breast Cancer Cells Proliferation: Role of IL6/IL8 Ratio. *Cytokine* (2017) 91:13–29. doi: 10.1016/j.cyto.2016.12.001
214. Cifaldi L, Prencipe G, Caiello I, Bracaglia C, Locatelli F, De Benedetti F, et al. Inhibition of Natural Killer Cell Cytotoxicity by Interleukin-6: Implications for the Pathogenesis of Macrophage Activation Syndrome. *Arthritis Rheumatol* (2015) 67:3037–46. doi: 10.1002/art.39295
215. Wu J, Gao F, Wang C, Qin M, Han F, Xu T, et al. IL-6 and IL-8 Secreted by Tumour Cells Impair the Function of NK Cells *Via* the STAT3 Pathway in Oesophageal Squamous Cell Carcinoma. *J Exp Clin Cancer Res* (2019) 38:321. doi: 10.1186/s13046-019-1310-0
216. Kang Y-J, Jeung IC, Park A, Park Y-J, Jung H, Kim T-D, et al. An Increased Level of IL-6 Suppresses NK Cell Activity in Peritoneal Fluid of Patients With Endometriosis *Via* Regulation of SHP-2 Expression. *Hum Reprod* (2014) 29:2176–89. doi: 10.1093/humrep/deu172
217. Jin L, Tao H, Karachi A, Long Y, Hou AY, Na M, et al. CXCR1- or CXCR2-Modified CAR T Cells Co-Opt IL-8 for Maximal Antitumor Efficacy in Solid Tumors. *Nat Commun* (2019) 10:4016. doi: 10.1038/s41467-019-11869-4
218. Campbell LM, Maxwell PJ, Waugh DJJ. Rationale and Means to Target Pro-Inflammatory Interleukin-8 (CXCL8) Signaling in Cancer. *Pharmaceuticals (Basel)* (2013) 6:929–59. doi: 10.3390/ph6080929
219. Sabry M, Zubiak A, Hood SP, Simmonds P, Arellano-Ballestero H, Cournoyer E, et al. Tumor- and Cytokine-Primed Human Natural Killer Cells Exhibit Distinct Phenotypic and Transcriptional Signatures. *PloS One* (2019) 14:e0218674. doi: 10.1371/journal.pone.0218674
220. Poznanski SM, Lee AJ, Nham T, Lusty E, Larché MJ, Lee DA, et al. Combined Stimulation With Interleukin-18 and Interleukin-12 Potently Induces Interleukin-8 Production by Natural Killer Cells. *JIN* (2017) 9:511–25. doi: 10.1159/000477172
221. Gonçalves S, Yin K, Ito Y, Chan A, Olan I, Gough S, et al. COX2 Regulates Senescence Secretome Composition and Senescence Surveillance Through PGE2. *Cell Rep* (2021) 34:108860. doi: 10.1016/j.celrep.2021.108860
222. Davalli P, Mitic T, Caporali A, Lauriola A, D'Arca DROS, Senescence C. And Novel Molecular Mechanisms in Aging and Age-Related Diseases. *Oxid Med Cell Longevity* (2016) 2016:e3565127. doi: 10.1155/2016/3565127
223. Sabbatinelli J, Prattichizzo F, Olivieri F, Procopio AD, Rippo MR, Giuliani A. Where Metabolism Meets Senescence: Focus on Endothelial Cells. *Front Physiol* (2019) 10:1523. doi: 10.3389/fphys.2019.01523
224. Harmon C, Robinson MW, Hand F, Almuaili D, Mentor K, Houlihan DD, et al. Lactate-Mediated Acidification of Tumor Microenvironment Induces Apoptosis of Liver-Resident NK Cells in Colorectal Liver Metastasis. *Cancer Immunol Res* (2019) 7:335–46. doi: 10.1158/2326-6066.CIR-18-0481
225. Duwe AK, Werkmeister J, Roder JC, Lauzon R, Payne U. Natural Killer Cell-Mediated Lysis Involves an Hydroxyl Radical-Dependent Step. *J Immunol* (1985) 134:2637–44.
226. Soriani A, Iannitto ML, Ricci B, Fionda C, Malgarini G, Morrone S, et al. Reactive Oxygen Species- and DNA Damage Response-Dependent NK Cell Activating Ligand Upregulation Occurs at Transcriptional Levels and Requires the Transcriptional Factor E2F1. *J Immunol* (2014) 193:950–60. doi: 10.4049/jimmunol.1400271
227. Yamamoto K, Fujiyama Y, Andoh A, Bamba T, Okabe H. Oxidative Stress Increases MICA and MICB Gene Expression in the Human Colon Carcinoma Cell Line (CaCo-2). *Biochim Biophys Acta* (2001) 1526:10–2. doi: 10.1016/s0304-4165(01)00099-x
228. Aydin E, Johansson J, Nazir FH, Hellstrand K, Martner A. Role of NOX2-Derived Reactive Oxygen Species in NK Cell-Mediated Control of Murine Melanoma Metastasis. *Cancer Immunol Res* (2017) 5:804–11. doi: 10.1158/2326-6066.CIR-16-0382
229. Romero AI, Thorén FB, Brune M, Hellstrand K. Nkp46 and NKG2D Receptor Expression in NK Cells With CD56dim and CD56bright Phenotype: Regulation by Histamine and Reactive Oxygen Species. *Br J Haematol* (2006) 132:91–8. doi: 10.1111/j.1365-2141.2005.05842.x
230. Martin-Antonio B, Najjar A, Robinson SN, Chew C, Li S, Yvon E, et al. Transmissible Cytotoxicity of Multiple Myeloma Cells by Cord Blood-Derived NK Cells is Mediated by Vesicle Trafficking. *Cell Death Differ* (2015) 22:96–107. doi: 10.1038/cdd.2014.120
231. Lgpl P, Ig O TT, Jl K. Senescent Cell Clearance by the Immune System: Emerging Therapeutic Opportunities. *Semin Immunol* (2018) 40:101275. doi: 10.1016/j.smim.2019.04.003
232. Hinojosa CA, Akula Suresh Babu R, Rahman MM, Fernandes G, Boyd AR, Orihuela CJ. Elevated A20 Contributes to Age-Dependent Macrophage Dysfunction in the Lungs. *Exp Gerontol* (2014) 54:58–66. doi: 10.1016/j.exger.2014.01.007
233. Ogata Y, Yamada T, Hasegawa S, Sanada A, Iwata Y, Arima M, et al. SASP-Induced Macrophage Dysfunction may Contribute to Accelerated Senescent Fibroblast Accumulation in the Dermis. *Exp Dermatol* (2021) 30:84–91. doi: 10.1111/exd.14205
234. Mazzoni M, Mauro G, Erreni M, Romeo P, Minna E, Vizioli MG, et al. Senescent Thyrocytes and Thyroid Tumor Cells Induce M2-Like Macrophage Polarization of Human Monocytes *via* a PGE2-Dependent Mechanism. *J Exp Clin Cancer Res* (2019) 38:208. doi: 10.1186/s13046-019-1198-8
235. Lujambio A, Akkari L, Simon J, Grace D, Tschaharganeh DF, Bolden JE, et al. Non-Cell-Autonomous Tumor Suppression by P53. *Cell* (2013) 153:449–60. doi: 10.1016/j.cell.2013.03.020
236. Lesina M, Wörmann SM, Morton J, Diakopoulos KN, Korneeva O, Wimmer M, et al. Rela Regulates CXCL1/CXCR2-Dependent Oncogene-Induced Senescence in Murine Kras-Driven Pancreatic Carcinogenesis. *J Clin Invest* (2016) 126:2919–32. doi: 10.1172/JCI86477
237. Karmakar M, Minns M, Greenberg EN, Diaz-Aponte J, Pestonjamasp K, Johnson JL, et al. N-GSDMD Trafficking to Neutrophil Organelles Facilitates IL-1β Release Independently of Plasma Membrane Pores and Pyroptosis. *Nat Commun* (2020) 11:2212. doi: 10.1038/s41467-020-16043-9
238. Liu Y, Fang Y, Chen X, Wang Z, Liang X, Zhang T, et al. Gasdermin E-Mediated Target Cell Pyroptosis by CAR T Cells Triggers Cytokine Release Syndrome. *Sci Immunol* (2020) 5(43):eaax7969. doi: 10.1126/sciimmunol.aax7969
239. Xia X, Wang X, Cheng Z, Qin W, Lei L, Jiang J, et al. The Role of Pyroptosis in Cancer: Pro-Cancer or Pro-"Host"? *Cell Death Dis* (2019) 10:1–13. doi: 10.1038/s41419-019-1883-8
240. Guo B, Fu S, Zhang J, Liu B, Li Z. Targeting Inflammasome/IL-1 Pathways for Cancer Immunotherapy. *Sci Rep* (2016) 6:36107. doi: 10.1038/srep36107
241. Weichand B, Popp R, Dziumbla S, Mora J, Strack E, Elwakeel E, et al. S1PR1 on Tumor-Associated Macrophages Promotes Lymphangiogenesis and Metastasis *via* NLRP3/IL-1β. *J Exp Med* (2017) 214:2695–713. doi: 10.1084/jem.20160392
242. Kovacs SB, Miao EA. Gasdermins: Effectors of Pyroptosis. *Trends Cell Biol* (2017) 27:673–84. doi: 10.1016/j.tcb.2017.05.005

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frontiers in Immunology

REVIEW
published: 20 August 2021
doi: 10.3389/fimmu.2021.704942

The Challenge of ICIs Resistance in Solid Tumours: Could Microbiota and Its Diversity Be Our Secret Weapon?

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The human microbiota and its functional interaction with the human body were recently returned to the spotlight of the scientific community. In light of the extensive implementation of newer and increasingly precise genome sequencing technologies, bioinformatics, and culturomic, we now have an extraordinary ability to study the microorganisms that live within the human body. Most of the recent studies only focused on the interaction between the intestinal microbiota and one other factor. Considering the complexity of gut microbiota and its role in the pathogenesis of numerous cancers, our aim was to investigate how microbiota is affected by intestinal microenvironment and how microenvironment alterations may influence the response to immune checkpoint inhibitors (ICIs). In this context, we show how diet is emerging as a fundamental determinant of microbiota's community structure and function. Particularly, we describe the role of certain dietary factors, as well as the use of probiotics, prebiotics, postbiotics, and antibiotics in modifying the human microbiota. The modulation of gut microbiota may be a secret weapon to potentiate the efficacy of immunotherapies. In addition, this review sheds new light on the possibility of administering fecal microbiota transplantation to modulate the gut microbiota in cancer treatment. These concepts and how these findings can be translated into the therapeutic response to cancer immunotherapies will be presented.

Keywords: microbiota, immunotherapy, immune checkpoint inhibitors (ICIs), fecal microbiota transplantation (FMT), diet, nutrients

OPEN ACCESS

Edited by:
Lorenzo Mortara,
University of Insubria, Italy

Reviewed by:
Steven F. Gameiro,
McMaster University, Canada
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Specialty section:
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

Received: *04 May 2021*
Accepted: *03 August 2021*
Published: *20 August 2021*

Citation:
Roberto M, Carconi C, Cerreti M, Schipilliti FM, Botticelli A, Mazzuca F and Marchetti P (2021) The Challenge of ICIs Resistance in Solid Tumours: Could Microbiota and Its Diversity Be Our Secret Weapon? Front. Immunol. 12:704942. doi: 10.3389/fimmu.2021.704942

INTRODUCTION

Over the past few decades, significant progress has been achieved in cancer treatment, with immunotherapy becoming a research hotspot in recent years (1). The last years have seen unprecedented clinical responses and rapid drug development, accumulating reports of advanced cancer patients defying the odds and achieving complete remissions with immunotherapy treatments (2).

Immunotherapy is a powerful strategy to treat cancer by harnessing the body's immune system to generate or augment an immune response against it (3). This is accomplished by either training resident immune cells to recognize and eliminate cells bearing tumor specific antigens, providing external stimuli to enhance immune mediated tumor cell lysis or abrogating signals directed by tumor cells to dampen immune responsiveness (4). Both cellular and molecular components of the tumor microenvironment can affect the efficacy of immunotherapy (5).

The tumor microenvironment has been recognized as a key factor in tumor development and progression (6). Many of its components influence cancer cell malignant behavior, within its three-dimensional structure (1, 2). Non-malignant cells include immune cells, cells of the vasculature and lymphatic system, cancer-associated fibroblasts, pericytes, and adipocytes (7). The communication between cell types is driven by an extremely complex network of cytokines, chemokines, growth factors, other inflammatory mediators, and matrix remodeling enzymes (8).

The intestinal microbiota is the collection of all microorganisms (eukaryotes, bacteria, virus) living in human gastrointestinal tract. Microbiome may be very different between individuals, and it is constantly influenced by age, nutrition, antibiotic use, smoking, alcohol. There is a continuous interaction and interplay between microbiome and the immune system, and the microbiota seems to play a role in the pathogenesis of various inflammatory diseases such as NASH, inflammatory bowel disease and obesity (9).

The human microbiome has recently been described as a component of various tumor microenvironments, due to its ability to impair tumor cell metabolism by maintaining a healthy mucosal barrier, to induce inflammation, and to produce genotoxins and different bacterial metabolites (10). It has been estimated that the total number of bacteria in the 70 kg average human male is $3.8{\cdot}10^{13}$ and that 10% of metabolites found in mammalian blood are derived from the gut microbiota (11, 12). Indeed, humans and their microbiome are considered to form a composite organism, a so-called holobiont, that defines humans together with their connected microbial network, instead of merely autonomous eukaryotic organisms (13, 14). Furthermore, a clear interplay between the local microbiome, the intestinal epithelium, and resident immune cells has recently begun to emerge, where all participants actively foster gastrointestinal homeostasis. In this system, bacterially derived metabolites serve as important signals that continuously contribute to the proper function of the epithelial barrier and immune cells (14).

Over the last decade, researchers have found a consistent connection between a dysfunctional gut microbiota (dysbiosis) and various cancers, such as cancers of the urinary tract, cervix, skin, airways, colon, breast, and lymphomas (10, 15). Considering that the primary characteristics of microbiota dysbiosis are alterations of bacterial species and the increase of pathogenic bacteria (16), studying the microbial communities in the tumor microenvironment may shed light on the role of host-bacteria interactions in cancer.

The relation between cancer and microbiota is also influenced by other factors. Out of the multiple host-endogenous and host-exogenous factors involved in the modulation of the composition of gut microbiota, such as diseases, drugs, and smoke (17), diet emerges as a pivotal determinant of its community structure and function (18). Considering that the populations of dominant species within the human colonic microbiota can potentially be modified by dietary intake to influence health (19), the responses of the gut microbiota to various factors are considered to be a valuable tool to exploit in order to develop new strategies to promote human health.

Therefore, it is important to identify gut resident bacteria. Metagenomics and culturomics are the tools used to study human microbiota, to understand and detect gut microbes, to identify their specific role in the microenvironment and correlate all data with clinical specifical situations (20, 21).

Considering the increasing interest in the microbiota composition of oncological patients, the aim of this review is to analyze the role of microbiota in cancer promotion, its effects on the immune system and its emerging role as a response modulator to immunotherapy-based cancer treatments. In this perspective, this review focuses on understanding how the diet and the use of probiotics, prebiotics, postbiotics and antibiotics might modify the composition of the gut microbiota and, consequently, the therapeutic response to cancer immunotherapies (**Figure 1**).

THE ROLE OF MICROBIOTA IN TUMORIGENESIS

Given the variability of gut microbiota between individuals due to external influences such as diet (22), host genetic background and other environmental factors, many studies employed both tumor and normal tissue samples taken from the same individual, in order to provide a more accurate view of the tumor-associated shifts in the microbiome (22, 23). The general conclusion is that tumor microenvironments harbor microbiomes distinct from those of normal tissue microenvironments. Various analyses consistently showed variation in the bacterial phyla abundance when comparing the matched normal and tumor tissues, demonstrating that there is indeed a cancer-associated signature in the tumor microbiome (24–26).

Gut microbiota can be divided into 3 clusters according to the effects of the microbes on the human body: beneficial, neutral, and pathogenic (27). The first group comprehends *Bifidobacterium* and *Lactobacillus*, which can protect the intestinal tract, produce beneficial metabolites, and detoxify the human gut. Neutral microbes, such as *Enterococcus*, have dual characteristics, being beneficial to human health in normal growth conditions and being able to cause different degrees of diseases when exceeding a certain standard growth or transferred to other parts of the body (28). Pathogenic microbes, such as *Salmonella* and *Helicobacter pylori*, secrete toxins and thus might cause disease (29).

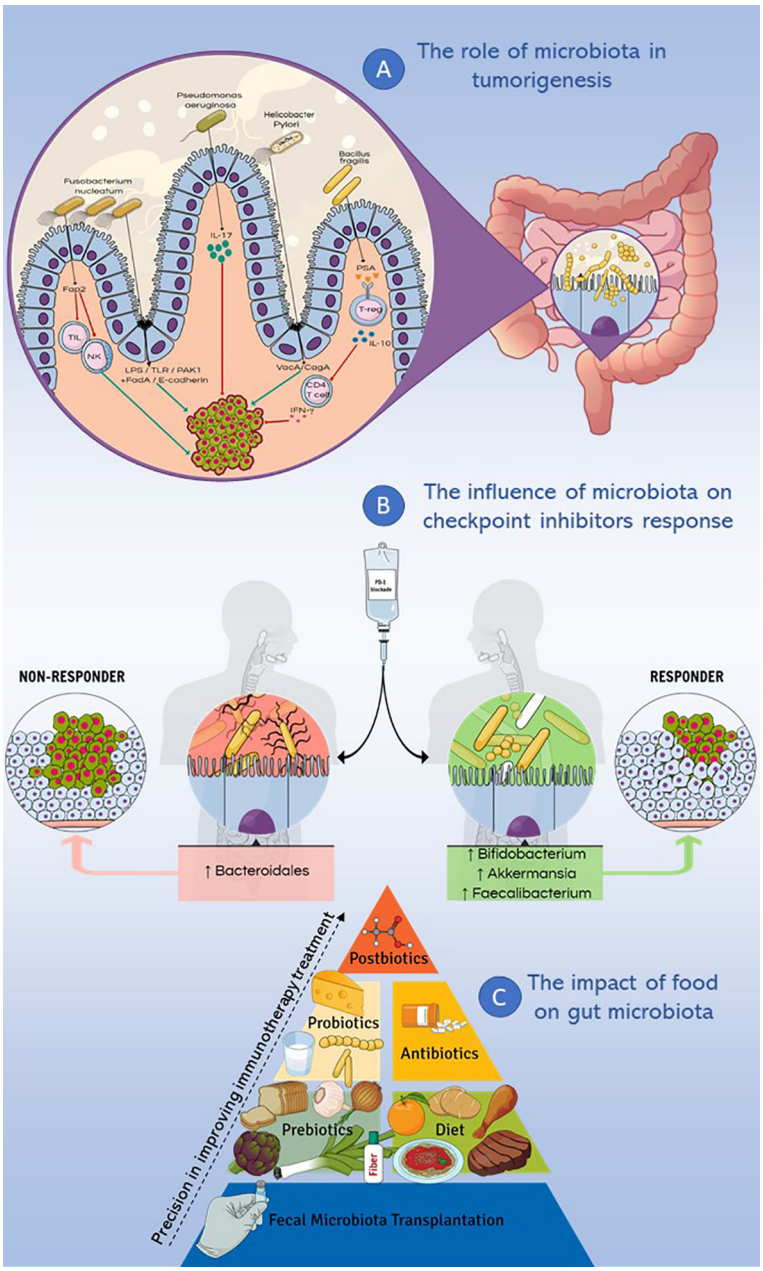

FIGURE 1 | Microbiota and immunotherapy resistance. This figure summarizes the main topics discussed in the review. **(A)** Different genera such as *Fusobacterium nucleatum, Pseudomonas aeruginosa, Helicobacter Pylori* and *Bacillus fragilis* were studied for their implication in cancer pathogenesis, causing inflammatory and/or immune response, DNA damage and modulating cell proliferation. **(B)** Microbiota influences the response to checkpoint inhibitors therapy: the enrichment of fecal microbiota with *Akkermansia muciniphila, Faecalibacterium* spp and *Bifidobacterium* spp correlates with a positive response to PD-1 immune-checkpoint blockade, while a higher abundance of *Bacteroidales* correlates with a deficient response to the same treatment. **(C)** Different dietary nutrients modify the response to immunotherapy, ranging from fecal microbiota transplantation to the use of postbiotics, with increasingly precise effects on the treatment response.

The gut microbiota has differential effects on tumorigenesis, in fact bacteria may be tumour suppressive for cancer, especially at distal sites by releasing metabolites and immune modulators such as histone deacetylase (HDACi), hypoxia induced factor (HIF), interkeukin-10 (IL-10) that enrich gut barrier function and have an antioxidant effect (30). Moreover, it is important to consider the role of TME and the gut mucosal barrier: the increased permeability of gut mucosal barrier is correlated with inflammation and development of cancer. Literature data describes a link between integrity of gut mucosal barrier and differential faecal bacteria (31).

Lacking bacterial diversity in the intestine is the key feature for many intestinal and extraintestinal disorders. Considering

the evident differences in the nutrient composition of the tumor microenvironment and the metabolic activity of microbiota, there is an unquestionable metabolic interaction between the tumor and its own microbiota (32). It is suggested that tumorigenesis is promoted by a combination of intestinal microbiota alterations (e.g., increased abundance of *Escherichia coli* and *Fusobacterium nucleatum*), rather than a difference in the abundance of a specific strain (33).

New evidence points to the association between the gut microbiota and the development and progression of gastrointestinal cancers such as colorectal cancer and hepatocellular carcinoma (34), as well as cancers of the respiratory system, where microbiota's dysbiosis in heavy smokers, together with the epithelial integrity loss, could initiate inflammation in lung cancer (35). Moreover, the relationship between human microbiota and other types of cancers, such as breast cancer, is starting to emerge (36).

As an example of the role of microbiota in cancerogenesis, here it is described the hypothesis that emerged to explain the contribution of bacteria to colorectal cancer (CRC) carcinogenesis. On one hand, the presence of a dysbiotic microbial community with pro-carcinogenic features can remodel the microbiome towards pro-inflammatory responses and epithelial cell transformation, thus leading to cancer. On the other hand, the "driver-passenger" theory states that the so-called "bacteria drivers" could initiate CRC by inducing epithelial DNA damage leading to tumors with indigenous ability to promote the proliferation of "passenger bacteria", by means of a growth advantage in the tumoral microenvironment (37, 38). These bacteria hardly colonize a healthy colon and cannot breach the intact colon wall, but they can easily invade a broken colon wall in the context of adenoma or carcinoma (37, 39). A highly diverse gut microbiota might be a key feature of a healthy gut, a balance between driver and passenger bacteria might create a species-rich ecosystem which is able to deal with environmental stresses that promote CRC (40).

Different studies aimed to identify potential "driver" bacteria. *Bradyrhizobium japonicum* was found to be increased in lung cancer patients with early-stage tumors (stages I and II) when compared to patients with advanced-stage tumors (III and IV) (41). Moreover, in patients with breast cancer, the analysis of 16S rRNA showed a higher relative abundance of *Bacillus* spp. compared with healthy samples, and *Methanobacteriaceae* was richer in malignant disease compared to benign disease (42, 43). The abundances of driver and passenger bacteria may serve as a primary indicator of cancer initiation risk and development.

Suspected Role-Players in Carcinogenesis

The human gut microbiota is dominated by 3 primary phyla: *Firmicutes* (30%-50%), *Bacteroidetes* (20%-40%) and *Actinobacteria* (1% - 10%). Some strict anaerobes, as well as *Bacteroides*, *Eubacterium*, *Bifidobacterium*, *Fusobacterium*, *Peptostreptococcus* and *Atopobium* (44), constitute a major portion of the gut microbiota, while facultative anaerobes, such as *Lactobacilli*, *Enterococci*, *Streptococci* and *Enterobacteriaceae*, represent a minor proportion (45).

During their phylogenetic evolution, bacteria progressively acquired virulence factors that conferred pathogenicity. In this regard, bacteria developed the ability to penetrate the gut mucosal barrier, as well as the ability to adhere to and invade intestinal epithelial cells, using flagella, pili, and adhesins (46–48). These virulence factors are considered to be one of the elements that determine disease-promoting and pro-carcinogenic effects of pathogens (49).

Intestinal bacteria contribute to carcinogenesis in different ways, causing inflammatory and/or immune response, DNA damage and modulating cell proliferation. Different genera were studied to prove their implication in cancer pathogenesis, especially in CRC. A recent study showed how colorectal cancer samples were dominated by *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* (22). Tumors showed an enrichment of *Proteobacteria* and a depletion of *Firmicutes* and *Bacteroidetes*, underlining the evident and significant changes in these phyla between the normal and cancer states. There was also an increase in the phylum *Fusobacteria* in the tumor-associated microbiome (22). The important findings were that two of the genera that have been found to be enriched in the tumor microbiome, *Providencia* and *Fusobacteria*, are already known to be pathogenic. Moreover, *Fusobacteria* has been implicated in CRC by many other studies (50, 51). The presence of species belonging to the genera *Providencia* and *Fusobacterium* in the tumor microenvironment may suggest that they could have a role in oncogenesis or tumor progression, or that the tumor's niche favors them.

Several studies suggest that *Fusobacteria* is likely a cancer driver and its carcinogenic mechanism has been unveiled (52, 53). The discovery of *Providencia* in the tumor microbiome is interesting as it produces an immunogenic lipopolysaccharide that participates in epithelial barrier dysfunction and endothelial apoptosis (54). These factors generally lead to gastroenteritis, but its association with the tumor environment may suggest that it should be studied as a cancer-promoting pathogen. Interestingly, *Fusobacteria* and *Providencia* share many important phenotypic characteristics such as the ability to damage colorectal tissue and to encode several virulence genes that are responsible for lipopolysaccharide biosynthesis, which are also significantly increased in the tumor microenvironment (22).

In the same way, certain CRC-associated *Escherichia coli* strains acquired virulence factors, such as the afa and eae adhesins, which conferred the ability to adhere to and invade the intestinal epithelium (55, 56). *E. Coli* is indeed a common gut commensal bacterium, but it has been shown to be able to colonize the colonic mucosa; it increases mucosal permeability through the activation of Wnt mitogenic signaling, it damages the DNA and interferes with the DNA repair process, hence inducing CRC development (57).

Other common pathogenic bacteria have been studied for their association with carcinogenesis. A study showed that CRC patients and precancerous lesions had a higher expression level of *Salmonella* flagella antibodies than healthy controls, with diet differences being one of the mediating factors, suggesting a potential link between *Salmonella* and CRC (58). Furthermore,

Salmonella can secrete the effector protein AvrA to promote acetylation and ubiquitination of target proteins. AvrA inhibits β-catenin degradation, maintains β-catenin stability, and promotes intestinal epithelial cell proliferation, thereby facilitating tumorigenesis, increasing tumor diversity, and driving tumor progression (59).

THE INFLUENCE OF MICROBIOTA ON CHECKPOINT INHIBITORS RESPONSE

It has recently been shown that gut microbiota influences the host immune response to different cancer therapies, such as radiotherapy, chemotherapy, stem cell transplant and immunotherapy, by upsetting drug metabolism, the anti-tumor effects and the toxicity of the medications currently used (60).

ICIs immunotherapy is based on using natural and artificial components in order to promote or induce the natural immune system to neutralize cancer cells (61, 62). Since the introduction of ICIs, there has been a change in the treatment of advanced cancer by introducing immunotherapy as a recognized first and second-line therapies. ICIs are monoclonal antibodies which target inhibitory receptors on the surface of T cells. Checkpoint blockade therapies release the inhibitory mechanism that control T-cell mediated immunity. The immune checkpoints are inhibitory pathways of immune cell that are important to regulate immune response and maintaining self-tolerance.

Once T cells are activated, they strengthen the immune system and boost an immune-mediated eradication of cancer cells (63). Immune checkpoints expressed on cytotoxic and regulatory T cells include programmed cell death protein-1 (PD-1 or CD279) and cytotoxic T lymphocyte associated antigen 4 (CTLA-4 or CD152) (64, 65) that interact with ligands cluster differential 80 (CD80), cluster differential 86 (CD86) and programmed death ligand-1 (PDL-1) on antigen presenting cells (APCs). ICIs prevent receptors and ligands from binding to each other, interrupting signals. In line with these considerations, the host immune system provides a powerful therapeutic target, thanks to its ability to precisely focus on tumor cells (66).

Despite the abovementioned advantages of immunotherapy, patients respond to ICIs heterogeneously and with a short-term efficacy (67). The reason why some tumors lack response is still unclear, although it probably depends on antigenicity and adjuvanticity defects, which are key factors in shaping the immunogenicity of tumor cells (68). Despite the fact that several biomarkers (PD-L1 expression, tumor-infiltrating lymphocytes, mutational burden, immune gene signatures and microsatellite instability) have been proposed, their sensibility and sensitivity are limited (69). Given that tumors with a high number of somatic mutations are more responsive to immunotherapies than the ones with a lower rate, the level of somatic mutations seems to be a crucial factor (70).

Preliminary data indicate that enteric microbiota may affect the efficiency of immunotherapy (71). It is well known that gut microbiota can modulate the peripheral immune system and that its diversity plays a crucial role in the maturation, development and function of both the innate and the adaptive immune systems (66, 72). Given the crosstalk between gut microbiota and immunity and considering that T cell infiltration of solid tumors, such as metastatic melanoma, is associated with favorable outcomes (73), microbiota could be considered as an important modulator of response to immunotherapy.

Along these lines, remarkable studies have demonstrated how the gut microbiota and its composition play a major role in the response to immunotherapy with ICIs, targeting the PD-1 and the CTLA-4 (74, 75).

With regards to the influence of gut microbiota on therapies targeting the PD-1/PD-L1 axis, Sivan et al. have provided important insights from murine models in 2015 (74). Indeed, they have demonstrated how genetically similar mice with different microbiota composition exhibited significant immune-mediated differences in melanoma growth rate. The intratumoral CD8+ T cell accumulation was found to be significantly lower in mice with a more aggressive tumor growth and a remarkable reduction in the difference of antitumor immunity was shown after cohousing, suggesting an environmental influence. Moreover, fecal suspensions derived from mice with less aggressive tumor growth were able to delay tumor growth and to enhance the induction and infiltration of tumor-specific CD8+ T cells in the other group of mice, thus supporting a microbe-derived effect. Microbiota composition could also influence the response to immunotherapy with antibodies targeting PD-L1. These abovementioned data support the idea that microbiota might be a source of intersubjective heterogeneity regarding spontaneous antitumor immunity and therapeutic effects of antibodies targeting the PD-1/PD-L1 axis.

A related research revealed how the antitumor effects of CTLA-4 blockade depend on distinct *Bacteroides* species, with a lack of response to CTLA-4 blockade in antibiotic-treated or germ-free mice (75). The analysis of microbiota composition showed *Bifidobacterium* being positively associated with antitumor T cell responses. Furthermore, *Bifidobacterium*-treated mice showed better tumor surveillance compared to their non-*Bifidobacterium* treated counterparts, together with a high increase of tumor-specific T cells in the periphery and a significant increase of antigen-specific CD8+ T cells within the tumor (74).

On the other hand, the treatment itself may affect microbiota composition. Indeed, in patients with metastatic melanoma, Ipilimumab can alter the abundance of gut *Bacteroides* spp. with an immunogenic power, especially *B. thetaiotaomicron* and *B. fragilis*, which, in turn, can affect its therapeutic effect. Feces rich in *B. fragilis* (except *B. distasonis* or *B. uniformis*) were negatively associated with tumor dimension after the therapy. Hence, the efficacy of CTLA-4 blockade is influenced by the microbiota composition (75). The gut microbiome and antibiotic therapies appear to impact the response to adoptive cell therapies in murine models (76, 77) and preliminary studies on haematological and solid tumor case series seem to align with this data (78).

Recent studies on humans have reported an unexpected role of specific members of the gut microbiota as predictors of response to immunotherapy in a distinctive series of epithelial tumors (NSCLC, renal cell carcinoma, and urothelial carcinoma) and melanoma patients (79–81). Routy et al. recently demonstrated how patients with epithelial tumors that responded to PD-1 blockade had differential composition of gut bacteria, being enriched in *Akkermansia* and *Alistipes*. Moreover, by performing a fecal microbial transplantation in mice it was demonstrated how there were enhanced responses related to the responders' fecal material. In addition, the efficacy of anti-PD-1 in GF mice receiving non-responders' transplantation could be restored by the administration of *Akkermansia muciniphila* alone or in combination with *Enterococcus hirae* (79). Regarding metastatic melanoma, a study by Gopalakrishan et al. revealed that responders to anti-PD-1 therapy not only had a significantly higher diversity of bacteria in their gut microbiota, but also had a higher relative abundance of *Clostridiales, Ruminococcaceae*, and *Faecalibacterium* spp. On the other hand, non-responders had significantly lower diversity of gut bacteria and a higher abundance of *Bacteroidales*. The composition of microbiota was related to the expression of cytotoxic T cell markers and the mechanism of antigen processing and presentation, which was increased in the first group of patients (80). In addition, another study has shown how the transplantation of stool to germ-free mice could improve the efficacy of anti-PD-L1 immunotherapy in mice that received responder-stool by increasing the density of CD8+ T-cells and reducing FoxP3+ CD4+ Tregs in the tumor microenvironment.

Given the recent findings of the microbiota being a significant modulator of response to ICIs, important insights are provided into the possibility of intervening on the composition of the intestinal microbiota to affect the ability to modulate antitumor immune responses. The crosstalk between microbiota and the immune system may allow a microbiota-based selection of patients that might benefit from a specific immunotherapy treatment, boosting their anticancer response. The prospect of being able to manipulate gut microbiota in order to modify the response to checkpoint inhibitors, serves as a continuous stimulus future research.

The Microbiota Modulation of Drug Resistance

Besides regulating the response to checkpoint blockade therapies, gut microbiota can also take part in resistance to this kind of treatment, crowding out its therapeutic benefits. Xiaochang Xue et al. indicated that commensal bacteria act in a direct way on our immune cells, down-regulating the intestinal miR-10a expression. As they have shown, *E. coli* and flagellated A4 commensal bacteria manage to recognize and engage TLR1/2, TLR4, TLR5, TLR9 and NOD2 on dendritic cells (DCs), resulting in a down-regulation of miR-10a *via* the MyD88-dependent pathway (82). Considering that miR-10a inhibits DC production of IL-12/IL-23p40, miR-10a itself acts as a negative regulator of both innate and adaptive immune responses to microbiota (82). It is known that IL-12/IL-23p40 gene has a key role in the stimulation of Th1 cell-mediated immune responses and cytotoxic activity of CD8+ T and natural killer cells (83). Thus, their absence threatens the effectiveness of the anticancer immune response.

Furthermore, both Gram-positive and Gram-negative bacteria are able to produce extracellular vesicles (EVs), which carry carbohydrates, signaling molecules, metabolites, proteins, DNA, RNA, in order to create a cell-to-cell communication through the transport of their content (84). Bacterial EVs contain short RNAs (85) (sRNAs) and miRNA-sized sRNAs (msRNAs) (86), which have regulatory functions as well as miRNA in eukaryotic cells. Different studies (87, 88) confirm that the exchange of information between bacterial EVs and host cells through the modulation of the gene expression, might be involved in inducing resistance to chemotherapy and immunotherapy. On the other hand, even human intestinal epithelial cells release miRNAs encapsulated in EVs, which, as it has been demonstrated by S. Liu et al., may promote the growth *F. nucleatus* and *E. coli*, in order to maintain a physiological balance of our intestinal microbiota (89).

In conclusion, it is clear that there is a mutual influence between bacteria and human host cells, thus, it is conceivable that further studies could provide additional findings to better understand EV-mediated inter-cell communication and, perhaps, a new opportunity to reduce the resistance to cancer therapies by using specific probiotics, antibiotics or focusing on the composition of microbiome to personalize therapies.

THE IMPACT OF FOOD ON GUT MICROBIOTA

Diet

The contribution of diet to the modulation of microbiota and its crucial role in orchestrating the host–microbiota crosstalk is evident since the beginning of a human life when there is a microbiota-dependent relationship between milk oligosaccharides and growth promotion (90). This crosstalk between diet and microbiota continues and becomes more complex with the increased bacterial richness associated with the introduction of solid foods (91), and keeps affecting our lives until the end, with a decreased richness in the microbiota of frail ageing populations living in long-stay care, probably due to reduced food diversity (92).

A study demonstrated how the gut microbiome can respond to dietary interventions in humans in a rapid, diet-specific manner and how a diet composed entirely of animal products is able to trigger enrichment in bile-tolerant bacteria (*Alistipes, Bilophila* and *Bacteroides*) and depletion in *Firmicutes* that metabolize plant polysaccharides (*Roseburia, Eubacterium rectale* and *Ruminococcus bromii*) (93). Some more metagenomic and metabolomic analyses confirmed this trade-off between protein fermentation and degradation in protein-rich, animal-based diets, as opposed to carbohydrate fermentation and amino acid biosynthesis in plant-based diets (94). For example, the elimination of animal fats in the human diet was associated with a decrease in harmful *Bacteroidales* bacteria (95).

One of the dietary components that has shown to have a significant impact on the microbiota's composition is fiber. Indeed, taking into consideration the different diet styles, it was shown how administering to mice a typical Western-style diet, that contains a relatively lower amount of fiber, could reduce the amount of *Bifidobacterium* and the gut microbiota diversity, leading to increased penetrability, and a reduced production rate of the inner mucus layer (96). Another study in healthy human volunteers (97), showed how the reduction in the amount of fiber intake led to a statistically significant reduction in the abundance of *Faecalibacterium prausnitzii* and *Roseburia* spp, which were positively correlated with the proportion of butyrate during both baseline normal diets. Moreover, a chronic lack of dietary fiber intake could lead to a reduced diversity in the gut microbiota (98). Preliminary data suggest that diet fiber intake could even impact the likelihood of response to anti-PD-1 treatment (99), providing interesting insights into the possible role of diet in the response to cancer therapies.

Many other dietary nutrients were studied for their roles in the modulation of gut microbiota, for example major groups of polyphenols assayed in both *in vitro* and preclinical studies have shown their ability to modulate the gut microbiota to a beneficial pool characterized by the abundance of *Bifidobacterium, Lactobacillus, Akkermansia*, and *Faecalibacterium* sp (100). Resveratrol is a naturally occurring polyphenol produced by some dietary botanicals, including red grapes (101), as a self-defence agent. Together with its cardio-protective and neuro-protective properties, it also serves as an antitumoral agent (102) which has shown the ability to induce antioxidant enzymes that attenuate oxidative stress (103).

Given the importance of these bacteria and their implications in cancer therapy, it is possible that diet could improve the patients' outcomes through the modulation of their microbiome. Furthermore, considering that diet interacts with the human 'holobiont' in a person-specific way, being able to obtain multiple parameters from the host and its resident microbiota could assist in devising precision dietary interventions (104). This would provide a safe and simple opportunity for assessing the implication of microbiota and downstream immune manipulation in cancer patient populations.

Ongoing trials are currently exploring the impact that diet could have on the gut microbiota of oncologic patients. A randomized clinical trial that started in 2013 (NCT02079662) is currently studying how an integrative oncological program, that aims to make changes in the patients' lifestyles and behaviors, including dietary recommendations and meal delivery, could influence long-term treatment results in patients with stage III breast cancer initiating radiotherapy. Interestingly, longitudinal gut and oral microbiome samples, along with a battery of questionnaires, are listed as secondary outcomes in order to better gauge how the microbiome might change in relation to behavioral patterns in cancer patients. A second trial (105) was designed to investigate fiber supplementation in patients with a previous history of colorectal cancer, through supplementation of beans into the normal diet for 8 weeks, to measure shifts in bacterial populations after a diet alteration. Even though both studies are not finalized yet, they will provide valuable information on how lifestyle factors can modulate the gut microbiome and its interaction with diet. A better understanding of the impact that diet has on microbiota will likely be key to the future of clinical and public health approaches to cancer.

Probiotics

Despite the impact of dietary nutrients seems relatively simple and fast to design, it may be hard to monitor the patient's compliance in dietary description intake; the effect of food on the microbiota might be modest and heavily host related. An alternative method that could provide much more control towards microbial manipulation could be the administration of probiotics.

Probiotics are living microorganisms that, when balanced in terms of quantity, grant beneficial effects to the host (106). It is well-established that probiotics act in different ways to prevent the colonization of pathogens, such as *Clostridium difficile* and *Staphylococcus aureus*, and, consequently, dysbiosis (107). Indeed, probiotics antagonize pathogen colonization by competing for nutrients (108), sticking to the epithelial cell surfaces or to the mucus (109) and creating clusters with pathogens themselves (110). They also have a role in producing metabolites, such as lactic acid, acetic acid and bacteriocins, which are able to lower luminal pH (111) and unleash a direct antimicrobial activity (112), in order to inhibit pathogen growth.

There has been an increasing interest towards probiotics potential role in improving antitumor immunity, considering their ability to repress colonic inflammation and to stimulate immunosurveillance (113).

Bifidobacterium and *Lactobacillus* are two of the most active probiotics, which have been identified as regulators of gut homeostasis (114, 115). Moreover, other probiotics improve gut barrier function, by restoring epithelial integrity (116). An innovative approach could consist of administering probiotics before, during, or after potentially "microbiota-disrupting" or "microbiota-modulated" treatments. There have been several clinical trials administering probiotics in CRC patients. One that was completed in 2017 (117), aimed to unveil the change in fecal and tumor microbiota from the baseline, after using probiotics containing strains of *L. acidophilus* and *B. lactis*. The results showed an increased abundance of butyrate-producing bacteria (above all *Faecalibacterium* and other *Clostridiales*) within the tumor, and its associated non-tumor colonic mucosa and stool. This is a demonstration that probiotic therapy can change colonic mucosa. Some other ongoing trials are assessing the impact of probiotic therapy on different types of cancer, including the change on CD8+ T cell infiltrate in patients with stage I-III breast cancer (NCT03358511), and thus, providing a perspective for a future better understanding of their influence on microbiome.

Nevertheless, even though probiotics are deemed safe and well-tolerated by healthy subjects, in patients with damaged

intestinal barrier or compromised immunity, such as cancer patients, their physiological protection may fail (118), resulting in bacteremia, fungemia, endocarditis, liver abscess and pneumonia (119). In fact, many of the ongoing trials mentioned before, have focused on safety endpoints. There is definitely wide variability regarding the stability and composition of the available probiotic therapies' formulations (120), and despite caution should be taken towards their use in cancer patients, the use of probiotics is not absolutely forbidden (113).

Prebiotics

Prebiotics, introduced by Gibson and Roberfroid in 1995, are non-viable food components, which can stimulate the growth and the activity of specific gut bacteria, improving the host's health (121).

Probiotics produce some kinds of prebiotics, such as short-chain fatty acids (SCFAs) (122). SCFAs are indeed produced by several bacteria in the gut that ferment fibers. Many SCFAs, such as acetate, butyrate and propionate, are important in maintaining intestinal homeostasis (123). Because of their ubiquitous presence, they are being studied for their potential as universal metabolic regulators of the immune system. Among them, it has been noticed that butyrate has a relevant role in CRC patients, inducing the apoptosis of cancer cells and inhibiting inflammation as well as oxidative stress (124). Though, it needs to be considered that every host has a different genetic background, which may interfere with butyrate beneficial effects (125).

Furthermore, prebiotic oligosaccharides with a low grade of polymerization may induce CD4+ T cells to produce IFN-γ and IL-10 (126). Besides, two different studies in which mice with a transplantable liver tumor have received inulin or oligofructose together with subtherapeutic doses of six chemotherapeutics, pointed out boosted chemotherapeutic effects and observed an increased lifespan (127, 128).

Despite the positive effects mentioned above, Singh et al. have also reported a harmful microbial fermentation as a result of prebiotic supplementation (129). Firstly, they tried to examine whether inulin has a mitigating effect towards metabolic syndrome in Toll-like receptor 5 (TLR5) knockout mice. Unfortunately, even though a long-term inulin enriched diet alleviates metabolic dysfunctions, concurrently, it promotes cholestasis and necroinflammation, and therefore it can induce hepatocellular carcinoma (HCC). However, a constant supplementation of inulin in drinking water revealed to trigger hepatic inflammation and fibrosis, but it did not promote tumor development. Additionally, similar effects have been induced by other soluble fiber, such as pectin and fructo-oligosaccharide, in contrast with some non-fermentable and insoluble fiber, such as cellulose, for instance. Interestingly, *Clostridia* species are highly present in mice which develop an HCC and a depletion in butyrate-producing bacteria has been reported to reduce the incidence of the hepatocellular carcinoma in TLR5 knockout mice (129).

In conclusion, the above submissions suggest that prebiotic fermentation and butyrate production have a partial contribution in the hepatocellular carcinoma development, although not being the decisive driver (113).

Postbiotics

In addition to probiotics and prebiotics, an interesting role in the modulation of gut homeostasis and patients' outcome is played by postbiotics, which are soluble products and metabolites derived from microorganisms (130). Instead of relying on bacteria supported by prebiotics or introduced through probiotics, postbiotics represent the microbial product itself, thus surpassing the bacteria (131). Despite the advantage of not being dependent on the cultivation of specific microbiota compositions, further characterization of postbiotic mechanism of action is still required.

In fact, it has been noted that *S. thermophilus* (132) and *E. coli* (133) generate supernatants, which protect rat gut from 5-FU-induced mucositis. In addition, p40, a soluble protein produced by *Lactobacillus rhamnosus* GG, avoids cytokine-induced epithelial apoptosis, prevents gut barrier dissolution (134, 135) and raises immunoglobulin A secretion (136). Moreover, an example of a molecule that can induce an immune phenotype in the absence of the microorganism is polysaccharide A (PSA) derived from *Bacterioides fragilis*. A study reported how this prominent human commensal can direct the conversion of CD4+ T cells into Foxp3+ Treg cells with the immunomodulatory molecule being polysaccharide A. Interestingly, polysaccharide A administration alone was sufficient to induce expansion of Tregs and to increase the production of anti-inflammatory IL-10 in mice *via* TLR2 activation. Furthermore, PSA was not only able to prevent, but also cure experimental colitis in animals (137). Despite microbial products are considered to be adjuvants stimulating the immune response, this study provides an insight into their ability to promote immune suppression as well.

Moreover, as mentioned before, SCFAs are gut microbiota-derived bacterial fermentation products that are being studied for their effect on the immune system. A study demonstrated how short-chain fatty acids regulate the size and function of the colonic Treg pool and protect against colitis in a Ffar2-dependent manner in mice (138). Another study showed that butyrate, produced by commensal microorganisms during starch fermentation, facilitated extrathymic generation of Treg cells and *de novo* Treg-cell generation in the periphery was potentiated by propionate (139).

In oncologic patients, postbiotics induce antitumor effects (140). In support of this possibility, a study published by Konishi et al. in 2016, showed that *Lactobacillus casei* ATCC334 supernatant contained a powerful tumor-suppressive molecule, identified as ferrichrome. Ferrichrome treatment could induce apoptosis through the activation of c-jun N-terminal kinase (JNK). Interestingly, despite the tumor-suppressive effect of ferrichrome on colon cancer cells was found to be greater than or equal to that of conventional CRC drugs, this postbiotic showed less of an effect on healthy intestinal cells (140).

Overall, these data demonstrate that exogenous bacterial metabolites mediate the communication between the commensal microbiota and the immune system and can be

utilized to influence immune activity in order to maintain homeostasis and promote health.

The putative mechanisms of actions of probiotics, prebiotics and postbiotics are shown in **Figure 2**.

Antibiotics

Even though probiotics and prebiotics bring numerous modifications to the human gut microbiota, unluckily, all their benefits are transient (141–144). Evidence sustains that intestinal microbiota alterations, provided by antibiotics injection, result in an enduring loss of the original human microbiota diversity (145). Considering that patients' response to immunotherapy partly depends on the varied composition of their microbiota, a loss in terms of abundance and types of microorganism species could affect therapeutic outcome.

A retrospective study investigated the negative association between the administration of antibiotics and ICIs. Patients that were recently given antibiotic therapy (ATB) had shorter Progression Free Survival (PFS) and Overall Survival (OS) when compared to those who did not receive ATB (146). Furthermore, the combination of ATBs and proton pump inhibitors has also been associated with gut dysbiosis, decreased bacterial richness, and the promotion of T-cell tolerance (147). It seems that antibiotic treatment might reduce the efficacy of ICIs by modifying the patient's microbiota (80).

Ipilimumab is a wholly human monoclonal antibody against CTLA-4 that was approved in 2011 for the treatment of unresectable and metastatic melanoma, as well as adjuvant treatment for melanoma (148). It was found that patients on treatment with Ipilimumab developed antibodies against some elements of gut microbiota (149). On the other hand, a combination of broad-spectrum antibiotics, such as Ampicillin, Colistin and Streptomycin could compromise the antitumoral effects of CTLA–4–specific antibodies, suggesting that gut microbiota is crucial to set up the best anticancer treatment outcome through CTLA-4 blockade (75). Indeed, it has been shown that the administration of antibiotics interferes with the

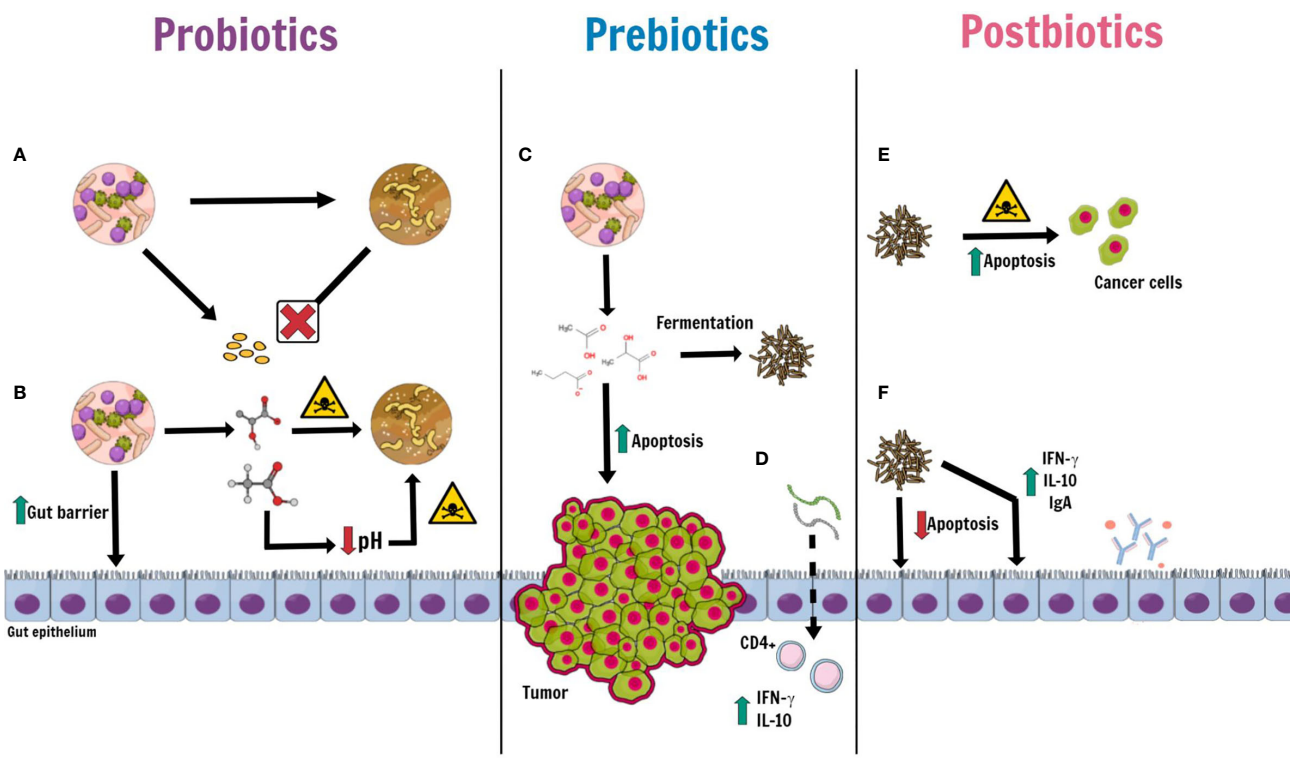

FIGURE 2 | Putative mechanisms of actions of probiotics, prebiotics and postbiotics. Some kinds of probiotic, such as *Bifidubacterium, Lactobacillus, Faecalibacterium* and *Clostridiales*, may take an active role in maintaining gut homeostasis by: **(A)** preventing the proliferation and colonization of pathogens by competing for nutrients and microenvironment; **(B)** releasing antimicrobial peptides (such as lactic acid, acetic acid and bacteriocins) with a direct bactericidal effect and, indirectly, by lowering luminal pH. Moreover, probiotics induce an increase of mucin production, promote epithelial restoration and can enhance the expression of tight junctions. Prebiotics (inulin, oligofructose, soybean and oat fiber, pectin and non-digestible carbohydrates), derived from probiotics, **(C)** produce postbiotics through a fermentation process. Among prebiotics, Butyrate not only has an anti-inflammatory and antioxidative effect, but also an apoptotic effect against cancer cells, in CRC patients. **(D)** Oligosaccharides with a low grade of polymerization, directly absorbed by gut epithelium, stimulate T-cell CD4+ to release IFN-γ and IL-10. Postbiotics, prebiotics-derived products, on the one hand, **(E)** play a cytotoxic role against cancer cells, which increase their apoptosis; on the others **(F)** *Lactobacillus rhamnosus GG* and *Bacteriodes fragilis*, for instance, provide the wellness of the intestinal epithelium by inhibiting apoptosis of normal epithelial cells and raising the level of Ig A, IFN-γ and IL-10.

clinical benefit of anti-CTLA-4 therapy in mouse models and also PD-1-based immunotherapy both in mice and in humans (75, 79, 150). In a study involving a group of 74 patients with a stage IV melanoma, 10 of them received ATB 30 days prior to the administration of ICI, while the rest of the group has been treated with a single-agent ICI, among Pembrolizumab, Nivolumab and Ipilimumab, as first-line therapy. Patients of the ATB group had a PFS and an OS meaningfully shorter than those in the non-ATB group (151).

Another study examined the impact of broad-spectrum antibiotic treatments administered 1 month before the initiation ICI to 3 months thereafter, in patients with metastatic non-small cell lung cancer. Interestingly, a shorter duration of ATB did not impact patient prognosis when compared with a longer course, bringing light on the potential importance of the duration of antibiotic treatments (152). The abovementioned data suggest that the duration of broad-spectrum antibiotic treatments with respect to the initiation of ICI-based immunotherapy is important.

In conclusion, it needs to be considered that patients that need antibiotic therapies may have an enfeebled immune system and are therefore more likely to be subjected to bacterial infections and to be refractory to anticancer immunotherapy. Consequently, in order to reduce the negative impact of ATB on ICI treatments, it will be important to define the specific antibiotics that are more likely to negatively impact on the clinical outcome. Thus, using prebiotics and probiotics during ATB might be solicited to reduce the negative impact on microbiome composition induced by antibiotic therapy.

Fecal Microbiome Transplantation

Fecal microbiota transplantation (FMT) represents the most direct way to affect microbiota, using complete normal human flora as a therapeutic probiotic mixture of living organisms. This type of bacteriotherapy has a longstanding history in animal health and is used against chronic infections of the bowel, including those infected by *Clostridium difficile* resistant to conventional therapies as well as other patient populations (153). Nonetheless, fecal microbiota transplantation is also one of the most used ways to prove that microbiota is able to upset the outcome of immunotherapy (74, 75, 80, 154–156).

Several studies aimed to show the impact of fecal microbiota transplantation in mice. Germ-free or antibiotic-treated mice that had received a fecal microbiota transplantation from patients who had a response to immune-checkpoint blockade, were enriched in CD45+ and CD8+ T cells, indeed correlating with a positive response to PD-1 immune-checkpoint blockade (80, 157) (**Figure 3**). On the other hand, fecal microbiota transplantation with feces from non-responders led to resistance to ICIs, with tumors having a high density of immunosuppressive CD4+ Treg cells (157).

Moreover, mice transplanted with feces from responders developed a higher response to anti-PD-L1 therapy (80, 154). It is noteworthy that, when fecal microbiota is enriched with *A. muciniphila*, as well as with *Faecalibacterium* spp and *Bifidobacterium* spp (80, 157), it correlates with a positive response to PD-1 immune-checkpoint blockade in patients with various types of tumors. Thus, *Bifidobacterium* in the gut is positively related to anti-tumor activity, especially by stimulating CD8+ T cells and DCs (60). In line with these observations, the use of antibiotics is related to lower clinical efficiency of immune-checkpoint blockade in different kinds of tumor tested in mice and patients (157).

Furthermore, clinical FMT trials are being considered in patients with both hematologic malignancies and solid tumors. The single-arm study "ODYSSEE" (158), explored the use of autologous fecal microbiota transplantation in acute myeloid leukemia patients treated with intensive chemotherapy and antibiotics. The aim was to restore the balance of their intestinal microbiome and thereby eradicate treatment-induced multidrug resistant bacteria, infection-related complications, as well as sequelae to the gastrointestinal tract. Moreover, in a Phase 1 clinical trial, FMT from patients that responded to immunotherapy is being administered to refractory patients with metastatic melanoma and unresectable stage III melanoma who failed at least one line of PD-1 blockade (159).

Recently, Baruch et al. reported the first-in-human clinical trials to test whether fecal microbiota transplantation can affect the response to anti–PD-1 immunotherapy in melanoma patients. In their phase 1 clinical trial, they investigated the safety and feasibility of FMT and the combination of FMT and reinduction of anti–PD-1 immunotherapy in 10 patients with anti–PD-1–refractory metastatic melanoma. They observed clinical responses in three patients, with FMT being associated with favorable changes in immune cell infiltrates and gene expression profiles in both the gut lamina propria and the tumor microenvironment (160). The design of new additional trials is currently underway, in order to test the hypothesis that the modulation of the gut microbiota can improve the response to treatment with ICIs (80).

These interesting preliminary findings offer compelling evidence for the ability of FMT to affect immunotherapy response in cancer patients, supporting the concept of overcoming resistance to immunotherapy by modulating the gut microbiota.

CONCLUSIONS AND FUTURE PERSPECTIVES

The microbiome era has begun, and we have obtained substantial results on the influence of microbiota on cancer progression and treatment, including ICIs. The crosstalk between the host immune system and microbiota may allow a microbiota-based selection of patients that might benefit from a specific immunotherapy treatment, boosting their anticancer response. However, more studies on the topic are needed in order to better elucidate the microbial communities that colonize the tumor microenvironment, as well as the approaches to modulate the composition of gut microbiota.

Many dietary nutrients were studied for modulating gut microbiota, with fiber having shown a significant impact on the maintenance of microbiota diversity and the response to

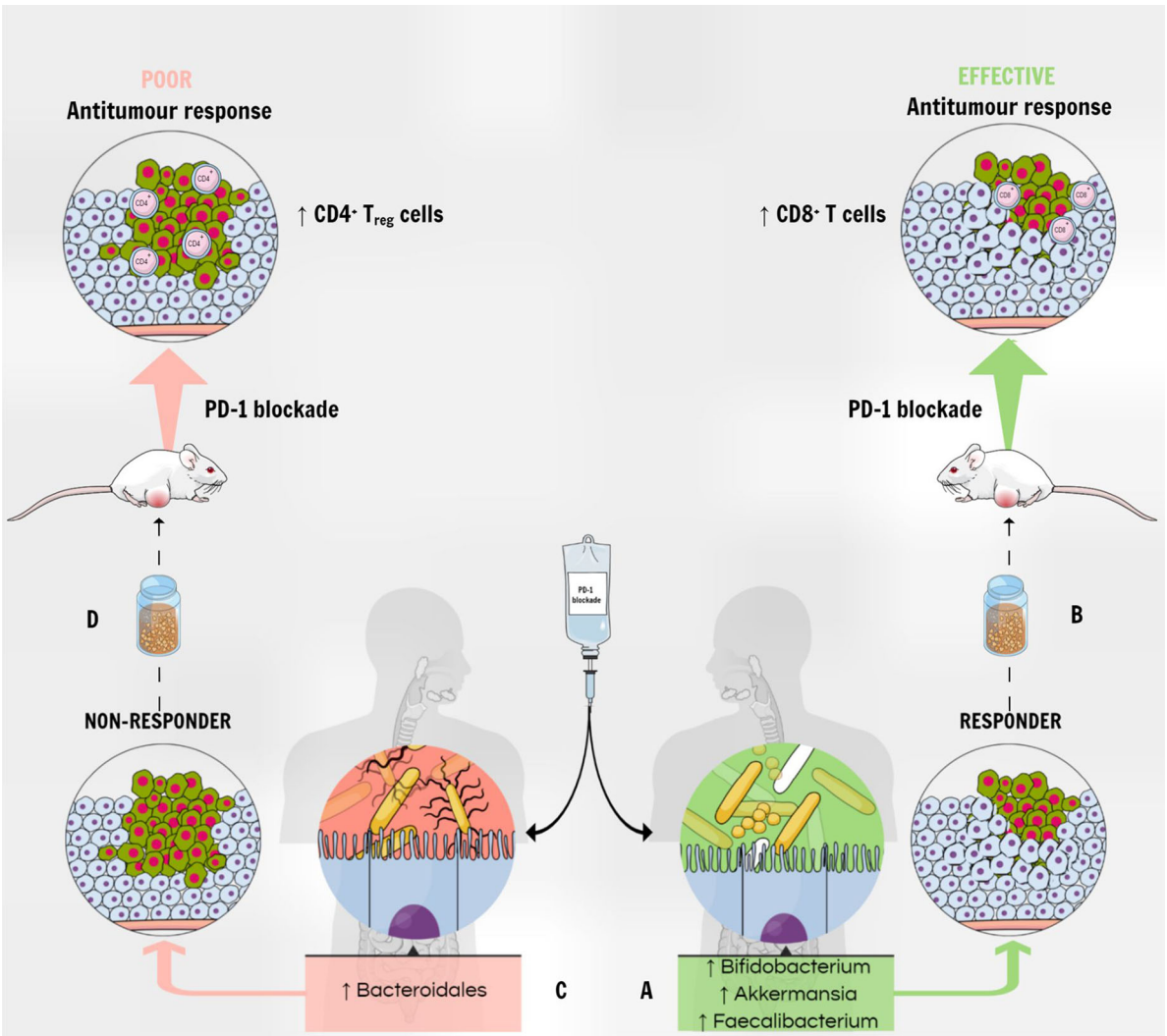

FIGURE 3 | The gut microbiota modulates the response to PD-1 blockade therapy. **(A)** The enrichment of fecal microbiota with *Akkermansia muciniphila, Faecalibacterium* spp and *Bifidobacterium* spp correlates with a positive response to PD-1 immune-checkpoint blockade in patients with various types of tumors. **(B)** A fecal microbiota transplantation from responders into tumor-bearing mice correlates with increased antitumor CD8+ T cells in the tumor and improved response to anti–PD-1 therapy. **(C)** On the other hand, the higher abundance of *Bacteroidales* correlates with a deficient response to PD-1 blockade therapy in humans. **(D)** Mice receiving FMT from non-responders show poor anti-tumor response to anti–PD-1 therapy, and tumors show a higher density of immunosuppressive CD4+ T_{reg} cells.

anti-PD-1 treatment. Since patients' compliance might be hard to monitor and the effect of food on microbiota might be modest and heavily host related. An alternative method that could provide control towards gut homeostasis could be the use of prebiotic, postbiotic, probiotic and the administration of specific therapeutic schemes, for example with antibiotics. However, broader research is needed to determine the impact of these environmental factors on cancer therapy.

Satisfactory results offer compelling evidence on the ability of FMT to affect immunotherapy response in cancer patients. Further clinical trials with the use of FMT in cancer patients during ICIs are needed to better identify a strategy to overcome resistance to immunotherapy and improve patients' outcomes.

Exploring the individual microbial profile and having a clear understanding of its interactions with various environmental factors could be a useful step to better modulate the gut microbiota. The prospect of being able to manipulate gut microbiota in order to modify the response to checkpoint inhibitors and set up personalized strategies serves as a continuous stimulus future research.

AUTHOR CONTRIBUTIONS

The authors contributed equally to this review. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Marin-Acevedo JA, Soyano AE, Dholaria B, Knutson KL, Lou Y. Cancer Immunotherapy Beyond Immune Checkpoint Inhibitors. *J Hematol Oncol* (2018) 11(1):8. doi: 10.1186/s13045-017-0552-6
2. Kelly PN. The Cancer Immunotherapy Revolution. *Sci (N Y NY)* (2018) 359 (6382):1344–5. doi: 10.1126/science.359.6382.1344
3. Khalil DN, Smith EL, Brentjens RJ, Wolchok JD. The Future of Cancer Treatment: Immunomodulation, CARs and Combination Immunotherapy. Nature Reviews. *Clin Oncol* (2016) 13(5):273–90. doi: 10.1038/nrclinonc.2016.25
4. Frankel T, Lanfranca MP, Zou W. The Role of Tumor Microenvironment in Cancer Immunotheraphy. *Advances in Experimental Medicine and Biology*. Springer Nature: Cham (2017) 1036: 51–64. doi: 10.1007/978-3-319-67577-0_4
5. Jiao S, Subudhi SK, Aparicio A, Ge Z, Guan B, Miura Y, et al. Differences in Tumor Microenvironment Dictate T Helper Lineage Polarization and Response to Immune Checkpoint Therapy. *Cell* (2019) 179(5):1177–1190.e13. doi: 10.1016/j.cell.2019.10.029
6. Chen F, Zhuang X, Lin L, Yu P, Wang Y, Shi Y, et al. New Horizons in Tumor Microenvironment Biology: Challenges and Opportunities. *BMC Med* (2015) 13:45. doi: 10.1186/s12916-015-0278-7
7. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. *Cell* (2011) 144(5):646–74. doi: 10.1016/j.cell.2011.02.013
8. Balkwill FR, Capasso M, Hagemann T. The Tumor Microenvironment at a Glance. *J Cell Sci* (2012) 125(Pt 23):5591–6. doi: 10.1242/jcs.116392
9. Jain T, Sharma P, Are AC, Vickers SM, Dudeja V. New Insights Into the Cancer-Microbiome-Immune Axis: Decrypting a Decade of Discoveries. *Front Immunol* (2021) 12:622064. doi: 10.3389/fimmu.2021.622064
10. Kovács T, Mikó E, Ujlaki G, Sári Z, Bai P. The Microbiome as a Component of the Tumor Microenvironment. In: A Birbrair, editor. *Tumor Microenvironment. Advances in Experimental Medicine and Biology*, vol. 1225 . Cham: Springer (2020). doi: 10.1007/978-3-030-35727-6_10.
11. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PloS Biol* (2016) 14(8):e1002533. doi: 10.1371/journal.pbio.1002533
12. Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, et al. Metabolomics Analysis Reveals Large Effects of Gut Microflora on Mammalian Blood Metabolites. *Proc Natl Acad Sci United States America* (2009) 106(10):3698–703. doi: 10.1073/pnas.0812874106
13. Bordenstein SR, Theis KR. Host Biology in Light of the Microbiome: Ten Principles of Holobionts and Hologenomes. *PloS Biol* (2015) 13:e1002226. doi: 10.1371/journal.pbio.1002226
14. Postler TS, Ghosh S. Understanding the Holobiont: How Microbial Metabolites Affect Human Health and Shape the Immune System. *Cell Metab* (2017) 26:110–30. doi: 10.1016/j.cmet.2017.05.008
15. Nakatsu G, Li X, Zhou H, Sheng J, Wong SH, Wu WKK, et al. Gut Mucosal Microbiome Across Stages of Colorectal Carcinogenesis. *Nat Commun* (2015) 6:8727. doi: 10.1038/ncomms9727
16. Holmes E, Li JV, Athanasiou T, Ashrafian H, Nicholson JK. Understanding the Role of Gut Microbiome-Host Metabolic Signal Disruption in Health and Disease. *Trends Microbiol* (2011) 19(7):349–59. doi: 10.1016/j.tim.2011.05.006
17. Zhernakova A, Kurilshikov A, Bonder MJ, Tigchelaar EF, Schirmer M, Vatanen T, et al. Population-Based Metagenomics Analysis Reveals Markers for Gut Microbiome Composition and Diversity. *Sci (N Y NY)* (2016) 352 (6285):565–9. doi: 10.1126/science.aad3369
18. Zmora N, Suez J, Elinav E. You are What You Eat: Diet, Health and the Gut Microbiota. *Nat Rev Gastroenterol Hepatol* (2019) 16:35–56. doi: 10.1038/s41575-018-0061-2
19. Walker AW, Ince J, Duncan SH, Webster LM, Holtrop G, Ze X, et al. Dominant and Diet-Responsive Groups of Bacteria Within the Human Colonic Microbiota. *ISME J* (2011) 5(2):220–30. doi: 10.1038/ismej.2010.118
20. Chang Y, Hou F, Pan Z, Huang Z, Han N. Optimization of Culturomics Strategy in Human Fecal Samples. *Front Microbiol* (2019) 10:2891. doi: 10.3389/fmicb.2019.02891
21. Lagier JC, Khelaifia S, Alou MT, Ndongo S, Dione N. Culture of Previously Uncultured Members of the Human Gut Microbiota by Culturomics. *Nat Microbiol* (2016) 1:16203. doi: 10.1038/nmicrobiol.2016.203
22. Burns MB, Lynch J, Starr TK, Knights D, Blekhman R. Virulence Genes are a Signature of the Microbiome in the Colorectal Tumor Microenvironment. *Genome Med* (2015) 7(1):55. doi: 10.1186/s13073-015-0177-8
23. Mira-Pascual L, Cabrera-Rubio R, Ocon S, Costales P, Parra A, Suarez A, et al. Microbial Mucosal Colonic Shifts Associated With the Development of Colorectal Cancer Reveal the Presence of Different Bacterial and Archaeal Biomarkers. *J Gastroenterol* (2015) 50(2):167–79. doi: 10.1007/s00535-014-0963-x
24. Bonnet M, Buc E, Sauvanet P, Darcha C, Dubois D, Pereira B, et al. Colonization of the Human Gut by E. Coli and Colorectal Cancer Risk. *Clin Cancer Res: Off J Am Assoc Cancer Res* (2014) 20(4):859–67. doi: 10.1158/1078-0432.CCR-13-1343
25. Kostic AD, Gevers D, Pedamallu CS, Michaud MM, Duke F, Earl AM, et al. Genomic Analysis Identifies Association of Fusobacterium With Colorectal Carcinoma. *Genome Res* (2012) 22(2):292–8. doi: 10.1101/gr.126573.111
26. Wu N, Yang X, Zhang R, Li J, Xiao C, Hu Y, et al. Dysbiosis Signature of Fecal Microbiota in Colorectal Cancer Patients. *Microbial Ecol* (2013) 66 (2):462–70. doi: 10.1007/s00248-013-0245-9
27. Kich DM, Vincenzi A, Majolo F, Volken de Souza CF, Goettert MI. Probiotic: Effectiveness Nutrition in Cancer Treatment and Prevention. *Nutricion Hospitalaria* (2016) 33(6):1430–7. doi: 10.20960/nh.806
28. The Human Microbiome Project Consortium, Methé B, Nelson K, et al. A Framework for Human Microbiome Research. *Nature* (2012) 486:215–21. doi: 10.1038/nature11209
29. Gagnière J, Raisch J, Veziant J, Barnich N, Bonnet R, Buc E, et al. Gut Microbiota Imbalance and Colorectal Cancer. *World J Gastroenterol* (2016) 22(2):501–18. doi: 10.3748/wjg.v22.i2.501
30. Bhatt AP, Redinbo MR, Bultman SJ. The Role of the Microbiome in Cancer Development and Therapy. *CA: Cancer J Clin* (2017) 67(4):326–44. doi: 10.3322/caac.21398
31. Liu X, Cheng Y, Shao L, Ling Z. Alterations of the Predominant Fecal Microbiota and Disruption of the Gut Mucosal Barrier in Patients With Early-Stage Colorectal Cancer. *BioMed Res Int* (2020) 2020:2948282. doi: 10.1155/2020/2948282
32. Yuan C, Subramanian S. microRNA-Mediated Tumor-Microbiota Metabolic Interactions in Colorectal Cancer. *DNA Cell Biol* (2019) 38 (4):281–5. doi: 10.1089/dna.2018.4579
33. Tilg H, Adolph TE, Gerner RR, Moschen AR. The Intestinal Microbiota in Colorectal Cancer. *Cancer Cell* (2018) 33:954–64. doi: 10.1016/j.ccell.2018.03.004
34. Jia W, Xie G, Jia W. Bile Acid-Microbiota Crosstalk in Gastrointestinal Inflammation and Carcinogenesis. *Nat Rev Gastroenterol Hepatol* (2018) 15 (2):111–28. doi: 10.1038/nrgastro.2017.119
35. Carbone C, Piro G, Di Noia V, D'Argento E, Vita E , Ferrara M, et al. Lung and Gut Microbiota as Potential Hidden Driver of Immunotherapy Efficacy in Lung Cancer. *Mediators Inflammation 2019* (2019) 2019:7652014. doi: 10.1155/2019/7652014
36. Fernández MF, Reina-Pérez I, Astorga JM, Rodríguez-Carrillo A, Plaza-Díaz J, Fontana L. Breast Cancer and Its Relationship With the Microbiota. *Int J Environ Res Public Health* (2018) 15(8):1747. doi: 10.3390/ijerph15081747
37. Sears CL, Garrett WS. Microbes, Microbiota, and Colon Cancer. *Cell Host Microbe* (2014) 15(3):317–28. doi: 10.1016/j.chom.2014.02.007
38. Tjalsma H, Boleij A, Marchesi JR, Dutilh BE. A Bacterial Driver-Passenger Model for Colorectal Cancer: Beyond the Usual Suspects. Nature Reviews. *Microbiology* (2012) 10(8):575–82. doi: 10.1038/nrmicro2819
39. Proctor LM. The Human Microbiome Project in 2011 and Beyond. *Cell Host Microbe* (2011) 10(4):287–91. doi: 10.1016/j.chom.2011.10.001
40. Wang Y, Zhang C, Hou S, Wu X, Liu J, Wan X. Analyses of Potential Driver and Passenger Bacteria in Human Colorectal Cancer. *Cancer Manage Res* (2020) 12:11553–61. doi: 10.2147/CMAR.S275316
41. Jin J, Gan T, Liu H, Wang Z, Yuan J, Deng T, et al. Diminishing Microbiome Richness and Distinction in the Lower Respiratory Tract of Lung Cancer Patients: A Multiple Comparative Study Design With Independent Validation. *Lung Cancer (Amsterdam Netherlands)* (2019) 136:129–35. doi: 10.1016/j.lungcan.2019.08.022
42. Eslami-S Z, Majidzadeh-A K, Halvaei S, Babapirali F, Esmaeili R. Microbiome and Breast Cancer: New Role for an Ancient Population. *Front Oncol* (2020) 10:120. doi: 10.3389/fonc.2020.00120

43. Meng S, Chen B, Yang J, Wang J, Zhu D, Meng Q, et al. Study of Microbiomes in Aseptically Collected Samples of Human Breast Tissue Using Needle Biopsy and the Potential Role of *In Situ* Tissue Microbiomes for Promoting Malignancy. *Front Oncol* (2018) 8:318. doi: 10.3389/fonc.2018.00318
44. Tlaskalová-Hogenová H, Štěpánková R, Hudcovic T, Tučková L, Cukrowska B, Lodinová-Žádníková R, et al. Commensal Bacteria (Normal Microflora), Mucosal Immunity and Chronic Inflammatory and Autoimmune Diseases. *Immunol Lett* (2004) 93(2-3):97–108. doi: 10.1016/j.imlet.2004.02.005
45. Gagnière J, Raisch J, Veziant J, Barnich N, Bonnet R, Buc E, et al. Gut Microbiota Imbalance and Colorectal Cancer. *World J Gastroenterol* (2016) 22(2):501–18. doi: 10.3748/wjg.v22.i2.501
46. Darfeuille-Michaud A, Boudeau J, Bulois P, Neut C, Glasser AL, Barnich N, et al. High Prevalence of Adherent-Invasive Escherichia Coli Associated With Ileal Mucosa in Crohn's Disease. *Gastroenterology* (2004) 127(2):412–21. doi: 10.1053/j.gastro.2004.04.061
47. Escobar-Páramo P, Grenet K, Le Menac'h A, Rode L, Salgado E, Amorin C, et al. Large-Scale Population Structure of Human Commensal Escherichia Coli Isolates. *Appl Environ Microbiol* (2004) 70(9):5698–700. doi: 10.1128/AEM.70.9.5698-5700.2004
48. Le Gall T, Clermont O, Gouriou S, Picard B, Nassif X, Denamur E, et al. Extraintestinal Virulence is a Coincidental by-Product of Commensalism in B2 Phylogenetic Group Escherichia Coli Strains. *Mol Biol Evol* (2007) 24 (11):2373–84. doi: 10.1093/molbev/msm172
49. Schwabe RF, Jobin C. The Microbiome and Cancer. *Nat Rev Cancer* (2013) 13(11):800–12. doi: 10.1038/nrc3610
50. Luzzaro F, Mezzatesta M, Mugnaioli C, Perilli M, Stefani S, Amicosante G, et al. Trends in Production of Extended-Spectrum Beta-Lactamases Among Enterobacteria of Medical Interest: Report of the Second Italian Nationwide Survey. *J Clin Microbiol* (2006) 44(5):1659–64. doi: 10.1128/JCM.44.5.1659-1664.2006
51. Marchesi JR, Dutilh BE, Hall N, Peters WH, Roelofs R, Boleij A, et al. Towards the Human Colorectal Cancer Microbiome. *PloS One* (2011) 6(5): e20447. doi: 10.1371/journal.pone.0020447
52. Castellarin M, Warren RL, Freeman JD, Dreolini L, Krzywinski M, Strauss J, et al. Fusobacterium Nucleatum Infection is Prevalent in Human Colorectal Carcinoma. *Genome Res* (2012) 22(2):299–306. doi: 10.1101/gr.126516.111
53. Asakura H, Momose Y, Ryu C-H, Kasuga F, Yamamoto S, Kumagai S, et al. Providencia Alcalifaciens Causes Barrier Dysfunction and Apoptosis in Tissue Cell Culture: Potent Role of Lipopolysaccharides on Diarrheagenicity. *Food Addit Amp Contam Part A Chem Anal Control Expo Risk Assess* (2013) 30:1459–66. doi: 10.1080/19440049.2013.790086
54. Chen T, Li Q, Zhang X, Long R, Wu Y, Wu J, et al. TOX Expression Decreases With Progression of Colorectal Cancers and is Associated With CD4 T-Cell Density and Fusobacterium Nucleatum Infection. *Hum Pathol* (2018) 79:93–101. doi: 10.1016/j.humpath.2018.05.008
55. Maddocks OD, Short AJ, Donnenberg MS, Bader S, Harrison DJ. Attaching and Effacing Escherichia Coli Downregulate DNA Mismatch Repair Protein *In Vitro* and are Associated With Colorectal Adenocarcinomas in Humans. *PloS One* (2009) 4(5):e5517. doi: 10.1371/journal.pone.0005517
56. Prorok-Hamon M, Friswell MK, Alswied A, Roberts CL, Song F, Flanagan PK, et al. Colonic Mucosa-Associated Diffusely Adherent Afac+ Escherichia Coli Expressing lpfA and Pks are Increased in Inflammatory Bowel Disease and Colon Cancer. *Gut* (2014) 63(5):761–70. doi: 10.1136/gutjnl-2013-304739
57. Raskov H, Burcharth J, Pommergaard HC. Linking Gut Microbiota to Colorectal Cancer. *J Cancer* (2017) 8(17):3378–95. doi: 10.7150/jca.20497
58. Kato I, Boleij A, Kortman GA, Roelofs R, Djuric Z, Severson RK, et al. Partial Associations of Dietary Iron, Smoking and Intestinal Bacteria With Colorectal Cancer Risk. *Nutr Cancer* (2013) 65(2):169–77. doi: 10.1080/01635581.2013.748922
59. Liu X, Lu R, Wu S, Sun J. Salmonella Regulation of Intestinal Stem Cells Through the Wnt/β-Catenin Pathway. *FEBS Lett* (2010) 584(5):911–6. doi: 10.1016/j.febslet.2010.01.024
60. Singh RP, Bashir H, Kumar R. Emerging Role of Microbiota in Immunomodulation and Cancer Immunotherapy. *Semin Cancer Biol* (2020) 70:37–52. doi: 10.1016/j.semcancer.2020.06.008
61. Klener P.JR, Otáhal P, Lateckova L, Klener P. Immunotherapy Approaches in Cancer Treatment. *Curr Pharm Biotechnol* (2015) 16(9):771–81. doi: 10.2174/1389201016666150619114554
62. Alatrash G, Jakher H, Stafford PD, Mittendorf EA. Cancer Immunotherapies, Their Safety and Toxicity. *Expert Opin Drug Saf* (2013) 12(5):631–45. doi: 10.1517/14740338.2013.795944
63. Matzinger P, Kamala T. Tissue-Based Class Control: The Other Side of Tolerance. *Nat Rev Immunol* (2011) 11(3):221–30. doi: 10.1038/nri2940
64. Leach DR, Krummel MF, Allison JP. Enhancement of Antitumor Immunity by CTLA-4 Blockade. *Sci (N Y NY)* (1996) 271(5256):1734–6. doi: 10.1126/science.271.5256.1734
65. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, et al. CTLA-4 Control Over Foxp3+ Regulatory T Cell Function. *Sci (N Y NY)* (2008) 322(5899):271–5. doi: 10.1126/science.1160062
66. Schluter J, Peled JU, Taylor BP, Markey KA, Smith M, Taur Y, et al. The Gut Microbiota is Associated With Immune Cell Dynamics in Humans. *Nature* (2020) 588(7837):303–7. doi: 10.1038/s41586-020-2971-8
67. Naidoo J, Page DB, Li BT, Connell LC, Schindler K, Lacouture ME, et al. Toxicities of the Anti-PD-1 and Anti-PD-L1 Immune Checkpoint Antibodies. *Ann Oncol: Off J Eur Soc Med Oncol* (2015) 26(12):2375–91. doi: 10.1093/annonc/mdv383
68. Galluzzi L, Buqué A, Kepp O, Zitvogel L, Kroemer G. Immunogenic Cell Death in Cancer and Infectious Disease. *Nat Rev Immunol* (2017) 17:97–111. doi: 10.1038/nri.2016.107
69. Gibney GT, Weiner LM, Atkins MB. Predictive Biomarkers for Checkpoint Inhibitor-Based Immunotherapy. *Lancet Oncol* (2016) 17:e542–51. doi: 10.1016/S1470-2045(16)30406-5
70. Australian Pancreatic Cancer Genome Initiative, ICGC Breast Cancer Consortium, ICGC MMML-Seq Consortium and ICGC PedBrain, Alexandrov LB, Nik-Zainal S, et al. Signatures of Mutational Processes in Human Cancer. *Nature* (2013) 500:415–21. doi: 10.1038/nature12477
71. Fessler J, Matson V, Gajewski TF. Exploring the Emerging Role of the Microbiome in Cancer Immunotherapy. *J Immunother Cancer* (2019) 7 (1):108. doi: 10.1186/s40425-019-0574-4
72. Macpherson AJ, Harris NL. Interactions Between Commensal Intestinal Bacteria and the Immune System. Nature Reviews. *Immunology* (2004) 4 (6):478–85. doi: 10.1038/nri1373
73. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 Blockade Induces Responses by Inhibiting Adaptive Immune Resistance. *Nature* (2014) 515(7528):568–71. doi: 10.1038/nature13954
74. Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, et al. Commensal Bifidobacterium Promotes Antitumor Immunity and Facilitates Anti–PD-L1 Efficacy. *Science* (2015) 350:1084–9. doi: 10.1126/science.aac4255
75. Vétizou M, Pitt JM, Daillère R, Lepage P, Waldschmitt N, Flament C, et al. Anticancer Immunotherapy by CTLA-4 Blockade Relies on the Gut Microbiota. *Sci (N Y NY)* (2015) 350(6264):1079–84. doi: 10.1126/science.aad1329
76. Kuczma MP, Ding Z-C, Li T, Habtetsion T, Chen T, Hao Z, et al. The Impact of Antibiotic Usage on the Efficacy of Chemoimmunotherapy Is Contingent on the Source of Tumor-Reactive T Cells. *Oncotarget* (2017) 8:111931–42. doi: 10.18632/oncotarget.22953
77. Uribe-Herranz M, Bittinger K, Rafail S, Guedan S, Pierini S, Tanes C, et al. Gut Microbiota Modulates Adoptive Cell Therapy. *Via CD8α Dendritic Cells IL-12 JCI Insight* (2018) 3(4). doi: 10.1172/jci.insight.94952
78. Smith M, Littmann ER, Slingerland JB, Clurman A, Slingerland AE. Intestinal Microbiota Composition Prior to CAR T Cell Infusion Correlates With Efficacy and Toxicity. *Blood* (2018) 132:3492. doi: 10.1182/blood-2018-99-118628
79. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillère R, et al. Gut Microbiome Influences Efficacy of PD-1–Based Immunotherapy Against Epithelial Tumors. *Science* (2018) 359:91–7. doi: 10.1126/science.aan3706
80. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, et al. Gut Microbiome Modulates Response to Anti–PD-1 Immunotherapy in Melanoma Patients. *Science* (2017) 359:97–103. doi: 10.1126/science.aan4236

81. Matson V, Fessler J, Bao R, Chongsuwat T, Zha Y, Alegre M-L, et al. The Commensal Microbiome is Associated With Anti–PD-1 Efficacy in Metastatic Melanoma Patients. *Science* (2018) 359:104–8. doi: 10.1126/science.aao3290
82. Xue X, Feng T, Yao S, Wolf KJ, Liu CG, Liu X, et al. Microbiota Downregulates Dendritic Cell Expression of miR-10a, Which Targets IL-12/IL-23p40. *J Immunol (Baltimore Md: 1950)* (2011) 187(11):5879–86. doi: 10.4049/jimmunol.1100535
83. Anfossi S, Calin GA. Gut Microbiota: A New Player in Regulating Immune- and Chemo-Therapy Efficacy. *Cancer Drug Resist (Alhambra Calif)* (2020) 3:356–70. doi: 10.20517/cdr.2020.04
84. van Niel G, D'Angelo G, Raposo G. Shedding Light on the Cell Biology of Extracellular Vesicles. Nature Reviews. *Mol Cell Biol* (2018) 19(4):213–28. doi: 10.1038/nrm.2017.125
85. Gottesman S. Micros for Microbes: non-Coding Regulatory RNAs in Bacteria. *Trends Genet: TIG* (2005) 21(7):399–404. doi: 10.1016/j.tig.2005.05.008
86. Kang SM, Choi JW, Lee Y, Hong SH, Lee HJ. Identification of microRNA-Size, Small RNAs in Escherichia Coli. *Curr Microbiol* (2013) 67(5):609–13. doi: 10.1007/s00284-013-0411-9
87. Koeppen K, Hampton TH, Jarek M, Scharfe M, Gerber SA, Mielcarz DW, et al. A Novel Mechanism of Host-Pathogen Interaction Through sRNA in Bacterial Outer Membrane Vesicles. *PloS Pathog* (2016) 12(6):e1005672. doi: 10.1371/journal.ppat.1005672
88. Choi JW, Kim SC, Hong SH, Lee HJ. Secretable Small RNAs *via* Outer Membrane Vesicles in Periodontal Pathogens. *J Dental Res* (2017) 96 (4):458–66. doi: 10.1177/0022034516685071
89. Liu S, da Cunha AP, Rezende RM, Cialic R, Wei Z, Bry L, et al. The Host Shapes the Gut Microbiota *via* Fecal MicroRNA. *Cell Host Microbe* (2016) 19 (1):32–43. doi: 10.1016/j.chom.2015.12.005
90. Charbonneau MR, O'Donnell D, Blanton LV, Totten SM, Davis JC, Barratt MJ, et al. Sialylated Milk Oligosaccharides Promote Microbiota-Dependent Growth in Models of Infant Undernutrition. *Cell* (2016) 164(5):859–71. doi: 10.1016/j.cell.2016.01.024
91. Laursen MF, Bahl MI, Michaelsen KF, Licht TR. First Foods and Gut Microbes. *Front Microbiol* (2017) 8:356. doi: 10.3389/fmicb.2017.00356
92. Claesson M, Jeffery I, Conde S, Power SE, O'Connor EM, Cusack S, et al. Gut Microbiota Composition Correlates With Diet and Health in the Elderly. *Nature* (2012) 488:178–84. doi: 10.1038/nature11319
93. David L, Maurice C, Carmody R, Gootenberg DB, Button JE, Wolfe BE, et al. Diet Rapidly and Reproducibly Alters the Human Gut Microbiome. *Nature* (2014) 505:559–63. doi: 10.1038/nature12820
94. Muegge BD, Kuczynski J, Knights D, Clemente JC, González A, Fontana L, et al. Diet Drives Convergence in Gut Microbiome Functions Across Mammalian Phylogeny and Within Humans. *Sci (N Y NY)* (2011) 332 (6032):970–4. doi: 10.1126/science.1198719
95. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The Human Microbiome Project. *Nature* (2007) 449(7164):804–10. doi: 10.1038/nature06244
96. Schroeder BO, Birchenough GM, Ståhlman M, Arike L, Johansson ME, Hansson GC, et al. Bifidobacteria or Fiber Protects Against Diet-Induced Microbiota-Mediated Colonic Mucus Deterioration. *Cell Host Microbe* (2018) 23:27–40. doi: 10.1016/j.chom.2017.11.004
97. Benus RF, van der Werf TS, Welling GW, Judd PA, Taylor MA, Harmsen HJ, et al. Association Between Faecalibacterium Prausnitzii and Dietary Fibre in Colonic Fermentation in Healthy Human Subjects. *Br J Nutr* (2010) 104(5):693–700. doi: 10.1017/S0007114510001030
98. Prajapati B, Rajput P, Kumar Jena P, Seshadri S. Investigation of Chitosan for Prevention of Diabetic Progression Through Gut Microbiota Alteration in Sugar Rich Diet Induced Diabetic Rats. *Curr Pharm Biotechnol* (2016) 17:173–84. doi: 10.2174/1389201017666151029110505
99. Spencer CN, Gopalakrishnan V, McQuade J, Andrews MC, Helmink B, Khan MAW, et al. (2019). The Gut Microbiome (GM) and Immunotherapy Response are Influenced by Host Lifestyle Factors, in: *Proceedings: AACR Annual Meeting 2019*, Atlanta, GA, USA: American Association for Cancer Research 29 March–3 April doi: 10.1158/1538-7445.AM2019-2838.
100. Yang Q, Liang Q, Balakrishnan B, Belobrajdic DP, Feng Q-J, Zhang W. Role of Dietary Nutrients in the Modulation of Gut Microbiota: A Narrative Review. *Nutrients* (2020) 12:381. doi: 10.3390/nu12020381
101. Singh CK, Liu X, Ahmad N. Resveratrol, in its Natural Combination in Whole Grape, for Health Promotion and Disease Management. *Ann New Y Acad Sci* (2015) 1348(1):150–60. doi: 10.1111/nyas.12798
102. Gatouillat G, Balasse E, Joseph-Pietras D, Morjani H, Madoulet C. Resveratrol Induces Cell-Cycle Disruption and Apoptosis in Chemoresistant B16 Melanoma. *J Cell Biochem* (2010) 110(4):893–902. doi: 10.1002/jcb.22601
103. Di Renzo L, Marsella LT, Carraro A, Valente R, Gualtieri P, Gratteri S, et al. Changes in LDL Oxidative Status and Oxidative and Inflammatory Gene Expression After Red Wine Intake in Healthy People: A Randomized Trial. *Mediators Inflamm* (2015) 2015:317–48. doi: 10.1155/2015/317348
104. Zmora N, Suez J, Elinav E. You are What You Eat: Diet, Health and the Gut Microbiota. *Nat Rev Gastroenterol Hepatol* (2019) 16:35–56. doi: 10.1038/s41575-018-0061-2
105. Zhang X, Browman G, Siu W, Basen-Engquist KM, Hanash SM, Hoffman KL, et al. The BE GONE Trial Study Protocol: A Randomized Crossover Dietary Intervention of Dry Beans Targeting the Gut Microbiome of Overweight and Obese Patients With a History of Colorectal Polyps or Cancer. *BMC Cancer* (2019) 19(1):1233. doi: 10.1186/s12885-019-6400-z
106. Geier MS, Butler RN, Howarth GS. Probiotics, Prebiotics and Synbiotics: A Role in Chemoprevention for Colorectal Cancer? *Cancer Biol Ther* (2006) 5 (10):1265–9. doi: 10.4161/cbt.5.10.3296
107. Mills JP, Rao K, Young VB. Probiotics for Prevention of Clostridium Difficile Infection. *Curr Opin Gastroenterol* (2018) 34(1):3–10. doi: 10.1097/MOG.0000000000000410
108. Kamada N, Kim YG, Sham HP, Vallance BA, Puente JL, Martens EC, et al. Regulated Virulence Controls the Ability of a Pathogen to Compete With the Gut Microbiota. *Sci (N Y NY)* (2012) 336(6086):1325–9. doi: 10.1126/science.1222195
109. Tuomola EM, Ouwehand AC, Salminen SJ. The Effect of Probiotic Bacteria on the Adhesion of Pathogens to Human Intestinal Mucus. *FEMS Immunol Med Microbiol* (1999) 26(2):137–42. doi: 10.1111/j.1574-695X.1999.tb01381.x
110. Campana R, van Hemert S, Baffone W. Strain-Specific Probiotic Properties of Lactic Acid Bacteria and Their Interference With Human Intestinal Pathogens Invasion. *Gut Pathog* (2017) Mar 69:12. doi: 10.1186/s13099-017-0162-4
111. Fayol-Messaoudi D, Berger CN, Coconnier-Polter MH, Liévin-Le Moal V, Servin AL. pH-, Lactic Acid-, and Non-Lactic Acid-Dependent Activities of Probiotic Lactobacilli Against Salmonella Enterica Serovar Typhimurium. *Appl Environ Microbiol* (2005) 71(10):6008–13. doi: 10.1128/AEM.71.10.6008-6013.2005
112. Gillor O, Etzion A, Riley MA. The Dual Role of Bacteriocins as Anti- and Probiotics. *Appl Microbiol Biotechnol* (2008) 81(4):591–606. doi: 10.1007/s00253-008-1726-5
113. Fong W, Li Q, Yu J. Gut Microbiota Modulation: A Novel Strategy for Prevention and Treatment of Colorectal Cancer. *Oncogene* (2020) 39 (26):4925–43. doi: 10.1038/s41388-020-1341-1
114. van Baarlen P, Wells JM, Kleerebezem M. Regulation of Intestinal Homeostasis and Immunity With Probiotic Lactobacilli. *Trends Immunol* (2013) 34(5):208–15. doi: 10.1016/j.it.2013.01.005
115. Ruiz L, Delgado S, Ruas-Madiedo P, Sánchez B, Margolles A. Bifidobacteria and Their Molecular Communication With the Immune System. *Front Microbiol* (2017) 8:2345. doi: 10.3389/fmicb.2017.02345
116. Kumar M, Kissoon-Singh V, Coria AL, Moreau F, Chadee K. Probiotic Mixture VSL3 Reduces Colonic Inflammation and Improves Intestinal Barrier Function in Muc2 Mucin-Deficient Mice. *Am J Physiol Gastrointestinal Liver Physiol* (2017) 312(1):G34–45. doi: 10.1152/ajpgi.00298.2016
117. Hibberd AA, Lyra A, Ouwehand AC, Rolny P, Lindegren H, Cedgård L, et al. Intestinal Microbiota is Altered in Patients With Colon Cancer and Modified by Probiotic Intervention. *BMJ Open Gastroenterol* (2017) 4(1):e000145. doi: 10.1136/bmjgast-2017-000145
118. Cannon JP, Lee TA, Bolanos JT, Danziger LH. Pathogenic Relevance of Lactobacillus: A Retrospective Review of Over 200 Cases. *Eur J Clin Microbiol Infect Dis: Off Publ Eur Soc Clin Microbiol* (2005) 24(1):31–40. doi: 10.1007/s10096-004-1253-y
119. Doron S, Snydman DR. Risk and Safety of Probiotics. *Clin Infect Dis: Off Publ Infect Dis Soc America* (2015) 60 Suppl 2(Suppl 2):S129–34. doi: 10.1093/cid/civ085

120. Huys G, Botteldoorn N, Delvigne F, De Vuyst L, Heyndrickx M, Pot B, et al. Microbial Characterization of Probiotics–Advisory Report of the Working Group "8651 Probiotics" of the Belgian Superior Health Council (SHC). *Mol Nutr Food Res* (2013) 57(8):1479–504. doi: 10.1002/mnfr.201300065
121. Gibson GR, Roberfroid MB. Dietary Modulation of the Human Colonic Microbiota: Introducing the Concept of Prebiotics. *J Nutr* (1995) 125 (6):1401–12. doi: 10.1093/jn/125.6.1401
122. Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ. Colonic Health: Fermentation and Short Chain Fatty Acids. *J Clin Gastroenterol* (2006) 40 (3):235–43. doi: 10.1097/00004836-200603000-00015
123. Parada Venegas D, de la Fuente MK, Landskron G, González MJ, Quera R, Dijkstra G, et al. Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases. *Front Immunol* (2019) 10:277. doi: 10.3389/fimmu.2019.00277
124. Canani RB, Costanzo MD, Leone L, Pedata M, Meli R, Calignano A. Potential Beneficial Effects of Butyrate in Intestinal and Extraintestinal Diseases. *World J Gastroenterol* (2011) 17(12):1519–28. doi: 10.3748/wjg.v17.i12.1519
125. Bultman SJ, Jobin C. Microbial-Derived Butyrate: An Oncometabolite or Tumor-Suppressive Metabolite? *Cell Host Microbe* (2014) 16(2):143–5. doi: 10.1016/j.chom.2014.07.011
126. Ito H, Takemura N, Sonoyama K, Kawagishi H, Topping DL, Conlon MA, et al. Degree of Polymerization of Inulin-Type Fructans Differentially Affects Number of Lactic Acid Bacteria, Intestinal Immune Functions, and Immunoglobulin A Secretion in the Rat Cecum. *J Agric Food Chem* (2011) 59(10):5771–8. doi: 10.1021/jf200859z
127. Taper HS, Roberfroid MB. Nontoxic Potentiation of Cancer Chemotherapy by Dietary Oligofructose or Inulin. *Nutr Cancer* (2000) 38(1):1–5. doi: 10.1207/S15327914NC381_1
128. Taper HS, Roberfroid MB. Possible Adjuvant Cancer Therapy by Two Prebiotics–Inulin or Oligofructose. *In Vivo (Athens Greece)* (2005) 19 (1):201–4. https://iv.iiarjournals.org/content/19/1/201.long
129. Singh V, Yeoh BS, Chassaing B, Xiao X, Saha P, Aguilera Olvera R, et al. Dysregulated Microbial Fermentation of Soluble Fiber Induces Cholestatic Liver Cancer. *Cell* (2018) 175(3):679–694.e22. doi: 10.1016/j.cell.2018.09.004
130. Panebianco C, Andriulli A, Pazienza V. Pharmacomicrobiomics: Exploiting the Drug-Microbiota Interactions in Anticancer Therapies. *Microbiome* (2018) 6(1):92. doi: 10.1186/s40168-018-0483-7
131. Aguilar-Toalá JE, Garcia-Varela R, Garcia HS, Mata-Haro V, González-Córdova AF, Vallejo-Cordoba B, et al. Postbiotics: An Evolving Term Within the Functional Foods Field. *Trends Food Sci Technol* (2018) 75:105–14. doi: 10.1016/j.tifs.2018.03.009
132. Whitford EJ, Cummins AG, Butler RN, Prisciandaro LD, Fauser JK, Yazbeck R, et al. Effects of Streptococcus Thermophilus TH-4 on Intestinal Mucositis Induced by the Chemotherapeutic Agent, 5-Fluorouracil (5-Fu). *Cancer Biol Ther* (2009) 8(6):505–11. doi: 10.4161/cbt.8.6.7594
133. Prisciandaro LD, Geier MS, Butler RN, Cummins AG, Howarth GS. Probiotic Factors Partially Improve Parameters of 5-Fluorouracil-Induced Intestinal Mucositis in Rats. *Cancer Biol Ther* (2011) 11(7):671–7. doi: 10.4161/cbt.11.7.14896
134. Wang L, Cao H, Liu L, Wang B, Walker WA, Acra SA, et al. Activation of Epidermal Growth Factor Receptor Mediates Mucin Production Stimulated by P40, a Lactobacillus Rhamnosus GG-Derived Protein. *J Biol Chem* (2014) 289(29):20234–44. doi: 10.1074/jbc.M114.553800
135. Yan F, Polk DB. Characterization of a Probiotic-Derived Soluble Protein Which Reveals a Mechanism of Preventive and Treatment Effects of Probiotics on Intestinal Inflammatory Diseases. *Gut Microbes* (2012) 3 (1):25–8. doi: 10.4161/gmic.19245
136. Wang Y, Liu L, Moore DJ, Shen X, Peek RM, Acra SA, et al. An LGG-Derived Protein Promotes IgA Production Through Upregulation of APRIL Expression in Intestinal Epithelial Cells. *Mucosal Immunol* (2017) 10 (2):373–84. doi: 10.1038/mi.2016.57
137. Round JL, Mazmanian SK. Inducible Foxp3+ Regulatory T-Cell Development by a Commensal Bacterium of the Intestinal Microbiota. *Proc Natl Acad Sci United States America* (2010) 107(27):12204–9. doi: 10.1073/pnas.0909122107
138. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly- Y, et al. The Microbial Metabolites, Short-Chain Fatty Acids, Regulate Colonic Treg Cell Homeostasis. *Sci (N Y NY)* (2013) 341(6145):569–73. doi: 10.1126/science.1241165
139. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, et al. Metabolites Produced by Commensal Bacteria Promote Peripheral Regulatory T-Cell Generation. *Nature* (2013) 504(7480):451–5. doi: 10.1038/nature12726
140. Konishi H, Fujiya M, Tanaka H, Ueno N, Moriichi K, Sasajima J, et al. Probiotic-Derived Ferrichrome Inhibits Colon Cancer Progression via JNK-Mediated Apoptosis. *Nat Commun* (2016) 7:12365. doi: 10.1038/ncomms12365
141. Manichanh C, Reeder J, Gibert P, Varela E, Llopis M, Antolin M, et al. Reshaping the Gut Microbiome With Bacterial Transplantation and Antibiotic Intake. *Genome Res* (2010) 20(10):1411–9. doi: 10.1101/gr.107987.110
142. Jernberg C, Löfmark S, Edlund C, Jansson JK. Long-Term Ecological Impacts of Antibiotic Administration on the Human Intestinal Microbiota. *ISME J* (2007) 1(1):56–66. doi: 10.1038/ismej.2007.3
143. Antonopoulos DA, Huse SM, Morrison HG, Schmidt TM, Sogin ML, Young VB. Reproducible Community Dynamics of the Gastrointestinal Microbiota Following Antibiotic Perturbation. *Infection Immun* (2009) 77(6):2367–75. doi: 10.1128/IAI.01520-08
144. Dethlefsen L, Huse S, Sogin ML, Relman DA. The Pervasive Effects of an Antibiotic on the Human Gut Microbiota, as Revealed by Deep 16S rRNA Sequencing. *PloS Biol* (2008) 6(11):e280. doi: 10.1371/journal.pbio.0060280
145. Löfmark S, Jernberg C, Jansson JK, Edlund C. Clindamycin-Induced Enrichment and Long-Term Persistence of Resistant Bacteroides Spp. And Resistance Genes. *J Antimicrobial Chemother* (2006) 58(6):1160–7. doi: 10.1093/jac/dkl420
146. Derosa L, Hellmann MD, Spaziano M, Halpenny D, Fidelle M, Rizvi H, et al. Negative Association of Antibiotics on Clinical Activity of Immune Checkpoint Inhibitors in Patients With Advanced Renal Cell and non-Small-Cell Lung Cancer. *Ann Oncol: Off J Eur Soc Med Oncol* (2018) 29 (6):1437–44. doi: 10.1093/annonc/mdy103
147. Chalabi M, Cardona A, Nagarkar DR, Dhawahir Scala A, Gandara DR, Rittmeyer A, et al. Efficacy of Chemotherapy and Atezolizumab in Patients With non-Small-Cell Lung Cancer Receiving Antibiotics and Proton Pump Inhibitors: Pooled Post Hoc Analyses of the OAK and POPLAR Trials. *Ann Oncol* (2020) 31:525–31. doi: 10.1016/j.annonc.2020.01.006
148. Peggs KS, Quezada SA, Korman AJ, Allison JP. Principles and Use of Anti-CTLA4 Antibody in Human Cancer Immunotherapy. *Curr Opin Immunol* (2006) 18(2):206–13. doi: 10.1016/j.coi.2006.01.011
149. Berman D, Parker SM, Siegel J, Chasalow SD, Weber J, Galbraith, et al. Blockade of Cytotoxic T-Lymphocyte Antigen-4 by Ipilimumab Results in Dysregulation of Gastrointestinal Immunity in Patients With Advanced Melanoma. *Cancer Immun* (2010) 10:11. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2999944/
150. Derosa L, Routy B, Mezquita L, Naltet C, Enot D, Fidelle M, et al. Antibiotics Prescription to Decrease Progression-Free Survival (PFS) and Overall Survival (OS) in Patients With Advanced Cancers Treated With PD1/PDL1 Immune Checkpoint Inhibitors. *J Clin Oncol* (2017) 35(15):3015–5. doi: 10.1200/JCO.2017.35.15_suppl.3015
151. Elkrief A, El Raichani L, Richard C, Messaoudene M, Belkaid W, Malo J, et al. Antibiotics are Associated With Decreased Progression-Free Survival of Advanced Melanoma Patients Treated With Immune Checkpoint Inhibitors. *Oncoimmunology* (2019) 8(4):e1568812. doi: 10.1080/2162402X.2019.1568812
152. Galli MPG, Poggi M, Fucà G, Imbimbo M, Proto C, Signorelli D, et al. Effects of Antibiotic Use During Immunotherapy in Metastatic non Small Cell Lung Cancer. *Ann Oncol* (2018) 29:493–547. doi: 10.1093/annonc/mdy292.088
153. Borody TJ, Warren EF, Leis SM, Surace R, Ashman O, Siarakas S. Bacteriotherapy Using Fecal Flora: Toying With Human Motions. *J Clin Gastroenterol* (2004) 38 (6):475–83. doi: 10.1097/01.mcg.0000128988.13808.dc
154. Gopalakrishnan V, Helmink BA, Spencer CN, Reuben A, Wargo JA. The Influence of the Gut Microbiome on Cancer, Immunity, and Cancer Immunotherapy. *Cancer Cell* (2018) 33(4):570–80. doi: 10.1016/j.ccell.2018.03.015
155. Elkrief A, Derosa L, Zitvogel L, Kroemer G, Routy B. The Intimate Relationship Between Gut Microbiota and Cancer Immunotherapy. *Gut Microbes* (2019) 10(3):424–8. doi: 10.1080/19490976.2018.1527167

156. Routy B, Gopalakrishnan V, Daillère R, Zitvogel L, Wargo JA, Kroemer G, et al. The Gut Microbiota Influences Anticancer Immunosurveillance and General Health. *Nat Rev Clin Oncol* (2018) 15:382–96. doi: 10.1038/s41571-018-0006-2
157. Chaput N, Lepage P, Coutzac C, Soularue E, Le Roux K, Monot C, et al. Baseline Gut Microbiota Predicts Clinical Response and Colitis in Metastatic Melanoma Patients Treated With Ipilimumab. *Ann Oncol: Off J Eur Soc Med Oncol* (2017) 28(6):1368–79. doi: 10.1093/annonc/mdx108
158. Mohty M, Malard F, Vekhoff A, Lapusan S, Isnard F, D'Incan E, et al. The Odyssee Study: Prevention of Dysbiosis Complications With Autologous Fecal Microbiota Transfer (FMT) in Acute Myeloid Leukemia (AML) Patients Undergoing Intensive Treatment: Results of a Prospective Multicenter Trial. *Blood* (2018) 132:1444–4. doi: 10.1182/blood-2018-99-112825
159. Baruch EN, Youngster I, Ortenberg R, Ben-Betzalel G, et al. Abstract CT042: Fecal Microbiota Transplantation (FMT) and Re-Induction of Anti-PD-1 Therapy in Refractory Metastatic Melanoma Patients - Preliminary Results From a Phase I Clinical Trial (NCT03353402). (2019), CT042–2. doi: 10.1158/1538-7445.SABCS18-CT042
160. Baruch EN, Youngster I, Ben-Betzalel G, Ortenberg R, Lahat A, Katz L, et al. Fecal Microbiota Transplant Promotes Response in Immunotherapy-Refractory Melanoma Patients. *Science* (2021) 371(6529):602–9. doi: 10.1126/science.abb5920

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frontiers in Immunology

ORIGINAL RESEARCH
published: 14 September 2021
doi: 10.3389/fimmu.2021.694490

Novel Immune Infiltrating Cell Signature Based on Cell Pair Algorithm Is a Prognostic Marker in Cancer

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OPEN ACCESS

Edited by:
Salem Chouaib, Institut Gustave Roussy, France

Reviewed by:
Ajit Johnson Nirmal, Dana-Farber Cancer Institute, United States
Claudine Kieda, CNRS Centre for Molecular Biophysics, France

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Specialty section:
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

Received: *13 April 2021*
Accepted: *11 August 2021*
Published: *14 September 2021*

Citation:
Zhang H, Wang Z, Dai Z, Wu W, Cao H, Li S, Zhang N and Cheng Q (2021) Novel Immune Infiltrating Cell Signature Based on Cell Pair Algorithm Is a Prognostic Marker in Cancer. Front. Immunol. 12:694490. doi: 10.3389/fimmu.2021.694490

Tumor-infiltrating immune cells (TIICs) have become an important source of markers for predicting the clinical outcomes of cancer patients. However, measurements of cellular heterogeneity vary due to the frequently updated reference genomes and gene annotations. In this study, we systematically collected and evaluated the infiltration pattern of 65 immune cells. We constructed the Immune Cell Pair (ICP) score based on the cell pair algorithm in 3,715 samples and across 12 independent cancer types, among which, the ICP score from six cancer types was further validated in 2,228 GEO samples. An extensive tumorigenic and immunogenomic analysis was subsequently conducted. As a result, the ICP score showed a robust reliability and efficacy in predicting the survival of patients with gliomas, in pan-cancer samples, and six independent cancer types. Notably, the ICP score was correlated with the genomic alteration features in gliomas. Moreover, the ICP score exhibited a remarkable association with multiple immunomodulators that could potentially mediate immune escape. Finally, the ICP score predicted immunotherapeutic responses with a high sensitivity, allowing a useful tool for predicting the overall survival and guiding immunotherapy for cancer patients.

Keywords: immune cell, glioma microenvironment, cell pair algorithm, immunotherapy, prognostic model

Abbreviations: TIICs, tumor-infiltrating immune cells; ICP, immune cell pair; NK, natural killer; GEO, Gene Expression Omnibus; TCGA, The Cancer Genome Atlas; CGGA, Chinese Glioma Genome Atlas; RMA, robust multichip average; FPKM, fragments per kilobase million; TPM, transcripts per kilobase million; SNV, single-nucleotide variant; SNP, single-nucleotide polymorphism; INS, insertion; DEL, deletion; ROC, receiver operating characteristic; AUC, area under the curve; CNV, copy number variation; MSI, microsatellite instability; HRD, homologous recombination deficiency; CTA, cancer testis antigen; APM, antigen processing and presenting machinery; TCR, T cell receptor; IFNG, interferon gamma; ISG.RS, interferon stimulated genes resistance signature; IFNG.GS, IFNG hallmark gene set; CYT, cytotoxic activity; GEP, T cell-inflamed gene expression profile; ICB, immune checkpoint blockage.

INTRODUCTION

Tumor-infiltrating immune cells (TIICs), including T cells, B cells, macrophages, and natural killer (NK) cells, represent the major components of immune response against a tumor (1). TIICs not only regulate the immunosurveillance and survival of cancer (2), but also accelerate tumor progression by creating a permissive microenvironment that stimulates tumor growth (3). Accumulating evidences have demonstrated that TIICs were associated with the clinical outcomes in various cancer types, including breast cancer (4), ovarian cancer (5), pancreatic tumor (6), lung adenocarcinoma (7), head and neck squamous cell carcinoma (8), and melanoma (9). However, efforts are still needed for a deep understanding of the immune activity of TIICs in the tumor microenvironment. So far, classic methods estimating TIICs include flow cytometry, immunofluorescence, and RNAseq. However, unified results may appear due to the different intervention factors in each method or different reference genomes. It should be noted that the fraction of each TIICs is within a relatively stable range. Thus, investigating the ratio of different TIICs is interesting and promising in optimizing the research about tumor microenvironment.

Previous studies have elucidated the tumor microenvironment in different cancer types, among which, glioma is one of the most common and malignant brain tumor with leading cancer-caused death rates. Currently, the clinical outcome of glioma patients is still dismal (10). Notably, glioma patients with similar clinical features tend to have a different prognosis due to the high level of heterogeneity, which greatly sets back the prospect for the prognosis of glioma patients. Previous studies have successfully extracted the TIICs from gliomas, aiming to provide a convincing evidence of the existence of abundant TIICs in gliomas microenvironment and provide important insights into immunotherapeutic approaches (11). The abundant available datasets of gliomas also facilitate the investigation on gliomas. Altogether, developing a TIIC-based signature in glioma and some other malignant cancer types can help in determining the prognostic value of TIICs, furthermore, improve the efficiency of immunotherapeutic approaches that activate the tumor-specific immune response.

In this study, 65 immune cell types were incorporated into the construction of the prognostic signature, the abundance of which was estimated in the glioma cohort and six independent cancer types to identify the immune cell types with an optimal prognostic value. Then, the immune cell pair (ICP) score was constructed based on the infiltration level of the identified results. ICP score was found to significantly correlate with the overall survival in glioma patients, six independent cancer types, and pan-cancer samples. Moreover, the ICP score profoundly correlates with various tumorigenic mutations in glioma patients and could sensitively predict the response to immunotherapy targeting immune checkpoints. This novel immune scoring system enables an in-depth understanding of tumor infiltrating immune cells and improves the clinical management of glioma patients.

MATERIALS AND METHODS

Datasets Collecting and Preprocessing

Pan-cancer data and the corresponding clinical datasets were collected from the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) and The Cancer Genome Atlas (TCGA; https://xenabrowser.net/). The glioma gene expression profiles and the corresponding clinical datasets were collected from the GEO, TCGA, and Chinese Glioma Genome Atlas (CGGA; http://www.cgga.org.cn/). A total of 5,230 pan-cancer samples of 12 independent cancer types were included in this study. A total of 3,715 glioma patient samples were collected from 14 cohorts. Cohorts with more than 50 glioma samples were included and cohorts with incomplete information on the overall survival of patients were excluded. In total, 2,228 samples of 12 cohorts consisting of 6 independent cancer types were from the GEO. The information of the platforms and numbers of samples of each cohort is provided in **Table S1**.

Raw data from the GEO datasets were generated using Affymetrix and Agilent. The robust multichip average (RMA) algorithm was used to perform quantile normalization and background correction of the raw data from Affymetrix. The consensus median polish algorithm was used for the final summarizing of oligonucleotides for each transcript in the Affymetrix software. Limma software was used for processing the raw data from Agilent. RNA-sequencing data were downloaded from the TCGA and CGGA data portals, and the fragments per kilobase million (FPKM) values were transformed into transcripts per kilobase million (TPM) values that had a similar signal intensity with the RMA-standardized values from the GEO datasets (12). R package sva was used to remove the computational batch effect among each cohort. Each cohort was processed and normalized independently.

Immune Cell Signature Collection

Immune cell signatures were collected from diverse publicly available resources through a manually extensive literature search (13–22). Literatures with the reference genome of immune cells over the last 15 years were screened out. A total of 65 immune cell signatures were finally integrated by combining the gene sets of the same immune cell type from different literatures and excluding non-immune and non-stromal cell types. These 65 immune and stromal cells included B cells, CD8 T cells, DCs, Macrophages, Neutrophils, Th1 cells, Th2 cells, Mast cells, NK cells, Erythrocytes, Melanocytes, Megakaryocytes, Fibroblasts, Astrocytes, Basophils, Monocytes, Endothelial cells, et al. (**Table S2**). Thus, this immune cell signature was considered to be reliable and comprehensive.

Development of a Reliable Prognostic Signature in Glioma

A prognostic signature was constructed based on stable immune infiltrating cells. The R package GSVA was applied to implement the single sample gene set enrichment analysis (ssGSEA) for calculating the immune enrichment score of 65 immune cell signatures in three glioma datasets, TCGALGGGBM-RNAseq

(656 samples), CGGA311 (311 samples), and GSE108474 (414 samples), respectively (23). Univariate Cox analysis was performed on the 65 immune cell signatures to select the overlapped prognosis-associated immune cell types whose expression was significantly associated with patient OS ($P < 0.05$) in these three glioma datasets. Prognosis-associated immune cell types (Ci) were paired with all immune infiltrating cell types (Cj). For a cell pair started with Ci, Ci and Cj, Score_ij = 1 (exp_Ci – exp_Cj > 0) and Score_ij = 0 (exp_Ci – exp_Cj < 0). C-index was adopted to estimate the performance of each Score_ij and find out the Score_ij with the statistically significant p-value and highest C-index (16). For each Ci, Score_ij was identified with the highest C-index. For the obtained cell pairs, cell pairs were sorted with the HR > 1 and duplicate cell pairs were removed. Then, the ICP score was calculated as the sum of these selected Score_ij:

$$\text{ICP score} = \Sigma \text{ Score\_ij}$$

ICP score was then validated in all included 14 glioma cohorts and the Xiangya cohort.

Genomic Alterations in the Immune Cell Pair Score

Somatic mutations and somatic copy number alternations (CNAs) which corresponded to the glioma samples with RNA-seq data were downloaded from TCGA. GISTIC analysis was adopted to determine the genomic event enrichment. CNAs associated with the two ICP score groups and the threshold copy number at alteration peaks were obtained using GISTIC 2.0 analysis (https://gatk.broadinstitute.org). The R package TCGAbiolinks was used for downloading the somatic mutation data derived from the WES data acquired by Mutect2 (24). Somatic mutations including the single-nucleotide variant (SNV), single-nucleotide polymorphism (SNP), insertion (INS), and deletion (DEL) were analyzed and visualized using the R package maftools (25). Based on the ascending order of the p-value, 30 most differentially mutated genes were detected using Fisher's exact test. CoMEt algorithm was used to detect the co-occurrence and mutually exclusive mutations.

Prediction of the Immune Cell Pair Score in Immunotherapy Response

The IMvigor210 cohort, a urothelial carcinoma cohort treated with the anti-PD-L1 antibody atezolizumab, was included for the prediction of response to immunotherapy (26). The melanoma dataset (GSE78220) was also used to predict the response to anti-PD-1 (pembrolizumab or nivolumab) immunotherapy (27). Based on the Creative Commons 3.0 License, complete expression data and clinical data were downloaded from http://research-pub.Gene.com/IMvigor210CoreBiologies. Raw data were normalized using the DEseq2 R package, and the count value or FPKM normalized value were transformed into the TPM value. ICP score was then constructed independently in these two datasets.

Development of a Reliable Prognostic Signature in Other Cancer Types

Subsequently, the prognostic signature was constructed independently based on stable immune infiltrating cells in 12 cancer types from the pan-cancer data in TCGA. Univariate Cox analysis was used to select the prognosis-associated immune cell types whose expression was significantly associated with patient OS in each of the 12 cancer types ($P < 0.05$), respectively. Prognosis-associated immune cell types (Ci) were paired with all immune infiltrating cell types (Cj). For a cell pair starting with Ci, Ci and Cj, Score_ij = 1 (exp_Ci – exp_Cj > 0) and Score_ij = 0 (exp_Ci – exp_Cj < 0). C-index was adopted to estimate the performance of each Score_ij and find out the Score_ij with the statistically significant p-value and highest C-index (16). For each Ci, Score_ij was identified with the highest C-index. For the obtained cell pairs, cell pairs were sorted with the HR > 1 and duplicate cell pairs were removed. Then, the ICP score was calculated as the sum of these selected Score_ij:

$$\text{ICP score} = \Sigma \text{ Score\_ij}$$

Twelve datasets of six representative cancer types were selected for further validation of the ICP score established in each cancer type.

RNA Sequencing

RNAstore-fixed tumor tissues from 48 glioma patients were collected for sequencing. RNA was sheared followed by sequencing library preparation using the NEBNext Ultra RNA Library Prep Kit. The Phusion High-Fidelity RNA polymerase, the Index (X) Primer and the Universal PCR primers. After target region capture by biotin-labeled probes, the captured libraries were sequenced on an Illumina Hiseq platform to generate 125/150 bp paired-end reads. In-house perlscripts were used to process raw data (raw reads). Then, reads containing adapter and ploy-N, and low-quality reads were removed to obtain clean data (clean reads). Reference genome and gene model annotation files were obtained from the genome website. Index of the reference genome was built using Hisat2 v2.0.5 and paired-end clean reads were aligned to the reference genome. FeatureCounts v1.5.0-p3 was then used to count the read numbers mapped to each gene. TPM value of each gene was calculated on the basis of the gene length and reads count.

Statistical Analysis

Kaplan-Meier curves with log-rank test were used to assess survival difference between groups. The univariate and multivariate Cox regression analyses were performed to detect the prognostic factors. Pearson correlation analyses were used to calculate correlation coefficients. The cutoff value of ICP scores was calculated using the R package survminer. Based on the dichotomized ICP scores, patients were grouped as with high or low ICP score in each data set. Data was visualized using the R package ggplot2. OncoPrint was used to delineate the mutation landscape of TCGA by the maftools R package (28).

All survivorship curves were generated using R package survminer. All statistical analyses were conducted using R software. $P < 0.05$ was considered statistically significant.

RESULT

Construction of the Immune Cell Pair Score and Its Prognostic Value

A total of 65 immune cell types were collected from publicly available resources and analyzed for the construction of ICP score. In total, 38 overlapped prognosis-associated immune cell types were identified by univariate Cox analysis performed on the 65 immune cell types in TCGA, CGGA, and GSE108474, respectively (**Table S3**). ICP score was calculated based on the predictive performance of each cell pair constituted from 38 prognosis-associated immune cell types and all 65 immune cell types (**Figure 1A**). Glioma patients were classified into high ICP score group and low ICP score group based on the cutoff value of the ICP scores calculated using the R package survminer. High ICP score was a prognostic marker for poor clinical outcomes in pan-glioma samples from TCGA, CGGA, and GSE108474 (log-rank test, $p < 0.001$; **Figures 1B–D**, respectively). High ICP score was also a prognostic marker for poor clinical outcomes in LGG, and GBM samples from TCGA (log-rank test, $p < 0.001$, $p = 0.00195$, respectively; **Figure S2A**), CGGA (log-rank test, $p < 0.001$, respectively; **Figure S2B**), and GSE108474 (log-rank test, $p < 0.001$, $p = 0.05947$; **Figure S2C**). Moreover, a high ICP score correlated with a worse survival probability in the Xiangya cohort (log-rank test, $p < 0.001$; **Figure 1E**, **Table S4**). The receiver operating characteristic (ROC) analyses with the Area Under the Curve (AUC) of 0.795 confirmed that ICP score was a prognostic biomarker in predicting the survival status of glioma patients (**Figure 1F**). Further, ICP score was a prognostic biomarker in predicting the 1-year, 3-year, and 5-year survival of glioma patients, which the AUC of ROC curve was 0.868, 0.879, and 0.801, respectively (**Figure 1G**). The prognostic value of ICP score was further verified in all 3,715 glioma samples included in this study (**Figure 2A**) and in each of the glioma datasets (**Figure 2B**). ICP score could significantly stratify the survival of glioma patients. The univariate Cox analyses confirmed that ICP score was a hazardous factor in glioma (**Figure 3A**).

Validation of the Immune Cell Pair Score in Other Cancer Types

To further confirm the efficacy and stability of the prognostic signature from the 65 immune cell types, ICP score was developed in 12 cancer types from TCGA, respectively. ICP score predicted a worse survival outcome in all of the 12 cancer types included (**Figure 3C**), and the univariate Cox analyses confirmed that ICP score was a hazardous factor in all of the 12 cancer types (**Figure 3B**). We then performed the validation of ICP score in six most representative cancer types (**Table S5**). As expected, ICP score was associated with a worse overall survival in breast cancer (**Figure 4A**), melanoma samples (**Figure 4B**), Head and Neck squamous cell carcinoma samples (**Figure 4C**), Pancreatic adenocarcinoma samples (**Figure 4D**), Lung adenocarcinoma samples (**Figure 4E**), and Liver hepatocellular carcinoma samples (**Figure 4F**).

Genomic Features of the Immune Cell Pair Score Groups in Glioma

Somatic mutation analysis and copy number variation (CNV) were performed using the TCGA dataset to explore the genomic traits of the two ICP score groups. A global CNV profile was obtained by comparing the two ICP score groups (**Figure 5A**, **Table S6**). According to somatic mutation analysis, mutations in EGFR (30%), TTN (24%), PTEN (29%), and TP53 (23%) were most highly enriched in the high ICP score group (**Figure 5B**). In comparison, IDH1 (77%), TP53 (48%), ARTX (33%), and CIC (20%) mutations were enriched in the low ICP score group (**Figure 5C**). Missense mutation was the predominant gene alteration type in all these genes except for ATRX, in which frame-shifting deletion was the most common type.

Different types of somatic mutations, including the single-nucleotide variant (SNV), single-nucleotide polymorphism (SNP), insertion, deletion, and intergenic region (IGR), were analyzed using the R package maftools. Silent, nonsense, missense, intronic, 5' and 3' UTR mutations were more common in the high ICP score group than in the low ICP score group (**Figure S3A**). While the frequencies of insertion and deletion were not statistically different between the two ICP score groups, SNPs were significantly more common in the high ICP score group (**Figure S3B**). Among the detected SNVs, C>T appeared to be the most common mutation in the high ICP score group (**Figure S3C**). The T to A, C to T,t and C to A mutations occurred more frequently in the high ICP score group than in the low ICP score group. The top 30 most differentially expressed mutated cancer-related genes between the two ICP groups are listed in **Figure S3D**. Common carcinogenic pathways were more active in the high ICP score group (**Figure S3E**). The strongest co-occurrent pairs of gene alteration in the high ICP score group were PTEN-TP53, RB1-TP53, TTN-CALN1, and TTN-FLG, which showed that TP53, PTEN, RB1, and TTN are functionally linked (**Figure S3F**). On the other hand, the most mutually exclusive pairs in the low ICP score group were CIC-TP53 and EGFR-IDH1 (**Figure S3F**). Furthermore, the AUC of ICP score for predicting the mutation status of IDH, CALN1, RB1, EGFR, and PTEN were 0.936, 0.826, 0.835, 0.81, and 0.841, respectively (**Figure 5D**).

Potential Intrinsic Immune Escape Mechanisms Related to the Immune Cell Pair Score

The intrinsic immune escape mechanism was reported to mainly include three aspects: immune checkpoint molecules, tumor immunogenicity, and antigen presentation capacity (29). We first explored the association between ICP score and immune checkpoint molecules which are classified into seven groups, including antigen-presenting, co-stimulator, co-inhibitor, and cell adhesion proteins and receptors, ligands, and others (3, 26). The increasing ICP score positively correlated with the expression of most immune checkpoint molecules (**Figure 6A**). In addition,

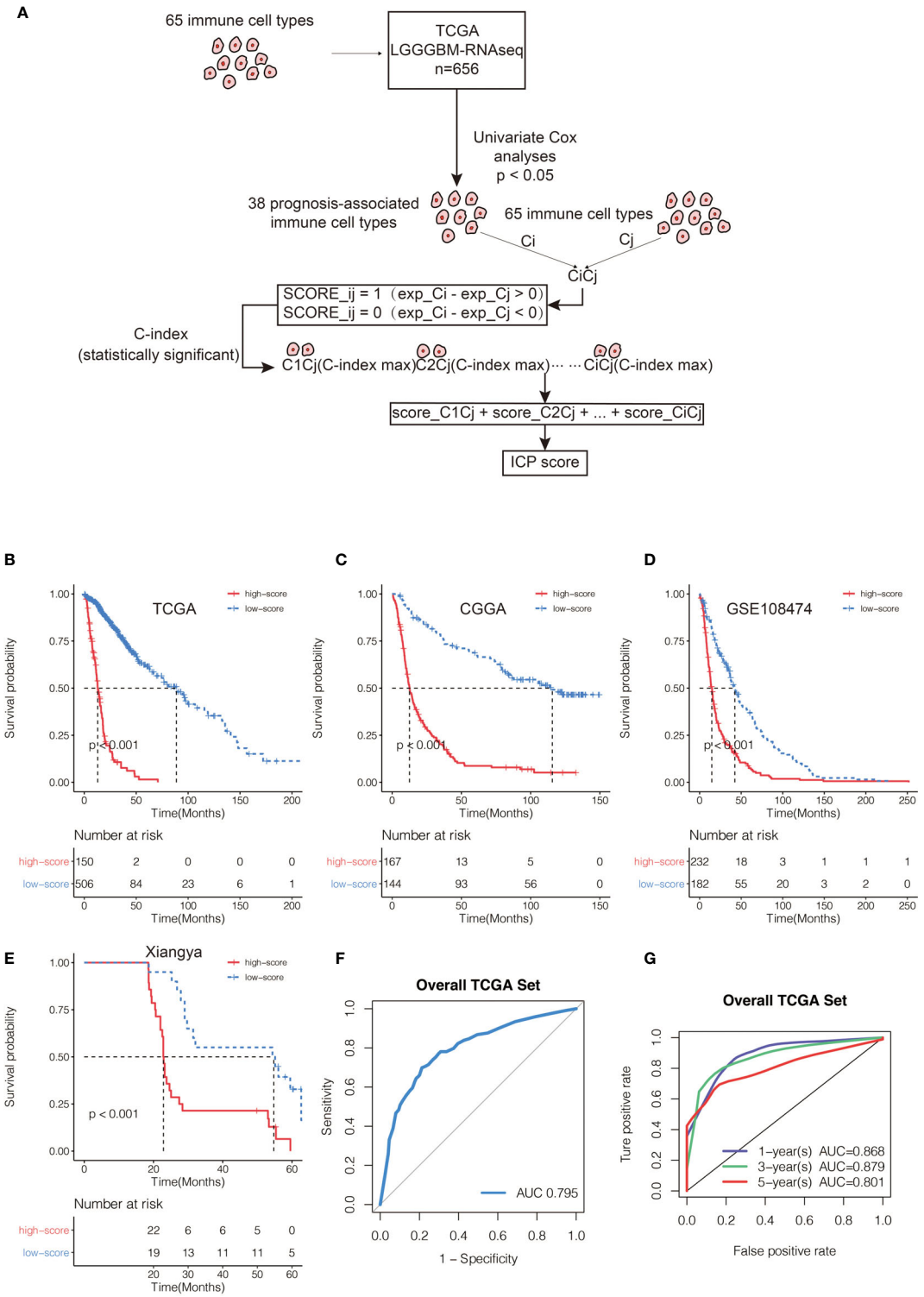

FIGURE 1 | **(A)** Flow diagram of the cell pair algorithm. Kaplan–Meier curves for the two ICP score groups in **(B)** TCGA, **(C)** CGGA, and **(D)** GSE108474, respectively. Log-rank test, P < 0.001. **(E)** Kaplan–Meier curves for the two ICP score groups in the Xiangya cohort. Log-rank test, P < 0.001. **(F)** ROC curve measuring the sensitivity of ICP score in predicting the survival status of the patients. The area under the ROC curve was 0.795. **(G)** ROC curve measuring the sensitivity of ICP score in predicting the 1-year, 3-year, and 5-year survival of the patients. The area under the ROC curve was 0.868, 0.879, and 0.801, respectively.

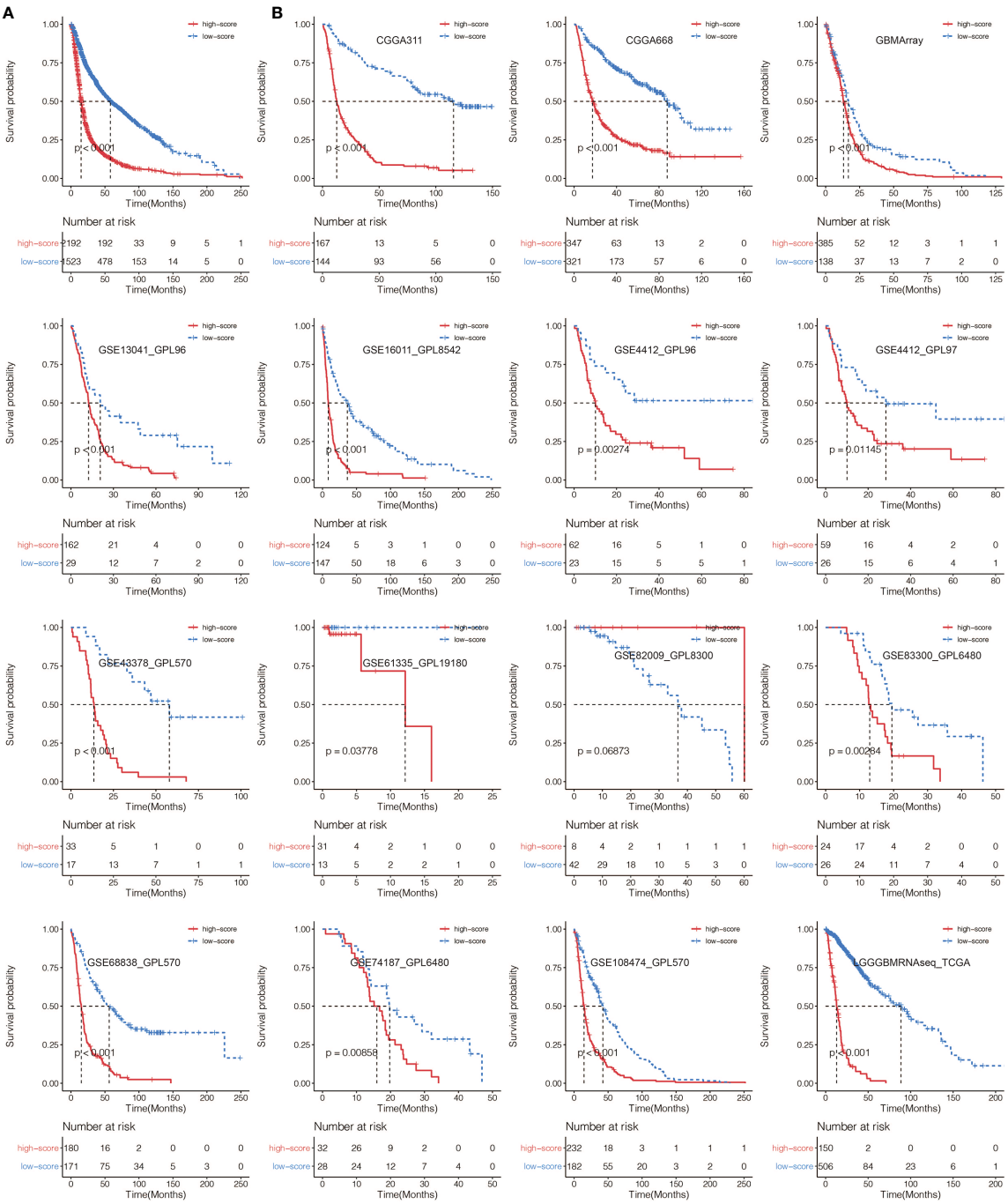

FIGURE 2 | **(A)** Kaplan–Meier curves for the two ICP score groups in all glioma samples. Log-rank test, P < 0.001. **(B)** Kaplan–Meier curves for the two ICP score groups in all collected glioma datasets. Log-rank test, P < 0.05.

ICP score had a significant positive relationship with some classical immune checkpoint molecules, including PDCD1, CD274, PDCD1LG2, TIGIT, HAVCR2, IDO1, and LAG3 in Xiangya cohort (**Figure 6B**).

A series of factors associated with tumor immunogenicity was then assessed (**Table S7**). The high ICP score group exhibited a lower microsatellite instability (MSI) and homologous recombination deficiency (HRD) (**Figure 7A**, **Figure S4A**). High ICP score group presented a higher level of intratumor heterogeneity, nonsilent mutation rate, number of segments, aneuploidy score, and fraction altered, all of which were significant indicators for genome alteration (**Figure 7B**,

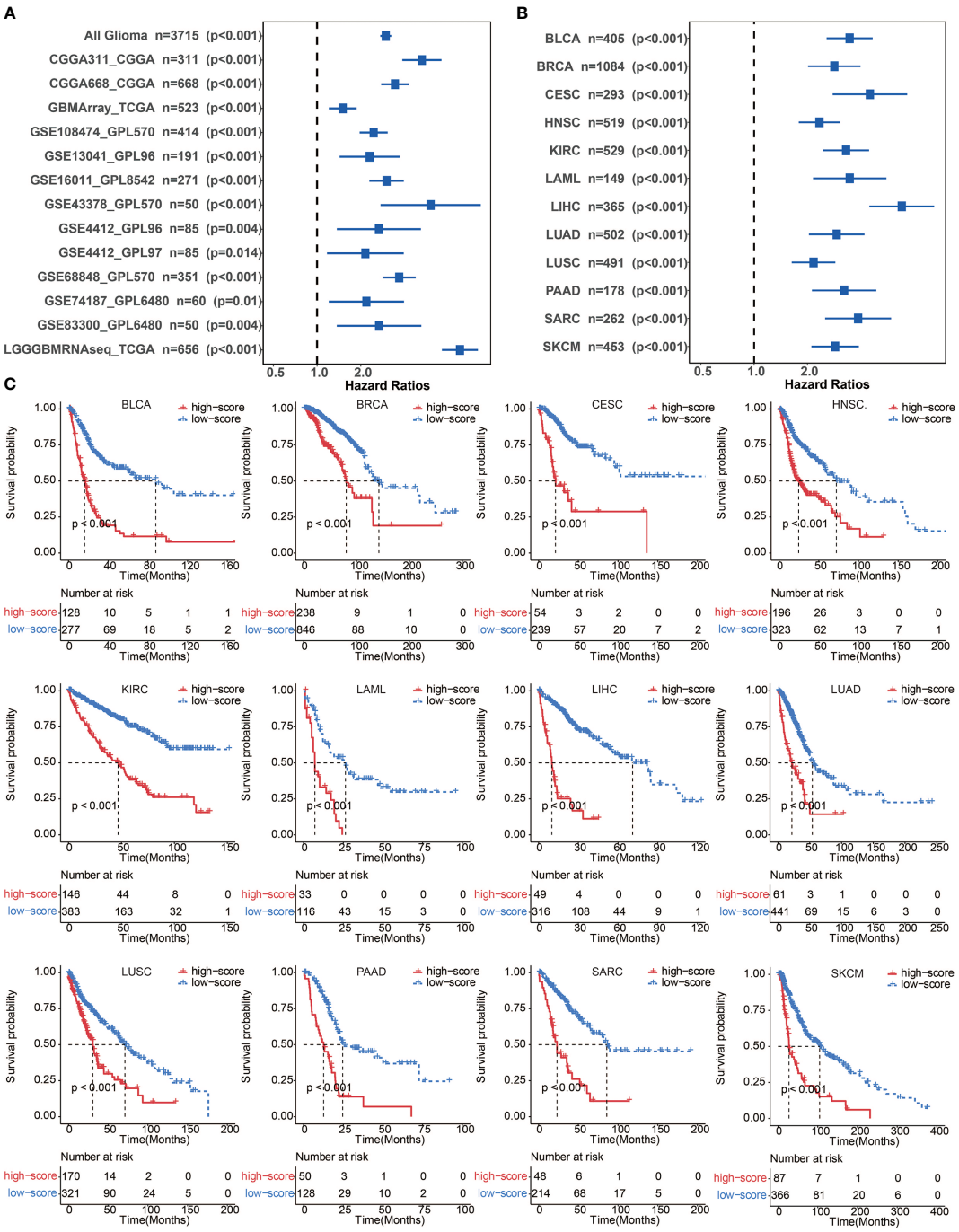

FIGURE 3 | **(A)** Univariate cox regression analyses to estimate the clinical prognostic value between the low/high ICP score groups in independent glioma datasets. **(B)** Univariate cox regression analyses to estimate clinical prognostic value between low/high ICP score groups in 12 independent cancer types in TCGA. The length of the horizontal line represents a 95% confidence interval for each group. The vertical dotted line represents the hazard ratio (HR) in all patients. **(C)** ICP score was developed in 12 independent cancer types in TCGA. Kaplan–Meier curves for two ICP score groups in 12 cancer types. Log-rank test, P < 0.001. BLCA, Bladder Urothelial Carcinoma; BRCA, breast invasive carcinoma; CESC, Cervical squamous cell carcinoma and endocervical adenocarcinoma; HNSC, Head and Neck squamous cell carcinoma; KIRC, Kidney renal clear cell carcinoma; LAML, Acute Myeloid Leukemia; LIHC, Liver hepatocellular carcinoma; LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma; MESO, Mesothelioma; PAAD, Pancreatic adenocarcinoma; SARC, Sarcoma; SKCM, Skin Cutaneous Melanoma.

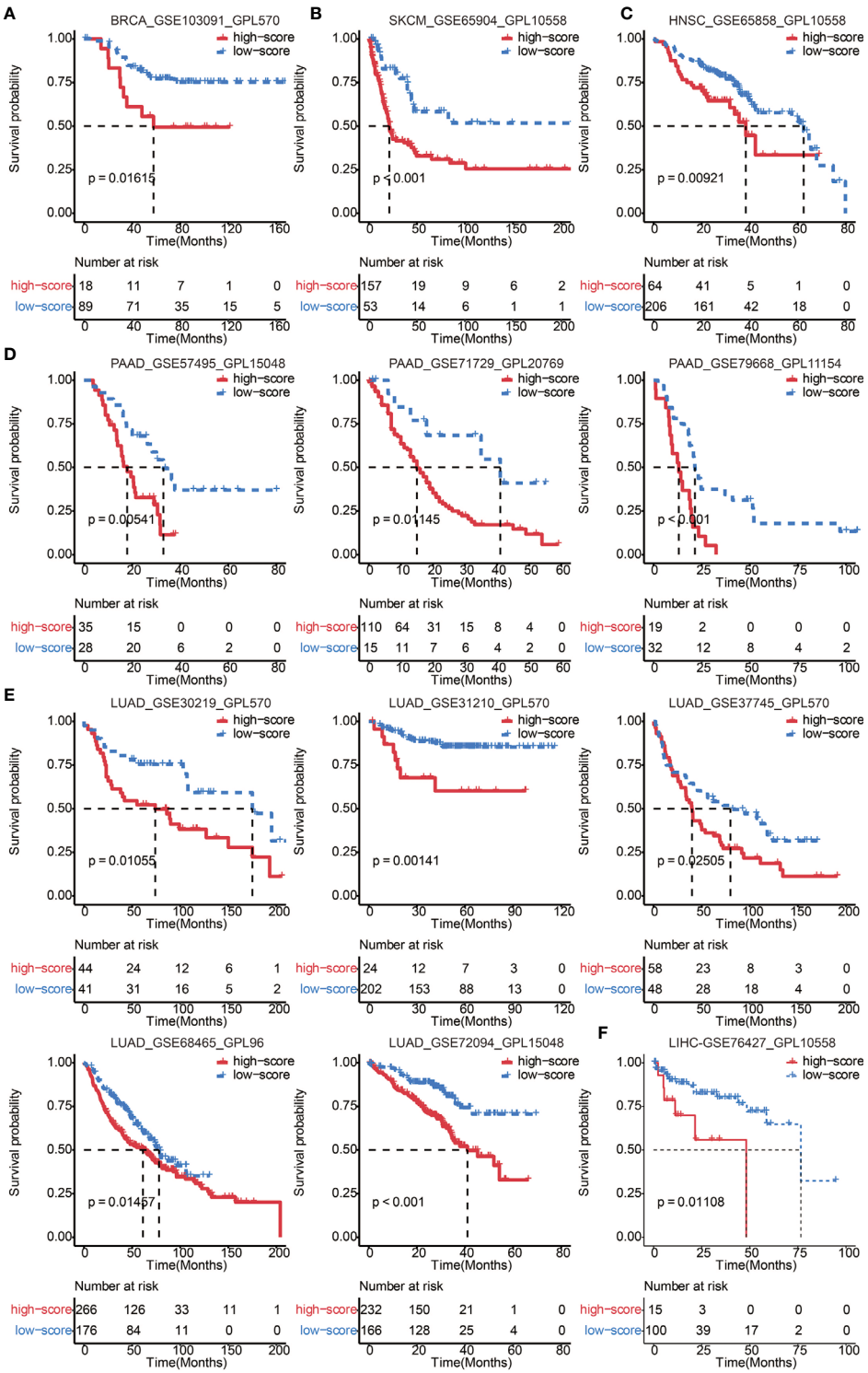

FIGURE 4 | Validation of ICP score in 6 representative cancer types. **(A)** Kaplan–Meier curves for the two ICP score groups in the BRCA dataset, GSE103091. Log-rank test, P = 0.01615. **(B)** Kaplan–Meier curves for the two ICP score groups in the SKCM dataset, GSE65904. Log-rank test, P < 0.001. **(C)** Kaplan–Meier curves for the two ICP score groups in the HNSC dataset, GSE65858. Log-rank test, P = 0.00921. **(D)** Kaplan–Meier curves for the two ICP score groups in the PAAD datasets, GSE57495, GSE71729, and GSE79668. Log-rank test, P = 0.00541, P = 0.01145, and P < 0.001, respectively. **(E)** Kaplan–Meier curves for the two ICP score groups in the LUAD datasets, GSE30219, GSE31210, GSE37745, GSE68465, and GSE72094. Log-rank test, P = 0.01055, P = 0.00141, P = 0.02505, P = 0.01457, and P < 0.001, respectively. **(F)** Kaplan–Meier curves for the two ICP score groups in the LIHC dataset, GSE76427. Log-rank test, P = 0.01108.

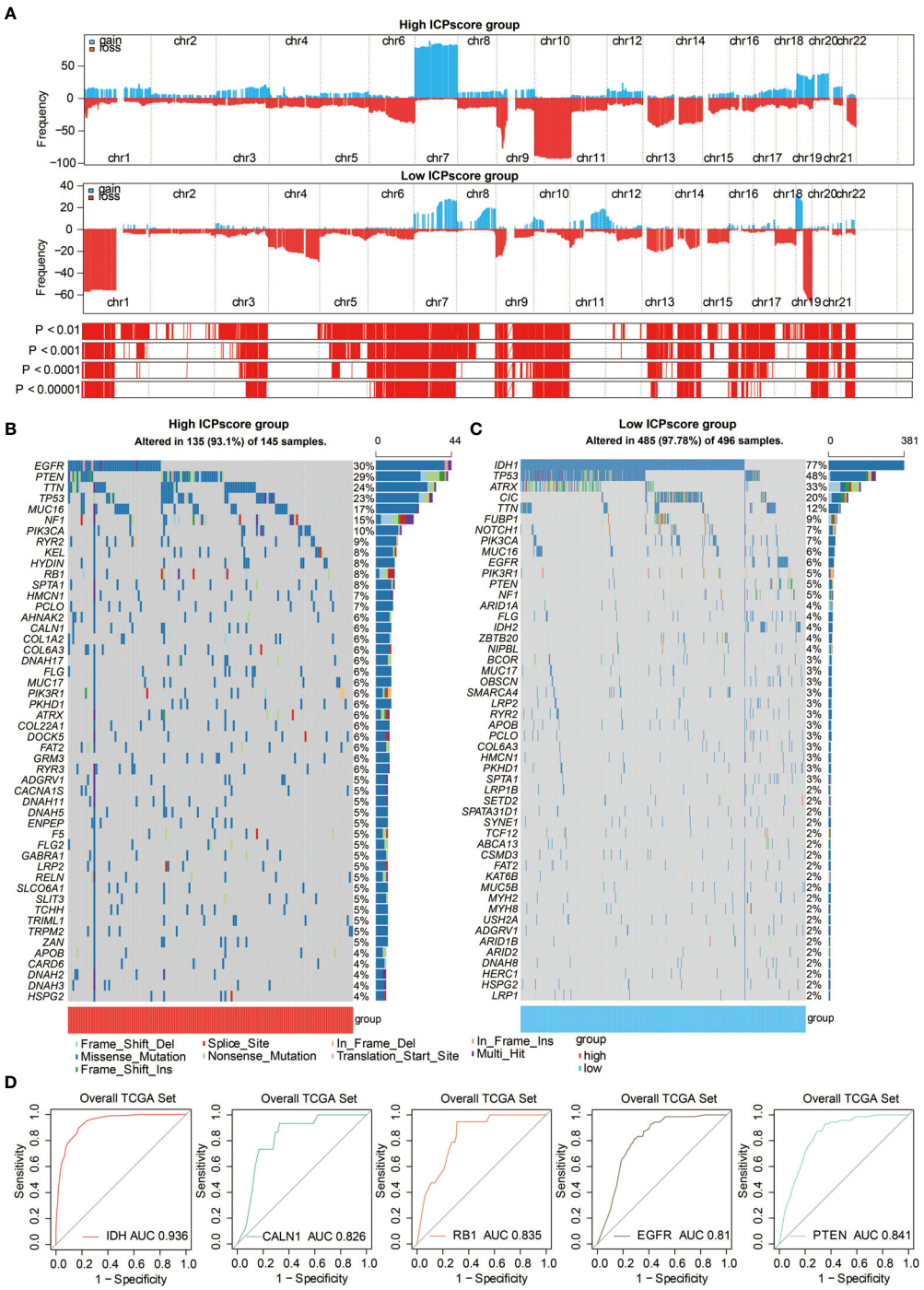

FIGURE 5 | Genomic features of ICP score. **(A)** GISTIC 2.0 distribution of gain or loss of function mutation in gliomas with high and low ICP score. Chromosomal locations of peaks of significantly recurring focal amplifications (red) and deletions (blue) are presented. **(B)** List of the most frequently altered somatic mutation genes in the high ICP score group. **(C)** List of the most frequently altered somatic mutation genes in the low ICP score group. **(D)** ROC curve measuring the sensitivity of ICP score in predicting IDH, CALN1, RB1, EGFR, and PTEN mutation status. The area under the ROC curve was 0.936, 0.826, 0.835, 0.81, and 0.841, respectively.

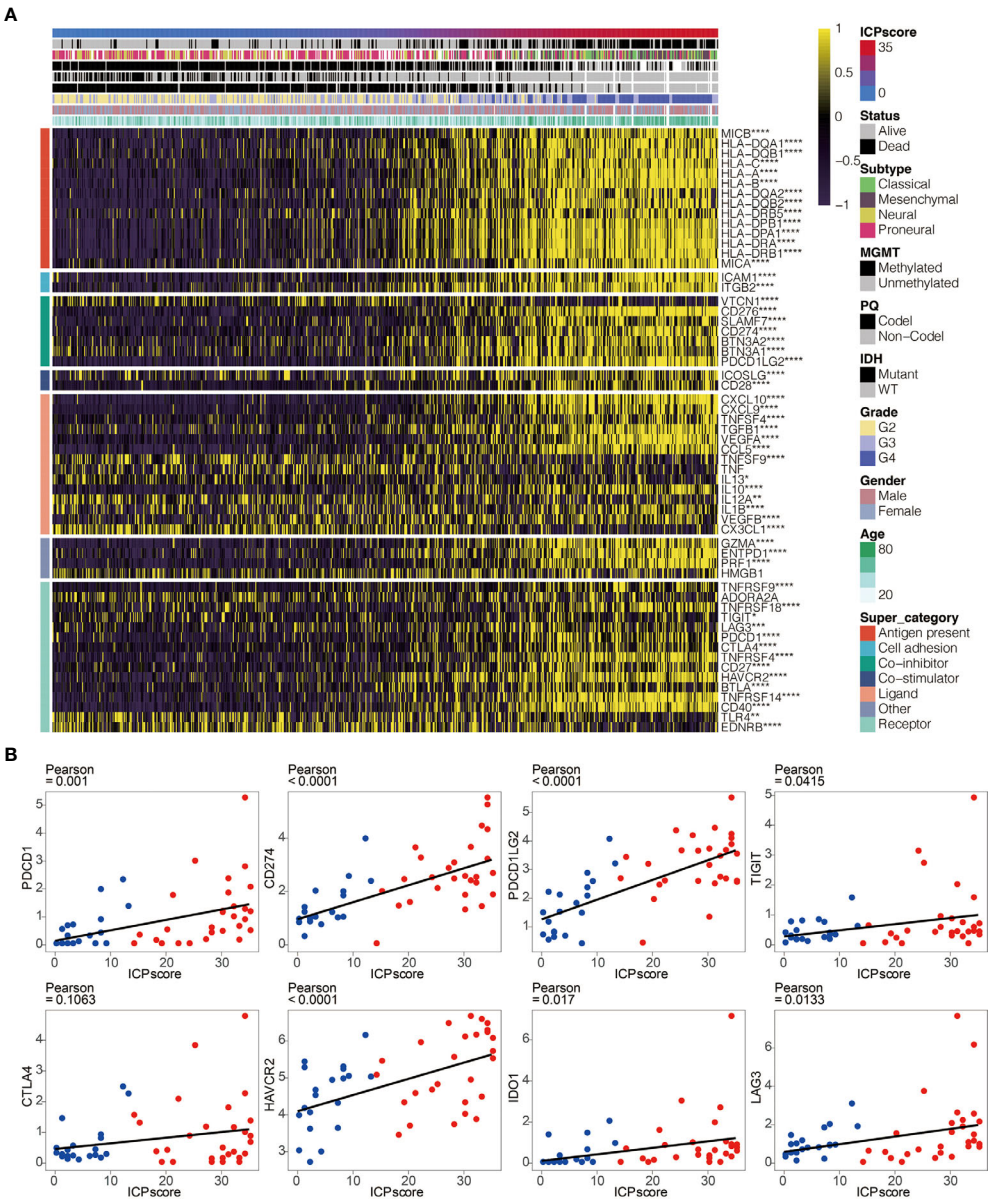

FIGURE 6 | ICP score correlated with immune checkpoints. **(A)** Heatmap illustrating the expression pattern of immune checkpoints in ICP score. **(B)** Scatter plots depicting a positive correlation between ICP score and eight classical immune checkpoints, including PDCD1, CD274, PDCD1LG2, TIGIT, CTLA4, HAVCR2, IDO1, and LAG3. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

Figures S4B–D). Cancer testis antigen (CTA) and neoantigens were a vital source of tumor-specific antigens, and they were significantly different between the ICP score groups (**Figures S4F–H**). In term of antigen presentation capacity (**Table S7**), the high ICP score group presented a higher antigen processing and presenting machinery (APM) score and T cell receptor (TCR) (**Figure 7C**, **Figures S4I, J**). Stroma signatures including TGF-beta response, leukocyte fraction, CD8, interferon gamma (IFNG), interferon stimulated genes resistance signature (ISG.RS), and IFNG hallmark gene set (IFNG.GS) were higher in the high ICP score group (**Figures S4K–P**).

Immune Cell Pair Score Predicted Immunotherapeutic Responses

Immunotherapy is innovating the treatment of several solid cancer types. The response rates of tumor to PD-1 inhibition are reported to be correlated with the TMB (30), Cytotoxic activity (CYT) (31), and T cell-inflamed gene expression profile (GEP) (32). To explore the predictive value of ICP score in immunotherapeutic response, we analyzed the correlation between ICP score and the above three immune markers. High ICP score group was found to have a higher TMB level (**Figure 7D**), CYT level (**Figure 7E**), and GEP level (**Figure 7G**). Furthermore, ICP score had a significantly

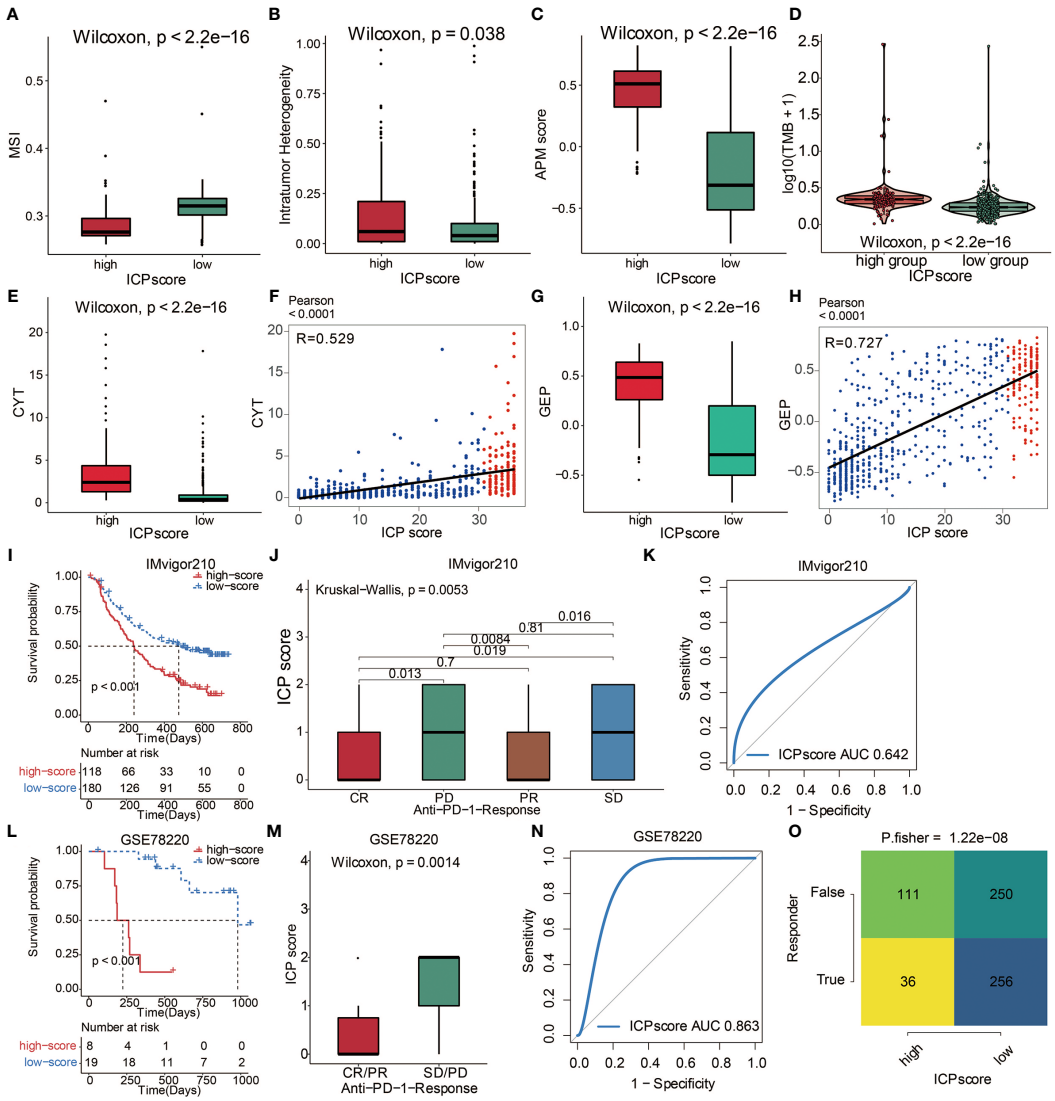

FIGURE 7 | The predictive value of ICP score in immunotherapy. **(A)** MSI score in high and low ICP score. **(B)** APM score in high and low ICP score. **(C)** Intratumor Heterogeneity in high and low ICP score. **(D)** TMB expression differences in high and low ICP score. Differences between groups were compared through the Wilcoxon test (Wilcoxon, P < 0.001). **(E)** CYT and **(G)** GEP expression differences in high and low ICP score. Differences between groups were compared through the Wilcoxon test (Wilcoxon, P < 0.001). Scatter plots depicting a positive correlation between ICP score and **(F)** CYT and **(H)** GEP. Pearson Correlation Coefficient R = 0.529 and 0.727, respectively. **(I)** Kaplan–Meier curves for the two ICP score groups in the IMvigor210 dataset. Log-rank test, P < 0.001. **(J)** ICP score in groups with different anti–PD-1 clinical response status (CR, PR, SD, PD). Differences between groups were compared by Kruskal-Wallis test (Kruskal-Wallis, P = 0.0053). **(K)** ROC curve measuring the sensitivity of ICP score in predicting the survival status of patients in the IMvigor210 dataset. The area under the ROC curve was 0.642. **(L)** Kaplan–Meier curves for the two ICP score groups in the GSE78220 dataset. Log-rank test, P < 0.001. **(M)** ICP score in groups with different anti–PD-1 clinical response status (CR/PR and SD/PD). Differences between groups were compared by Wilcoxon test (Wilcoxon, P = 0.0014). **(N)** ROC curve measuring the sensitivity of ICP score in predicting the survival status of patients in the GSE78220 dataset. The area under the ROC curve was 0.863. **(O)** TIDE value and response to immunotherapy of patients with ICP score. Fisher's test, P < 0.001.

positive correlation with CYT (**Figure 7F**) and GEP (**Figure 7H**). The ability of the ICP score to predict the response of patients to immune-checkpoint therapy was explored by assigning the IMvigor210 cohort patients (urothelial carcinoma dataset) to different ICP score groups (**Table S8**). Patients receiving atezolizumab as the anti-PD-L1 therapy with a high ICP score exhibited a significantly shorter OS compared to patients with a low ICP score (**Figure 7I**). Patients with a low ICP score exhibited better immunotherapeutic responses (**Figure 7J**). ICP score was a prognostic biomarker in predicting patient survival status in the IMvigor210 cohort (**Figure 7K**). In the melanoma dataset, GSE78220, patients receiving either pembrolizumab or nivolumab as the anti-PD-1 therapy with a high ICP score also exhibited a significantly shorter OS compared to patients with a low ICP score (**Figure 7L**; **Table S9**). Likewise, patients with a low ICP score exhibited better immunotherapeutic responses

(**Figure 7M**). ICP score was also a prognostic biomarker in predicting patient survival status in the GSE78220 dataset (**Figure 7N**). Meanwhile, the TIDE analyses proved that a high ICP score was less sensitive to anti-PD1 therapy and anti-CTLA4 therapy (**Figure 7O**).

DISCUSSION

Tumor infiltrating immune cells have been critical in tumorigenesis by exerting the two-sided effect that both regulates the immunosurveillance of cancer and creates a favorable microenvironment for cancer cell survival. Previous studies have demonstrated the prognostic value of several TIICs in different cancer types (33, 34). However, the overall survival under the influence of TIICs in cancers have not been adequately determined and a consensus-oriented prognostic signature regarding TIICs has not been reached. Moreover, considering the differences in the reference genomes and gene signatures of immune cells used for quantifying RNA-sequencing data, multiple previous prognostic models may have limitations in the cross-validation of different transcriptional datasets or different cancer types. The measurements of cellular heterogeneity vary due to the frequent updated version of annotation for immune cells and reference genome, which may impede their extensive application and set back the prospect for clinical practice (**Figure S5**) (35, 36). To resolve this issue, we collected and integrated 65 immune cells to establish a robust and comprehensive prognostic signature with the concept of cell pair. As mentioned in the method section, we focused on the relative expression level of immune cells for the quantification of the ICP score, which extensively reduced the effect of the updated annotation of the reference genome, eliminated the need for data normalization, and increased the accuracy in designing the signature.

In this study, given the malignancy of gliomas and abundant publicly available datasets, ICP score was first established in glioma samples. ICP score could significantly stratify the overall survival of glioma patients from TCGA and CGGA. Based on the sequencing data from Xiangya, high ICP score was associated with a worse survival in glioma patients. Consistently, high ICP score predicted a worse survival in the other 15 external glioma datasets. The independent establishment of ICP score was performed in 12 representative cancer types including BLCA, BRCA, CESC, HNSC, KIRC, LAML, LIHC, LUAD, LUSC, MESO, PAAD, SARC, and SKCM, all of which proved the predictive value of ICP score. The univariate cox regression analysis proved that ICP score was a hazardous marker in both glioma samples and 12 independent cancer types. Furthermore, six most representative cancer types including BRCA, SKCM, HNSC, PAAD, LUAD, and LIHC were selected for the validation of the ICP score. As expected, ICP score served as a hazardous marker, and the predictive value of ICP score was stable in all of the 12 GEO datasets. The findings above proved the generality and reliability of ICP score in predicting the prognosis of cancer patients.

Furthermore, the genomic features of ICP score were annotated in gliomas. The present study finds that the IDH1 missense mutations are overrepresented in the low ICP score group (77%), in accordance with previous findings that IDH mutations are more enriched in low grade gliomas and confer better survival outcomes in glioma patients (37). Likewise, tumor suppressor TP53, inhibiting GBM malignancy (38), was found to be more frequently mutated in the low ICP score group (48%). Conversely, EGFR, which is the most enriched mutated gene in the high ICP score group (30%) and whose alteration occurs in less than 6% of the low ICP score group as identified by somatic mutation analysis, has been reported to be frequently activated in GBM and predict worse survival outcomes in glioma patients (39). Another critical oncogene, PTEN (33), also had higher mutation rates in the high ICP score group (29%), implying a more malignant feature of the high ICP score group. Commonly mutated cancer-related genes were found to be more frequently expressed in high ICP score group, with PTEN-TP53, RB1-TP53, TTN-CALN1, and TTN-FLG being the strongest co-occurrent pairs of gene alteration. PTEN (40), TP53, RB1 (41), CALN1 (42), EGFR (43), and TTN (44) have been previously reported to play a role in tumorogenesis, in which ICP score exhibited a high sensitivity in predicting the mutation status of IDH, CALN1, RB1, EGFR, and PTEN. Thus, ICP score may be a potential predictor for the oncogenic process.

The potential immune escape mechanisms of ICP score were summarized and underlined. Immune checkpoint blockage (ICB) therapy targeting immune checkpoint molecules have demonstrated remarkable benefits (45). The significant correlation between ICP score and classical immune checkpoint molecules such as PDCD1, CD274, TIGIT, and LAG3 suggested that ICP score could be an effective indicator for immune checkpoint blockage (ICB) therapy (46–49). Moreover, high ICP score prominently participated in the regulation of immunomodulators for tumor immunogenicity and antigen presentation capacity. Low MSI, a diagnostic phenotype with more malignancy of cancer (50), was more significantly correlated with a high ICP score. High ICP score was also detected with higher Intratumor Heterogeneity, a diagnostic phenotype with more malignancy of cancer (51). Additionally, a high ICP score had the distinct biological characteristics regarding stroma signatures such as TGF-beta response, leukocyte fraction, and ISG.RS compared with a low ICP score, and these stroma signatures have previously been proved to facilitate the immune escape of cancer (52). The findings above suggested a novel orientation for the inclusion of ICP score as the indicators of immunosuppression.

Immunotherapy, represented by ICB, has become increasingly promising in tumor treatment. Notably, the IMvigor210 cohort and the melanoma dataset (GSE78220) treated with the anti-PD-L1 antibody atezolizumab have demonstrated remarkable clinical outcomes (26, 27). ICP score was then validated in these two datasets regarding its predictive value of the response to immunotherapy. As expected, a high ICP score correlated with a worse survival in both cohorts and predicted a worse response to immunotherapy. Further, high ICP score correlated with higher

levels of TMB, CYT, and GEP, all of which are valuable markers in predicting immunotherapeutic response. Taken together, our findings revealed the robust value of ICP score in predicting immunotherapy efficacy.

Of note, more comprehensive analysis of multi-omics analysis about the functional annotation of immune signature will greatly complement the findings in this study and ensure the prospective application of the ICP scoring system. To the best of our knowledge, we are the first one to collect the comprehensive immune cell types in cancer and introduce the concept of cell pair for the establishment of a robust immune signature. The relative stable ratio of TIICs regarding their abundance in tumor microenvironment ensures the extensive application and high sensitivity of this immune signature, and will undeniably help understand tumor microenvironment and TIICs effects on immunotherapy.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

HZ, QC, NZ, and ZW designed and drafted the manuscript. HZ, QC, NZ, HC, SL, WW, and ZD wrote figure legends and revised the manuscript. QC, HZ, and NZ conducted the data analysis. All authors contributed to the article and approved the submitted version.

FUNDING

Financial support was provided by the National Natural Science Foundation of China (NO. 82073893, 81703622), China Postdoctoral Science Foundation (NO. 2018M633002), Hunan Provincial Natural Science Foundation of China (NO. 2018JJ3838), and Hunan Provincial Health Committee Foundation of China (C2019186). Xiangya Hospital Central South University postdoctoral foundation. Fundamental Research Funds for the Central Universities of Central South University (2021zzts1027).

ACKNOWLEDGMENTS

We appreciate the contributions of Dr. Liyang Zhang to establish and manage the XYNS cohort. We acknowledge TCGA, CGGA and GEO database for providing their platforms and contributors for uploading their meaningful datasets.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.694490/full#supplementary-material

REFERENCES

1. Hiraoka N. Tumor-Infiltrating Lymphocytes and Hepatocellular Carcinoma: Molecular Biology. *Int J Clin Oncol* (2010) 15:544–51. doi: 10.1007/s10147-010-0130-1
2. Beatty GL, Gladney WL. Immune Escape Mechanisms as a Guide for Cancer Immunotherapy. *Clin Cancer Res* (2015) 21:687–92. doi: 10.1158/1078-0432.CCR-14-1860
3. Schreiber RD, Old LJ, Smyth MJ. Cancer Immunoediting: Integrating Immunity's Roles in Cancer Suppression and Promotion. *Science* (2011) 331:1565–70. doi: 10.1126/science.1203486
4. Althobiti M, Aleskandarany MA, Joseph C, Toss M, Mongan N, Diez-Rodriguez M, et al. Heterogeneity of Tumour-Infiltrating Lymphocytes in Breast Cancer and its Prognostic Significance. *Histopathology* (2018) 73:887–96. doi: 10.1111/his.13695
5. Santoiemma PP, Powell DJJr. Tumor Infiltrating Lymphocytes in Ovarian Cancer. *Cancer Biol Ther* (2015) 16:807–20. doi: 10.1080/15384047.2015.1040960
6. Hall M, Liu H, Malafa M, Centeno B, Hodul PJ, Pimiento J, et al. Expansion of Tumor-Infiltrating Lymphocytes (TIL) From Human Pancreatic Tumors. *J Immunother Cancer* (2016) 4:61. doi: 10.1186/s40425-016-0164-7
7. Guo X, Zhang Y, Zheng L, Zheng C, Song J, Zhang Q, et al. Global Characterization of T Cells in Non-Small-Cell Lung Cancer by Single-Cell Sequencing. *Nat Med* (2018) 24:978–85. doi: 10.1038/s41591-018-0045-3
8. Wu L, Mao L, Liu JF, Chen L, Yu GT, Yang LL, et al. Blockade of TIGIT/CD155 Signaling Reverses T-Cell Exhaustion and Enhances Antitumor Capability in Head and Neck Squamous Cell Carcinoma. *Cancer Immunol Res* (2019) 7:1700–13. doi: 10.1158/2326-6066.CIR-18-0725
9. Verdegaal EM, de Miranda NF, Visser M, Harryvan T, van Buuren MM, Andersen RS, et al. Neoantigen Landscape Dynamics During Human Melanoma-T Cell Interactions. *Nature* (2016) 536:91–5. doi: 10.1038/nature18945
10. Zhang H, Wang R, Yu Y, Liu J, Luo T, Fan F. Glioblastoma Treatment Modalities Besides Surgery. *J Cancer* (2019) 10:4793–806. doi: 10.7150/jca.32475
11. Liu Z, Meng Q, Bartek JJr., Poiret T, Persson O, Rane L, et al. Tumor-Infiltrating Lymphocytes (TILs) From Patients With Glioma. *Oncoimmunology* (2017) 6:e1252894. doi: 10.1080/2162402X.2016.1252894
12. Wagner GP, Kin K, Lynch VJ. Measurement of mRNA Abundance Using RNA-Seq Data: RPKM Measure Is Inconsistent Among Samples. *Theory Biosci* (2012) 131:281–5. doi: 10.1007/s12064-012-0162-3
13. Aran D, Hu Z, Butte AJ. Xcell: Digitally Portraying the Tissue Cellular Heterogeneity Landscape. *Genome Biol* (2017) 18:220. doi: 10.1186/s13059-017-1349-1
14. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, et al. Spatiotemporal Dynamics of Intratumoral Immune Cells Reveal the Immune Landscape in Human Cancer. *Immunity* (2013) 39:782–95. doi: 10.1016/j.immuni.2013.10.003
15. Finotello F, Trajanoski Z. Quantifying Tumor-Infiltrating Immune Cells From Transcriptomics Data. *Cancer Immunol Immunother* (2018) 67:1031–40. doi: 10.1007/s00262-018-2150-z
16. Harrell FE Jr., Lee KL, Mark DB. Multivariable Prognostic Models: Issues in Developing Models, Evaluating Assumptions and Adequacy, and Measuring and Reducing Errors. *Stat Med* (1996) 15:361–87. doi: 10.1002/(SICI)1097-0258(19960229)15:4<361::AID-SIM168>3.0.CO;2-4
17. Hendrickx W, Simeone I, Anjum S, Mokrab Y, Bertucci F, Finetti P, et al. Identification of Genetic Determinants of Breast Cancer Immune Phenotypes

by Integrative Genome-Scale Analysis. *Oncoimmunology* (2017) 6:e1253654. doi: 10.1080/2162402X.2016.1253654
18. Iglesia MD, Vincent BG, Parker JS, Hoadley KA, Carey LA, Perou CM, et al. Prognostic B-Cell Signatures Using mRNA-Seq in Patients With Subtype-Specific Breast and Ovarian Cancer. *Clin Cancer Res* (2014) 20:3818–29. doi: 10.1158/1078-0432.CCR-13-3368
19. Monaco G, Lee B, Xu W, Mustafah S, Hwang YY, Carre C, et al. RNA-Seq Signatures Normalized by mRNA Abundance Allow Absolute Deconvolution of Human Immune Cell Types. *Cell Rep* (2019) 26:1627–40.e7. doi: 10.1016/j.celrep.2019.01.041
20. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, et al. Robust Enumeration of Cell Subsets From Tissue Expression Profiles. *Nat Methods* (2015) 12:453–7. doi: 10.1038/nmeth.3337
21. Palmer C, Diehn M, Alizadeh AA, Brown PO. Cell-Type Specific Gene Expression Profiles of Leukocytes in Human Peripheral Blood. *BMC Genomics* (2006) 7:115. doi: 10.1186/1471-2164-7-115
22. Rody A, Karn T, Liedtke C, Pusztai L, Ruckhaeberle E, Hanker L, et al. A Clinically Relevant Gene Signature in Triple Negative and Basal-Like Breast Cancer. *Breast Cancer Res* (2011) 13:R97. doi: 10.1186/bcr3035
23. Hanzelmann S, Castelo R, Guinney J. GSVA: Gene Set Variation Analysis for Microarray and RNA-Seq Data. *BMC Bioinf* (2013) 14:7. doi: 10.1186/1471-2105-14-7
24. Cibulskis K, Lawrence MS, Carter SL, Sivachenko A, Jaffe D, Sougnez C, et al. Sensitive Detection of Somatic Point Mutations in Impure and Heterogeneous Cancer Samples. *Nat Biotechnol* (2013) 31:213–9. doi: 10.1038/nbt.2514
25. Mayakonda A, Lin DC, Assenov Y, Plass C, Koeffler HP. Maftools: Efficient and Comprehensive Analysis of Somatic Variants in Cancer. *Genome Res* (2018) 28:1747–56. doi: 10.1101/gr.239244.118
26. Wang S, Zhang Q, Yu C, Cao Y, Zuo Y, Yang L. Immune Cell Infiltration-Based Signature for Prognosis and Immunogenomic Analysis in Breast Cancer. *Brief Bioinform* (2020) 22(2):2020–31. doi: 10.1093/bib/bbaa026
27. Hugo W, Zaretsky JM, Sun L, Song C, Moreno BH, Hu-Lieskovan S, et al. Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. *Cell* (2017) 168:542. doi: 10.1016/j.cell.2017.01.010
28. Gu Z, Eils R, Schlesner M. Complex Heatmaps Reveal Patterns and Correlations in Multidimensional Genomic Data. *Bioinformatics* (2016) 32:2847–9. doi: 10.1093/bioinformatics/btw313
29. Schumacher TN, Schreiber RD. Neoantigens in Cancer Immunotherapy. *Science* (2015) 348:69–74. doi: 10.1126/science.aaa4971
30. Yarchoan M, Hopkins A, Jaffee EM. Tumor Mutational Burden and Response Rate to PD-1 Inhibition. *N Engl J Med* (2017) 377:2500–1. doi: 10.1056/NEJMc1713444
31. Roh W, Chen PL, Reuben A, Spencer CN, Prieto PA, Miller JP, et al. Integrated Molecular Analysis of Tumor Biopsies on Sequential CTLA-4 and PD-1 Blockade Reveals Markers of Response and Resistance. *Sci Transl Med* (2017) 9(379):eaah3560. doi: 10.1126/scitranslmed.aah3560
32. Ayers M, Lunceford J, Nebozhyn M, Murphy E, Loboda A, Kaufman DR, et al. IFN-Gamma-Related mRNA Profile Predicts Clinical Response to PD-1 Blockade. *J Clin Invest* (2017) 127:2930–40. doi: 10.1172/JCI91190
33. Chen P, Zhao D, Li J, Liang X, Li J, Chang A, et al. Symbiotic Macrophage-Glioma Cell Interactions Reveal Synthetic Lethality in PTEN-Null Glioma. *Cancer Cell* (2019) 35:868–84.e6. doi: 10.1016/j.ccell.2019.05.003
34. Stanton SE, Disis ML. Clinical Significance of Tumor-Infiltrating Lymphocytes in Breast Cancer. *J Immunother Cancer* (2016) 4:59. doi: 10.1186/s40425-016-0165-6
35. Jalali S, Gandhi S, Scaria V. Navigating the Dynamic Landscape of Long Noncoding RNA and Protein-Coding Gene Annotations in GENCODE. *Hum Genomics* (2016) 10:35. doi: 10.1186/s40246-016-0090-2
36. Zeng D, Ye Z, Shen R, Yu G, Wu J, Xiong Y, et al. IOBR: Multi-Omics Immuno-Oncology Biological Research to Decode Tumor Microenvironment and Signatures. *Front Immunol* (2021) 12:687975. doi: 10.3389/fimmu.2021.687975
37. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, et al. IDH1 and IDH2 Mutations in Gliomas. *N Engl J Med* (2009) 360:765–73. doi: 10.1056/NEJMoa0808710
38. Zhang Y, Dube C, Gibert M Jr., Cruickshanks N, Wang B, Coughlan M, et al. The P53 Pathway in Glioblastoma. *Cancers (Basel)* (2018) 10(9):297. doi: 10.3390/cancers10090297
39. Network CGAR. Comprehensive Genomic Characterization Defines Human Glioblastoma Genes and Core Pathways. *Nature* (2008) 455:1061–8. doi: 10.1038/nature07385
40. Song MS, Salmena L, Pandolfi PP. The Functions and Regulation of the PTEN Tumour Suppressor. *Nat Rev Mol Cell Biol* (2012) 13:283–96. doi: 10.1038/nrm3330
41. Knudsen ES, Pruitt SC, Hershberger PA, Witkiewicz AK, Goodrich DW. Cell Cycle and Beyond: Exploiting New RB1 Controlled Mechanisms for Cancer Therapy. *Trends Cancer* (2019) 5:308–24. doi: 10.1016/j.trecan.2019.03.005
42. Li H, Yu B, Li J, Su L, Yan M, Zhu Z, et al. Overexpression of lncRNA H19 Enhances Carcinogenesis and Metastasis of Gastric Cancer. *Oncotarget* (2014) 5:2318–29. doi: 10.18632/oncotarget.1913
43. Eskilsson E, Rosland GV, Solecki G, Wang Q, Harter PN, Graziani G, et al. EGFR Heterogeneity and Implications for Therapeutic Intervention in Glioblastoma. *Neuro Oncol* (2018) 20:743–52. doi: 10.1093/neuonc/nox191
44. Yang Y, Zhang J, Chen Y, Xu R, Zhao Q, Guo W. MUC4, MUC16, and TTN Genes Mutation Correlated With Prognosis, and Predicted Tumor Mutation Burden and Immunotherapy Efficacy in Gastric Cancer and Pan-Cancer. *Clin Transl Med* (2020) 10:e155. doi: 10.1002/ctm2.155
45. Zhang H, Dai Z, Wu W, Wang Z, Zhang N, Zhang L, et al. Regulatory Mechanisms of Immune Checkpoints PD-L1 and CTLA-4 in Cancer. *J Exp Clin Cancer Res* (2021) 40:184. doi: 10.1186/s13046-021-01987-7
46. Zhang H, Zhou Y, Cheng Q, Dai Z, Wang Z, Liu F, et al. PDIA3 Correlates With Clinical Malignant Features and Immune Signature in Human Gliomas. *Aging (Albany NY)* (2020) 12:15392–413. doi: 10.18632/aging.103601
47. Zhang H, Fan F, Yu Y, Wang Z, Liu F, Dai Z, et al. Clinical Characterization, Genetic Profiling, and Immune Infiltration of TOX in Diffuse Gliomas. *J Transl Med* (2020) 18:305. doi: 10.1186/s12967-020-02460-3
48. Zhang H, Cui B, Zhou Y, Wang X, Wu W, Wang Z, et al. B2M Overexpression Correlates With Malignancy and Immune Signatures in Human Gliomas. *Sci Rep* (2021) 11:5045. doi: 10.1038/s41598-021-84465-6
49. Zhang H, He J, Dai Z, Wang Z, Liang X, He F, et al. PDIA5 Is Correlated With Immune Infiltration and Predicts Poor Prognosis in Gliomas. *Front Immunol* (2021) 12:628966. doi: 10.3389/fimmu.2021.628966
50. Hause RJ, Pritchard CC, Shendure J, Salipante SJ. Classification and Characterization of Microsatellite Instability Across 18 Cancer Types. *Nat Med* (2016) 22:1342–50. doi: 10.1038/nm.4191
51. McGranahan N, Swanton C. Biological and Therapeutic Impact of Intratumor Heterogeneity in Cancer Evolution. *Cancer Cell* (2015) 27:15–26. doi: 10.1016/j.ccell.2014.12.001
52. Tauriello DVF, Palomo-Ponce S, Stork D, Berenguer-Llergo A, Badia-Ramentol J, Iglesias M, et al. TGFbeta Drives Immune Evasion in Genetically Reconstituted Colon Cancer Metastasis. *Nature* (2018) 554:538–43. doi: 10.1038/nature25492

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frontiers in Immunology

REVIEW
published: 07 January 2022
doi: 10.3389/fimmu.2021.799455

Therapeutic Implications of Tumor Microenvironment in Lung Cancer: Focus on Immune Checkpoint Blockade

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OPEN ACCESS

Edited by:
Lorenzo Mortara, University of Insubria, Italy

Reviewed by:
Daniel Olive, Aix Marseille Université, France
Maria Rosaria Galdiero, University of Naples Federico II, Italy

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Specialty section:
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

Received: *21 October 2021*
Accepted: *03 December 2021*
Published: *07 January 2022*

Citation:
Genova C, Dellepiane C, Carrega P, Sommariva S, Ferlazzo G, Pronzato P, Gangemi R, Filaci G, Coco S and Croce M (2022) Therapeutic Implications of Tumor Microenvironment in Lung Cancer: Focus on Immune Checkpoint Blockade. Front. Immunol. 12:799455. doi: 10.3389/fimmu.2021.799455

In the last decade, the treatment of non-small cell lung cancer (NSCLC) has been revolutionized by the introduction of immune checkpoint inhibitors (ICI) directed against programmed death protein 1 (PD-1) and its ligand (PD-L1), or cytotoxic T lymphocyte antigen 4 (CTLA-4). In spite of these improvements, some patients do not achieve any benefit from ICI, and inevitably develop resistance to therapy over time. Tumor microenvironment (TME) might influence response to immunotherapy due to its prominent role in the multiple interactions between neoplastic cells and the immune system. Studies investigating lung cancer from the perspective of TME pointed out a complex scenario where tumor angiogenesis, soluble factors, immune suppressive/regulatory elements and cells composing TME itself participate to tumor growth. In this review, we point out the current state of knowledge involving the relationship between tumor cells and the components of TME in NSCLC as well as their interactions with immunotherapy providing an update on novel predictors of benefit from currently employed ICI or new therapeutic targets of investigational agents. In first place, increasing evidence suggests that TME might represent a promising biomarker of sensitivity to ICI, based on the presence of immune-modulating cells, such as Treg, myeloid derived suppressor cells, and tumor associated macrophages, which are known to induce an immunosuppressive environment, poorly responsive to ICI. Consequently, multiple clinical studies have been designed to influence TME towards a pro-immunogenic state and subsequently improve the activity of ICI. Currently, the mostly employed approach relies on the association of "classic" ICI targeting PD-1/PD-L1 and novel agents directed

on molecules, such as LAG-3 and TIM-3. To date, some trials have already shown promising results, while a multitude of prospective studies are ongoing, and their results might significantly influence the future approach to cancer immunotherapy.

Keywords: NSCLC, PD-1/PD-L1, CTLA-4, tumor microenvironment (TME), immune checkpoint inhibitors, dysfunctional T cells, immunotherapy

1 INTRODUCTION

In the last decades, a remarkable shift in the clinical management of non-small cell lung cancer (NSCLC) patients has been driven by the introduction of immune checkpoint inhibitors (ICI) targeting the axis involving programmed death protein 1 (PD-1) and its ligand (PD-L1). The introduction of these agents brought to unprecedented durability in the responses compared to chemotherapy. Notably, the most relevant benefit with single-agent ICI in NSCLC is observed in the case of patients whose tumor is characterized by high expression of PD-L1 (≥50%). Indeed, the anti-PD-1 agents pembrolizumab and cemiplimab, as well as the anti-PD-L1 agent atezolizumab, have achieved improved outcomes in terms of response and survival compared to chemotherapy in randomized phase III trials involving previously untreated patients affected by advanced NSCLC with high PD-L1 expression; conversely when PD-L1 expression is lower than 50% the advantage of PD-1 or PD-L1 inhibitors employed as single agent in first-line over platinum-based chemotherapy is limited, and this observation was confirmed in sub-group analyses of patients with PD-L1 between 1-49% enrolled in the KEYNOTE 042 and IMPOWER 110 trials (1–4). In order to improve the outcomes of patients with low or absent PD-L1 expression, anti-PD-1/PD-L1 agents have been employed in combination with either chemotherapy or with other ICI, such as agents targeting the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). In the randomized, phase III KEYNOTE 189 and KEYNOTE 407 trials, which involved patients with advanced non-squamous and squamous NSCLC, respectively, the addition of pembrolizumab to platinum-based chemotherapy resulted in improved outcomes in terms of response and survival over chemotherapy alone (5, 6). Notably, the advantage deriving from the combination of immunotherapy and chemotherapy was independent from the expression of PD-L1, including those patients whose tumor did not express PD-L1 at all (7, 8). More recently, the combination of the anti-PD-1 agent nivolumab and anti-CTLA-4 agent ipilimumab associated with two cycles of platinum-based chemotherapy achieved improved outcomes compared to first-line chemotherapy in the randomized, phase III CheckMate 9LA trial. Even in this case, the experimental combination achieved superior results irrespective of PD-L1 expression (7).

In spite of these impressive results, patients receiving ICI, either alone or as part of combination regimens, are destined to eventually experience disease progression associated with acquired resistance; furthermore, a non-negligible proportion of patients receiving ICI do not respond to treatment in spite of high PD-L1 expression. Indeed, response rate with single-agent pembrolizumab was 44.8% in KEYNOTE 024 (hence more than half of the patient population did not achieve partial response) (2); furthermore, in EMPOWER-LUNG 1, 18% of the patients randomized in the cemiplimab arm experienced disease progression as best response during treatment in spite of high PD-L1 expression (1). Hence, new combination approaches are warranted. Tumor microenvironment (TME) represents an element of increasing interest for the development of cancer immunotherapy as potential source of predictive factors for treatment with ICI or even as an additional therapeutic target by itself. TME consists of a heterogeneous population of cancer cells, immune cells, vessels, stroma, signaling mediators and extracellular matrix proteins (8). The presence of a chronic inflammatory environment in lung cancer (9) may alter or deviate immune cell differentiation, resulting in an imbalance of anti-tumor activity, thus favoring tumor evasion (8) and later on, resistance to ICI (10). In this context, TME might represent a relevant source of predictive biomarkers for ICIs, as well as a potential target for novel therapeutic strategies. Therefore, in this review we will point out the role of TME in the treatment of NSCLC with immunotherapy, either as a predictor of benefit from currently employed ICI or as therapeutic target from investigational agents. Furthermore, we will explore the potential impact of combinations including "classic" ICI and novel agents under clinical investigation. To this aim, we evaluated indexed publications on PubMed and abstracts presented at the most relevant scientific meetings.

2 TUMOR MICROENVIRONMENT

Studies on NSCLC TME based on histological and immunological analyses of the primary tumor have been difficult due to the limited availability of tissue because the majority of patients are diagnosed in advanced disease and are therefore inoperable. Nevertheless, different studies described a TME characterized by the presence of tumor infiltrating lymphocytes (TILs), which have been exploited to define prediction tools for patient's survival and response to therapy. The presence of lymphocytes in the tumor area represents an independent prognostic factor for patient's survival, with intense lymphocytic infiltration predicting longer survival (11, 12). In particular, CD8+T cells and M1-macrophages correlate with positive prognosis (12). The distribution of lymphocytes within the tumor evaluated through tissue microarrays revealed that high density of T lymphocytes (CD4+ and CD8+) in the tumor stroma correlated with better prognosis (12, 13). Beside this, it has been suggested that the presence of high density CD8+T cells

in resected NSCLC may be considered as an additional marker to the tumor–node–metastasis classification (TNM-Immunoscore) (14, 15).

It is getting clearer that the reasons for the resistance to ICI must be sought in the tumor tissue, in the complex network of interactions that exist between tumor cells and TME (10). The presence of TILs, macrophages and dendritic cells (DC) may recall a hot TME potentially responsive to immunotherapy. Unfortunately, only a proportion of patients possess a hot TME, while more frequently cold (very few TILs) or 'altered' (TILs mainly at the edge of the tumor) TME have been observed (16). Spatial histology combined with exome and RNA-sequencing analyses on 100 patients from the TRACERx cohort helped to define that tumors with more than one immune cold region had a higher risk of relapse, regardless of tumor size and stage (17). Low TILs are also correlated with limited efficacy of ICI treatment and resistance to immunotherapy (14).

2.1 T and NK Cells Exhaustion

NSCLC is characterized by high levels of somatic non-synonymous mutations defined as tumor mutation burden (TMB), with higher numbers of mutations in metastases than in primary lung tumors (18–20). Mutations may originate neo antigens, which may be recognized by cytotoxic T cells in the TME, resulting in the development of an antitumor response. Although high infiltrated tumors might be advantaged in recognizing neo antigens, the presence of high TILs rather immunosuppressive or dysfunctional abolishes the possibility of that responses. In a recent published paper CD8+PD-L1+ TILs were associated with increased tumor burden constituting a hot but immunosuppressive TME, but patients with these characteristics were more likely to obtain a good response to anti-PD-1 therapy (21, 22). Using single-cell transcriptomics, Caushi et al., studied the transcriptional programs of mutation-associated neoantigens (MANA)-specific TILs from tumors of 20 patients, which received nivolumab +/- ipilimumab, enrolled in the clinical trial NCT02259621. MANA-specific CD8+ T cells were more numerous in the tumor than in normal lung. MANA-specific T cells from responsive patients showed higher expression of genes associated with memory (*IL7R* and *TCF7*) and effector functions (*GZMK*), while MANA-specific T cells from non-responsive patients expressed mainly genes associated with T cell dysfunction such as *TOX2*, *CTLA4*, *HAVCR2* and *ENTPD1* (22).

The presence of alternative immune checkpoint receptors leading to a progressive and profound T-cell exhaustion has been correlated with resistance to ICI (**Figure 1**). Dysfunctional, 'burned-out' CD8+ TILs (Ebo) were identified using single-cell mass cytometry and tissue imaging technologies from 25 patients with resectable and 35 patients with advanced NSCLC. Ebo TILs accumulated in the TME, show high proliferation rate and activation markers but produce low amount of interferon-gamma (IFNγ). The presence of these cells expressing high levels of PD-1, TIM-3 and LAG-3 was associated with resistance to cancer immunotherapy (23). The lymphocyte activation gene-3 (LAG-3; CD223) is an inhibitory immune receptor expressed on NK, activated T and B cells and exerts its inhibitory action by binding class II MHC. Regulatory T cells (Treg) cells expressing LAG-3 are more active, while LAG-3 expression in cytotoxic T lymphocytes (CTL) is associated with decreased proliferation and activity. T cell immunoglobulin and mucin domain protein 3 (TIM-3), similarly to LAG-3, is an inhibitory receptor frequently detected upregulated on NSCLC TILs during tumor progression and is associated with an exhausted, burned phenotype of TILs and resistance to ICI (23, 24).

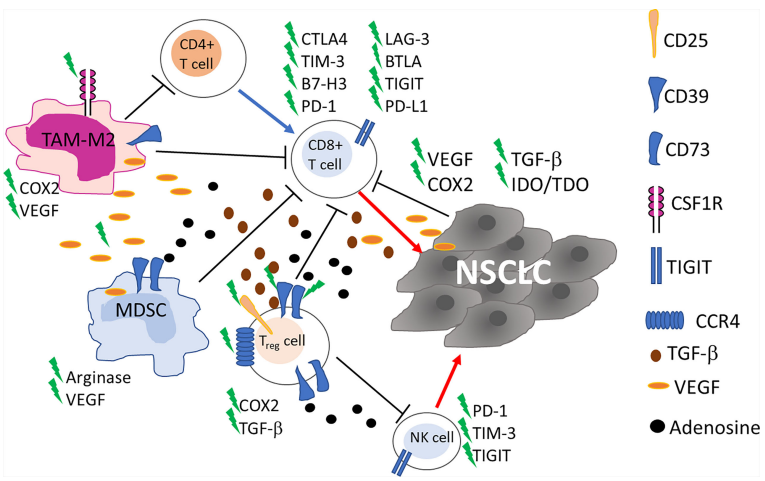

FIGURE 1 | Schematic representation of the main cells in tumor microenvironment involved in NSCLC resistance to ICI. Up-regulation of alternative immune checkpoints on cytotoxic CD8+ T cells impairs recognition and killing of tumor cells. Myeloid derived suppressor cells (MDSC), tumor associated macrophages (TAM)-M2 and CD4+ T Regulatory (Treg) cells through cytokine and soluble factors contribute to the inhibition of the immune responses. Blue and red arrows indicate stimulation and killing, respectively. New targets for on-going clinical trials are highlighted by a green flash.

In patients with NSCLC PD-1, TIM-3, CTLA-4, LAG-3, and BTLA inhibitory receptors were detected on TILs with a gradual and continuous upregulation during tumor progression, in 24 tumor lesions (24).

In NSCLC the accumulation of NK cells is observed, mainly constituted by non-cytotoxic $CD56^{bright}CD16^{-}$ NK cells, a subset endowed with immunoregulatory properties (25, 26). NK cell dysfunction, as well as T cell exhaustion, has also been observed (**Figure 1**). PD-1 is expressed not only on activated T cells, but also on NK cells, and its interaction with anti-PD-1 ICI enhances immune function. In a randomized controlled trial in patients with PD-L1+ NSCLC the combination of *in vitro* expanded allogenic NK cells with anti-PD-1 improved overall survival (OS) and progression-free survival (PFS), compared to single anti-PD-1 treatment, without adverse events associated with NK cell therapy [NCT02843204 (27)]. Killer-cell immunoglobulin-like receptors (KIR) are molecules expressed on the surface of NK cells that, through the engagement of MHC class I ligands expressed on cancer cells, generate inhibitory signals to NK cells. The final result of such interaction is NK cell inactivation (28). He et al. showed that among 11 NSCLC patients treated with nivolumab, 45.5% (n=5) displayed KIR expression in the tumor tissue and in 2 out of 5 increased after treatment with anti-PD-1 ICI (29). However, the authors do not clearly identify NK cells among TILs and analyzed only a small number of patients, thus further studies are needed to point out a real role for KIR in ICI resistance.

2.2 Immunosuppression

Frequently, TME is characterized by the presence of cells endowed with immune suppressive activities and an association with resistance to ICI has been reported, in cancer (10, 30, 31). Treg, myeloid derived suppressor cells (MDSC), and tumor associated macrophages (TAM)-M2 through a cytokine network contribute to the inhibition of the immune responses thus inducing immune suppression (**Figure 1**). Treg cells inhibit T cell responses in different ways, and, in general, are associated with poor clinical outcomes in lung cancer patients (32). Recently, an increase in PD-1+Treg has been detected in patients non-responsive to anti-PD-1/PD-L1 ICI in a study evaluating patients with NSCLC (n=27) and other solid cancers. The authors demonstrated that the balance of PD-1 expression between CD8+ T cells and Treg cells in the TME can predict the clinical effectiveness of ICI therapies better than PD-L1 expression or TMB. Anti-PD-1/PD-L1 ICI, while recovering dysfunctional PD-1+CD8+ T cells, may enhance PD-1+ Treg cell-mediated immunosuppression (33). In a previous study on 73 NSCLC patients treated with anti-PD-1/PD-L1 ICI, the density of PD-L1+ Treg in the TME was indicated as an additional prediction biomarker of response to ICI (34), thus Treg warrant consideration as a therapeutic target to augment the clinical efficacy of ICI in lung cancer.

MDSC can affect TME inducing immunosuppression in many different ways: i) producing nitric oxide (NO) and reactive oxygen species (ROS); ii) eliminating key nutrition factors for T cells from the microenvironment, such as L-arginine, and L-tryptophan; iii) interfering with T cells homing and trafficking; iv) inducing up-regulation of checkpoint; v) and releasing immune regulatory molecules, such as adenosine, Vascular endothelial growth factor (VEGF)-alpha and inhibitory cytokine (interleukin (IL)-10) (35). MDSC, like Treg cells, express CD39 and CD73 ectonucleotidases that in tandem convert ATP into adenosine which is considered an important mediator of immune suppression in the TME (36) (**Figure 1**). MDSC expressing CD39 and CD73 were found in tumor tissue of NSCLC patients and positively correlated to disease progression but chemotherapy significantly reduced these cells (37). The role of MDSC in lung cancer outgrowth and ICI therapy has been deeply investigated in preclinical studies in mice (38–40). These studies show that MDSC promote lung cancer metastasis and that their inhibition may overcome resistance to ICI.

The role of TAM has been explored in a cohort of 187 NSCLC patients, mostly treated with ICI. CD163+CD33+PD-L1+ M2-TAM were detected in lesions of patients experiencing hyperprogression. These cells possess an epithelioid morphology (alveolar macrophage-like) and form clusters within neoplastic foci (41). Low CD8+PD-L1+ T cells, and low CD68+CD163+ M2-TAM were predictive for positive response in 33 stage II-IV NSCLC patients treated with ICI (42). By DNA-based quantitative immunofluorescence and confocal microscopy, most PD-L1+ cells are CD68+ macrophages and high cell counts of PD-L1+CD68+ macrophages in the TME has been associated with better OS in 81 patients treated with anti-PD-1 (YTMA404 cohort) (43).

Kargl et al., found that neutrophil content in the TME negatively correlated with the presence of CD8+ and CD4+ T cells and with Th1 and Th17 subsets, but not with Treg cells, implicating a potential immune suppressive role for neutrophils in NSCLC (44, 45). Data from preclinical studies in *IL-17:K-Ras* mutated transgenic mice demonstrated that resistance to anti-PD-1 therapy is abrogated by neutrophil depletion, reconstituting T cell activation (46). The role of neutrophils in the resistance to ICI in NSCLC patients still remains to be addressed.

2.3 Angiogenesis

Angiogenesis, with abnormal vasculature is part of TME and is a hallmark of cancer associated with development, proliferation and metastasis (47–49). Vascular endothelial growth factor/vascular endothelial growth factor receptor (VEGF/VEGFR) are a family of proteins that play an essential role in tumor induced angiogenesis promoting vascular permeability by regulating the differentiation, migration, proliferation and survival of microvascular endothelial cells (48). VEGF proteins can inhibit the maturation, differentiation, and antigen presentation of professional Antigen Presenting Cells (APC), DC, NK, and T cells, while improving the suppressive effect of Treg, TAM, and MDSC (**Figure 1**). A comprehensive review on VEGF and its targeting in association with ICI has been published yet in 2021 by Ren et al. (48). Targeting VEGF-A has been exploited in patients to reduce resistance to immunotherapy by combining bevacizumab (anti-VEGFA antibody) with atezolizumab (NCT02366143) and chemotherapy, showing a significant improvement of PFS and

OS of patients with metastatic lung cancer (50). This clinical response was independent from PD-L1 expression and genetic alteration status of tumors, and strongly supports a role of angiogenesis in the resistance to ICI.

2.4 Tertiary Lymphoid Structures

NSCLC are often associated with the presence of 'Tertiary Lymphoid Structures' (TLS). TLS may occur at both the margins and the core of tumors, are spatially well-organized and are composed of T and B cell zones and germinal centers (51). Some authors have correlated the presence of B cells in TLS with favorable outcomes (52–56). In particular, Tang et al. observed an increase in TLS area and B cell proportion within TLS in lung cancer patients with resectable tumors and found a correlation with longer survival rates (56). Since presence and composition of TLS might be influenced by chronic inflammation, TLS from patients who had undergone resection for lung cancer were analyzed, comparing patients with chronic obstructive pulmonary disease (COPD) and those without. Notably, the samples from patients with underlying COPD were characterized by reduced TLS and reduced germinal centers compared to samples from patients without COPD. Follow-up demonstrated poorer survival for patients with fewer TLS, especially among COPD patients (56). These findings imply that chronic inflammation might result in reduced immunological responses against tumorigenesis, but studies on TLS role in ICI resistance need to be pursued for NSCLC patients.

2.5 Tumor Driver Mutations and TME

Tumor intrinsic mechanisms, such as specific driver mutations may affect NSCLC resistance to ICI therapy. In particular, D' Incecco et al. found that PD-1+ tumors are characterized by *KRAS* mutations, whereas PD-L1+ tumors are mainly *EGFR* mutated (57). *EGFR* mutated NSCLC exhibited reduced CD8+ lymphocyte infiltration, while *KRAS* mutant displayed higher CD8+ T cells, as detected using tissue microarray (58). By single-cell RNA sequencing on NSCLC tissue harboring *EGFR* mutation, myeloid and T cells, mainly exhausted, and Treg, were the most abundant immune cells identified (59). The reasons for the weak response of *EGFR*-positive NSCLC patients to ICI are still not fully understood. *EGFR* mutated tumors have lower somatic mutations and number of neoantigens (60), display an uninflamed TME, which may explain the poor efficacy of ICI compared to *EGFR*-wild type (61). The role of *EGFR* mutation on the upregulation of PD-L1 expression is still controversial (62). *STK11/LKB1* alterations confer to NSCLC resistance to PD-1 blockade, in a study conducted on 66 patients with PD-L1+ tumors receiving anti-PD-1/PD-L1 therapy (63). In particular, *STK11/LKB1* alterations were frequently associated to *KRAS* mutations and with low TILs, reduced PD-L1 expression and high TMB (63). In a genetic engineered mouse model bearing *KRAS* and *STK11/LKB1* mutations a massive recruitment of immunosuppressive neutrophils and increase in the expression of exhaustion marker on T cells was detected (64).

Resistance to ICI may also be driven by loss of antigen presentation occurring in antigen presenting cells or cancer cells within the TME, and is frequently associated with acquired genetic mutations, such as loss of heterozygosity (LOH) in HLA loci, mutation of HLA genes, and modulation of HLA gene expression (65, 66).

Recently, Bagaev et al. have developed a multi-omics and robust analytical platform to classify, reconstruct, and visualize the entire tumor composition (67). They took into consideration genomic and transcriptomic analyses that evaluate the tumor (mutations of DNA repair genes, and cell cycle regulation) and the TME (the major functional components and immune, stromal, and other cellular populations) as a whole for different cancers. They defined four distinct TME subtypes predictive of response to immunotherapy [Immune-Enriched, Fibrotic (IE/F); Immune-Enriched, Non Fibrotic (IE); Fibrotic (F); Depleted (D)] based on melanoma that were conserved across at least 20 additional cancers, including lung cancer [n=27 (67)]. Subtype IE had significantly longer OS and PFS compared to F and D, with F being the worst, in melanoma. Lung cancer patients with TME subtype IE demonstrated the longest OS. Genetic alterations, such as *EGFR* in lung cancer, were associated to F and D TME subtypes.

3 IMMUNE RELATED SIGNATURES

In the last decade great efforts have been made to identify reliable predictive TME-based signatures for lung cancer immunotherapy. Currently, one of the most powerful prognostic tools in oncology is "immunoscore" (IS) based on the numbering of T lymphocytes within the tumor (68). This tool is a digital tumor tissue-based test that estimates patient's prognosis on immune cell infiltration (*i.e.*, CD3/CD45RO, CD3/CD8, or CD8/CD45RO). Specifically, IS measures the subpopulations of T cells in the center and periphery of the tumor and provides a score ranging from IS 0 with a low density of immune cells to IS 4 with a high density in both regions. This test, initially validated on colorectal cancers (68), has shown great promise as a supplement to the classification of lymph node metastases (TNMs) in a number of cancers, including NSCLC (69). In particular, numerous studies have shown that a high IS score correlates with better survival (70–73). In addition, CD8+ TIL has also been described as a powerful biomarker in discriminating patients with a significantly longer PFS after ICI treatment; this association was strengthened when IS was integrated with tumor PD-L1 expression, suggesting that the combination of these markers could be a reliable biomarker for immunotherapy (74). Gene signatures, an alternative approach to characterize the TME on the transcriptomic profiling, have recently gained a great interest in the scientific community. The TME signature consists of lists of genes indicative of the presence of a given population of immune/stromal cells and/or descriptive of a particular state of TME-cell activation.

With the advent of high-throughput technologies (*i.e.* microarray and more recently RNA seq) capable of screening the whole transcriptome of the tumor bulk, an increasing

number of computational algorithms have been developed for the prediction of non-cancer cell infiltration (**Table 1**) (75, 79, 82, 85, 86, 88, 89).

Despite each algorithm varies in terms of computational approach, the output consists of a score based on tumor-infiltrating immune and/or stromal cells, allowing a better comprehension of the mechanisms underlying cancer immunity and their potential role in the response to ICI. The output scores consist of TME signature allowing a comprehension of the intra tumoral heterogeneity as well as the inter-sample comparisons. Among the most relevant studies on the evaluation of the cancer immune landscape using the gene expression profile, the Cancer Genome Atlas Network project deserves to be mentioned (90). The consortium performed a large immuno-genomic study of over 10,000 tumors across 33 cancers by integrating the mRNA expression profile with DNA copy number and mutational status. Then, applying a combination of computational algorithms, the authors characterized the TME in six major immune subtypes defined as follows: 1) wound healing, 2) IFN-dominant, 3) inflammatory, 4) lymphocyte depletion, 5) immunologically silent, and 6) TGF-β dominant. Lung neoplasms were mainly enriched in the first three subtypes; in particular, squamous cell carcinomas (SCCs) showed an enrichment of 'wound healing' (defined by high angiogenic gene expression, elevated proliferation rate and Th2 cell bias for adaptive immune infiltrate) and 'IFN-dominant' (depicted by high M1/M2 macrophage ratio polarization and a strong CD8 signal such as a high diversity TCR) subtypes. In contrast, lung adenocarcinoma (ADC) showed greater enrichment of 'INF dominant' and 'inflammatory' (characterized by elevated Th17 and Th1 genes, low/moderate tumor cell proliferation, and low levels of aneuploidy) subtypes. A similar extensive bioinformatic strategy was also performed by Charoentong et al. who, by integrating DNA and RNA data over 8,000 patients across 20 solid cancers, defined an immunophenoscore, able to discriminate patients more responsive to ICI (81). In particular, the predictive score provides information on some relevant immunogenomic characteristics such as TIL composition, cancer antigen profiles and tumor heterogeneity. Another pan-cancer study that examined the TME gene profile aimed at predicting clinical response to PD-1 blockade, was performed by Ayers in 2017 (91). The authors, starting from a small pilot study including 19 patients with metastatic melanoma undergoing anti-PD-1 ICI, profiled the expression of 680 tumor and immune genes using the digital platform NanoString nCounter (91, 92). Through a rigorous multi-step validation, they defined an 18-gene score, named 'Tumor Inflammation Signature' (TIS), that included genes linked to cytotoxic cells, antigen presentation, and IFNγ activity. More recently, the prognostic value of the TIS score was also evaluated in the 9,083 tumor gene expression profiles downloaded from the Cancer Genome Atlas (TCGA) database (980 from lung cancers) (93). As already reported in the previous study, tumors with known clinical sensitivity to ICI such as NSCLC, showed generally higher TIS scores. In addition, the TIS score showed a stronger prediction for identifying patients with clinical sensitivity to ICI than TMB status, especially in tumors with low TMB variability, such as SCC. In the wake of these intriguing findings, an exponential number of studies have profiled TME genes on lung cancers by identifying highly specific and accurate signatures capable of predicting molecular subtypes more sensitive to anti-PD-L1/PD1-based therapies (94–97). For example, Higgs et al. identified an IFNγ signature, focused on 4 genes already included in the previous TIS such as *IFNγ, LAG3, CXCL9* and *PD-L1* (94). IFNγ-positive signature patients showed higher overall response rates and better PFS and OS with the anti-PD-L1 durvalumab, regardless of tissue PD-L1 status. In addition, several studies downloaded RNA datasets from public databases and using mathematical models each score was then tested in independent validation sets to improve prediction performance (97–101). Chaoqi Zhang et al. using more than 1,500 RNA data from ADC tumors, tested 60 costimulatory molecule genes on 502 cases. Then, applying a step-wise method, they filtered the combination of 5 genes which was validated on ten independent sets. The costimulatory molecule 5 gene-based signature identified two risk groups with distinct inflammatory profiles and immune infiltrate, through a computational method. 'High-risk' patients had a significantly higher proportion of activated NK cells, DC, neutrophils, macrophages M0, resting DC, and Treg. 'Low-risk' patients had a high proportion of memory B cells, resting CD4 memory T cells, and gamma delta T cells. According to the profiles, the authors indirectly predicted that high-risk patients could benefit from immunotherapy (98).

3.1 Novel Emerging Signature

Despite the impressive results, the tissue-based immune signatures require the collection of representative tumor specimens and can therefore be limited by inadequate samples or by intra-tumoral heterogeneity, commonly described in NSCLC. To date, radiomics represents one of the most promising across the emerging predictive biomarkers for ICI. Radiomics is a high-throughput extraction of features from medical images using computer algorithms, aimed at providing quantitative information on tissue composition that otherwise cannot be detected through simple observation (102, 103). Ideally, radiomics can be considered as a virtual biopsy with the advantage of being a totally non-invasive tool, which allows the evaluation of the tumor and its microenvironment, the characterization of intra-tumoral heterogeneity and a dynamic monitoring. One of the first application of the radiomics in the characterization of molecular heterogeneity of lung cancers dated in 2012. The authors compared images from preoperative computed tomography (CT) and Positron Emission Tomography/Computed Tomography (PET/CT) from a cohort of 26 NSCLC patients with tissue gene expression profiles (radiogenomics) identifying significant correlations (104). In the last decade, a growing number of studies have investigated the potential clinical utility of radiomic features (RFs) providing radiomic-based signatures for precision diagnosis as well as the prediction of gene mutations (105–107). In addition, the radiomic approach has also been applied to decipher lung TME (108, 109). Recently, Chen and colleagues, applying the least absolute shrinkage and selection operator (LASSO) and logistic regression to CT images

TABLE 1 | Current state-of-art computational tools.

Name	Year	Type	Output	Web-server	Code
CIBERSORT (75)	2015	DB	Fractions of the immune cell-types defined by the signature matrix provided in input and corresponding p-value	https://cibersort.stanford.edu/ (registration required)	External R package: https://github.com/icbi-lab/immunedeconv
CIBERSORTx (76, 77)	2019	DB	(i) custom gene signature matrix computed from scRNA-seq or bulk sorted RNA-seq data (ii) cell type proportion inferred from GEPs by using the computed (or provided) gene signature matrix (iii) cell-type specific GEPs.	https://cibersortx.stanford.edu (registration required)	N.A.
EPIC (78)	2017	DB	Fractions of (i) individual non-malignant cell-types for which a GEP is provided (ii) all the other non-characterized (cancer) cell types grouped together. The package provides reference GEPs for B, CD4 T, CD8 T, NK, CAFs, Endothelial, Macrophages, Monocytes, Neutrophils.	http://epic.gfellerlab.org	R package: https://github.com/GfellerLab/EPIC
ESTIMATE (79)	2013	SB	Two scores representing the level of immune and stromal cells. A derived level of tumor purity.	N.A.	R package: https://bioinformatics.mdanderson.org/estimate/
Gene signature of infiltrating Leukocytes (80)	2017	SB	60 GS for 14 immune cell types (B, CD45, Cytotoxic, Exhausted CD8, Macrophages, Mast cells, Neutrophils, NK, NK CD56dim, T, Th1, Treg, CD8, CD4) derived testing gene signatures from the literature.	N.A.	R code for reproducing the analysis as supplementary material of the paper.
Immunophenoscore (81)	2017	SB	782 GS for 28 immune cell types (T, Tcm, Tem, activated, central memory, CD4+, CD8+, gamma delta T, Th1, Th2, Th17, Treg, Tfh, activated, immature, and memory B, machrophage, monocytes, mast cells, esosinophils, neutophils, acitvated, monocytes, and immature DC, NK, NKT, MDSC). An aggregate score, tarmed immunophenoscore, quantifying tumour immunogenicity.	https://tcia.at	R package: https://github.com/icbi-lab/Immunophenogram
MCP-Counter (82)	2016	SB	Abundance score for 8 immune cell types (T cells, CD8+ T cells, NK cells, cytotoxic lymphocytes, B cell lineage, monocytic lineage cells, myeloid dendritic cells, and neutrophils) and 2 stromal cell types (endothelial cells and fibroblasts)	http://134.157.229.105:3838/webMCP/	R package: https://github.com/ebecht/MCPcounter
QuanTIseq (83)	2019	DB	Absolute fractions for 10 immune cell types (B cells, M1 and M2 macrophages, monocytes, neutrophils, NK cells, CD4+ T cells, CD8+ T cells, Treg cells, and myeloid dendritic cells) and abundance of the remaining uncharacterized cells.	N.A.	Pipeline: http://icbi.at/quantiseq (Raw FASTQ data allowed) R package: https://bioconductor.org/packages/release/bioc/html/quantiseqr.html
TIP (84)	2018	Both	(I) 23 immune activity score computed based on 178 signature genes. This score quantifies the activity status of the 7-step immunity cell-cycle. (II) Relative proportion of tumor-infiltrating immune cells computed by CIBERSORT. If microarray GEPs are provided the original signature matrix with 22 cell-types is used; if RNA-seq data are provided a dedicated signature matrix with 24 cell-types is used.	http://biocc.hrbmu.edu.cn/TIP/	R package: https://github.com/dengchunyu/TIP
TIMER (85, 86)	2016	DB	Relative abundance of 6 immune cell types: B cells, CD4 T cells, CD8 T cells, neutrophils, macrophages, dendritic cells.	https://cistrome.shinyapps.io/timer/	R package: http://cistrome.org/TIMER/download.html
TIMER 2.0 (87)	2020	Both	Results and comparison from TIMER, xCell, MCP-counter, CIBERSORT, EPIC, quanTIseq	http://timer.cistrome.org/	External R package: https://github.com/icbi-lab/immunedeconv
Xcell (88)	2017	SB	GS score for 64 immune and stroma cell types corrected for spillover effects.	https://xcell.ucsf.edu/	R package: https://github.com/dviraran/xCell

Two groups of methods exist namely signature-based (SB) and deconvolution-based (DB) approaches. SB approaches identify a set of genes whose expression is characteristic of a specific type of cell. Then, a score is defined to quantify the abundance of each cell type based on the expression of the corresponding signature genes. DB approaches formulate the problem as a mathematical deconvolution, that is the tissue gene expression profile (GEP) is written as the weighted sum of precomputed typical expression profiles of the considered cell-types. The unknown weights are then estimated by using a proper regression technique. For each tool we report: the year of publication of the paper; the method DB and SB approaches; the type of cells for which abundance is computed; possible available web-server and/or open-source package implementing the method. GS, gene signature; NA, not available.

from 120 patients, extracted 462 RFs. The combined model, including RFs, clinical and morphological data, showed an optimal prediction power for PD-L1 expression levels and TMB status (110). A number of studies also reported image-based signatures predictive to ICI response or outcome (111, 112). Very recently, Yang and colleagues used pretreatment CT images, from 92 patients treated with an ICI, to select 88 RFs. Then, the authors, developed two nomogram-based models, integrating RFs with clinical pathological characteristics and demonstrated good performances in identifying patients with a

durable response and a longer PFS (113). In another retrospective study, Khorrami et al. applying a machine learning approach, compared the delta radiomic texture (DelRADx) of CT patterns both in the tumor and peritumoral regions between the baseline and the post-treatment scans of 139 advanced patients receiving ICI. The combination of eight identified DelRADx features were predictive of response to ICI therapy and of OS (114). Similarly, a new algorithm 'TMB radiomic biomarker' (TMBRB) combining deep learning technology to CT images from 327 NSCLC patients distinguished tumors with a High-TMB versus a Low-TMB value. TMBRB, in a cohort of 123 patients treated with an ICI resulted an optimal predictor in terms of both OS (HR: 2.33, 95% CI: 1.14 to 4.77) and PFS (HR: 1.90, 95%CI: 1.14 to 3.19) (115). Recently, DelRADx features resulted predictive of response to ICI therapy, prognostic of improved OS, and correlated with TIL density (114).

4 IMMUNOBIOLOGY OF LUNG CANCER

Several lines of evidence highlight the roles of both innate and adaptive immune components in the elimination phase of cancer immunoediting process. The adaptive branch of the immune system has been demonstrated as the prominent mechanism able to eliminate cancer cells through the recognition of tumor antigen in the context of MHC complex (116).

Tumor associated antigens (TAA) overexpressed in lung cancer are MUC-1, CEA, NY-ESO, MAGE-A3 (117–119). Due to their expression in normal cells, these antigens are considered less immunogenic and more likely to induce tolerance, furthermore tumors expressing these antigens seem less responsive to ICI.

Conversely, tumor specific antigens (TSA) are unique to tumor cells and should result from non-synonymous somatic mutations thus represent the ideal antigens for cellular immunotherapy (120, 121). Several reports have demonstrated that tumors with a high TMB, like NSCLC, possess a high number of neoantigens. Among the various somatic mutations noted, some occur in driver genes including in *TP53, KRAS, CDKN2A, ARID1A, NOTCH1, MYC, SMARCA4* and *RB1* (122, 123). Neoantigens can be recognized by TILs. Accordingly, neoantigen density has been shown to correlate with a favorable prognosis and higher CTL content (124) as well as, with benefit from ICI (125). Despite being extremely challenging, neoantigen-specific cells have been successfully identified in NSCLC patients by using the Mutation Associated NeoAntigen Functional Expansion of Specific T-cells (MANAFEST) platform (126). CTL specific for peptides derived from oncogenic driver mutations such as *TP53* R248L (22), or *BRAF* N581I (127) have been found.

Cancer vaccines aim at boosting T cell and B cell-mediated response against TAA or TSA. Several clinical trials are currently evaluating different vaccines in lung cancer patients and specific target antigens (*e.g.* MAGE-A3, CEA, mesothelin, RAS, NY-ESO-1, telomerase, WT1), as well as immunomodulatory enzymes such as Indoleamine 2,3-dioxygenase (IDO) and Arginase-1 (119, 128). Interestingly, some of these cancer vaccines have been recently administered also in combination with ICI in phase I/II studies (i.e. NCT04908111, NCT02879760, NCT03562871), even if no data regarding effectiveness has been released yet.

Tumor neoantigens are highly specific to tumors of an individual patient and not expressed on normal cells, thus able to evoke robust tumor-specific T cell responses (129). To date, several clinical trials are ongoing investigating personalized neoantigen-based vaccines alone or in combination with anti-PD-1, -PD-L1 and/or -CTLA-4 antibodies in various tumor types, comprising NSCLC (130). Neoantigens can be identified by multiple bioinformatic technologies, mainly based on whole-exome sequencing computational algorithms for antigen prediction. Personalized vaccines are being developed and employed in different formulations, such as synthetic long peptide (SLP), DNA, RNA, DC-based, and associated to viral and bacterial vectors (131). Recently, data from a phase Ib trial of personalized neoantigen therapy (NEO-PV-01, NCT02897765) plus nivolumab in patients with Advanced Melanoma, NSCLC (n=18), or Bladder Cancer was released, demonstrating that this type of regimen was safe and did not lead to treatment-related serious adverse events. In addition, the data demonstrated that the vaccine was able to trigger an effective T cell response against neoantigens in all vaccinated patients. Interestingly, the vaccine evoked a T cell response also to neoantigens not included in the vaccine formulation (epitope spread) (132).

Targeting of tumor antigens has been also pursued by adoptive transfer of tumor-reactive T Cells (ACT). Upon isolation from the patient, natural or *in-vitro*-modified T cells are expanded ex vivo and reintroduced into the patient to enhance T cell responses and kill tumor cells. ACT therapies include the adoptive transfer of TILs, or of engineered T cells that possess retargeted specificity and higher affinities for tumor antigens, such as engineered affinity-enhanced αβTCR or chimeric antigen receptors (CAR). Compared to vaccine-based strategies, ACT provides patient with already competent effector cells, thus overcoming the requirement of T-cell priming in patients who are often immune compromised and tolerant to cancer antigens. Current strategies for targeting advanced NSCLC include adoptive transfer of engineered T cells directed against specific TAA, such as NY-ESO-1/LAGE-1, also in combination with ICI (NCT03709706), as well as personalized adoptive cell therapy where neoantigen-specific T cells from individual tumors are identified, expanded ex vivo, and then re-injected in patients (NCT04596033). Despite being very promising, TCR-based ACT may suffer from certain disadvantages. αβTCR-based targeting approaches remain susceptible to tumor escape arising through immunoediting processes that select tumor clones unable to present antigens due to impairment in MHC-class I expression or to interference with antigen presentation (66, 133). More recently, by analyzing next-generation sequencing data derived from previous early-stage NSCLC and matched brain metastases, McGranahan et al. found that 40% of early-stage NSCLC displayed LOH and that

metastases had an even higher prevalence of such genetic alteration. Interestingly, HLA-LOH in metastasis was associated with an elevated non-synonymous mutation rate, suggesting LOH as an immune escape mechanism that prevents presentation of neoantigens (134). To circumvent the loss of MHC and antigen presentation, transduction of patient's T cells with chimeric antigen receptors (CAR) recognizing intact cell surface proteins represents an alternative approach to redirect T cell specificity. However, exploitation of CAR T cell technology in solid tumors still presents many hurdles. In order to overcome these limitations, CAR-T cells have now been engineered to enhance tumor infiltration, induce the remodeling of the TME and endogenous immune response, and disrupt immunosuppressive axes (135). This is the case, for example, of an early phase I clinical trial which exploits the possibility to use CAR-T cells directed against mesothelin (MSLN) further engineered to secrete, locally, anti-PD-1 antibodies in NSCLC and mesothelioma patients [NCT04489862 (136)]. The possibility to target EGFR expressed by NSCLC cells has been also investigated by the use of anti-EGFR CAR T, further modified to express C-X-C Chemokine receptor type 5 (CXCR5), in a phase I clinical study (NCT04153799). Although these trials estimate to recruit small numbers of patients, results will be very important to define the safety and the toxicity of these approaches.

Besides T cells, also NK cells are suitable for engineering with CAR constructs. NK cells equipped with CAR have demonstrated safety, such as a lack or minimal cytokine release syndrome and neurotoxicity, in an autologous setting. CAR-NK cells can also kill targets in a CAR-independent manner (137). Clinical trials evaluating CAR-NK cells for the treatment of solid tumors have been started also in NSCLC (NCT02839954). This phase I/II trial uses CAR-NK cells specific for MUC-1 antigen expressed by different cancers, including NSCLC. Because activated NK cells, similarly to T cells, can express immune checkpoint molecules (*e.g.*, PD-1, LAG-3, and TIM-3) that might inhibit NK anti-tumor responses their blockade with ICI could be envisaged in order to reinvigorate cytotoxic activity (138–140).

5 NOVEL IMMUNOTHERAPEUTIC APPROACHES

Since TME is able to greatly influence immune response through complex pathways, its components represent promising targets for investigational agents. Current immune-oncology research is focusing on the association of "classic", acknowledged ICI, such as anti-PD-1/PD-L1 and anti-CTLA-4 agents, with investigational compounds, either directed at TME molecules or at newly discovered immune checkpoints. The aim of these novel combinations is to overcome the resistance to ICI and hence improve survival of NSCLC patients. The currently available information on these agents have been reported in the following sub-sections. Notably, as most clinical studies are still ongoing, they have been resumed in **Table 2**.

5.1 Targeting Emerging Immune Checkpoints

Recently, several novel immune checkpoints with potential therapeutic have been identified, and the most promising molecules appear to be LAG-3, TIM-3, B7-H3, and TIGIT.

LAG-3 direct targeting is exploited by the use of a soluble dimeric recombinant LAG-3 (Eftilagimod alpha or IMP321), that stimulates DC through MHC class II molecules and induces sustained immune responses together with anti-PD-1, in patients with previously untreated unresectable or metastatic NSCLC (NCT03625323). Other approaches use bispecific antibodies targeting on one hand LAG-3 and on the other PD-1 (NCT04140500; NCT03219268), rather than single-agent compounds (NCT03250832; NCT03849469). More recently, the anti-LAG-3 antibody relatlimab (BMS-986016) has been assessed in the randomized, phase III trial RELATIVITY-047 in which 714 treatment-naïve patients affected by metastatic melanoma were randomized to receive nivolumab plus relatlimab or nivolumab plus placebo. Median PFS (the primary end-point) was significantly longer in the combination arm compared to the control arm (10.1 vs. 4.6 months; HR= 0.75; p= 0.0055); furthermore, the combination was well tolerated in terms of safety with no unexpected toxicities. Notably, RELATIVITY-047 is the first randomized study to demonstrate clinical benefit of dual LAG-3 and PD-1 inhibition in a solid tumor (141). Following these results, additional studies involving the dual blockade in other solid tumors, including NSCLC, are currently ongoing (NCT04623775) (**Table 2**).

TIM-3, apart from CTL, NK and Treg, is also expressed on DC and macrophages (in which its expression favors M2 polarization) (142). Monoclonal antibodies targeting TIM-3 either alone or in association with anti-PD-1 are under investigations in different clinical trials in solid tumors (NCT03652077; NCT02608268) (**Table 2**). Additionally, the use of bispecific antibodies capable to bind to both TIM-3 and PD-1 is being explored in ongoing trials specifically involving NSCLC patients (NCT03708328; NCT04931654). The safety and tolerability of combinations including anti-TIM-3 and anti-PD-1 with platinum-based doublet chemotherapy are currently being assessed in NCT03307785, and data collection is still on-going. Combination therapies simultaneously targeting TIM-3, PD-1 and LAG-3 immune checkpoint have also been evaluated for advanced cancers (NCT04641871). To date, only few clinical data are available for NSCLC. In a single-arm, phase II dose-expansion part of a phase I/II study, 33 patients (including 16 patients with melanoma and 17 with NSCLC), who were progressing after PD-1/PD-L1 blockade, received MBG453 (anti-TIM-3) and spartalizumab (anti-PD-1) until progression, death, or unacceptable toxicity. The combination resulted generally safe, but with limited activity in the setting of NSCLC and melanoma patients who had previously received ICI (143). Although definitive data are still immature, other early reports suggest that the combination of anti-TIM-3 (TSR-022) and anti-PD-1

TABLE 2 | Ongoing clinical trials.

NCT number	Trial	Status	Phase	Total Estimated enrollment	Investigator	First Submitted Date	Last Update Posted Date
			anti-LAG3 and ICI				
NCT03625323	Combination Study With Soluble LAG-3 Fusion Protein Eftilagimod Alpha (IMP321) and Pembrolizumab in Patients With Previously Untreated Unresectable or Metastatic NSCLC, or Recurrent PD-X Refractory NSCLC or With Recurrent or Metastatic HNSCC (TACTI-002) - TACTI-002 Keynote-PN798 (Other Identifier: Merck Sharp & Dohme Corp)	Recruiting	Phase II	183	Frederic Triebel	August 10, 2018	April 9, 2021
NCT04140500	Dose Escalation Study of a PD1-LAG3 Bispecific Antibody in Patients With Advanced and/or Metastatic Solid Tumors	Recruiting	Phase I	320	Reference Study ID: NP41300 www.roche.com/about_roche/roche_worldwide.htm	October 28, 2019	July 22, 2021
NCT03219268	A Study of MGD013 in Patients With Unresectable or Metastatic Neoplasms	Active, not recruiting	Phase I	353	Bradley Sumrow, MD MacroGenics	July 17, 2017	August 9, 2021
NCT03250832	Study of TSR-033 With an Anti-programmed Cell Death-1 Receptor (PD-1) in Participants With Advanced Solid Tumors (CITRINO)	Active, not recruiting	Phase I	111	GSK Clinical Trials Glaxo SmithKlin	August 16, 2017	May 18, 2021
NCT04641871	Sym021 in Combination With Either Sym022 or Sym023 in Patients With Advanced Solid Tumor Malignancies	Active, not recruiting	Phase I	200	Nehal Lakhani, MD START Midwest	November 24, 2020	May 14, 2021
NCT03849469	A Study of XmAb®22841 Monotherapy & in Combination w/Pembrolizumab in Subjects w/ Selected Advanced Solid Tumors (DUET-4)	Recruiting	Phase I	242	Benjamin Thompson, MD, PhD Xencor, Inc.	February 21, 2019	May 5, 2021
NCT04623775	A Study of Relatlimab Plus Nivolumab in Combination With Chemotherapy vs. Nivolumab in Combination With Chemotherapy as First Line Treatment for Participants With Stage IV or Recurrent Non-small Cell Lung Cancer (NSCLC)	Recruiting	Phase II	520	Bristol-Myers-Squibb	November 10, 2020	August 25, 2021
			anti-TIM-3 and ICI				
NCT03708328	A Dose Escalation and Expansion Study of RO7121661, a PD-1/TIM-3 Bispecific Antibody, in Participants With Advanced and/or Metastatic Solid Tumors	Recruiting	Phase I	280	Clinical Trials Hoffmann-La Roche	October 17, 2018	July 19, 2021
NCT04931654	A Study to Assess the Safety and Efficacy of AZD7789 in Participants With Advanced or Metastatic Solid Cancer	Not yet recruiting	Phase I	81	AstraZeneca	June 18, 2021	July 16, 2021
NCT03652077	A Safety and Tolerability Study of INCAGN02390 in Select Advanced Malignancies	Active, not recruiting	Phase I	40	John Janik, MD Incyte Corporation	August 29, 2018	March 17, 2021
NCT04641871	Sym021 in Combination With Either Sym022 or Sym023 in Patients With Advanced Solid Tumor Malignancies	Active, not recruiting	Phase I	200	Nehal Lakhani, MD START Midwest	November 24, 2020	May 14, 2021
NCT02817633	A Study of TSR-022 in Participants With Advanced Solid Tumors (AMBER)	Recruiting	Phase I	369	GSK Clinical Trials GlaxoSmithKline	June 29, 2016	June 8, 2021
NCT03307785	Previous Study | Return to List | Next Study Study of Niraparib, TSR-022, Bevacizumab, and Platinum-Based Doublet Chemotherapy in Combination With TSR-042	Active, not recruiting Has results	Phase I	58	GSK Clinical Trials GlaxoSmithKline	October 12, 2017	May 10, 2021
NCT02608268	Phase I-Ib/II Study of MBG453 as Single Agent and in Combination With PDR001 in Patients With Advanced Malignancies	Active, not recruiting	Phase I Phase II	252	Novartis Pharmaceuticals	November 18, 2015	July 19, 2021
NCT03099109	A Study of LY3321367 Alone or With LY3300054 in Participants With Advanced Relapsed/Refractory Solid Tumors	Active, not recruiting	Phase I	275	Eli Lilly and Company	April 12, 2017	September 5, 2021

(Continued)

TABLE 2 | Continued

NCT number	Trial	Status	Phase	Total Estimated enrollment	Investigator	First Submitted Date	Last Update Posted Date
			anti-B7-H3 and ICI				
NCT02475213	Safety Study of Enoblituzumab (MGA271) in Combination With Pembrolizumab or MGA012 in Refractory Cancer	Active, not recruiting	Phase I	145	Stacie Goldberg, M.D. MacroGenics	June 18, 2015	April 14, 2021
NCT02381314	Safety Study of Enoblituzumab (MGA271) in Combination With Ipilimumab in Refractory Cancer	Completed	Phase I	24	Stacie Goldberg, M.D. MacroGenics	March 6, 2015	March 25, 2019
NCT03729596	MGC018 With or Without MGA012 in Advanced Solid Tumors	Recruiting	Phase I Phase 2	182	Chet Bohac, PharmD MD MSc MacroGenics	November 2, 2018	April 28, 2021
			anti-TIGIT and ICI				
NCT04995523	A Study to Assess the Safety and Efficacy of AZD2936 in Participants With Advanced or Metastatic Non-small Cell Lung Cancer (NSCLC) (ARTEMIDE-01)	Not yet recruiting	Phase I Phase II	147	AstraZeneca	August 9, 2021	August 9, 2021
NCT04952597	Study of Ociperlimab Plus Tislelizumab Plus Chemoradiotherapy in Participants With Untreated Limited-Stage Small Cell Lung Cancer	Recruiting	Phase II	120	BeiGene	July 7, 2021	July 30, 2021
NCT04746924	A Study of Ociperlimab With Tislelizumab Compared to Pembrolizumab in Participants With Untreated Lung Cancer	Recruiting	Phase III	605	Mark Socinski, MD Advent Health Orlando	February 10, 2021	June 14, 2021
NCT04672356	A Study to Evaluate the Safety, Tolerability and Efficacy of IBI939 in Combination With Sintilimab in Patients With Advanced Lung Cancer	Recruiting	Phase I	20	Ying Cheng Jilin Province Cancer Hospital	December 17, 2020	February 21, 2021
NCT04294810	A Study of Tiragolumab in Combination With Atezolizumab Compared With Placebo in Combination With Atezolizumab in Patients With Previously Untreated Locally Advanced Unresectable or Metastatic PD-L1-Selected Non-Small Cell Lung Cancer (SKYSCRAPER-01)	Recruiting	Phase III	560	Hoffmann-La Roche	March 4, 2020	July 20, 2021
NCT04791839	Safety and Efficacy of Zimberelimab (AB122) in Combination With Domvanalimab (AB154) and Etrumadenant (AB928) in Patients With Previously Treated Non-Small Cell Lung Cancer	Recruiting	Phase II	30	Daniel MorgenszternM.D. Washington University School of Medicine	March 10, 2021	August 11, 2021
NCT04672369	A Study to Evaluate the Efficacy of IBI939 in Combination With Sintilimab in Patients With Advanced NSCLC	Not yet recruiting	Phase I	42	Ying Cheng Jilin Province Cancer Hospital	December 17, 2020	December 17, 2020
NCT04866017	Tislelizumab Plus BGB-A1217 Versus Tislelizumab Versus Durvalumab When Co-administered With Concurrent Chemoradiotherapy (cCRT) in Lung Cancer	Recruiting	Phase III	900	Yalan Yang, MD BeiGene	April 29, 2021	July 1, 2021
			anti-KIRs and ICI				
NCT03347123	A Study of Epacadostat and Nivolumab in Combination With Immune Therapies in Subjects With Advanced or Metastatic Malignancies (ECHO-208)	Completed	Phase I Phase II	11	Incyte Corporation	November 20, 2017	April 19, 2021
			anti-NKG2A and ICI				
NCT03822351	Durvalumab Alone or in Combination With Novel Agents in Subjects With NSCLC (COAST)	Active, not recruiting	Phase II	189	AstraZeneca	December 19, 2018	August 4, 2021

(Continued)

TABLE 2 | Continued

NCT number	Trial	Status	Phase	Total Estimated enrollment	Investigator	First Submitted Date	Last Update Posted Date
			Targeting immune suppression and ICI				
NCT03621982	Study of ADCT-301 in Patients With Selected Advanced Solid Tumors	Recruiting	Phase I	95	ADC Therapeutics	August 9, 2018	July 13, 2021
NCT04396535	Docetaxel With or Without Bintrafusp Alfa for the Treatment of Advanced Non-small Cell Lung Cancer	Recruiting	Phase II	80	Alex A Adjei Mayo Clinic in Rochester	May 20, 2020	May 4, 2021
NCT02903914	Arginase Inhibitor INCB001158 as a Single Agent and in Combination With Immune Checkpoint Therapy in Patients With Advanced/Metastatic Solid Tumors	Active, not recruiting	Phase I Phase II	260	Sven Gogov, MD Incyte Corporation	September 16, 2016	March 23, 2021
NCT03322540	Pembrolizumab Plus Epacadostat vs Pembrolizumab Plus Placebo in Metastatic Non-Small Cell Lung Cancer (KEYNOTE-654-05/ECHO-305-05)	Completed	Phase II	154	Lance Leopold, MD Incyte Corporation	October 26, 2017	January 6, 2021
NCT03343613	A Study of LY3381916 Alone or in Combination With LY3300054 in Participants With Solid Tumors	Terminated (Study terminated due to strategic business decision by Eli Lilly and Company.)	Phase I	60	Eli Lilly and Company	November 17, 2017	June 9, 2020
NCT02298153	A Study of Atezolizumab (MPDL3280A) in Combination With Epacadostat (INCB024360) in Subjects With Previously Treated Stage IIIB or Stage IV Non-Small Cell Lung Cancer and Previously Treated Stage IV Urothelial Carcinoma (ECHO-110)	Terminated (Study halted prematurely and will not resume; participants are no longer being examined or receiving intervention.)	Phase I	29	Hiroomi Tada, MD Incyte Corporation	November 21, 2014	December 11, 2017
NCT03562871	IO102 With Pembrolizumab, With or Without Chemotherapy, as First-line Treatment of Metastatic NSCLC	Active, not recruiting	Phase I Phase II	108	James Spicer, MD ProfGuy's Hospital	June 20, 2018	May 19, 2021
NCT03502330	APX005M With Nivolumab and Cabiralizumab in Advanced Melanoma, Non-small Cell Lung Cancer or Renal Cell Carcinoma	Recruiting	Phase I	120	Harriet Kluger, MD Yale University	April 18, 2018	December 22, 2020
NCT04306900	TTX-030 in Combination With Immunotherapy and/or Chemotherapy in Subjects With Advanced Cancers	Recruiting	Phase I	185	Trishula Therapeutics, Inc.	March 13, 2020	September 30, 2021
NCT03884556	TTX-030 Single Agent and in Combination With Immunotherapy or Chemotherapy for Patients With Advanced Cancers	Recruiting	Phase I	100	Trishula Therapeutics, Inc.	March 1, 2019	May 3, 2021
			Targeting Angiogenesis and ICI				
NCT04900363	A Trial of AK112 (PD-1/VEGF Bispecific Antibody) in Patients With NSCLC	Recruiting	Phase I/II	360	Caicun Zhou, MD	May 25, 2021	May 25, 2021
			Targeting cancer cell death and ICI				
NCT03775486	Study of Durvalumab+ Olaparib or Durvalumab After Treatment With Durvalumab and Chemotherapy in Patients With Lung Cancer (ORION)	Active, not recruiting	Phase II	401	Myung-Ju Ahn, MD	December 14, 2018	April 28, 2020
NCT03976323	Study of Pembrolizumab With Maintenance Olaparib or Maintenance Pemetrexed in First-line (1L) Metastatic Nonsquamous Non-Small-Cell Lung Cancer (NSCLC) (MK-7339-006, KEYLYNK-006)	Active, not recruiting	Phase III	792	Merck Sharp & Dohme Corp.	June 6, 2019	May 18, 2021
NCT03976362	A Study of Pembrolizumab (MK-3475) With or Without Maintenance Olaparib in First-line Metastatic Squamous Non-small Cell Lung Cancer (NSCLC, MK-7339-008/KEYLYNK-008)	Recruiting	Phase III	735	Merck Sharp & Dohme Corp.	June 6, 2019	October 1, 2021
NCT03307785	Study of Niraparib, TSR-022, Bevacizumab, and Platinum-Based Doublet Chemotherapy in Combination With TSR-042	Active, not recruiting	Phase I	58	Tesaro, Inc.	October 12, 2017	May 10, 2021

(TSR-042) has shown activity in NSCLC patients progressing on previous anti-PD-1 therapy (142). Additionally, the anti-TIM-3 agent LY3321367 was employed alone (23 patients) or in combination with the anti-PD-1 antibody LY3300054 (18 patients) in a phase Ia/Ib trial (NCT03099109) (**Table 2**). Both combination and single-agent were well tolerated, and single-agent treatment with LY3321367 achieved > 20% tumor regression in two patients, one of which, affected by small cell lung cancer, was later confirmed as a partial response (144).

B7-H3, also known as CD276, is a transmembrane protein frequently expressed by cancer cells, and is considered an immune-checkpoint molecule exploited by cancer cells to escape immune system recognition. B7-H3 expression was hypothesized to be potentially involved in resistance to anti-PD-1/PD-L1 blockade in NSCLC (145). So far, 3 clinical trials assessed the possible use of an antibody to target B7-H3 in association with anti-PD-1 or anti-CTLA-4 in advanced, previously treated solid tumors (NCT03729596; NCT02475213; NCT02381314), while other studies are exploring the possibility to target B7-H3 by using Chimeric Antigen Receptor T Cells (CAR-T) (NCT03198052; NCT04842812). All these studies are currently ongoing.

T-cell immunoreceptor with Ig and ITIM domains (TIGIT) is expressed by activated CD8+ and CD4+ T cells, NK, Treg, and potently inhibits innate and adaptive immunity (146). While the mechanism of action of TIGIT has to be elucidated yet, the molecule is known to bind CD155, thus preventing its binding to the immune activator receptor CD226, down-regulating NK and T cells function. Furthermore, TIGIT is known to induce M2 macrophage differentiation (147). To date, the most promising anti-TIGIT agent in NSCLC is represented by tiragolumab. Recently, this agent has been evaluated in combination with atezolizumab in the CITYSCAPE trial. In this randomized, double-blind, phase II study, 135 previously untreated patients with advanced NSCLC positive for PD-L1 expression (≥1%) were randomized to receive tiragolumab plus atezolizumab or placebo plus atezolizumab as first-line treatment. In the intent-to-treat (ITT) population, objective response rate (ORR) was higher in the tiragolumab-atezolizumab arm compared to placebo-atezolizumab (37% vs. 21%). In sub-group analyses, the ORR advantage was confirmed in the subset of patients with PD-L1 expression ≥50% (ORR: 66% vs. 24%), while in the sub-group of patients with PD-L1 expression ranging from 1-49%, no advantage in terms of ORR was observed for the combination compared to placebo arm (16% vs. 18%). Similarly, a significant advantage in PFS was observed in the sub-group with PD-L1 ≥50% (median PFS not reached in the experimental arm compared to 4.11 months in the placebo arm; HR= 0.30), while no difference was observed in the sub-group with PD-L1 ranging from 1-49% (4.04 months vs. 3.58 months; HR= 0.89) (148).

With regards to other investigational agents, a currently ongoing phase II study aims to set safety and efficacy of zimberelimab (anti-PD-1) in combination with domvanalimab (anti-TIGIT) and etrumadenant (selective, A2A and A2B adenosine receptor, small-molecule antagonist) in previously treated 30 NSCLC patients (NCT04791839) (**Table 2**). This is an interesting approach to reduce inhibition of T and NK cells due to immune checkpoints and reduce adenosine mediated immunosuppression.

KIR expression in NSCLC was correlated to resistance to anti-PD-1 ICI (149). In a phase I-II clinical trial safety, tolerability, and efficacy of Epacadostat (IDO1 inhibitor), nivolumab (anti-PD-1), and lirilumab (anti-KIRD2) combination was evaluated on 11 patients with solid tumors (NCT03347123) (**Table 2**). Results are awaited with interest, though the number of patients included in the trial is small. Notably, increasing interest has raised towards the Natural-killer group 2 member A (NKG2A) receptor, which is typically expressed on NK cells and is characterized by inhibitory functions, although its mechanism of action is not yet fully disclosed (150). Recently, in the open-label, randomized, phase II COAST trial, 189 patients affected by inoperable, stage III NSCLC candidate for maintenance after chemo-radiation were randomized to receive either durvalumab (the current standard of care anti-PD-L1 agent) alone, durvalumab plus oleclumab (an anti-CD73), or durvalumab plus monalizumab (an anti-NKG2A). In the experimental arm including durvalumab plus monalizumab, ORR (the primary end-point) was superior than the standard arm including durvalumab alone (37.1% vs. 25.4%; Odds Ratio= 1.77). Similarly, durvalumab plus monalizumab achieved longer PFS compared to durvalumab alone at the interim analysis (15.1 vs. 6.3 months; HR= 0.65), thus suggesting a promising clinical role for the combination of PD-L1 and NKG2A inhibition (151).

5.2 Targeting Immune Suppression

Since the immune system is regulated by several immunosuppressive mechanisms, which represent interesting targets for novel agents designed to improve the activity of "classic" ICI. Such mechanisms and pathways are globally mediated by inflammatory regulators, metabolic regulators, as well as immunosuppressive cells within the TME, such as Treg and TAM (**Table 2** and **Figure 1**).

5.2.1 Manipulation of Inflammatory Regulators

Cyclooxygenase (COX)-2 is frequently expressed by NSCLC and is required for prostaglandins synthesis, which are known to induce FoxP3+ Treg cells (152). Targeting COX-2 to inhibit Treg cells expansion and mediated immunosuppression has been exploited in several clinical trials using inhibitors in association with chemotherapy. Unfortunately, results did not meet the expectations. More specifically, in the GEmcitabine-COxib in NSCLC (GECO) study, the addition of oral rofecoxib to cisplatin-gemcitabine was associated with significantly increased rate of adverse events, including diarrhea, weight loss, constipation, fatigue and pain, as well as severe cardiac ischemia, without evidence of survival advantage (153). In the CALGB 30801 trial, 312 patients affected by unresectable NSCLC expressing COX-2 at immunohistochemistry assay were randomized to receive platinum-based chemotherapy with either celecoxib or placebo; the study was closed early due to futility as the addition of celecoxib failed to improve PFS over chemotherapy plus placebo (154).

While prospective data involving the use of ICI and COX inhibitors are limited, in a recent paper Wang et al. reported that the concomitant usage of COX inhibitors during ICI therapy for patients with NSCLC improved patients' outcomes in terms of response (ORR at 6 months 73.7% vs 33.3%, p=0.036) and time to progression (HR 0.45; 95% CI 0.21 to 0.97; p=0.042), albeit these results were observed retrospectively in a cohort of 37 patients (155).

Targeting of TGF-β in association with ICI has been investigated using a bifunctional fusion protein (bintrafusp; M7824) consisting of the extracellular domain of TGF-β receptor II fused to an anti-PD-L1 in patients with NSCLC in a phase I trial. The expansion cohort of the trial included 80 NSCLC patients previously treated with platinum-based chemotherapy who were randomized at a one-to-one ratio to receive either bintrafusp alfa 500 mg or the recommended phase 2 dosage of 1200 mg every 2 weeks. The ORR was 17.5% and 25% in the 500 mg and 1200 mg dose, respectively; notably, ORR was higher in the sub-group of patients with PD-L1 expression ≥80% (ORR: 85.7%). The treatment was relatively well tolerated, with 69% of patients experiencing adverse events, including 23 out of 80 patients experiencing grade ≥3 adverse events (156). Other new studies are ongoing: in a phase II trial (NCT04396535) (**Table 2**) docetaxel is administered with or without bintrafusp alfa in treating patients after progressing on a combination of anti-PD-1/PD-L1 and chemotherapy; in a phase III trial (NCT03631706) the efficacy of bintrafusp alfa will be compared with pembrolizumab in patients with high PD-L1-tumor expression and no genetic alterations.

5.2.2 Manipulation of Metabolic Mediators

Notably, some metabolic mediators, such as adenosine, arginine, and tryptophan (and its catabolic products) are involved in several immune-regulatory pathways, usually with immunosuppressive activity; hence, the pathways involving these molecules represent a promising target for immune-modulating treatments.

CD39/CD73 expressed on Treg and MDSC cells are considered another potential therapeutic target (36), indeed, multiple clinical trials designed to explore the activity of antibodies targeting either CD39 or CD73 in association with ICI alone or with chemotherapy are currently active and recruiting (NCT04306900, NCT03884556). Recently, results of the aforementioned COAST trial have been reported at the European Society of Medical Oncology (ESMO) Congress 2021; one of the treatment arms included in the trial was durvalumab plus oleclumab (an anti-CD73). This investigational combination was superior to durvalumab alone both in terms of ORR (38.3% vs. 25.4%; Odds Ratio= 1.83) and PFS (median not reached vs. 6.3 months; HR= 0.44) (151).

Arginase depletes arginine from tumor milieu and is produced by MDSC and neutrophils. Arginine is a fundamental amino acid which is required for optimal T cell functions (35); therefore, inhibition of arginase in association with ICI is apparently a potentially useful therapeutic approach for cancer immunotherapy. INCB001158 is a new inhibitory molecule of arginase, currently under investigation in a phase I clinical trial both as a single agent and in combination with "classic" ICI in patients with advanced/metastatic solid tumors [(157) NCT02903914] (**Table 2**). Results involving NSCLC have not been published yet, but the first data from patients with colorectal cancer indicate a good tolerability of INCB001158 in association with pembrolizumab and an increase in CD8+ T cells accumulation within the tumor (158, 159).

IDO1 and tryptophan 2,3-dioxygenase 2 (TDO2) catalyze the kynurenine metabolic pathway which leads, through tryptophan depletion in TME, to the generation of immune-tolerant DC and Treg, while the catabolic products kynurenines exert toxic activity on cytotoxic T cells (160, 161). Combination of epacadostat and pembrolizumab have largely disattended previous expectations in melanoma, and subsequently a phase II clinical trial investigating its potential activity in combination with pembrolizumab alone for treatment-naïve PD-L1 high (≥50%) NSCLC patients has been discontinued due to lack of advantage compared to pembrolizumab alone (NCT03322540). However, combinations of anti-PD-1 with other IDO-1 inhibitors (BMS-986205, NLG-919, navoximod/GDC-0919), dual IDO/TDO inhibitors (RG70099 and IOM-D) as well as indoximod are in clinical development (NCT03343613, NCT03322540, NCT02298153, NCT03562871) (**Table 2**).

5.2.3 Manipulation of Immunoregulatory Cells

One possible approach for improving immune response to tumor relies in the modulation of immunoregulatory cells within the TME, with specific reference to immunosuppressive cells, which might be managed either directly (*e.g.* by depletion) or by reducing their proliferation (*e.g.* by use of inhibitors).

Since Treg are the immunosuppressive cells more frequently associated to resistance to ICI, the possibility of disrupting Treg function in association with ICI has been pursued. One possible approach is represented by the use of anti-CD25 antibody to deplete Treg in cancer. Currently, a single-arm phase Ib clinical trial exploiting the inhibition of Treg in association with pembrolizumab in different cancers, including NSCLC, is open for recruitment (NCT03621982). Patients enrolled in this study will receive ADCT-301/Camidanlumab tesirine, which is an anti-CD25 antibody–drug conjugate; the agent will be employed either alone or in combination with pembrolizumab. Preclinical studies demonstrated that this molecule would efficiently deplete Treg and cause immunogenic cell death and would concomitantly increase the number of activated tumor-infiltrating CD8+ T effector cells (162).

Recently, the results of a phase I trial involving the CD40 agonist APX005M (sotigalimab) and cabiralizumab, an inhibitor of colony stimulating factor-1 receptor (CSF1R), were published. Notably, CSF1R signaling is known to facilitate recruitment and activation of TAM and is associated with lower levels of cytotoxic lymphocytes, thus favoring an immunosuppressive environment (163); CD40 is a member of the TNF receptor super-family and is known to facilitate T cell activation and support a pro-inflammatory environment, including macrophage polarization towards M1 (164). In the trial, 26 patients with solid tumors, including 12 melanomas, 1 NSCLC, and 13 renal cell carcinomas, who had progressed on anti-PD-1/PD-L1 treatment, were

treated in dose-escalating cohorts of APX005M with fixed doses of cabiralizumab, with or without nivolumab. The combination was generally tolerated and the observed results globally encourage further research involving combinations designed to polarize TME towards a pro-inflammatory pattern (164–166).

Another promising therapeutic target is represented by chemokine receptor type 4 (CCR4) known to stimulate the enrollment of Treg, thus promoting an immunosuppressive TME; hence, inhibiting CCR4 might result in Treg depletion and reversion towards immunogenic microenvironment. Mogamulizumab (anti-CCR4 antibody) has been evaluated in combination with anti-PD-1/PD-L1 or anti-CTLA-4 in two phase I trials. In the first trial, 96 patients with solid tumors received nivolumab plus escalating doses of mogamulizumab; the combination was generally safe, with mostly mild and moderate adverse events and no unexpected toxicities, and moderately active in terms of response, with 4 out of 15 patients with hepatocellular carcinoma achieving partial response. Among the 15 patients with NSCLC, 3 partial responses and 3 disease stabilizations were observed as best response (167). In the other phase I trial, 40 patients with solid tumors were included in dose-escalation cohorts of mogamulizumab concurrently with dose escalation of durvalumab or tremelimumab, and further 24 patients were included in dose-expansion cohorts. Although the combination treatment was generally well tolerated, the observed antitumor activity of mogamulizumab with either durvalumab or tremelimumab was modest across the different solid tumors involved (168).

5.3 Targeting Angiogenesis

Anti-angiogenic agents have been a mainstay among cancer therapies, with several compounds approved for multiple malignancies, either as "pure" anti-angiogenic agents, such as antibodies (bevacizumab, ramucirumab), or as multi-targeted agents active on angiogenesis as well as different molecular pathways (nintedanib, sunitinib, and others). The cornerstone of anti-angiogenic agents is currently represented by activity on VEGF and its receptors. Following the large use of angiogenesis-disrupting agents, great interest has developed toward the use of combinations of ICI and anti-angiogenic drugs. One notable difficulty associated with this approach lies in the necessity of equilibrium when formation of blood vessels is manipulated. Indeed, neo-angiogenesis promoted by tumor cells is typically chaotic and composed by disorganized and tortuous blood vessels characterized by excessive permeability, which results in increased interstitial fluid pression and ultimately reduced perfusion and oxygenation. Disrupting this process might result in transient normalization of blood circulation, thus facilitating the recruitment of lymphocytes. On the other hand, when anti-angiogenesis effects proceed, leucocytes have more difficulties in terms of accessibility to tumor mass, potentially resulting in less TILs (169). Notably, it has also been observed that high expression of VEGF results in increased proportion of immature DC, which promote immune tolerance, and Treg; furthermore, it has been suggested that VEGF might have a role in polarizing macrophages to M2 phenotype (169, 170). Recent updates on pre-clinical rationale and clinical experience with anti-VEGF agents and ICI have been comprehensively summarized by Ren et al. (48). The combination of the anti-VEGFR2 (ramucirumab) plus pembrolizumab in NSCLC was evaluated in a phase Ia/Ib trial. In an expansion cohort of the study, 11 out of 26 NSCLC patients (42.3%) experienced grade ≥3 treatment-related adverse events, the most frequent being hypertension (4 patients; 15.4%), which was consistent with the expected toxicity from ramucirumab; furthermore, 2 patients (7.7%) experienced myocardial infarction. Notably, ORR was 42.3% in the whole cohort, and patients with PD-L1 ≥50% achieved an ORR= 56.3%, compared to 22.2% achieved by the other patients. Similarly, median PFS was not reached for high PD-L1 expressors, while it was 4.9 months for patients with PD-L1 = 1-49% (171).

The combination of bevacizumab plus chemo-immunotherapy with atezolizumab, carboplatin, and paclitaxel was assessed in the large, randomized, phase III trial Impower150. In this study, which enrolled 1202 patients, the combination including bevacizumab achieved superior outcomes compared to the arm containing only bevacizumab, carboplatin, and paclitaxel, both in terms of PFS (8.3 vs. 6.8 months; HR= 0.62; p<0.001) and OS (19.2 vs. 14.7 months; HR= 0.78; p=0.02) (172). Notably, the trial included a small, although non-negligible sub-group of patients with activating mutations of *EGFR*, which are known to be associated with poor response to ICI. In this sub-population (124 patients), the combination of chemo-immunotherapy plus bevacizumab was associated with increased OS (median not reached at the time of analysis) over chemotherapy and bevacizumab alone (18.7 months), thus suggesting a potential effect of anti-angiogenesis plus ICI and chemotherapy in a population typically not suitable for treatment with ICI alone (173).

Finally a new and interesting approach targeting VEGF investigated the possible therapeutic efficacy of AK112, a PD-1/VEGF bispecific antibody, in patients with advanced NSCLC. The study is currently recruiting and its results are awaited (NCT04900363).

5.4 Targeting Cancer Cell Death

The possibility to target and inhibit Poly (ADP-ribose) polymerases (PARPs), thus triggering cell death in association with ICI to activate T cells represents an additional promising therapeutic approach; however, published data in NSCLC are still limited so far. In the phase II, JASPER trial, 38 patients affected by advanced NSCLC were divided in two cohorts (cohort 1: PD-L1 ≥50%; cohort 2: PD-L1=1-49%) and received first-line treatment with pembrolizumab plus niraparib. The primary end-point, ORR, was 56.3% in cohort 1 (9/16 evaluable patients) and 20.0% in cohort 2 (4/20 evaluable patients); with regards to survival outcomes in cohort 1 and cohort 2, median PFS was 8.4 months and 4.2 months, respectively, while OS was not reached and 7.7 months, respectively. Notably, 35.3% and 23.8% of patients in cohort 1 and cohort 2 experienced serious treatment-related adverse events. The authors concluded that the combination of pembrolizumab and niraparib is active in advanced NSCLC with high PD-L1 expression (174).

While published data involving PARP-inhibitors and ICI are still limited, several clinical trials are currently ongoing and might

produce interesting results in the upcoming months. With regards to olaparib, the ongoing phase II ORION trial (NCT03775486), is evaluating the efficacy and safety of a maintenance with olaparib plus durvalumab combination compared to durvalumab alone in patients affected by stage IV NSCLC not progressing after a first-line of platinum-based chemotherapy plus durvalumab. Furthermore, two other phase III trials are evaluating the combination of pembrolizumab plus olaparib in NSCLC patients (NCT03976323, NCT03976362) (**Table 2**).

Finally, an ongoing phase I clinical trial aims to study the combination of niraparib (another PARP-inhibitor), TSR-022 (anti-TIM-3), bevacizumab, and platinum-based doublet chemotherapy with TSR-042 (anti-PD-1) in advanced or metastatic cancers, including NSCLC (NCT03307785) (**Table 2**). The mail goal of this study is to determine tolerability and safety of such combinations for subsequent phase II development.

6 DISCUSSION

To date, ICI are the standard of care, either as monotherapy or in combination, for advanced non-oncogene-addicted NSCLC patients. However, a portion of patients do not benefit from these treatments and it is increasingly clear that reverting T or NK cytotoxic cell dysfunctional state with anti-PD-1/PD-L1 and/or anti-CTLA-4 may not be enough and needs to be improved. Indeed, increasing evidences sustain the role of new additional inhibitory immune checkpoint molecules, such as TIM-3, LAG-3, and TIGIT, in order to overcome the resistance to ICI (141, 144, 148). More importantly, the presence of an immune suppressive TME, mainly composed by Treg, MDSC and M2-TAM, in which cytotoxic cells reinvigorated by ICI act, is still a limitation for their anti-tumor activity, thus being acknowledged as another mechanism of resistance to ICI (32, 33, 37, 42, 43). Nonetheless, the identification of TILs with antigen specificity in the TME indicates that tumor recognition may occur and may lead to tumor growth control in the presence of an appropriate immune context (22). Further studies using multiplex histopathological, immunofluorescence and single-cell transcriptomics analyses are required to better define additional soluble mediators, cell to cell, and spatial relationships within the TME, that might collaborate to confer resistance to ICI. Moreover, an open question is how to select which patient will respond to treatment. Consequently, defining reliable biomarkers capable of predicting efficacy is a fundamental requirement. Currently, a number of TME-based scores both directly on tumor tissue visualization or indirectly through deductive techniques (*e.g.* gene expression profiles and radiomic feature extraction), have been tested as predictor of ICI efficacy in the lung cancer. Among these, PD-L1 expression by immunohistochemistry is still the only valid bio-marker widely used for the selection of suitable patients for anti-PD-1 treatment. Unfortunately, a number of issues are unresolved, such as the high intra-tumoral heterogeneity of PD-L1 which could prevent proper evaluation in small tumor biopsies, and pathologist interpretation is still a relevant factor (175). Gene signatures are now under development and show, for example, how an inflammatory state or the enrichment of the IFN pathway are predictors of a benefit from anti-PD-1/PD-L1 treatments. These predictive models have shown an optimal ability to retrospectively discriminate a benefit in disease response or progression, but prospective multi-institutional studies on larger patient cohorts are needed to ensure their reliability in a clinical setting.

To date multiple trials are currently ongoing with the aim of evaluating the use of novel agents in combination with ICI to overcome resistance (141–144, 146, 148). While these agents vary in terms of specific mechanism of action and some are explicitly designed to target additional immune checkpoints, other compounds are more specifically designed to interfere with TME (151, 153–156, 167, 171, 174). These approaches pursue the stimulation of a more pro-inflammatory microenvironment, usually by manipulating the proportion of immune cells populating the TME. More specifically their aim is the reduction of Treg and immature DC, while simultaneously favoring macrophage polarization toward an M1 differentiation rather than M2. It is important to stress that much of our current knowledge on resistance mechanisms and its biomarkers is derived from melanoma studies, and further studies, specific to lung cancer, are required.

Most clinical data are still limited so far, however, some interim results and safety data from phase I trials are already available and appear to be quite encouraging, especially when multi-modality approaches involving combinations of "classic" anti-PD-1/PD-L1 or CTLA-4 agents and novel immune-modulating drugs are employed. Data from ongoing clinical trials identify new interesting and promising drugs, such as tiragolumab (anti-TIGIT antibody) that in association with atezolizumab demonstrated higher ORR compared to placebo-atezolizumab (148). Other promising agents include monalizumab (anti-NKG2A antibody) and oleclumab (anti-CD73 antibody), both demonstrating to be superior to durvalumab alone, in terms of ORR and PFS (151). Similarly, other innovative immunotherapies, such as CAR-T or CAR-NK with selected tumor antigen specificity seem promising, and might represent a novel and effective approach to solid tumors (NCT04489862, NCT04153799, NCT02839954) (136, 137).

In the near future, we can expect that at least some of the currently investigated novel agents targeting additional immune checkpoints or components of the TME will proceed toward late phases of clinical research and eventually be approved. One potential issue will be represented by proper patient selection for receiving one among the different regimens that are available (single-agent PD-1 or PD-L1 inhibitor, chemotherapy plus PD-1/PD-L1 inhibitor, dual checkpoint blockade with PD-1 and CTLA-4 inhibitor plus chemotherapy), or among the regimens that might become available in the following months or years (such as PD-1 inhibitor plus TIGIT inhibitor, PD-1 inhibitor plus PARP inhibitor, among others). This is a most likely scenario for the next future, and we can also hypothesize that one strong focus of the upcoming research will be dedicated to the identification of predictive

biomarkers of efficacy for the current and future regimens, eventually in addition or in replacement of PD-L1 expression. While the approach considering specific biomarkers and agents is intuitive *(e.g.* BRCA mutations and PARP inhibitors) and is easily accepted and adopted by pulmonary oncologists we have to consider that the addition of novel tissue-based biomarkers to the current panels of molecular alterations (which are expected to enlarge in their turn) might be severely limited by the amount of available adequate samples, especially since tissue will be consumed by routine molecular analyses. Furthermore, small biopsies might not be effectively representative of the complex interactions between the whole tumor and the immune system, and these interactions may change during time.

In conclusion, the exploitation of TME for the development of novel therapeutic strategies involving the components of TME, might represent the future of cancer immunotherapy. Moreover, the development of algorithms integrating clinical, histological, genetic, and radiomic features could help clinicians in patient management in defining specific personalized therapies comparable to what has been successfully done in oncogene-driven NSCLC.

AUTHOR CONTRIBUTIONS

CG, CD, and PP, introduction and clinical trials. PC and GuF, immunobiology. SS and SC, signatures. RG, GiF, and MC, tumor microenvironment. CG, SC, RG, and MC, discussion. All authors contributed to the article and approved the submitted version.

FUNDING

The authors wish to thank the Italian Ministry of Health (5x1000 funds, Ricerca Corrente, CO-2016-02361470), Compagnia di San Paolo (2017–0529), AIRC 5x1000 2018 Id:21073, and Bristol-Myers-Squibb, which provided grants in support to our research in cancer immunotherapy. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

REFERENCES

1. Sezer A, Kilickap S, Gümüş M, Bondarenko I, Özgüroğlu M, Gogishvili M, et al. Cemiplimab Monotherapy for First-Line Treatment of Advanced Non-Small-Cell Lung Cancer With PD-L1 of at Least 50%: A Multicentre, Open-Label, Global, Phase 3, Randomised, Controlled Trial. *Lancet* (2021) 397:592–604. doi: 10.1016/S0140-6736(21)00228-2
2. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Updated Analysis of KEYNOTE-024: Pembrolizumab Versus Platinum-Based Chemotherapy for Advanced Non-Small-Cell Lung Cancer With PD-L1 Tumor Proportion Score of 50% or Greater. *J Clin Oncol* (2019) 37:537–46. doi: 10.1200/JCO.18.00149
3. Herbst RS, Giaccone G, de Marinis F, Reinmuth N, Vergnenegre A, Barrios CH, et al. Atezolizumab for First-Line Treatment of PD-L1-Selected Patients With NSCLC. *N Engl J Med* (2020) 383:1328–39. doi: 10.1056/NEJMoa1917346
4. Mok TSK, Wu Y-L, Kudaba I, Kowalski DM, Cho BC, Turna HZ, et al. Pembrolizumab Versus Chemotherapy for Previously Untreated, PD-L1-Expressing, Locally Advanced or Metastatic Non-Small-Cell Lung Cancer (KEYNOTE-042): A Randomised, Open-Label, Controlled, Phase 3 Trial. *Lancet* (2019) 393:1819–30. doi: 10.1016/S0140-6736(18)32409-7
5. Gandhi L, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, Angelis FD, et al. Pembrolizumab Plus Chemotherapy in Metastatic Non–Small-Cell Lung Cancer. *N Engl J Med* (2018) 378:2078–92. doi: 10.1056/NEJMoa1801005
6. Paz-Ares L, Luft A, Vicente D, Tafreshi A, Gümüş M, Mazières J, et al. Pembrolizumab Plus Chemotherapy for Squamous Non-Small-Cell Lung Cancer. *N Engl J Med* (2018) 379:2040–51. doi: 10.1056/NEJMoa1810865
7. Paz-Ares L, Ciuleanu T-E, Cobo M, Schenker M, Zurawski B, Menezes J, et al. First-Line Nivolumab Plus Ipilimumab Combined With Two Cycles of Chemotherapy in Patients With Non-Small-Cell Lung Cancer (CheckMate 9LA): An International, Randomised, Open-Label, Phase 3 Trial. *Lancet Oncol* (2021) 22:198–211. doi: 10.1016/S1470-2045(20)30641-0
8. Murciano-Goroff YR, Warner AB, Wolchok JD. The Future of Cancer Immunotherapy: Microenvironment-Targeting Combinations. *Cell Res* (2020) 30:507–19. doi: 10.1038/s41422-020-0337-2
9. Tan Z, Xue H, Sun Y, Zhang C, Song Y, Qi Y. The Role of Tumor Inflammatory Microenvironment in Lung Cancer. *Front Pharmacol* (2021) 12:688625. doi: 10.3389/fphar.2021.688625
10. O'Donnell JS, Teng MWL, Smyth MJ. Cancer Immunoediting and Resistance to T Cell-Based Immunotherapy. *Nat Rev Clin Oncol* (2019) 16:151–67. doi: 10.1038/s41571-018-0142-8
11. Brambilla E, Le Teuff G, Marguet S, Lantuejoul S, Dunant A, Graziano S, et al. Prognostic Effect of Tumor Lymphocytic Infiltration in Resectable Non–Small-Cell Lung Cancer. *J Clin Oncol* (2016) 34:1223–30. doi: 10.1200/JCO.2015.63.0970
12. Fridman WH, Zitvogel L, Sautès–Fridman C, Kroemer G. The Immune Contexture in Cancer Prognosis and Treatment. *Nat Rev Clin Oncol* (2017) 14:717–34. doi: 10.1038/nrclinonc.2017.101
13. Al-Shibli KI, Donnem T, Al-Saad S, Persson M, Bremnes RM, Busund L-T. Prognostic Effect of Epithelial and Stromal Lymphocyte Infiltration in Non-Small Cell Lung Cancer. *Clin Cancer Res* (2008) 14:5220–7. doi: 10.1158/1078-0432.CCR-08-0133
14. Donnem T, Hald SM, Paulsen E-E, Richardsen E, Al-Saad S, Kilvaer TK, et al. Stromal CD8+ T-Cell Density—A Promising Supplement to TNM Staging in Non-Small Cell Lung Cancer. *Clin Cancer Res* (2015) 21:2635–43. doi: 10.1158/1078-0432.CCR-14-1905
15. Kilvaer TK, Paulsen E-E, Andersen S, Rakaee M, Bremnes RM, Busund L-TR, et al. Digitally Quantified CD8+ Cells: The Best Candidate Marker for an Immune Cell Score in Non-Small Cell Lung Cancer? *Carcinogenesis* (2020) 41:1671–81. doi: 10.1093/carcin/bgaa105
16. Galon J, Bruni D. Approaches to Treat Immune Hot, Altered and Cold Tumours With Combination Immunotherapies. *Nat Rev Drug Discov* (2019) 18:197–218. doi: 10.1038/s41573-018-0007-y
17. AbdulJabbar K, Raza SEA, Rosenthal R, Jamal-Hanjani M, Veeriah S, Akarca A, et al. Geospatial Immune Variability Illuminates Differential Evolution of Lung Adenocarcinoma. *Nat Med* (2020) 26:1054–62. doi: 10.1038/s41591-020-0900-x
18. Rizvi H, Sanchez-Vega F, La K, Chatila W, Jonsson P, Halpenny D, et al. Molecular Determinants of Response to Anti-Programmed Cell Death (PD)-1 and Anti-Programmed Death-Ligand 1 (PD-L1) Blockade in Patients With Non-Small-Cell Lung Cancer Profiled With Targeted Next-Generation Sequencing. *J Clin Oncol* (2018) 36:633–41. doi: 10.1200/JCO.2017.75.3384
19. Samstein RM, Lee C-H, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, et al. Tumor Mutational Load Predicts Survival After Immunotherapy Across Multiple Cancer Types. *Nat Genet* (2019) 51:202–6. doi: 10.1038/s41588-018-0312-8
20. Bravaccini S, Bronte G, Ulivi P. TMB in NSCLC: A Broken Dream? *Int J Mol Sci* (2021) 22:6536. doi: 10.3390/ijms22126536
21. Zhang L, Chen Y, Wang H, Xu Z, Wang Y, Li S, et al. Massive PD-L1 and CD8 Double Positive TILs Characterize an Immunosuppressive Microenvironment With High Mutational Burden in Lung Cancer. *J Immunother Cancer* (2021) 9:e002356. doi: 10.1136/jitc-2021-002356

22. Caushi JX, Zhang J, Ji Z, Vaghasia A, Zhang B, Hsiue EH-C, et al. Transcriptional Programs of Neoantigen-Specific TIL in Anti-PD-1-Treated Lung Cancers. *Nature* (2021) 598(7881):E1. doi: 10.1038/s41586-021-03752-4
23. Sanmamed MF, Nie X, Desai SS, Villaroel-Espindola F, Badri T, Zhao D, et al. A Burned-Out CD8+ T-Cell Subset Expands in the Tumor Microenvironment and Curbs Cancer Immunotherapy. *Cancer Discov* (2021) 11:1700–15. doi: 10.1158/2159-8290.CD-20-0962
24. Thommen DS, Schreiner J, Müller P, Herzig P, Roller A, Belousov A, et al. Progression of Lung Cancer Is Associated With Increased Dysfunction of T Cells Defined by Coexpression of Multiple Inhibitory Receptors. *Cancer Immunol Res* (2015) 3:1344–55. doi: 10.1158/2326-6066.CIR-15-0097
25. Carrega P, Morandi B, Costa R, Frumento G, Forte G, Altavilla G, et al. Natural Killer Cells Infiltrating Human Nonsmall-Cell Lung Cancer Are Enriched in CD56brightCD16– Cells and Display an Impaired Capability to Kill Tumor Cells. *Cancer* (2008) 112:863–75. doi: 10.1002/cncr.23239
26. Russick J, Joubert P-E, Gillard-Bocquet M, Torset C, Meylan M, Petitprez F, et al. Natural Killer Cells in the Human Lung Tumor Microenvironment Display Immune Inhibitory Functions. *J Immunother Cancer* (2020) 8: e001054. doi: 10.1136/jitc-2020-001054
27. Lin M, Luo H, Liang S, Chen J, Liu A, Niu L, et al. Pembrolizumab Plus Allogeneic NK Cells in Advanced Non-Small Cell Lung Cancer Patients. *J Clin Invest* (2020) 130:2560–9. doi: 10.1172/JCI132712
28. Pende D, Falco M, Vitale M, Cantoni C, Vitale C, Munari E, et al. Killer Ig-Like Receptors (KIRs): Their Role in NK Cell Modulation and Developments Leading to Their Clinical Exploitation. *Front Immunol* (2019) 10:1179. doi: 10.3389/fimmu.2019.01179
29. He Y, Liu S, Mattei J, Bunn PA, Zhou C, Chan D. The Combination of Anti-KIR Monoclonal Antibodies With Anti-PD-1/PD-L1 Monoclonal Antibodies Could be a Critical Breakthrough in Overcoming Tumor Immune Escape in NSCLC. *Drug Des Devel Ther* (2018) 12:981–6. doi: 10.2147/DDDT.S163304
30. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer Immunoediting: From Immunosurveillance to Tumor Escape. *Nat Immunol* (2002) 3:991–8. doi: 10.1038/ni1102-991
31. Schreiber RD, Old LJ, Smyth MJ. Cancer Immunoediting: Integrating Immunity's Roles in Cancer Suppression and Promotion. *Science* (2011) 331:1565–70. doi: 10.1126/science.1203486
32. Principe DR, Chiec L, Mohindra NA, Munshi HG. Regulatory T-Cells as an Emerging Barrier to Immune Checkpoint Inhibition in Lung Cancer. *Front Oncol* (2021) 0:684098. doi: 10.3389/fonc.2021.684098
33. Kumagai S, Togashi Y, Kamada T, Sugiyama E, Nishinakamura H, Takeuchi Y, et al. The PD-1 Expression Balance Between Effector and Regulatory T Cells Predicts the Clinical Efficacy of PD-1 Blockade Therapies. *Nat Immunol* (2020) 21:1346–58. doi: 10.1038/s41590-020-0769-3
34. Wu S-P, Liao R-Q, Tu H-Y, Wang W-J, Dong Z-Y, Huang S-M, et al. Stromal PD-L1-Positive Regulatory T Cells and PD-1-Positive CD8-Positive T Cells Define the Response of Different Subsets of Non-Small Cell Lung Cancer to PD-1/PD-L1 Blockade Immunotherapy. *J Thorac Oncol* (2018) 13:521–32. doi: 10.1016/j.jtho.2017.11.132
35. Veglia F, Sanseviero E, Gabrilovich DI. Myeloid-Derived Suppressor Cells in the Era of Increasing Myeloid Cell Diversity. *Nat Rev Immunol* (2021) 21:485–98. doi: 10.1038/s41577-020-00490-y
36. Allard B, Longhi MS, Robson SC, Stagg J. The Ectonucleotidases CD39 and CD73: Novel Checkpoint Inhibitor Targets. *Immunol Rev* (2017) 276:121–44. doi: 10.1111/imr.12528
37. Li J, Wang L, Chen X, Li L, Li Y, Ping Y, et al. CD39/CD73 Upregulation on Myeloid-Derived Suppressor Cells *via* TGF-β-mTOR-HIF-1 Signaling in Patients With Non-Small Cell Lung Cancer. *Oncoimmunology* (2017) 6: e1320011. doi: 10.1080/2162402X.2017.1320011
38. Lin S, Zhang X, Huang G, Cheng L, Lv J, Zheng D, et al. Myeloid-Derived Suppressor Cells Promote Lung Cancer Metastasis by CCL11 to Activate ERK and AKT Signaling and Induce Epithelial-Mesenchymal Transition in Tumor Cells. *Oncogene* (2021) 40:1476–89. doi: 10.1038/s41388-020-01605-4
39. Li R, Salehi-Rad R, Crosson W, Momcilovic M, Lim RJ, Ong SL, et al. Inhibition of Granulocytic Myeloid-Derived Suppressor Cells Overcomes Resistance to Immune Checkpoint Inhibition in LKB1-Deficient Non–Small Cell Lung Cancer. *Cancer Res* (2021) 81:3295–308. doi: 10.1158/0008-5472.CAN-20-3564
40. Orillion A, Hashimoto A, Damayanti N, Shen L, Adelaiye-Ogala R, Arisa S, et al. Entinostat Neutralizes Myeloid-Derived Suppressor Cells and Enhances the Antitumor Effect of PD-1 Inhibition in Murine Models of Lung and Renal Cell Carcinoma. *Clin Cancer Res* (2017) 23:5187–201. doi: 10.1158/1078-0432.CCR-17-0741
41. Lo Russo G, Moro M, Sommariva M, Cancila V, Boeri M, Centonze G, et al. Antibody-Fc/FcR Interaction on Macrophages as a Mechanism for Hyperprogressive Disease in Non-Small Cell Lung Cancer Subsequent to PD-1/PD-L1 Blockade. *Clin Cancer Res* (2019) 25:989–99. doi: 10.1158/1078-0432.CCR-18-1390
42. Li L, Lu G, Liu Y, Gong L, Zheng X, Zheng H, et al. Low Infiltration of CD8+ PD-L1+ T Cells and M2 Macrophages Predicts Improved Clinical Outcomes After Immune Checkpoint Inhibitor Therapy in Non-Small Cell Lung Carcinoma. *Front Oncol* (2021) 0:658690. doi: 10.3389/fonc.2021.658690
43. Liu Y, Zugazagoitia J, Ahmed FS, Henick BS, Gettinger SN, Herbst RS, et al. Immune Cell PD-L1 Colocalizes With Macrophages and Is Associated With Outcome in PD-1 Pathway Blockade Therapy. *Clin Cancer Res* (2020) 26:970–7. doi: 10.1158/1078-0432.CCR-19-1040
44. Kargl J, Busch SE, Yang GHY, Kim K-H, Hanke ML, Metz HE, et al. Neutrophils Dominate the Immune Cell Composition in Non-Small Cell Lung Cancer. *Nat Commun* (2017) 8:14381. doi: 10.1038/ncomms14381
45. Aloe C, Wang H, Vlahos R, Irving L, Steinfort D, Bozinovski S. Emerging and Multifaceted Role of Neutrophils in Lung Cancer. *Transl Lung Cancer Res* (2021) 10:2806–18. doi: 10.21037/tlcr-20-760
46. Akbay EA, Koyama S, Liu Y, Dries R, Bufe LE, Silkes M, et al. Interleukin-17a Promotes Lung Tumor Progression Through Neutrophil Attraction to Tumor Sites and Mediating Resistance to PD-1 Blockade. *J Thorac Oncol* (2017) 12:1268–79. doi: 10.1016/j.jtho.2017.04.017
47. Rajabi M, Mousa SA. The Role of Angiogenesis in Cancer Treatment. *Biomedicines* (2017) 5:E34. doi: 10.3390/biomedicines5020034
48. Ren S, Xiong X, You H, Shen J, Zhou P. The Combination of Immune Checkpoint Blockade and Angiogenesis Inhibitors in the Treatment of Advanced Non-Small Cell Lung Cancer. *Front Immunol* (2021) 12:689132. doi: 10.3389/fimmu.2021.689132
49. Tamura R, Tanaka T, Akasaki Y, Murayama Y, Yoshida K, Sasaki H. The Role of Vascular Endothelial Growth Factor in the Hypoxic and Immunosuppressive Tumor Microenvironment: Perspectives for Therapeutic Implications. *Med Oncol* (2019) 37:2. doi: 10.1007/s12032-019-1329-2
50. Socinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, et al. Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. *N Engl J Med* (2018) 378:2288–301. doi: 10.1056/NEJMoa1716948
51. Jacquelot N, Tellier J, Nutt SL, Belz GT. Tertiary Lymphoid Structures and B Lymphocytes in Cancer Prognosis and Response to Immunotherapies. *OncoImmunology* (2021) 10:1900508. doi: 10.1080/2162402X.2021.1900508
52. Germain C, Gnjatic S, Tamzalit F, Knockaert S, Remark R, Goc J, et al. Presence of B Cells in Tertiary Lymphoid Structures Is Associated With a Protective Immunity in Patients With Lung Cancer. *Am J Respir Crit Care Med* (2014) 189:832–44. doi: 10.1164/rccm.201309-1611OC
53. Dieu-Nosjean M-C, Antoine M, Danel C, Heudes D, Wislez M, Poulot V, et al. Long-Term Survival for Patients With Nn-Small-Cell Lung Cancer With Intratumoral Lymphoid Structures. *J Clin Oncol* (2008) 26:4410–7. doi: 10.1200/JCO.2007.15.0284
54. Sautès-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary Lymphoid Structures in the Era of Cancer Immunotherapy. *Nat Rev Cancer* (2019) 19:307–25. doi: 10.1038/s41568-019-0144-6
55. Vanhersecke L, Brunet M, Guégan J-P, Rey C, Bougouin A, Cousin S, et al. Mature Tertiary Lymphoid Structures Predict Immune Checkpoint Inhibitor Efficacy in Solid Tumors Independently of PD-L1 Expression. *Nat Cancer* (2021) 2:794–802. doi: 10.1038/s43018-021-00232-6
56. Tang J, Ramis-Cabrer D, Curull V, Wang X, Mateu-Jiménez M, Pijuan L, et al. B Cells and Tertiary Lymphoid Structures Influence Survival in Lung Cancer Patients With Resectable Tumors. *Cancers (Basel)* (2020) 12:E2644. doi: 10.3390/cancers12092644
57. D'Incecco A, Andreozzi M, Ludovini V, Rossi E, Capodanno A, Landi L, et al. PD-1 and PD-L1 Expression in Molecularly Selected Non-Small-Cell

Lung Cancer Patients. *Br J Cancer* (2015) 112:95–102. doi: 10.1038/bjc.2014.555
58. Busch SE, Hanke ML, Kargl J, Metz HE, MacPherson D, Houghton AM. Lung Cancer Subtypes Generate Unique Immune Responses. *J Immunol* (2016) 197:4493–503. doi: 10.4049/jimmunol.1600576
59. He D, Wang D, Lu P, Yang N, Xue Z, Zhu X, et al. Single-Cell RNA Sequencing Reveals Heterogeneous Tumor and Immune Cell Populations in Early-Stage Lung Adenocarcinomas Harboring EGFR Mutations. *Oncogene* (2021) 40:355–68. doi: 10.1038/s41388-020-01528-0
60. Schumacher TN, Schreiber RD. Neoantigens in Cancer Immunotherapy. *Science* (2015) 348:69–74. doi: 10.1126/science.aaa4971
61. Qiao M, Jiang T, Liu X, Mao S, Zhou F, Li X, et al. Immune Checkpoint Inhibitors in EGFR-Mutated NSCLC: Dusk or Dawn? *J Thorac Oncol* (2021) 16:1267–88. doi: 10.1016/j.jtho.2021.04.003
62. Lin A, Wei T, Meng H, Luo P, Zhang J. Role of the Dynamic Tumor Microenvironment in Controversies Regarding Immune Checkpoint Inhibitors for the Treatment of Non-Small Cell Lung Cancer (NSCLC) With EGFR Mutations. *Mol Cancer* (2019) 18:139. doi: 10.1186/s12943-019-1062-7
63. Skoulidis F, Goldberg ME, Greenawalt DM, Hellmann MD, Awad MM, Gainor JF, et al. STK11/LKB1 Mutations and PD-1 Inhibitor Resistance in KRAS-Mutant Lung Adenocarcinoma. *Cancer Discov* (2018) 8:822–35. doi: 10.1158/2159-8290.CD-18-0099
64. Koyama S, Akbay EA, Li YY, Aref AR, Skoulidis F, Herter-Sprie GS, et al. STK11/LKB1 Deficiency Promotes Neutrophil Recruitment and Proinflammatory Cytokine Production to Suppress T-Cell Activity in the Lung Tumor Microenvironment. *Cancer Res* (2016) 76:999–1008. doi: 10.1158/0008-5472.CAN-15-1439
65. Chowell D, Morris LGT, Grigg CM, Weber JK, Samstein RM, Makarov V, et al. Patient HLA Class I Genotype Influences Cancer Response to Checkpoint Blockade Immunotherapy. *Science* (2018) 359:582–7. doi: 10.1126/science.aao4572
66. Gettinger S, Choi J, Hastings K, Truini A, Datar I, Sowell R, et al. Impaired HLA Class I Antigen Processing and Presentation as a Mechanism of Acquired Resistance to Immune Checkpoint Inhibitors in Lung Cancer. *Cancer Discov* (2017) 7:1420–35. doi: 10.1158/2159-8290.CD-17-0593
67. Bagaev A, Kotlov N, Nomie K, Svekolkin V, Gafurov A, Isaeva O, et al. Conserved Pan-Cancer Microenvironment Subtypes Predict Response to Immunotherapy. *Cancer Cell* (2021) 39:845–65.e7. doi: 10.1016/j.ccell.2021.04.014
68. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, et al. Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome. *Science* (2006) 313:1960–4. doi: 10.1126/science.1129139
69. Galon J, Mlecnik B, Bindea G, Angell HK, Berger A, Lagorce C, et al. Towards the Introduction of the "Immunoscore" in the Classification of Malignant Tumours. *J Pathol* (2014) 232:199–209. doi: 10.1002/path.4287
70. Feng W, Li Y, Shen L, Zhang Q, Cai X-W, Zhu Z-F, et al. Clinical Impact of the Tumor Immune Microenvironment in Completely Resected Stage IIIA(N2) Non-Small Cell Lung Cancer Based on an Immunoscore Approach. *Ther Adv Med Oncol* (2021) 13:1758835920984975. doi: 10.1177/1758835920984975
71. Zeng Z, Yang F, Wang Y, Zhao H, Wei F, Zhang P, et al. Significantly Different Immunoscores in Lung Adenocarcinoma and Squamous Cell Carcinoma and a Proposal for a New Immune Staging System. *Oncoimmunology* (2020) 9:1828538. doi: 10.1080/2162402X.2020.1828538
72. Boscolo A, Fortarezza F, Lunardi F, Comacchio G, Urso L, Frega S, et al. Combined Immunoscore for Prognostic Stratification of Early Stage Non-Small-Cell Lung Cancer. *Front Oncol* (2020) 10:564915. doi: 10.3389/fonc.2020.564915
73. Zhao Z, Zhao D, Xia J, Wang Y, Wang B. Immunoscore Predicts Survival in Early-Stage Lung Adenocarcinoma Patients. *Front Oncol* (2020) 10:691. doi: 10.3389/fonc.2020.00691
74. Fumet J-D, Richard C, Ledys F, Klopfenstein Q, Joubert P, Routy B, et al. Correction: Prognostic and Predictive Role of CD8 and PD-L1 Determination in Lung Tumor Tissue of Patients Under Anti-PD-1 Therapy. *Br J Cancer* (2019) 121:283. doi: 10.1038/s41416-019-0512-8
75. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, et al. Robust Enumeration of Cell Subsets From Tissue Expression Profiles. *Nat Methods* (2015) 12:453–7. doi: 10.1038/nmeth.3337
76. Newman AM, Steen CB, Liu CL, Gentles AJ, Chaudhuri AA, Scherer F, et al. Determining Cell Type Abundance and Expression From Bulk Tissues With Digital Cytometry. *Nat Biotechnol* (2019) 37:773–82. doi: 10.1038/s41587-019-0114-2
77. Steen CB, Liu CL, Alizadeh AA, Newman AM. Profiling Cell Type Abundance and Expression in Bulk Tissues With CIBERSORTx. *Methods Mol Biol* (2020) 2117:135–57. doi: 10.1007/978-1-0716-0301-7_7
78. Racle J, de Jonge K, Baumgaertner P, Speiser DE, Gfeller D. Simultaneous Enumeration of Cancer and Immune Cell Types From Bulk Tumor Gene Expression Data. *Elife* (2017) 6:e26476. doi: 10.7554/eLife.26476
79. Yoshihara K, Shahmoradgoli M, Martínez E, Vegesna R, Kim H, Torres-Garcia W, et al. Inferring Tumour Purity and Stromal and Immune Cell Admixture From Expression Data. *Nat Commun* (2013) 4:2612. doi: 10.1038/ncomms3612
80. Danaher P, Warren S, Dennis L, D'Amico L, White A, Disis ML, et al. Gene Expression Markers of Tumor Infiltrating Leukocytes. *J Immunother Cancer* (2017) 5:18. doi: 10.1186/s40425-017-0215-8
81. Charoentong P, Finotello F, Angelova M, Mayer C, Efremova M, Rieder D, et al. Pan-Cancer Immunogenomic Analyses Reveal Genotype-Immunophenotype Relationships and Predictors of Response to Checkpoint Blockade. *Cell Rep* (2017) 18:248–62. doi: 10.1016/j.celrep.2016.12.019
82. Becht E, Giraldo NA, Lacroix L, Buttard B, Elarouci N, Petitprez F, et al. Estimating the Population Abundance of Tissue-Infiltrating Immune and Stromal Cell Populations Using Gene Expression. *Genome Biol* (2016) 17:218. doi: 10.1186/s13059-016-1070-5
83. Finotello F, Mayer C, Plattner C, Laschober G, Rieder D, Hackl H, et al. Molecular and Pharmacological Modulators of the Tumor Immune Contexture Revealed by Deconvolution of RNA-Seq Data. *Genome Med* (2019) 11:34. doi: 10.1186/s13073-019-0638-6
84. Xu L, Deng C, Pang B, Zhang X, Liu W, Liao G, et al. TIP: A Web Server for Resolving Tumor Immunophenotype Profiling. *Cancer Res* (2018) 78:6575–80. doi: 10.1158/0008-5472.CAN-18-0689
85. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, et al. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. *Cancer Res* (2017) 77:e108–10. doi: 10.1158/0008-5472.CAN-17-0307
86. Li B, Severson E, Pignon J-C, Zhao H, Li T, Novak J, et al. Comprehensive Analyses of Tumor Immunity: Implications for Cancer Immunotherapy. *Genome Biol* (2016) 17:174. doi: 10.1186/s13059-016-1028-7
87. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, et al. TIMER2.0 for Analysis of Tumor-Infiltrating Immune Cells. *Nucleic Acids Res* (2020) 48:W509–14. doi: 10.1093/nar/gkaa407
88. Aran D, Hu Z, Butte AJ. Xcell: Digitally Portraying the Tissue Cellular Heterogeneity Landscape. *Genome Biol* (2017) 18(1):220. doi: 10.1101/114165
89. Sturm G, Finotello F, Petitprez F, Zhang JD, Baumbach J, Fridman WH, et al. Comprehensive Evaluation of Transcriptome-Based Cell-Type Quantification Methods for Immuno-Oncology. *Bioinformatics* (2019) 35:i436–45. doi: 10.1093/bioinformatics/btz363
90. Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou Yang T-H, et al. The Immune Landscape of Cancer. *Immunity* (2018) 48:812–30.e14. doi: 10.1016/j.immuni.2018.03.023
91. Ayers M, Lunceford J, Nebozhyn M, Murphy E, Loboda A, Kaufman DR, et al. IFN-γ-Related mRNA Profile Predicts Clinical Response to PD-1 Blockade. *J Clin Invest* (2017) 127:2930–40. doi: 10.1172/JCI91190
92. Cesano A. Ncounter(®) PanCancer Immune Profiling Panel (NanoString Technologies, Inc., Seattle, Wa). *J Immunother Cancer* (2015) 3:42. doi: 10.1186/s40425-015-0088-7
93. Danaher P, Warren S, Lu R, Samayoa J, Sullivan A, Pekker I, et al. Pan-Cancer Adaptive Immune Resistance as Defined by the Tumor Inflammation Signature (TIS): Results From The Cancer Genome Atlas (TCGA). *J Immunother Cancer* (2018) 6:63. doi: 10.1186/s40425-018-0367-1
94. Higgs BW, Morehouse CA, Streicher K, Brohawn PZ, Pilataxi F, Gupta A, et al. Interferon Gamma Messenger RNA Signature in Tumor Biopsies Predicts Outcomes in Patients With Non-Small Cell Lung Carcinoma or Urothelial Cancer Treated With Durvalumab. *Clin Cancer Res* (2018) 24:3857–66. doi: 10.1158/1078-0432.CCR-17-3451
95. Thompson JC, Hwang W-T, Davis C, Deshpande C, Jeffries S, Rajpurohit Y, et al. Gene Signatures of Tumor Inflammation and Epithelial-to-

Mesenchymal Transition (EMT) Predict Responses to Immune Checkpoint Blockade in Lung Cancer With High Accuracy. *Lung Cancer* (2020) 139:1–8. doi: 10.1016/j.lungcan.2019.10.012

96. Hwang S, Kwon A-Y, Jeong J-Y, Kim S, Kang H, Park J, et al. Immune Gene Signatures for Predicting Durable Clinical Benefit of Anti-PD-1 Immunotherapy in Patients With Non-Small Cell Lung Cancer. *Sci Rep* (2020) 10:643. doi: 10.1038/s41598-019-57218-9
97. Zheng Y, Tian H, Zhou Z, Xiao C, Liu H, Liu Y, et al. A Novel Immune-Related Prognostic Model for Response to Immunotherapy and Survival in Patients With Lung Adenocarcinoma. *Front Cell Dev Biol* (2021) 9:651406. doi: 10.3389/fcell.2021.651406
98. Zhang C, Zhang Z, Sun N, Zhang Z, Zhang G, Wang F, et al. Identification of a Costimulatory Molecule-Based Signature for Predicting Prognosis Risk and Immunotherapy Response in Patients With Lung Adenocarcinoma. *Oncoimmunology* (2020) 9:1824641. doi: 10.1080/2162402X.2020.1824641
99. Budczies J, Kirchner M, Kluck K, Kazdal D, Glade J, Allgäuer M, et al. A Gene Expression Signature Associated With B Cells Predicts Benefit From Immune Checkpoint Blockade in Lung Adenocarcinoma. *Oncoimmunology* (2021) 10:1860586. doi: 10.1080/2162402X.2020.1860586
100. Yi M, Li A, Zhou L, Chu Q, Luo S, Wu K. Immune Signature-Based Risk Stratification and Prediction of Immune Checkpoint Inhibitor's Efficacy for Lung Adenocarcinoma. *Cancer Immunol Immunother* (2021) 70:1705–19. doi: 10.1007/s00262-020-02817-z
101. Han L, Shi H, Luo Y, Sun W, Li S, Zhang N, et al. Gene Signature Based on B Cell Predicts Clinical Outcome of Radiotherapy and Immunotherapy for Patients With Lung Adenocarcinoma. *Cancer Med* (2020) 9:9581–94. doi: 10.1002/cam4.3561
102. Aerts HJWL, Velazquez ER, Leijenaar RTH, Parmar C, Grossmann P, Carvalho S, et al. Decoding Tumour Phenotype by Noninvasive Imaging Using a Quantitative Radiomics Approach. *Nat Commun* (2014) 5:4006. doi: 10.1038/ncomms5006
103. Lambin P, Leijenaar RTH, Deist TM, Peerlings J, de Jong EEC, van Timmeren J, et al. Radiomics: The Bridge Between Medical Imaging and Personalized Medicine. *Nat Rev Clin Oncol* (2017) 14:749–62. doi: 10.1038/nrclinonc.2017.141
104. Gevaert O, Xu J, Hoang CD, Leung AN, Xu Y, Quon A, et al. Non-Small Cell Lung Cancer: Identifying Prognostic Imaging Biomarkers by Leveraging Public Gene Expression Microarray Data–Methods and Preliminary Results. *Radiology* (2012) 264:387–96. doi: 10.1148/radiol.12111607
105. Jia T-Y, Xiong J-F, Li X-Y, Yu W, Xu Z-Y, Cai X-W, et al. Identifying EGFR Mutations in Lung Adenocarcinoma by Noninvasive Imaging Using Radiomics Features and Random Forest Modeling. *Eur Radiol* (2019) 29:4742–50. doi: 10.1007/s00330-019-06024-y
106. Rossi G, Barabino E, Fedeli A, Ficarra G, Coco S, Russo A, et al. Radiomic Detection of EGFR Mutations in NSCLC. *Cancer Res* (2021) 81:724–31. doi: 10.1158/0008-5472.CAN-20-0999
107. Yoon HJ, Sohn I, Cho JH, Lee HY, Kim J-H, Choi Y-L, et al. Decoding Tumor Phenotypes for ALK, ROS1, and RET Fusions in Lung Adenocarcinoma Using a Radiomics Approach. *Med (Baltimore)* (2015) 94:e1753. doi: 10.1097/MD.0000000000001753
108. Yoon HJ, Kang J, Park H, Sohn I, Lee S-H, Lee HY. Deciphering the Tumor Microenvironment Through Radiomics in Non-Small Cell Lung Cancer: Correlation With Immune Profiles. *PloS One* (2020) 15:e0231227. doi: 10.1371/journal.pone.0231227
109. Trentini F, Mazzaschi G, Milanese G, Pavone C, Madeddu D, Gnetti L, et al. Validation of a Radiomic Approach to Decipher NSCLC Immune Microenvironment in Surgically Resected Patients. *Tumori* (2021), 3008916211000808. doi: 10.1177/03008916211000808
110. Wen Q, Yang Z, Dai H, Feng A, Li Q. Radiomics Study for Predicting the Expression of PD-L1 and Tumor Mutation Burden in Non-Small Cell Lung Cancer Based on CT Images and Clinicopathological Features. *Front Oncol* (2021) 11:620246. doi: 10.3389/fonc.2021.620246
111. Chen Q, Zhang L, Mo X, You J, Chen L, Fang J, et al. Current Status and Quality of Radiomic Studies for Predicting Immunotherapy Response and Outcome in Patients With Non-Small Cell Lung Cancer: A Systematic Review and Meta-Analysis. *Eur J Nucl Med Mol Imaging* (2021). doi: 10.1007/s00259-021-05509-7
112. Chetan MR, Gleeson FV. Radiomics in Predicting Treatment Response in non-Small-Cell Lung Cancer: Current Status, Challenges and Future Perspectives. *Eur Radiol* (2021) 31:1049–58. doi: 10.1007/s00330-020-07141-9
113. Yang B, Zhou L, Zhong J, Lv T, Li A, Ma L, et al. Combination of Computed Tomography Imaging-Based Radiomics and Clinicopathological Characteristics for Predicting the Clinical Benefits of Immune Checkpoint Inhibitors in Lung Cancer. *Respir Res* (2021) 22:189. doi: 10.1186/s12931-021-01780-2
114. Khorrami M, Prasanna P, Gupta A, Patil P, Velu PD, Thawani R, et al. Changes in CT Radiomic Features Associated With Lymphocyte Distribution Predict Overall Survival and Response to Immunotherapy in Non-Small Cell Lung Cancer. *Cancer Immunol Res* (2020) 8:108–19. doi: 10.1158/2326-6066.CIR-19-0476
115. He B, Dong D, She Y, Zhou C, Fang M, Zhu Y, et al. Predicting Response to Immunotherapy in Advanced Non-Small-Cell Lung Cancer Using Tumor Mutational Burden Radiomic Biomarker. *J Immunother Cancer* (2020) 8: e000550. doi: 10.1136/jitc-2020-000550
116. Chen DS, Mellman I. Oncology Meets Immunology: The Cancer-Immunity Cycle. *Immunity* (2013) 39:1–10. doi: 10.1016/j.immuni.2013.07.012
117. Raina D, Kosugi M, Ahmad R, Panchamoorthy G, Rajabi H, Alam M, et al. Dependence on the MUC1-C Oncoprotein in Non-Small Cell Lung Cancer Cells. *Mol Cancer Ther* (2011) 10:806–16. doi: 10.1158/1535-7163.MCT-10-1050
118. Ford CH, Stokes HJ, Newman CE. Carcinoembryonic Antigen and Prognosis After Radical Surgery for Lung Cancer: Immunocytochemical Localization and Serum Levels. *Br J Cancer* (1981) 44:145–53. doi: 10.1038/bjc.1981.164
119. Grah JJ, Katalinic D, Juretic A, Santek F, Samarzija M. Clinical Significance of Immunohistochemical Expression of Cancer/Testis Tumor-Associated Antigens (MAGE-A1, MAGE-A3/4, NY-ESO-1) in Patients With Non-Small Cell Lung Cancer. *Tumori* (2014) 100:60–8. doi: 10.1700/1430.15817
120. Li Y, Hermanson DL, Moriarity BS, Kaufman DS. Human iPSC-Derived Natural Killer Cells Engineered With Chimeric Antigen Receptors Enhance Anti-Tumor Activity. *Cell Stem Cell* (2018) 23:181–92.e5. doi: 10.1016/j.stem.2018.06.002
121. Kelderman S, Kvistborg P. Tumor Antigens in Human Cancer Control. *Biochim Biophys Acta* (2016) 1865:83–9. doi: 10.1016/j.bbcan.2015.10.004
122. Forde PM, Chaft JE, Pardoll DM. Neoadjuvant PD-1 Blockade in Resectable Lung Cancer. *N Engl J Med* (2018) 379:e14. doi: 10.1056/NEJMc1808251
123. Anagnostou V, Smith KN, Forde PM, Niknafs N, Bhattacharya R, White J, et al. Evolution of Neoantigen Landscape During Immune Checkpoint Blockade in Non-Small Cell Lung Cancer. *Cancer Discov* (2017) 7:264–76. doi: 10.1158/2159-8290.CD-16-0828
124. Brown SD, Warren RL, Gibb EA, Martin SD, Spinelli JJ, Nelson BH, et al. Neo-Antigens Predicted by Tumor Genome Meta-Analysis Correlate With Increased Patient Survival. *Genome Res* (2014) 24:743–50. doi: 10.1101/gr.165985.113
125. Rizvi NA, Peters S. Immunotherapy for Unresectable Stage III Non-Small-Cell Lung Cancer. *N Engl J Med* (2017) 377:1986–8. doi: 10.1056/NEJMe1711430
126. Danilova L, Anagnostou V, Caushi JX, Sidhom J-W, Guo H, Chan HY, et al. The Mutation-Associated Neoantigen Functional Expansion of Specific T Cells (MANAFEST) Assay: A Sensitive Platform for Monitoring Antitumor Immunity. *Cancer Immunol Res* (2018) 6:888–99. doi: 10.1158/2326-6066.CIR-18-0129
127. Smith KN, Llosa NJ, Cottrell TR, Siegel N, Fan H, Suri P, et al. Persistent Mutant Oncogene Specific T Cells in Two Patients Benefitting From Anti-PD-1. *J Immunother Cancer* (2019) 7:40. doi: 10.1186/s40425-018-0492-x
128. Oliveres H, Caglevic C, Passiglia F, Taverna S, Smits E, Rolfo C. Vaccine and Immune Cell Therapy in Non-Small Cell Lung Cancer. *J Thorac Dis* (2018) 10:S1602–14. doi: 10.21037/jtd.2018.05.134
129. Jiang T, Shi T, Zhang H, Hu J, Song Y, Wei J, et al. Tumor Neoantigens: From Basic Research to Clinical Applications. *J Hematol Oncol* (2019) 12:93. doi: 10.1186/s13045-019-0787-5
130. Blass E, Ott PA. Advances in the Development of Personalized Neoantigen-Based Therapeutic Cancer Vaccines. *Nat Rev Clin Oncol* (2021) 18:215–29. doi: 10.1038/s41571-020-00460-2

131. Sahin U, Türeci Ö. Personalized Vaccines for Cancer Immunotherapy. *Science* (2018) 359:1355–60. doi: 10.1126/science.aar7112
132. Ott PA, Hu-Lieskovan S, Chmielowski B, Govindan R, Naing A, Bhardwaj N, et al. A Phase Ib Trial of Personalized Neoantigen Therapy Plus Anti-PD-1 in Patients With Advanced Melanoma, Non-Small Cell Lung Cancer, or Bladder Cancer. *Cell* (2020) 183:347–62.e24. doi: 10.1016/j.cell.2020.08.053
133. Dhatchinamoorthy K, Colbert JD, Rock KL. Cancer Immune Evasion Through Loss of MHC Class I Antigen Presentation. *Front Immunol* (2021) 12:636568. doi: 10.3389/fimmu.2021.636568
134. McGranahan N, Rosenthal R, Hiley CT, Rowan AJ, Watkins TBK, Wilson GA, et al. Allele-Specific HLA Loss and Immune Escape in Lung Cancer Evolution. *Cell* (2017) 171:1259–71.e11. doi: 10.1016/j.cell.2017.10.001
135. Hong M, Clubb JD, Chen YY. Engineering CAR-T Cells for Next-Generation Cancer Therapy. *Cancer Cell* (2020) 38:473–88. doi: 10.1016/j.ccell.2020.07.005
136. Zeltsman M, Dozier J, McGee E, Ngai D, Adusumilli PS. CAR T-Cell Therapy for Lung Cancer and Malignant Pleural Mesothelioma. *Transl Res* (2017) 187:1–10. doi: 10.1016/j.trsl.2017.04.004
137. Xie G, Dong H, Liang Y, Ham JD, Rizwan R, Chen J. CAR-NK Cells: A Promising Cellular Immunotherapy for Cancer. *EBioMedicine* (2020) 59:102975. doi: 10.1016/j.ebiom.2020.102975
138. Buckle I, Guillerey C. Inhibitory Receptors and Immune Checkpoints Regulating Natural Killer Cell Responses to Cancer. *Cancers* (2021) 13:4263. doi: 10.3390/cancers13174263
139. Pesce S, Greppi M, Tabellini G, Rampinelli F, Parolini S, Olive D, et al. Identification of a Subset of Human Natural Killer Cells Expressing High Levels of Programmed Death 1: A Phenotypic and Functional Characterization. *J Allergy Clin Immunol* (2017) 139:335–46.e3. doi: 10.1016/j.jaci.2016.04.025
140. Carrega P, Ferlazzo G. Natural Killers Are Made Not Born: How to Exploit NK Cells in Lung Malignancies. *Front Immunol* (2017) 8:277. doi: 10.3389/fimmu.2017.00277
141. Lipson EJ, Tawbi HA-H, Schadendorf D, Ascierto PA, Matamala L, Gutiérrez EC, et al. Relatlimab (RELA) Plus Nivolumab (NIVO) Versus NIVO in First-Line Advanced Melanoma: Primary Phase III Results From RELATIVITY-047 (CA224-047). *JCO* (2021) 39:9503–3. doi: 10.1200/JCO.2021.39.15_suppl.9503
142. Acharya N, Sabatos-Peyton C, Anderson AC. Tim-3 Finds its Place in the Cancer Immunotherapy Landscape. *J Immunother Cancer* (2020) 8:e000911. doi: 10.1136/jitc-2020-000911
143. *Phase (Ph) II Study of MBG453 + Spartalizumab in Patients (Pts) With non-Small Cell Lung Cancer (NSCLC) and Melanoma Pretreated With Anti–PD-1/L1 T… | OncologyPRO*. Available at: https://oncologypro.esmo.org/meeting-resources/esmo-2019-congress/Phase-Ph-II-study-of-MBG453-spartalizumab-in-patients-pts-with-non-small-cell-lung-cancer-NSCLC-and-melanoma-pretreated-with-anti-PD-1-L1-therapy (Accessed October 19, 2021).
144. Harding JJ, Patnaik A, Moreno V, Stein M, Jankowska AM, Velez de Mendizabal N, et al. A Phase Ia/Ib Study of an Anti-TIM-3 Antibody (LY3321367) Monotherapy or in Combination With an Anti-PD-L1 Antibody (LY3300054): Interim Safety, Efficacy, and Pharmacokinetic Findings in Advanced Cancers. *JCO* (2019) 37:12–2. doi: 10.1200/JCO.2019.37.8_suppl.12
145. Yonesaka K, Haratani K, Takamura S, Sakai H, Kato R, Takegawa N, et al. B7-H3 Negatively Modulates CTL-Mediated Cancer Immunity. *Clin Cancer Res* (2018) 24:2653–64. doi: 10.1158/1078-0432.CCR-17-2852
146. Chauvin J-M, Zarour HM. TIGIT in Cancer Immunotherapy. *J Immunother Cancer* (2020) 8:e000957. doi: 10.1136/jitc-2020-000957
147. Chen X, Lu P-H, Liu L, Fang Z-M, Duan W, Liu Z-L, et al. TIGIT Negatively Regulates Inflammation by Altering Macrophage Phenotype. *Immunobiology* (2016) 221:48–55. doi: 10.1016/j.imbio.2015.08.003
148. Rodriguez-Abreu D, Johnson ML, Hussein MA, Cobo M, Patel AJ, Secen NM, et al. Primary Analysis of a Randomized, Double-Blind, Phase II Study of the Anti-TIGIT Antibody Tiragolumab (Tira) Plus Atezolizumab (Atezo) Versus Placebo Plus Atezo as First-Line (1L) Treatment in Patients With PD-L1-Selected NSCLC (CITYSCAPE). *JCO* (2020) 38:9503–3. doi: 10.1200/JCO.2020.38.15_suppl.9503
149. Trefny MP, Rothschild SI, Uhlenbrock F, Rieder D, Kasenda B, Stanczak MA, et al. A Variant of a Killer Cell Immunoglobulin-Like Receptor Is Associated With Resistance to PD-1 Blockade in Lung Cancer. *Clin Cancer Res* (2019) 25:3026–34. doi: 10.1158/1078-0432.CCR-18-3041
150. Pesce S, Greppi M, Grossi F, Del Zotto G, Moretta L, Sivori S, et al. PD/1-PD-Ls Checkpoint: Insight on the Potential Role of NK Cells. *Front Immunol* (2019) 10:1242. doi: 10.3389/fimmu.2019.01242
151. Martinez-Marti A, Majem M, Barlesi F, Carcereny Costa E, Chu Q, Monnet I, et al. LBA42 COAST: An Open-Label, Randomised, Phase II Platform Study of Durvalumab Alone or in Combination With Novel Agents in Patients With Locally Advanced, Unresectable, Stage III NSCLC. *Ann Oncol* (2021) 32:S1320. doi: 10.1016/j.annonc.2021.08.2121
152. Shimizu K, Nakata M, Hirami Y, Yukawa T, Maeda A, Tanemoto K. Tumor-Infiltrating Foxp3+ Regulatory T Cells Are Correlated With Cyclooxygenase-2 Expression and are Associated With Recurrence in Resected Non-Small Cell Lung Cancer. *J Thorac Oncol* (2010) 5:585–90. doi: 10.1097/JTO.0b013e3181d60fd7
153. Edelman MJ, Wang X, Hodgson L, Cheney RT, Baggstrom MQ, Thomas SP, et al. Phase III Randomized, Placebo-Controlled, Double-Blind Trial of Celecoxib in Addition to Standard Chemotherapy for Advanced Non-Small-Cell Lung Cancer With Cyclooxygenase-2 Overexpression: CALGB 30801 (Alliance). *J Clin Oncol* (2017) 35:2184–92. doi: 10.1200/JCO.2016.71.3743
154. Martinez-Marti A, Navarro A, Felip E. COX-2 Inhibitors in NSCLC: Never-Ending Story or Misplaced? In: *Translational Lung Cancer Research* (2018). Available at: https://tlcr.amegroups.com/article/view/21031 (Accessed August 5, 2021).
155. Wang S-J, Khullar K, Kim S, Yegya-Raman N, Malhotra J, Groisberg R, et al. Effect of Cyclo-Oxygenase Inhibitor Use During Checkpoint Blockade Immunotherapy in Patients With Metastatic Melanoma and Non-Small Cell Lung Cancer. *J Immunother Cancer* (2020) 8:e000889. doi: 10.1136/jitc-2020-000889
156. Paz-Ares L, Kim TM, Vicente D, Felip E, Lee DH, Lee KH, et al. Bintrafusp Alfa, a Bifunctional Fusion Protein Targeting TGF-β and PD-L1, in Second-Line Treatment of Patients With NSCLC: Results From an Expansion Cohort of a Phase 1 Trial. *J Thorac Oncol* (2020) 15:1210–22. doi: 10.1016/j.jtho.2020.03.003
157. Naing A, Bauer T, Papadopoulos KP, Rahma O, Tsai F, Garralda E, et al. 440o - Phase I Study of the Arginase Inhibitor INCB001158 (1158) Alone and in Combination With Pembrolizumab (PEM) in Patients (Pts) With Advanced/Metastatic (Adv/Met) Solid Tumours. *Ann Oncol* (2019) 30:v160. doi: 10.1093/annonc/mdz244.002
158. Calithera Biosciences, Inc. *New Data Presented at ESMO Congress 2019 From the Arginase Inhibitor INCB001158 Alone and in Combination With Pembrolizumab*. Available at: https://ir.calithera.com/news-releases/news-release-details/new-data-presented-esmo-congress-2019-arginase-inhibitor (Accessed August 9, 2021).
159. Javle MM, Bridgewater JA, Gbolahan OB, Jungels C, Cho MT, Papadopoulos KP, et al. A Phase I/II Study of Safety and Efficacy of the Arginase Inhibitor INCB001158 Plus Chemotherapy in Patients (Pts) With Advanced Biliary Tract Cancers. *JCO* (2021) 39:311–1. doi: 10.1200/JCO.2021.39.3_suppl.311
160. Cheong JE, Sun L. Targeting the IDO1/TDO2-KYN-AhR Pathway for Cancer Immunotherapy - Challenges and Opportunities. *Trends Pharmacol Sci* (2018) 39:307–25. doi: 10.1016/j.tips.2017.11.007
161. Frumento G, Rotondo R, Tonetti M, Damonte G, Benatti U, Ferrara GB. Tryptophan-Derived Catabolites are Responsible for Inhibition of T and Natural Killer Cell Proliferation Induced by Indoleamine 2,3-Dioxygenase. *J Exp Med* (2002) 196:459–68. doi: 10.1084/jem.20020121
162. Zammarchi F, Havenith K, Bertelli F, Vijayakrishnan B, Chivers S, van Berkel PH. CD25-Targeted Antibody-Drug Conjugate Depletes Regulatory T Cells and Eliminates Established Syngeneic Tumors *via* Antitumor Immunity. *J Immunother Cancer* (2020) 8:e000860. doi: 10.1136/jitc-2020-000860
163. Zhou K, Cheng T, Zhan J, Peng X, Zhang Y, Wen J, et al. Targeting Tumor-Associated Macrophages in the Tumor Microenvironment. *Oncol Lett* (2020) 20:234. doi: 10.3892/ol.2020.12097
164. Vonderheide RH. CD40 Agonist Antibodies in Cancer Immunotherapy. *Annu Rev Med* (2020) 71:47–58. doi: 10.1146/annurev-med-062518-045435
165. Wesolowski R, Sharma N, Reebel L, Rodal MB, Peck A, West BL, et al. Phase Ib Study of the Combination of Pexidartinib (PLX3397), a CSF-1R Inhibitor,

and Paclitaxel in Patients With Advanced Solid Tumors. *Ther Adv Med Oncol* (2019) 11:1758835919854238. doi: 10.1177/1758835919854238

166. Weiss SA, Djureinovic D, Jessel S, Krykbaeva I, Zhang L, Jilaveanu L, et al. A Phase I Study of APX005M and Cabiralizumab With or Without Nivolumab in Patients With Melanoma, Kidney Cancer, or Non–Small Cell Lung Cancer Resistant to Anti-PD-1/PD-L1. *Clin Cancer Res* (2021) 27(17):4757–67. doi: 10.1158/1078-0432.CCR-21-0903
167. Doi T, Muro K, Ishii H, Kato T, Tsushima T, Takenoyama M, et al. A Phase I Study of the Anti-CC Chemokine Receptor 4 Antibody, Mogamulizumab, in Combination With Nivolumab in Patients With Advanced or Metastatic Solid Tumors. *Clin Cancer Res* (2019) 25:6614–22. doi: 10.1158/1078-0432.CCR-19-1090
168. Zamarin D, Hamid O, Nayak-Kapoor A, Sahebjam S, Sznol M, Collaku A, et al. Mogamulizumab in Combination With Durvalumab or Tremelimumab in Patients With Advanced Solid Tumors: A Phase I Study. *Clin Cancer Res* (2020) 26:4531–41. doi: 10.1158/1078-0432.CCR-20-0328
169. Ramjiawan RR, Griffioen AW, Duda DG. Anti-Angiogenesis for Cancer Revisited: Is There a Role for Combinations With Immunotherapy? *Angiogenesis* (2017) 20:185–204. doi: 10.1007/s10456-017-9552-y
170. Wheeler KC, Jena MK, Pradhan BS, Nayak N, Das S, Hsu C-D, et al. VEGF may Contribute to Macrophage Recruitment and M2 Polarization in the Decidua. *PloS One* (2018) 13:e0191040. doi: 10.1371/journal.pone.0191040
171. Herbst RS, Arkenau HT, Bendell J, Arrowsmith E, Wermke M, Soriano A, et al. Phase 1 Expansion Cohort of Ramucirumab Plus Pembrolizumab in Advanced Treatment-Naive NSCLC. *J Thorac Oncol* (2021) 16:289–98. doi: 10.1016/j.jtho.2020.10.004
172. Socinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, et al. Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. *N Engl J Med* (2018) 378:2288–301. doi: 10.1056/NEJMoa1716948
173. Reck M, Mok TSK, Nishio M, Jotte RM, Cappuzzo F, Orlandi F, et al. Atezolizumab Plus Bevacizumab and Chemotherapy in Non-Small-Cell Lung Cancer (IMpower150): Key Subgroup Analyses of Patients With EGFR Mutations or Baseline Liver Metastases in a Randomised, Open-Label Phase 3 Trial. *Lancet Respir Med* (2019) 7:387–401. doi: 10.1016/S2213-2600(19)30084-0
174. Ramalingam SS, Thara E, Awad MM, Dowlati A, Haque B, Stinchcombe TE, et al. JASPER: Phase 2 Trial of First-Line Niraparib Plus Pembrolizumab in Patients With Advanced Non-Small Cell Lung Cancer. *Cancer* (2021). doi: 10.1002/cncr.33885
175. Topalian SL, Taube JM, Anders RA, Pardoll DM. Mechanism-Driven Biomarkers to Guide Immune Checkpoint Blockade in Cancer Therapy. *Nat Rev Cancer* (2016) 16:275–87. doi: 10.1038/nrc.2016.36

Conflict of Interest: CG declares honoraria from Astra Zeneca, Bristol-Myers-Squibb, Boehringer-Ingelheim, Merck-Sharp-Dohme, Roche, Takeda. CD declares honoraria from Astra Zeneca, Bristol-Myers-Squibb, Merck-Sharp-Dohme, Roche.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Immunology

REVIEW
published: 08 February 2022
doi: 10.3389/fimmu.2022.828875

The Effect of Hypoxia and Hypoxia-Associated Pathways in the Regulation of Antitumor Response: Friends or Foes?

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OPEN ACCESS

Edited by:
Daniel F. Alonso, National University of Quilmes, Argentina

Reviewed by:
Mariano Rolando Gabri, National University of Quilmes, Argentina
Jose Alejandro Chabalgoity, University of the Republic, Uruguay

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Specialty section:
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

Received: *04 December 2021*
Accepted: *19 January 2022*
Published: *08 February 2022*

Citation:
Abou Khouzam R, Zaarour RF, Brodaczewska K, Azakir B, Venkatesh GH, Thiery J, Terry S and Chouaib S (2022) The Effect of Hypoxia and Hypoxia-Associated Pathways in the Regulation of Antitumor Response: Friends or Foes? Front. Immunol. 13:828875. doi: 10.3389/fimmu.2022.828875

Hypoxia is an environmental stressor that is instigated by low oxygen availability. It fuels the progression of solid tumors by driving tumor plasticity, heterogeneity, stemness and genomic instability. Hypoxia metabolically reprograms the tumor microenvironment (TME), adding insult to injury to the acidic, nutrient deprived and poorly vascularized conditions that act to dampen immune cell function. Through its impact on key cancer hallmarks and by creating a physical barrier conducive to tumor survival, hypoxia modulates tumor cell escape from the mounted immune response. The tumor cell-immune cell crosstalk in the context of a hypoxic TME tips the balance towards a cold and immunosuppressed microenvironment that is resistant to immune checkpoint inhibitors (ICI). Nonetheless, evidence is emerging that could make hypoxia an asset for improving response to ICI. Tackling the tumor immune contexture has taken on an *in silico*, digitalized approach with an increasing number of studies applying bioinformatics to deconvolute the cellular and non-cellular elements of the TME. Such approaches have additionally been combined with signature-based proxies of hypoxia to further dissect the turbulent hypoxia-immune relationship. In this review we will be highlighting the mechanisms by which hypoxia impacts immune cell functions and how that could translate to predicting response to immunotherapy in an era of machine learning and computational biology.

Keywords: hypoxia, tumor microenvironment, immune microenvironment, tumor plasticity, genetic instability, immunogenicity, hypoxia signature

INTRODUCTION

Solid tumors manifest in a microenvironment that harbors an array of cellular and non-cellular factors, cycling through various environmental pressures, which contribute to shaping a tumor's immunological features (1). Hypoxia is an early event in tumor evolution that has been shown to both directly and indirectly impact this tumor immune microenvironment (TIME), with much of

the evidence leaning towards an immunosuppressive influence (2–4). In addition, this condition of low oxygen is implicated in enabling tumor aggressiveness by providing tumor cells with a metabolic advantage (5) and by modulating autophagy (6). Hypoxia also promotes stemness and epithelial-mesenchymal transition (EMT) (7), genomic instability (8), and angiogenesis (9), thus contributing to all cancer hallmarks.

The repercussions of hypoxia in the TME occur through both hypoxia inducible factor (HIF)-dependent and independent mechanisms. HIF proteins are heterodimers composed of a constitutive β-subunit and an inducible α-subunit (HIF-1α, HIF-2α or HIF-3α). Under normal oxygen tension, HIF-1α and HIF-2α subunits are hydroxylated by prolyl hydroxylase (PHD), resulting in their subsequent ubiquitylation by the Von Hippel-Lindau tumor-suppressor protein (VHL), a component of an E3 ubiquitin ligase complex, and degradation by the proteasome (10). In hypoxic cells, HIF proteins are stabilized and in turn regulate the transcription of downstream genes, thereby modulating the microenvironmental stimuli within a tumor. The result is an acidic, nutrient deprived and immune-hostile microenvironment that is resistant to immunotherapy (2, 11). Indeed, limiting hypoxia in the TME in preclinical models has shown considerable improvement in the response to immune checkpoint inhibitors (ICI) (12, 13).

While there is emerging evidence of HIFs enhancing the activation status of immune cells in the TME (14–16), hypoxia is also known to mitigate their infiltration rate and function (3, 4). The TME comprises a slew of immune cell types, including those derived from the innate arm of the immune system, namely natural killer cells (NK) cells, macrophages and dendritic cells (DCs); as well as those belonging to the adaptive arm, including CD8+ effector T cells and CD4+ helper T cells. NK cells provide major histocompatibility complex (MHC)-unrestricted cytotoxicity against tumor cells (17). They also contribute to the sensitization of tumor cells to effector T cell killing by secreting the interferon, IFN-γ which acts on tumor cells upregulating their MHC and immunoproteasome expression (18, 19). With respect to macrophages, there is mounting evidence on the marked heterogeneity among populations resulting in a spectrum of macrophage subtypes with varying transcriptional sates (20, 21). This is contrary to the original binary model, wherein it is generally accepted that tumor associated macrophages (TAMs) display the so-called M2-like phenotype, which exhibits pro-tumorigenic features; while M1 macrophages have tumoricidal function and are classically activated (20). Dendritic cells are another subset of innate immune cells, classified as antigen presenting cells, that given the presence of co-stimulatory molecules and the correct cytokine environment, work to prime and activate T cells against tumor cells (22, 23). With respect to adaptive immune cells, the CD8+ effector T cells, or cytolytic T cells (CTLs) take the reigns as they are involved in direct tumor cell death through the induction of apoptosis and through cytokine secretion (24). The CD4+ T cells, on the other hand, exist as various subsets, among which the T helper 1 (Th1) subset is the most studied and is known to contribute antitumor activity by both direct killing and cytokine release; while regulatory T cells (Treg) and T helper 2 (Th2) cells constitute immunosuppressive subsets (24).

Hypoxia's involvement in reprogramming the TME to one that is conducive to immune resistance has been evidenced multiple times, however exploring the intricacies of the hypoxia-immune cell relationship *in vivo* has been a challenge. The coupling of signatures reflecting the degree of a tumor's hypoxic state with computational algorithms that can delineate its respective immune composition could uncover unexplored pathways and mechanisms of immune resistance. This is crucial given that an in depth understanding of the interplay between hypoxia-driven tumor cell remodeling and the immune contexture could aid in the betterment of patient response to immunotherapy. In this review, a link will be woven between the survival strategies taken up by tumor cells under hypoxic conditions and their impact on the immune microenvironment. In addition, recent findings from *in silico* analysis and the application of such tools to address hypoxia and the TIME will be discussed.

HYPOXIA METABOLICALLY REPROGRAMS THE TUMOR MICROENVIRONMENT EXCLUDING AND PERTURBING IMMUNE CELL FUNCTION

The increased requirement for oxygen and nutrients within the hypoxic TME breeds a metabolic switch that works to nurture tumor survival, while posing as a functional barrier to the sustainability and activity of an anti-tumor immune response. When levels of molecular oxygen become too low to sustain mitochondrial adenosine triphosphate (ATP) production, a transition occurs from oxidative phosphorylation to glycolysis. This is supported by HIF-1α-induced upregulation of glucose transporters, which enhance the influx of glucose, that is in turn shuttled through the glycolytic pathway thanks to the HIF-1-mediated transactivation of key regulatory glycolytic enzymes, while inhibiting the tricarboxylic acid (TCA) cycle (5). As a byproduct of glycolysis, hypoxic cells concomitantly experience high levels of intracellular lactate and hydrogen ions. To overcome the eventual acidification of the cell, HIF-1α induces the expression of transporters and carbonic anhydrases to expel them (5, 25). The net effect is a glucose depleted and acidic TME with a pH as low as 5.8 to 6.5 (5, 26) and lactate concentrations reaching up to 30 mM, that is ten times higher than normal tissue (27). In such a TME, the anti-tumorigenic function of immune-activating cells is thwarted, while that of immunosuppressive cells is advocated.

Just like cancer cells, cytotoxic T cells also rely on glucose for aerobic glycolysis, which is itself necessary for their effector function (28); however, in the TME tumor cells outcompete T cells for glucose, thus inhibiting their antitumor activity (29). Glucose metabolism is further integral to the inflammatory phenotype in macrophages, the maturation and function of dendritic cells and NK cell activation (24, 30). Paradoxically, the immunosuppressive Tregs gain a metabolic advantage in a

glucose deprived TME since they are less dependent on glucose as an energy source (31). Similarly, while Treg is resistant to the high lactate levels in the extracellular milieu (31), both NK cells and CD8+ T cells are encumbered by it. *In vitro* studies have shown that NK cell cytotoxicity and cytokine production are suppressed by high lactate and low pH levels (32, 33), as is CTL survival and function (4, 33). Lactate was shown to perturb DC maturation (34) and to drive Treg polarization from naïve T cells (35). Other immunosuppressive cells are likewise affected by the high lactate concentrations. The infiltration level of the T cell- and NK cell- suppressor, myeloid-derived suppressor cells (MDSC) is increased by tumor lactate (32). Tumor-derived lactate also induces the polarization of TAMs into the M2-like immunosuppressive phenotype (36) and treatment of a macrophage cell line with lactate promoted gain of M2-like features and downregulated the expression of cytokines, TNF-α and IL-12, secreted by M1 TAM (37). In addition, the acid-labile interferon, IFN-γ is rendered dysfunctional in this hostile TME, which in turn halts the maturation of anti-tumor M1 macrophages and promotes the differentiation of T helper cells to tumor promoting Th2 cells (38).

Apart from its impact on glucose metabolism, hypoxia interferes with amino acid and lipid metabolism, which are also essential for fueling cancer cell's survival and modulating the immune contexture. The availability of the nonessential amino acid, glutamine, as well as other amino acids including tryptophan and arginine, which are vital for T cell function, can additionally be modulated by hypoxia. In particular, *in vitro* experiments showed that the deprivation of glucose or glutamine, which results in low α-ketoglutarate (α-KG), skewed CD4+ T cell differentiation in favor of immunosuppressive Treg cells (39). On the other hand, glutamine blockade *in vitro* and in tumor-bearing mice left CD8+ T cells metabolically intact and functional, while suppressing oxidative and glycolytic metabolism of cancer cells, leading to nutrient depletion, attenuated hypoxia, and acidosis in mice (40). This divergent response was attributed to the effector T cells using alternative sources as supplement for their long-lived, highly activated phenotype (24, 40). The opposite changes in cancer cells and effector T cells touch on a metabolic plasticity among the two that can be harnessed as an additional checkpoint for immunotherapy (24, 40). With respect to tryptophan, hypoxia has been shown to induce the expression of the rate limiting enzyme in its catabolism, indoleamine 2,3-dioxygenase 1 (IDO1). In macrophages, this resulted in suppressed T cell proliferation, coupled with enhanced expansion of immunosuppressive Tregs (41). Furthermore, IDO1 depletes tryptophan inducing an amino acid starvation response that promotes T cell anergy (42). While hypoxia was also shown to induce IDO-1 in DC (43), an opposite effect was reported in cancer cell lines of ovarian (44), cervical and glioblastoma (45) origins. Furthermore, the functional ortholog of IDO, TDO2 (tryptophan-2,3-dioxygenase), was found to be significantly downregulated in a HIF-1α dependent manner in glioblastoma cells exposed to hypoxia (46). TDO2 expressing cells in hypoxia were able to rescue T cell proliferation that is otherwise suppressed under normoxic conditions (46). The interplay between hypoxia and tryptophan metabolism is clearly riddled with controversial evidence, nonetheless, targeting IDO, as well as other players in the tryptophan catabolic pathway is being investigated in various clinical trials, alone or in combination with immune checkpoint inhibitors (47). In terms of arginine, it is metabolized rapidly by activated T cells and supplementing them with increased arginine levels was shown to enhance their anti-tumor activity *in vivo* (48). On the other hand, low arginine levels have been shown to suppress activating receptors of NK cells, like NKp30 and NKp46, to reduce the ability of NKs to produce IFN-γ and to impair their proliferation (24). Hypoxia has been shown to upregulate the expression of the two main enzymes in arginine metabolism, arginase 1 (ARG1) and the inducible nitric oxide synthase (iNOS), on MDSCs. This was in a HIF-1α-dependent manner and resulted in the differentiation of MDSCs into M2-like TAMs (49). MDSCs thereby compete with T cells for the utilization of this crucial amino acid, inhibiting T cell proliferation (50). Furthermore, *in vitro* coculture of macrophages with T cells in hypoxia promoted an increase in iNOS and ARG1 that resulted in T cell inhibition (51). Interestingly, another mechanism that leads to the induction of ARG1 on the surface of MDSCs may also be modulated by HIF1 and that is through the increased production of prostaglandin E (PGE). The inducible cyclooxygenase-2 (COX-2) in tumor cells leads to increased expression of PGE2, which has been shown to maintain the expression of ARG1 on the surface of MDSCs (52). COX-2 and the increase in PGE2 has also been shown to occur in a HIF-1α dependent manner, inhibiting the maturation of DC and enhancing the suppressive capacity of Tregs (53). Therefore, hypoxia could be compounding the depletion of arginine in the TME and the accompanying immunosuppression.

Along with its interference with nutrient uptake and their metabolism, hypoxia has been widely implicated in sending ATP metabolism into overdrive, which further feeds into an immunosuppressive outcome. In an inflammatory setting, extracellular ATP can be released by stressed and dying cells as well as activated monocytes and is involved in immune activation (54). The safety switch to halt the activated immune response and prevent damage of healthy tissue involves the phosphohydrolysis of extracellular ATP to adenosine; a process predominantly regulated by the two membrane-bound nucleotidases, CD39 and CD73 (54). Of interest, both ectonucleotidases are abundantly expressed in the TME and are additionally upregulated through a HIF-1α dependent mechanism in hypoxia (55). This is highly relevant in amplifying the immunosuppressive nature of the hypoxic TME, since adenosine possesses immune-dampening properties, repressing T cell effector function while stabilizing the suppressive function of Tregs (56). The immunosuppressive effects of extracellular adenosine have been well documented and are executed through the binding of this ligand to the Gs-protein-coupled receptors A2aR, expressed on the surface of monocytes, lymphocytes, NK cells and DC, as well as A2bR, which is most prominently expressed on DCs and macrophages (54, 56). Through these two purinergic receptors, adenosine

triggers cyclic AMP (cAMP) accumulation. Within immune effector cells an increase in this intracellular signaling molecule results in an accumulation of an array of immunosuppressive molecules, including IL-10, TGF-β, PD-1 and CTLA-4, as well as the downregulation of key effector factors, such as IL-2, IFN-γ and perforin, which ordinarily participate in a pro-immune response (57). Hypoxia can further modulate adenosine levels through HIF-1α-dependent inhibition of adenosine kinase activity required to generate adenosine monophosphate (AMP), which in turn maximizes extracellular adenosine accumulation and depletes ATP levels in the cell (58). Indeed, the Hypoxia-Adenosine-Adenosine receptor axis represents various pharmacological targets and preclinical data support the rationale of combining A2aR blockade with hypoxia targeting strategies to reinvigorate the NK and T cell mediated anti-tumor immune response (59). Present data thereby suggests supplementing this combinational approach to current immunotherapeutic options to potentiate their efficacy (59).

HYPOXIA-MEDIATED AUTOPHAGY PLAYS A DOUBLE ROLE IN THE IMMUNE RESPONSE

There is broad consensus that hypoxic stress in the tumor microenvironment activates autophagy mediated adaptation to low oxygen, however, autophagy outcome is still controversial and is observed as a double agent both promoting or suppressing tumor development commensurate to tumor type and staging which is strongly correlated to the therapeutic response. In fact, autophagy can mediate adaptive survival response to hypoxia (60–62) or a nonapoptotic programmed cell death called autophagy cell death (63, 64). Clinical data suggests a direct correlation between autophagy influx and tumor development. High Beclin-1 expression was linked with poor prognosis in advanced human nasopharyngeal carcinoma and colorectal adenocarcinoma samples (65, 66). Moreover, high autophagy turnover tumors were less sensitive to treatments in comparison with low autophagy turnover (67). As hypoxia-mediated autophagy induces more resistance to tumors in response to therapies than normoxic cells (68), a dual synergic treatment with autophagy inhibitors is suggested (69). During hypoxia, autophagy is induced through the activation of the HIF-1α which upregulated BCL2 Interacting Protein 3 (BNIP3) and BNIP3L, rendering Beclin-1 free to promote its interaction with VPS34 and the formation of autophagolysosomes (6). Alternatively, hypoxia induced autophagy can be mediated independently of HIF-1α through the Unfolded Protein Response (UPR) (70) or through the energy sensor AMP-activated protein kinase (AMPK) (64).

It has now been well documented that tumor lesions form, progress, and respond to therapy in the setting of a complicated interaction with the host immune system (71, 72). Data from genetically engineered mouse models demonstrated that autophagy influx influences the tumor cells as well as the immune cells in the TME (73, 74). Thus, autophagy machinery is suggested as a potential beneficial pharmacological and genetic target to mitigate anti-tumor immune responses (75–79) with some successful preclinical data. Notably, immune cells' activation, differentiation and proliferation can be modulated by autophagy, which mediates promotion or inhibition of tumor development. Under hypoxic condition, as in tumors, immune cells experience hypoxia and have to adjust their metabolic needs and may do so through autophagy machinery which plays a plethora of action of immune cells to regulate anti-tumor immune reaction. CD8+ cells differentiation to cytotoxic T lymphocytes (80), their infiltrating and stemness preservation (81), T cells differentiation to Th cells, iNKT cells survival, differentiation and proliferation (82), DCs and B cells development (82), Treg cells survival, stability and immune tolerance (83), monocytes differentiation into macrophages and polarization and the number of macrophages as well (84), MDSCs growth and the establishment of T cell memory (85–87) are enhanced by autophagy. However, autophagy negatively regulates neutrophils development and induces their degradation (88).

Similarly to its dual impact on tumors development, autophagy can be observed as immune-simulator or immune-suppressor in the context of immune-mediated tumor elimination (88). It even becomes more complex when tumors are submitted to hypoxic stress. Understanding the contribution of hypoxia-induced autophagy in immune response to tumors is instrumental for better shaping therapeutic strategies. Thus, many studies showed the implication of multiple signaling pathways in hypoxia inducing autophagy to downregulate immune responses. Hypoxia-induced autophagy can attenuate NK cells anti-tumor activity. Loss of HIF-1α in NK cells blocks tumor growth (89) and hypoxia upregulation of HIF-1α in NK cells is dependent on PI3K/mTOR signaling pathway activation in response to cytokine receptor gamma chain, reducing NK cells tumor suppressive function (90). Moreover, hypoxia can modify the transcriptome of NK cells, regulating their immunoactivity and influencing their migration which may profoundly influence their infiltrating capacity in tumor tissues (91). Once X-irradiated, NK cells became more resistant and maintain killing capacity under hypoxic conditions (92). Recently, data demonstrated a strong correlation between attenuated NK cell cytotoxicity and a decreasing level of phosphorylated STAT3 and ERK through protein tyrosine phosphatase SHP-1 (Src homology region 2 domain-containing phosphatase-1) activation (93). Both STAT3 and ERK phosphorylation is increased in pre-activated hypoxic NK cells which restores their proliferation under hypoxic conditions (94). In contrast, hypoxia-induced autophagy attenuates CTL-mediated tumor degradation by activating the Src Kinase, which phosphorylates STAT3 in a HIF-1α dependent manner (95, 96). Simultaneously, HIF-1α induces autophagy thought the Beclin-1- BNIP3- Bcl-2 axis, resulting in the degradation of the SQSTM1/p62 protein responsible for the degradation of p-STAT3 leading to its accumulation in cells (97, 98) and preventing CTL attacks. Concomitantly, STAT3 expression promotes HIF-1α expression and modulates hypoxia-induced EMT in esophageal squamous cell cancer (99). Moreover, hypoxia-induced

autophagy degrades NK-derived Granzyme B in tumor cells through the autophagy sensor Inositol 1,4,5-Trisphosphate Receptor Type 1 (ITPR1), thus impairing NK-mediated tumor cell degradation (100). Furthermore, hypoxia-induced autophagy has been associated with destabilization of the immune synapse between the NK and the tumor cells through decreasing the level of connexin 43 leading to impairment of NK killing efficacy (101, 102).

HYPOXIA-DRIVEN TUMOR PLASTICITY AND HETEROGENEITY INCITE IMMUNE ESCAPE

Epithelial to Mesenchymal Transition (EMT) and Stemness in the TME

Optimum oxygen levels are essential to maintain tissue homeostasis. When oxygen sensing mechanisms or when oxygen levels decrease, a cascade of molecular events escalates a multitude of responses. Stabilization of HIF-1α ensues molecular changes that initiate EMT. EMT is characterized by an increase in cell migration, invasion, production of extracellular matrix (ECM) and resistance to apoptosis (103). Specific transcription factors are activated by HIF-1α to mediate EMT phenotypes, these include SNAIL SLUG, TWIST1, ZEB1, SIP1/ZEB2 (104). Additional pathways that have been shown to be involved in the hypoxia-mediated EMT include TGF-β, Wnt/β-catenin, hedgehog and Notch (104).

The process of EMT in cancer cells endows them with stem cell features. These newly formed cancer stem cells (CSCs) result in a heterogeneous cancer cell population. CSCs just like normal tissue stem cells can adapt a quiescent cellular state, characterized by a cell cycle arrest with reduced metabolic activities (105). In addition, CSCs have the capacity to self-renew and differentiate, as such they are credited for tumor growth, invasive growth, and metastasis at distal sites (106). The quiescent state of CSCs contributes to their resistance to therapeutic drugs (105). However, targeting CSCs requires an understanding of the several developmental signaling pathways that function to mediate and maintain their self-renewal and differentiation, these include TGF-β/BMP, Notch, Wnt, Hedgehog, FGF, and IGF (107). These signaling pathways are interconnected and overlapping and are present at crossroads that feedback into the hypoxia axis: TGF-β/SMAD3 pathway can be activated by HIF1 and at the same time it results in the stabilization of HIF1 by suppressing PHD levels (104). Notch interacts with HIF-1α to turn on expression of genes important in maintaining the undifferentiated cell state (108). In addition, Notch signaling regulates SNAI1 as well as hypoxia-induced cell motility and invasion (108). Wnt/β-catenin signaling has a pro-EMT effect in cells under hypoxia (109). Furthermore, HIF-1α knockdown abolishes hedgehog pathway activation (110). Finally, FGF induces HIF-1α expression (111).

Identifying cancer stem cells relies on the expression of unique markers on their cell surface. Depending on the cancer type these include EpCAM, ALDHA1, Lgr5, CD13, CD24, CD26, CD47, CD49f/Integrin alpha 6, CD66c, CD90, CD166, CD271, CD105, CD44, CD133, CD117/c-kit, CD138, CD151 and CD166 (112). CD44 and CD133 are the most widely used markers. CD44 is a transmembrane glycoprotein that is expressed in solid and hematological cancers, it mediates stromal interaction and has different activation states upon binding to its ligand hyaluronan (HA). In this context, CD44 has been shown to be active on cancer cells and not in normal cells (113). CD44 constitutes a potential marker to enhance targeted therapy, indeed in breast cancer CD44-doxorubicin conjugated aptamers inhibited selective cell proliferation of CD44 expressing cells (114). CD133 is a transmembrane glycoprotein expressed in several tumors. A variety of promising immunotherapeutic strategies have been developed to target CD133 expressing cells (115).

Impact of Plasticity and Heterogeneity on Tumor Immune Escape

Hypoxia-driven tumor plasticity and heterogeneity may have substantial impact on immunosuppression and cancer immune evasion (116). The pioneer study of Ye and colleagues previously revealed that hypoxia-induced EMT of hepatocellular carcinoma cells can promote an immunosuppressive TME by stimulating the release of the CCL20, leading to the production of IDO by monocyte-derived macrophages, which in turn suppressed T cell proliferation and promoted the expansion of immunosuppressive regulatory T cells (41). In fact, there are many known potential factors such as TGF-β contributing to hypoxia-driven tumor immune escape (3, 7) evoking features of cancer stem cells and tumor epithelial-mesenchymal plasticity. We previously showed that the stemness-associated transcription factor NANOG is induced by hypoxic stress; not only conferring stemness properties to carcinoma cells, but it also increases TGF-β expression and secretion, thereby promoting infiltration of immunosuppressive cells in the murine B16 melanoma model (117). We also showed that hypoxic stress can promote EMT programs enhancing immune evasion of NSCLC carcinoma cells (118). In the human IGR-Heu model, the tumor population was found to be highly heterogeneous following hypoxic stress, with an important fraction of cells conserving marked epithelial features. The mesenchymal cancer clones were found to have increased intrinsic TGF-β pathway activity and increased capacity to resist attacks by immune cytotoxic effector cells compared to the more epithelial clones, as reflected by reduced cancer cell susceptibility to CTL and NK cell-mediated lysis. To note, heterogeneity also exists within the mesenchymal clones. For instance, the expression of the receptor tyrosine kinase AXL could mark cancer clones with pronounced immune evasion capacity in association with reduced expression of ICAM1, ULBP1, and MHC class I levels in cells (119). It is interesting to consider that AXL expression can be upregulated by many intrinsic as well as extrinsic factors including hypoxia (120, 121). Published data have been unclear across different cancer systems and models. Research is still needed to decrypt the regulatory events controlling the expression of AXL and more generally of the TAM (Tyro3 Axl Mertk) family receptors. On the other hand, AXL activity has been shown to

support a hypoxic state in carcinoma cells by stabilizing tumoral HIF-1α through cooperation with HER2 (122). In a murine Her2+ breast cancer model, this cooperative event greatly contributed to shaping an immunosuppressed TME, while Axl targeting led to improving anti-Pd-1 treatment efficacy.

Work by Zhang and colleagues revealed that HIF-1α can stimulate CD47 expression in breast cancer cells gaining stemness features, which also serves as a mechanism to evade phagocytosis by innate immune cells such as macrophages (123). The CD47 SIRP interaction hampers the "eat me signal" on macrophages impairing phagocytosis. Another study found that the CD47 gene is a direct target of EMT-associated transcription factors SNAI1 and ZEB1 (124). Moreover, CD47 and PD-L1 can act synergistically to sustain resistance and immunosuppression (123). Interestingly, carcinoma cells with stemness features certainly exhibit immunogenicity profiles that differ from well-differentiated carcinoma cells with consequences on tumor immunogenicity, neo-antigen expression and the anti-tumor immune response (125). Complex interactions between the different contingents should also be highlighted. For instance, Faget et al. showed that neutrophils in lung tumors alter angiogenesis and immunotherapy efficacy by promoting tumor hypoxia and partial EMT of carcinomas as events of a vicious cycle maintaining an immunosuppressed pro-tumoral microenvironment (126).

Thus, several studies have demonstrated the role of hypoxia mediated-EMT and plasticity on tumor immune escape, although with variability in terms of the mechanisms and cell types involved. It will be important to better integrate the intratumor heterogeneity parameter in future investigations. Another important challenge will be to translate this information into the clinic with safe effective strategies.

Hypoxia-Dependent Modulation of Cancer Cell Glycosylation as a Mediator of Immune Escape

Another aspect of cancer cells that is modified by hypoxia and plays a role in the modulation of the immune response is glycosylation. Addition of glycans is a posttranslational modification that regulates the activity of as many as 50% of human genes, making it one of the most important regulators of gene expression. In tumors, abnormal glycosylation gives rise to a glycol-profile that perpetuates key cancer hallmarks of proliferation, EMT, angiogenesis, invasion, and metastasis (127, 128). Moreover, immune response itself is highly controlled by glycosylation [as reviewed in (129)]. Altered glycan residues on cancer cell surface, usually related to increased or unusual sugar components, give rise to tumor-associated carbohydrate antigens (TACAs) which are weakly immunogenic, and thus may serve as an immune escape strategy (130–132). Glycosyltransferases and glycosidases that add and remove sugar residues, respectively, are highly modulated by hypoxia in a tumor-dependent fashion (127, 128). In addition, hypoxia contributes to increasing specific structures involved in tumor invasion and immune escape (127, 128). In particular, the highly hypoxic muscle-invasive bladder cancer (MIBC) overexpresses the cancer-associated carbohydrate antigen sialyl-Tn (STn), which has been reported to be at least in part due to a HIF-1α-dependent cell survival strategy that favors cell migration and invasion (133). Recently, through the application of glycoproteomics in bladder cancer, the same group demonstrated cell surface expression of an ordinarily intracellular protein, homer homolog 3 (HOMER3), carrying short-chain O-glycans that are characteristic of membrane proteins (134). They further reported that under glucose deprivation and hypoxic conditions, HOMER3 contributed to the tumor cell's invasive capacity. Of interest, cell-surface expression of this protein was associated with significantly worse survival in MIBC patients. Furthermore, while HOMER3 expression was not cancer-specific, STn and HOMER3 did not co-express in healthy tissue, suggesting that HOMER3-STn could be a tumor-specific biomarker that can be used to target the more aggressive cancer cell populations residing in the hypoxic TME (134). Similarly, some cancer-specific glycoconjugates, like N-glycolyl (NeuGc) GM3 gangliosides, are promising therapeutic targets, as their increased expression is characteristic in tumors and almost not present in healthy human tissues (135). Gangliosides are glycosphingolipids containing sialic acid residues and their expression can be induced by hypoxia (136). While the exact mechanism is unknown, hypoxia has been shown to induce the sialic acid transporter, sialin (137). In addition, despite humans lacking the functional enzyme responsible for N-glycolyl (NeuGc) GM3 synthesis, hypoxia upregulates the succinate dehydrogenase subunit B (SDHB) of the mitochondrial respiratory complex II, which is hypothesized to provide the deleted iron/sulfur catalytic domain of the enzyme, restoring its functionality (138). Gangliosides, and in particular GM3(NeuGc), have been showed to play a key role in suppressing the antitumor immune response and therefore serve as neoantigens that can be targeted by immunotherapy (135). Indeed, given that they can also induce antibody responses, several clinical trials are ongoing to use them as anti-cancer vaccine antigens, as has recently been reviewed (135, 139).

ROLE OF HYPOXIA IN ANGIOGENIC SWITCH AND PRO-ANGIOGENIC MILIEU MODULATION OF IMMUNE RESPONSE

Initially, a growing tumor remains avascularised and relies on the diffusion of oxygen and nutrients from surrounding tissues (140) or reprograms metabolically to survive hostile, O_2 and nutrient limited environment (as discussed above). However, upon progression a phenotypic change in cancer cells occurs, termed angiogenic switch when balance of secreted factors moves from anti- towards proangiogenic. This event causes dramatic change in the tumor milieu that primarily induces angiogenesis (141). Previously mentioned HIF-1α stabilization in response to hypoxia activates in cancer cells not only adaptation to low pO_2 but also production of one of the most potent proangiogenic factors, vascular endothelial growth factor (VEGF). Other proangiogenic factors secreted by cancer cells include fibroblast growth factor (FGF) family, interleukin-8 (IL-8), epidermal growth factor (EGF) and platelet-derived growth factor

(PDGF) (142). In response to such milieu [and, also hypoxia itself, reviewed elsewhere (143)], in order to supply the growing tumor, endothelial cells (ECs) from surrounding tissues are activated to form new vessels. However, due to dysregulation of proangiogenic response in cancer cells, the forming vessels are disturbed, as a consequence of pathological angiogenesis, contributing even more to cancer progression (144).

Pathological Endothelium and Consequences for Immune Homing

Cancer-associated, pathological vessels are characterized by leakiness and disturbed shape causing uneven vascularization of the tumor mass. Cellular and ECM composition of cancer-associated vessels are altered, which limits their barrier functions causing uncontrolled transport of nutrients, oxygen, and drugs. Consequently, due to abnormalities, newly formed vessels are not able to restore physiological level of oxygen within the tumor, and hypoxia sets in. Impaired perfusion and increased interstitial pressure of cancer vessels were shown to negatively affect leukocyte trafficking (145). Additionally, composition of immune homing receptors is altered in cancer-associated endothelial cells, which affects the infiltration of the tumor with leukocytes. In a steady state, ECs remain quiescent, regulate blood flow and barrier functions of the endothelium, however upon activation, for example in response to inflammation, expression of adhesion molecules changes, allowing leukocyte trafficking into the organ. On one hand, pro-inflammatory molecules, like TNF-α or IL-1α, which can be secreted by cancer cells, activate ECs. On the other, pro-angiogenic factors (VEGF, bFGF) were shown to reduce the amount of adhesion molecules on ECs (146). These dual effects are reflected in leukocyte trafficking through the tumor endothelium. It was shown that vessels present in the tumors lose P-selectin that limits the infiltration of leukocytes, making tumors inaccessible for the immune response (147). High VEGF levels were linked to lower levels of ICAM1 and T cell infiltration (145). This is affected by anti-cancer treatment, as ipilimumab plus bevacizumab could restore ICAM/VCAM expression on ECs, enhancing infiltration of cytotoxic lymphocytes (148). On the other hand, levels of E-selectin were increased in tumoral vessels in breast cancer and were also present in surrounding inflamed adipose tissue vasculature (149). Expression of this adhesion molecule allowed monocyte infiltration; however, these monocytes could be TAMs as their presence predicted poor survival. In pancreatic cancer, upregulation of adhesion proteins on the endothelium, including E-selectin, MAdCAM-1 and VCAM-1, allowed increased infiltration of Tregs (150). E-selectin was shown to favorably promote infiltration of non-protective Th2- polarized cells (151). Immune infiltrate can also be shaped due to the production of chemokines by tumor endothelial cells (TECs). ECs in the TME were characterized by downregulation of immuno-attractant molecules (CCL2, CCL18, IL-6) (152), additionally further limiting the leukocyte infiltration. At the same time, TECs were described to possess a specific secretory profile, including IL-4, -13, -6, -8, and TNF-α, which can modulate immune responses (153). Another way ECs can affect immune cells is by directly interacting with them, for example through PD-L1. It was shown that endothelial PD-L1 is increased in several cancers in comparison to healthy tissue, which coincided with lower infiltration of T cells and dominance of Tregs (154).

Therefore, alteration of adhesion molecule patterns and levels of secreted factors in TECs can mediate selective infiltration of immunosuppressive leukocytes promoting tumor growth or make the tumor impenetrable for protective immune cells (so called "cold", uninflamed tumors). This points to the role of ECs as contributors to shaping the immunosuppressive TME.

Immune-Modulating Role of VEGF and Other Pro-Angiogenic Factors

Apart from the modulatory role on ECs, proangiogenic factors were shown to affect immune cells' function. It was observed that VEGF can directly expand Tregs, recruit MDSC and inhibit DC maturation (155). VEGFR is selectively present on Tregs and not effector T cells, which explains homing of immunosuppressive cells into proangiogenic TME (156). It is also a known factor promoting Th2 responses that are usually not protective in cancer. Additionally, VEGF induces expression of immunosuppressive PD-1 on T lymphocytes (157). Another proangiogenic factor with strong immunomodulatory potential is FGF (158). It was shown to polarize macrophages into M2 subtype (159) and expand MDSCs (160). Interestingly, anti-FGF treatment caused broader T-cell receptor repertoire, probably due to increased cancer cells apoptosis (161) that shows an additional aspect that can be altered by proangiogenic and immunomodulatory molecules. Similarly, however less studied, activities were reported for PDGF. This growth factor is an important regulator of angiogenesis, especially during development (162), and tends to increase during EMT in cancer cells (163). It can inhibit maturation of DCs and induce IL-10 producing T cells with regulatory phenotype (164). A strongly angiogenic chemokine, IL-8 (165), affects several immune cells, mostly by promoting their adhesion to the endothelium and subsequent migration towards inflamed tissue. However, it was observed that IL-8 mediates recruitment into the tumor of MDSCs and N2, pro-tumoral neutrophils (166), pointing to the potential immunomodulating action of this chemokine.

To sum up, angiogenic switch and consequently pathological angiogenesis on several levels affect immune response. As factors shaping TME, they contribute to induction of immunosuppression and/or allow the tumor to remain immunoevasive, both by not alleviating hypoxia and maintaining a pro-angiogenic and immunomodulatory milieu.

THE HIDDEN POTENTIAL OF HYPOXIA-MODULATED GENETIC HETEROGENEITY IN EVOKING AN IMMUNE RESPONSE

In the tumor microenvironment, hypoxia is often associated with genomic instability through downregulation of DNA repair processes and replication signaling mechanisms. Although the

DNA damage sensing and signaling mechanisms are on high-alert for recognition of plausible DNA damage under acute/chronic hypoxia, the DNA repair pathways such as homologous recombination, Non-Homologous End Joining (NHEJ) and mismatch repair, are downregulated (8). The effect of hypoxia on DNA repair pathways and related genes has been reviewed elsewhere (167–169). In contrast, chronic hypoxia/anoxia for longer durations can induce replication stress due to downregulation of ribonucleotide reductase and depletion of deoxyribonucleotides (170). The downregulation of these processes contributes to genetic heterogeneity in tumors through induction of chromosomal instability, point mutations, and genome-doubling events (8). Hypoxia induced structural changes (large deletions, copy number aberrations, duplications, and truncations) are substantially higher than single nucleotide alterations, according to a recent study examining the pan-cancer data sets (171). Hypoxia is associated with increased mutational load and hypoxia associated early mutations occur in key driver genes like *BCL2*, *TP53*, *MYC*, *PTEN* and *VHL* (171). Although driver mutations contribute to clonal development of tumors, branching mutations are the major cause of intratumor genetic heterogeneity and play a key role in drug resistance (172). In a clinical setting, irrespective of the tumor type, branched evolution remains the norm and influence the tumor's evolutionary trajectory (173). The contribution of hypoxia to branched evolution of tumors can be extrapolated from a study done by Gerlinger and coworkers analyzing the effect of *VHL* driver mutations in clear cell renal cell carcinomas (ccRCC). *VHL* mutations are seen in 80% of the ccRCC and contribute to constant pseudohypoxia phenotype (174, 175). Using multi-region sequencing and phylogenetic analysis, the study revealed that the inactivation of the *VHL* gene through mutations/methylation were a founder event in the trunk of the phylogenetic trees (176) and showed a heterogeneity in genomic landscape among the subclones with wide-ranging clinical outcome. *In vitro* experimental studies have shown that hypoxia induces a panoply of single nucleotide variations and contributes to microevolution of tumors. However, in a clinical setting, it is noted that chromosomal instability is a major contributor of tumors heterogeneity and a major determinant of clinical outcome in cancers (177). Hypoxia exerts selection pressure to accelerate the adaptation of more competent chromosomally unstable tumor clones in several ways (178). Hypoxia triggers the selection of mutant clones (for example, *TP53*-mutated tumors) by allowing them to evade apoptotic mechanisms (179). A hypoxic microenvironment promotes cell competition and metastases by HIF-1α mediated epithelial-mesenchymal transition (178). Hypoxia drives the immune-escape of tumors by inducing the expression of immune checkpoint inhibitors and controlling the antigen presenting mechanisms (180). Genome-doubling events associated with hypoxia have been found *in vitro* in melanoma cells with an increase in levels of tetraploid cells, however, such events in clinical samples are not well understood (181).

Inactivation of DNA repair pathways can lead to significant increase in tumor mutational burden (182, 183). The contribution of DNA repair and replication processes to genomic instability under hypoxic conditions is clearly evident from the defective homologous recombination and defective mismatch repair related mutational signatures (171). Single base substitution signatures and insertion and deletion signature analysis reveals that high-hypoxia is associated with clonal mutations in tumors rather than subclonal mutations (171). Furthermore, hypoxia is associated with increase in APOBEC activity and cyclic hypoxia induced replication stress provides single stranded DNA substrates for APOBEC mediated mutagenesis in breast, lung, and colorectal cancers (184).

Increased neoantigen load renders the tumor immunogenic with increased infiltration of lymphocytes leading to better clinical response to ICI in non-small cell lung cancer, melanoma, colorectal adenocarcinoma (185). In a breast cancer model, recent research from our group found that tumor hypoxia increased tumor mutational load and potential neoantigens (186). Using publicly available datasets, Bhandari and coworkers revealed that high-hypoxia is associated with increased TMB at pan-cancer level (171). However, clinical evidence on hypoxia-induced TMB and neoantigen burden is lacking. On the contrary, tumor hypoxia leads to an 'immune-cold' environment. Hypoxic tumor microenvironment is associated with immune evasion through expression of immune checkpoints (programmed death ligand -1), downregulation of type-I interferon signaling, shedding of antigen presenting molecules (MHC class I), enrichment of immunosuppressive cytokines and aggregation of immune suppressive cells (MDSC and Tregs) in the TME (187). In this regard, recent attempts to target hypoxic cells selectively with hypoxia activated prodrugs have yielded encouraging results with a significant antitumor response to immune checkpoint blockade. Jayaprakash and coworkers, using transgenic adenocarcinoma of the mouse prostate (TRAMP-C2), demonstrated that hypoxia targeting through Evofosfamide restored the T cell infiltration within the tumor and enhanced the response to immune checkpoint blockade (12). A study by Lequeux and coworkers investigated the inhibition of HIF-1α activity on cytotoxic immune cell infiltration into B16-F10 melanoma, and found an increase in infiltration of NK and CD8+ effector T cells and a significantly increased response to anti-PD-1 blockade (13). A comprehensive understanding of hypoxia induced mutational burden, neoantigen load will be crucial for enhancing the immunotherapy response in ICI resistant tumors.

UNRAVELING THE HYPOXIA-IMMUNE CONTEXTURE *IN SILICO*: THE HITS AND THE MISSES

The methods utilized thus far to study the immune contexture and degree of hypoxia in tumors have mainly done so separately. Regarding tumor infiltrating immune populations, imaging techniques including immunohistochemistry (IHC) and fluorescence microscopy, as well as cytometry-based methods,

using cell/population-specific antibodies have been the standard approach (188). For hypoxia, in addition to IHC which is used to check hypoxia induced proteins (like CAIX and GLUT1), various imaging techniques, such as positron emission tomography (PET), oxygen-enhanced (OE) magnetic resonance imaging (MRI), as well as blood oxygen level dependent (BOLD) and tissue oxygen level dependent (TOLD) MRI, have been utilized (9, 106). The focus of this section, however, is the uprise of *in silico* approaches to simultaneously navigate both the immune and hypoxic aspects of the TME.

In the last decade, the application of hypoxia gene signatures to reflect the degree of tumor hypoxia has taken the literature by storm with published signatures covering almost every solid tumor type (9, 106). In addition to that, there has been an escalating number of papers focused not only on designating the hypoxic state of a tumor, but also interrogating the immune populations and immune activation status of that tumor depending on its hypoxic phenotype (189–197). The process generally entails first deriving a hypoxia signature in the cancer type of interest, which often takes the route of narrowing down a list of hypoxia-related differentially expressed genes (DEGs) between normal and tumor tissue, then determining which genes are correlated with patient prognosis, be it overall survival or disease-free survival. The top genes and the factor by which they influence survival are then put together in a formula to calculate the risk score. Each sample is then allocated to the high-risk or low-risk group depending on their expression of the signature genes and whether their score is greater or less than the median risk score of the entire cohort. The score in this case is not only reflective of the hypoxic state of the tumor but is also associated with worse patient prognosis. An alternate strategy has also been used to group patients into high and low hypoxia groups based on their hypoxia score (197). The hypoxia score is calculated according to their expression levels of the hypoxia signature genes alone, without incorporating a risk parameter. Here again, the distribution is based on the variation from the median expression of the signature genes, and higher score is associated with worse survival. In either case, the next step has been to apply different tools or immune signatures to compare the two groups to make conclusions on the immune microenvironment in the context of hypoxia.

Several computational tools exist that rely on a tumor's bulk transcriptomic data to enumerate its existing immune populations (188, 198). These tools employ both a selected statistical framework as well as a base signature matrix or gene set representing the immune cell types of interest to deduce the tumor's respective immune phenotype (188, 199). The statistical framework is a variation of one of two primary algorithms, enrichment, or deconvolution. Gene set enrichment gives a semiquantitative score describing the enrichment of a cell type of interest in a sample based on the ranking of cell-type specific marker genes compared to all other genes present (198). A variation of that algorithm is single-sample GSEA (ssGSEA), in which the enrichment score is computed to represent the coordinately upregulated or downregulated genes within a single sample (198, 200). On the other hand, deconvolution algorithms consider the transcriptome profile of a heterogenous sample as a linear mixture of gene expression levels of distinct cell types. The unknown cell fraction of interest can then be estimated by determining the weighted contribution of each gene to a signature matrix that includes the cell-type specific expression profiles (198). In this way, tools based on the deconvolution algorithm give quantitative estimates of relative cell fractions; however, given that in a heterogeneous sample cell types having higher amounts of total mRNA will have a stronger contribution to the mixture, such cell types may be overestimated (198).

An important determinant for the effective estimation of the immune cell populations is the quality and accuracy of the gene set or base signature matrix being incorporated by the computational tool (201, 202). For example, Estimation of STromal and Immune cells in MAlignant Tumours using Expression data (ESTIMATE) uses a gene set derived from the overlap between gene expression profiles (GEPs) of normal hematopoietic samples and genes associated with the quantity of immune cells infiltrating tumor tissue (203). This gene set constitutes the immune signature and is used to give a tumor sample an immune score. The tool also has a gene set representing the stromal signature and uses that to give the same sample a stromal score. The combination of the two scores indicates the ESTIMATE score, or tumor purity.

With respect to deconvolution-based tools, the first base signature matrices to be used were derived from microarray data conducted on FACS-derived subsets of cells originating from peripheral blood mononuclear cells (PBMCs) of healthy individuals, or *in vitro* stimulated and differentiated cells (204, 205). These result in suboptimal coverage of cellular phenotypes in complex tissues and prevent the discovery of possible new cellular states, as well as gene expression profiles that are cell-type specific (206). Furthermore, tools based on such base matrices, are only compatible with microarray derived gene expression profile of a tumor sample. Such tools, include Cell-type Identification By Estimating Relative Subsets Of RNA Transcripts (CIBERSORT), which can be applied with a leukocyte signature matrix representing 22 immune cells to deconvolute and resolve the relative fractions of these cells in complex tissue (205). To overcome the stated limitations, an upgraded computational framework of CIBERSORT, termed CIBERSORTx, has been formulated using cell type-specific reference profiles derived from single cell RNA sequencing, allowing cross-platform normalization and *in silico* cell purification (206). Starting form RNA expression profiles of intact whole tissue samples, the cell-type-specific GEPs and abundance of each cell type can then be accurately inferred (206). Indeed, this tool is heralded as a digital cytometer that negates the need for physical dissociation, living material or antibodies, yet manages to give a detailed portrait of tissue components from bulk RNA admixtures (206).

In addition to ESTIMATE, CIBERSORT and CIBERSORTx, other commonly used tools include Microenvironment Cell Populations (MCP)-counter, which computes an abundance estimate of eight different immune cell types and two stromal cells (fibroblasts and endothelial cells) (207); as well as the

webtool Tumor IMmune Estimation Resource (TIMER), which provides the proportions of six immune cells, CD4+ and CD8+ T cells, B cells, DCs, macrophages, and neutrophils in the tissue of 23 tumor types from The Cancer Genome Atlas (TCGA) (208). One more tool of interest is ImmuCellAI (Immune Cell Abundance Identifier) that uses signatures to give abundance estimates on 24 immune cells including 18 subtypes of T cells, as well as B cells, DCs, monocytes, NK cells, macrophages, and neutrophils. Both RNA-Seq and microarray expression data are compatible with this tool (209). A final tool worth mentioning here is TIDE, which stands for Tumor Immune Dysfunction and Exclusion. This tool goes beyond determining the immune status of a tumor and potential immune escape. It was developed to use gene expression profiles of cancer samples to predict response to ICI by reporting on both immune and stromal cellular elements, a tissue agnostic interferon gamma signature, as well as the enrichment scores of ICI biomarkers, microsatellite instability and PD-L1, among others (210).

Table 1 represents select studies that have used one or a combination of the computational approaches to annotate the tumor immune microenvironment and merged that with a hypoxia gene signature to distinguish the more hypoxic from the less hypoxic tumors. As evident from the table, every signature consists of its own set of genes, even if it was derived from the same tumor type, with minimal overlap with other signatures. The conclusions of most papers underscore the immunosuppressive power of hypoxia in bladder cancer (189, 213), breast cancer (214), colorectal cancer (CRC) (191, 216, 217), head and neck squamous cell carcinoma (218), hepatocellular carcinoma (HCC) (221–223), lung cancer (195, 225, 226), melanoma (196), oral squamous cell carcinoma (227), osteosarcoma (229), ovarian carcinoma (230) and pancreatic ductal adenocarcinoma (PDAC) (197, 231). In the case of bladder cancer, two other studies determined the presence of high infiltration of immune cells in the high-risk group and found positive correlations between immune score and the risk score (190, 212). The authors also showed that despite the presence of both tumor-promoting and tumor-antagonizing immune cells, the risk score was positively correlated with immune checkpoints. One study even went on to report a potentially enhanced response of the high-risk group to immunotherapy (190). With respect to CRC, the only contradictory study included a single cohort and only focused on GSEA and the ESTIMATE score, not considering immune populations (215). In terms of HCC, one study reported a higher immune score in the high-risk group (219) as well as a significant infiltration of immune cells in this group which also showed enhanced predicted response to ICI (220). In a study that integrated 11 independent HCC cohorts, not a single immune cell population could be significantly differentiated in a consistent manner between the low-risk and high-risk groups, highlighting the complexity of the factors determining immune cell infiltration (194). It is difficult to make any conclusion on the findings in renal cell carcinoma, as all three studies included a single cohort respectively and reported distinct findings (232–234) (**Table 1**).

It is clear that to resolve the discrepancies identified for even the same tumor type, a validated hypoxia gene signature should be utilized to score the tumor and the *in silico* analysis of the immune contexture should be done using signatures that reflect the immune state of the specific cancer type. The tools utilized thus far apply gene expression profiles obtained from immune cells of healthy PBMCs or tissue, meanwhile based on the complexity of mechanisms governing the activation state of immune cells, a cancer-specific approach would provide a more accurate representation of the immune contexture. This would ultimately enhance the quality of the findings being generated from these tools, making them more consistent and having higher accuracy. Furthermore, one downside remains with the inability of such tools to give information about the localization of the reported immune populations in the tumor mass. This spatial dimension is intercalated with the global functional state of the immune response and is now being investigated with advanced techniques, including single cell transcriptomics using slide sequencing (235), as well as multiplex immunofluorescence imaging using the CODEX® System (236). Therefore, despite the simplicity and ease of use of current computational tools, they should not be used as a standalone analysis platform to conclude on the immune activation state of a tumor.

DISCUSSION

To date, clinical benefit from cancer immunotherapy has been limited to a minority of patients. Achieving benefit in the majority of patients necessitates a wholistic understanding of anti-tumor response mechanisms and both the cell-intrinsic and extrinsic molecules involved in primary, adaptive, as well as acquired resistance to immunotherapy. In this regard, it has become clear that the TME is likely to play a crucial role in cancer response to treatment. In fact, the growth and progression of cancer cells depend not only on their malignant potential, but also on the multidirectional interactions of cellular and metabolic components of tumor microenvironment. It is widely admitted that novel and continuously evolving pathological entities arise as a result of the interactions among tumor cells and stromal cells during cancer progression.

As previously reported, many cellular, molecular, and metabolic elements of the TME are emerging as attractive targets for therapeutic approaches (47, 59, 115, 122). In this respect, the existence of hypoxia in solid tumors is associated, not only with tumor invasion and metastasis, but also with a heightened risk of treatment failure and patient mortality and is currently attracting significant interest. Accumulating evidence indicates that hypoxia plays a key role in promoting the acquisition of tumor resistance to various antitumor immune effectors (26). Tumor hypoxia allows tumor cells to escape CTL- and NK -mediated killing through in part the activation of autophagy (97, 237, 238) and modulates the composition and function of the immune infiltrate (97, 237, 238). Hypoxic zones in tumors have also been reported to attract immunosuppressive cells such as MDSCs, tumor-associated macrophages and regulatory T cells. In addition, the association of hypoxia with cancer stemness in the tumor microenvironment of different cancer types is widely

TABLE 1 | Studies applying computational tools to investigate the immune landscape of tumors classified based on hypoxia signatures.

s.n	Cancer	Hypoxia Signature	Cohort Number‡	Immune Investigation Method	High-Risk Group (Hypoxia-High)	Low-Risk Group (Hypoxia-Low)	Reference
1	**ACC***	3 genes (*CCNA2*, *COL5A1*, *EFNA3*)	1	CIBERSORT	Resting NK cell	Activated NK cell	(211)
2	**BLCA**	8 genes (*AKAP12*, *ALDOB*, *CASP6*, *DTNA*, *HS3ST1*, *JUN*, *KDELR3*, *STC1*)	3	CIBERSORT	M0 and M1 macrophages		(189)
3	**BLCA#**	4 genes (*ANXA2*, *COL5A1*, *GALK1*, *HS3ST1*)	1	ESTIMATE; ssGSEA	Immune and stromal scores positively correlated with risk score Activated CD4 T cell, activated CD8 T cell, central memory CD8 T cell, effector memory CD8 T cell, gamma delta T cell, follicular helper T cell, Th1, Th2, aDC, pDC, activated B cell, immature B cell, memory B cell, NK cell, NK T cell, Treg, macrophage, MDSC, mast cell, monocyte, neutrophil, eosinophil	Th17, CD56bright NK cell	(212)
4	**BLCA#**	16 genes (*AKAP12*, *ANKZF1*, *CASP6*, *CCNG2*, *GALK1*, *GAPDH*, *HDLBP*, *HEXA*, *HS3ST1*, *SDC4*, *SLC2A1*, *SLC2A3*, *SRPX*, *STC1*, *VEGFA*, *WISP2*)	2	ssGSEA	CD8 T cell, NK cell, DC, Th1 Risk score positively related to T cell inflamed score and enrichment scores of immunotherapy-positive gene signatures		(190)
5	**BLCA#**	7 genes (*ALDOB*, *EGFR*, *FOXO3*, *GPC1*, *SDC4*, *SLC2A3*, *VEGFA*)	1	CIBERSORT	Resting mast cell, neutrophil, resting CD4 memory T cell	Follicular helper T cell, CD8 T cell, plasma cell	(213)
6	**BC**	13 genes (*ADM*, *ALDOA*, *CDKN3*, *LDHA*, *MIF*, *MRPS17*, *NDRG1*, *P4HA1*, *PGAM1*, *SLC2A1*, *TPI1*, *TUBB6*, *VEGFA*)	1	ImmuCellAI	nTreg cell, iTreg cell	CD8 T cell, CD4 T cell	(214)
7	**CRC**	5 genes (*ARL4C*, *CARS2*, *PSMD12*, *PTTG1IP*, *SEC61G*)	1	GSEA; ESTIMATE	Enriched immune pathways Positively correlated with immune score and stromal score		(215)
8	**CRC**	12 genes (*CASP6*, *CYB5R3*, *DTX3L*, *FAM117B*, *IRF1*, *MBTD1*, *MINPP1*, *ORAI3*, *TNFAIP8*, *TRAF3*, *PRELID2*, *ZBTB44*)	2	ESTIMATE; CIBERSORT	Treg, M2 macrophage	Higher immune and stromal scores; CD4 T cell, M1 macrophage	(216)
9	**CRC**	4 genes (*ALDOB*, *ALDOC*, *GPC1*, *SLC2A3*)	2	CIBERSORT	M0 macrophage		(217)
10	**CRC**	356 genes	4	CIBERSORT	M0 and M2 macrophages	CD8 T cell, resting NK, resting CD4 memory T cell	(191)
11	**GC#**	2 genes (*EFNA3*, *SERPINE1*)	2	ESTIMATE; ssGSEA	Higher immune and stromal scores; Treg, macrophage, neutrophil, mast cell		(192)
12	**Glioma#**	5 genes (*GAPDH*, *HK2*, *JUN*, *LDHA*, *VEGFA*)	2	CIBERSORT	Resting CD4 memory T cell, Treg, resting NK cell, M0 macrophage, neutrophil		(193)
13	**HNSCC**	24 genes (*AMPD3*, *BHLHE40*, *COL5A1*, *CP*, *CSRP2*, *CXCR4*, *DDIT4*, *DUSP1*, *ERRFI1*, *F3*, *GPC4*, *HS3ST1*, *IL6*, *ISG20*, *MAFF*, *PGM2*, *PIM1*, *PLAC8*, *PPP1R3C*, *S100A4*, *SDC2*, *SELENBP1*, *SERPINE1*, *SRPX*)	1	CIBERSORT	Activated DC, M0 macrophage, eosinophil, activated mast cell, resting NK cell, resting CD4 memory T cell	Memory B cell, CD8 T cell, resting mast cell, Treg, follicular helper T cell, activated CD4 memory T cell, gamma delta T cell, plasma cell, activated NK cell	(218)
14	**HCC**	4 genes (*ENO1*, *GAPDH*, *LDHA*, *SLC2A1*)	1	ESTIMATE CIBERSORT	Higher immune score; Treg, M0 macrophage, neutrophil	Activated NK cell, M1 macrophage, resting mast cell	(219)
15	**HCC#**	24 genes (*ACOT7*, *ADM*, *ALDOA*, *ANGPTL4*, *BNC1*, *CA9*, *CDKN3*, *COL4A6*, *ENO1*, *FOSL1*, *GNAI1*, *LDHA*, *MIF*, *MRPS17*, *NDRG1*, *P4HA1*, *PGAM1*, *PGK1*, *SDC1*, *SLC16A1*,	1 meta-cohort (from 3 datasets)	MCP-counter; ssGSEA; TIDE	CD4 T cell, activated CD8 T cell, iDC, aDC, CD56bright and CD56dim NK cell, gamma delta T cell, immature B cell, macrophage, mast cell, MDSC, NK T cell, pDC, follicular helper T cell, Th1, Th2; Three times higher response to ICI	Eosinophil, neutrophil, Treg, Th17	(220)

(Continued)

TABLE 1 | Continued

s.n	Cancer	Hypoxia Signature	Cohort Number‡	Immune Investigation Method	High-Risk Group (Hypoxia-High)	Low-Risk Group (Hypoxia-Low)	Reference
		SLC2A1, *TPI1*, *TUBB6*, *VEGFA*)					
16	**HCC#**	21 genes (*ADM*, *BNIP3*, *BNIP3L*, *CA9*, *EGLN3*, *GDF15*, *GYS1*, *HCAR3*, *HILPDA*, *HK2*, *INSIG2*, *JUN*, *KDM3A*, *PFKFB4*, *PLIN2*, *PTPRH*, *SLC2A3*, *SMAD3*, *SPAG4*, *TMEM45A*, *WSB1*)	11	ESTIMATE; CIBERSORT	3 cohorts: higher stromal score; 6 cohorts: higher immune score; 6 cohorts: activated CD4 memory T cell; 5 cohorts: activated mast cell; 5 cohorts: M0 macrophage	6 cohorts: resting CD4 memory T cell; 5 cohorts: resting mast cell; 3 cohorts: NK cell	(194)
17	**HCC#**	3 genes (*CDCA8*, *PDSS1*, *SLC7A11*)	1	CIBERSORT	M0 macrophage, memory B cell, follicular helper T cell		(221)
18	**HCC**	10 genes (*APEX1*, *ATR*, *CTSA*, *DNAJC5*, *ENO1*, *EPO*, *HMOX1*, *LDHA*, *NDRG1*, *PER1*)	1	CIBERSORT	M0 macrophage, Treg, neutrophil, eosinophil	Resting mast cell, resting CD4 memory cell, M1 macrophage, monocyte	(222)
19	**HCC**	4 genes (*DCN*, *DDIT4*, *NDRG1*, *PRKCA*)	2	ssGSEA		Activated B cell, activated CD8 T cell, effector memory CD8 T cell, Treg, Th1, CD56bright NK cell, NK cell, NK T cell, eosinophil, macrophage, mast cell, MDSC, monocyte, pDC	(223)
20	**NSCLC***	11 genes (*AMPD3*, *DDX11*, *FANCI*, *HIF-3α*, *IDE*, *LRP8*, *NOLC1*, *PAIP1*, *PDCD2*, *PSMF1*, *SNAPC5*)	1	ESTIMATE; ssGSEA		Higher immune score; DCs, aDCs, iDCs, pDCs, HLA, B cell, mast cell, neutrophil, T helper cell, T cell co-inhibition, T cell co-stimulation, TILs, Type II IFN response	(224)
21	**NSCLC**	4 genes (*ANGPTL4*, *PFKP*, *SLC2A1*, *XPNPEP1*)	2	CIBERSORT	Activated CD4 memory T cell, resting NK cell, M0 and M1 macrophages	Memory B cell, resting CD4 memory T cell, monocyte, resting DC, resting mast cell	(195)
22	**NSCLC**	18 genes (*ADM*, *BIK*, *DDIT3*, *ENO1*, *EPAS1*, *FGF3*, *GAPDH*, *MIF*, *NFKB1*, *PFKP*, *PGK1*, *PLAUR*, *SPP1*, *STC1*, *TEK*, *TFRC*, *TGFA*, *XRCC6*)	1	ssGSEA	Activated CD4 T cell, CD56bright NK cell, memory B cell, Th2	Activated B cell, activated CD8 T cell, central memory CD4 T cell, effector memory CD8 T cell, eosinophil, immature B cell, iDC, pDCs, macrophage, mast cell, MDSC, monocyte, NK cell, neutrophil, follicular helper T cell, Th1, Th17	(225)
23	**NSCLC#**	7 lncRNAs (AC010980.2, AC022784.1, AC079949.2, AC090001.1, AL161431.1, LINC00707, LINC00941)	1	CIBERSORT	Neutrophil, M0 and M2 macrophages	Monocyte	(226)
24	**SKCM**	11 genes (*CP*, *DPYSL4*, *EGFR*, *FBP1*, *FOXO3*, *IGFBP1*, *ISG20*, *KIF5A*, *PPARGC1A*, *S100A4*, *SDC3*)	2	CIBERSORT	Treg, mast cell; 1 cohort: resting CD4 memory T cell, monocyte	Activated CD4 memory T cell, M1 macrophage; 1 cohort: CD8 T cell, plasma cell	(196)
25	**OSCC#**	4 genes (*ALDOA*, *P4HA1*, *PGK1*, *VEGFA*)	1	CIBERSORT	M0 macrophage, mast cell	Naïve B cell, CD8 T cell, follicular helper T cell, Treg, neutrophil	(227)
26	**OS***	2 genes (*P4HA1*, *DCN*)	2	ssGSEA		DC, pDC, macrophage, neutrophil, TIL	(228)
27	**OS**	4 genes (*EFNA1*, *P4HA1*, *STC2*, *MAFF*)	2	CIBERSORT	Resting CD4 memory T cell		(229)
28	**OVC#**	9 genes (*ALOX5AP*, *ANXA1*, *IGFBP2*, *LAG3*, *PLK3*, *SLC1A1*, *SREBF1*, *SREBF2*, *TGFB1*)	1	CIBERSORT; TIMER	Activated CD4 memory T cell, gamma-delta T cell, activated NK, neutrophil, M1 and M2 macrophages	Resting CD4 memory T cell, follicular helper T cell, Treg, aDC, resting mast cell; Higher MHC and antigen presenting molecules	(230)
29	**PDAC#**	8 genes (*DDIT4*, *LDHA*, *MXI1*, *NDRG1*, *P4HA1*, *PGK1*, *SLC2A1*, *VEGFA*)	2	CIBERSORTx	M0 macrophage	CD8 T cell; Higher immune score and cytolytic index§	(197)

(Continued)

TABLE 1 | Continued

s.n	Cancer	Hypoxia Signature	Cohort Number‡	Immune Investigation Method	High-Risk Group (Hypoxia-High)	Low-Risk Group (Hypoxia-Low)	Reference
30	**PDAC**	4 genes (*ENO3*, *LDHA*, *PGK1*, *PGM1*)	2	CIBERSORT	M2 macrophage, resting NK cell	CD8 T cell, naive B cell	(231)
31	**RCC**	9 lnRNA (AC002070.1, AC008760.2, AC084876.1, AC147651.1, FOXD2-AS1, ITPR1-DT, LINC00944, LINC01615, LINC02027)	1	CIBERSORT	Plasma cell, follicular helper T cell, Treg	M2 macrophage, resting DC, resting mast cell	(232)
32	**RCC**	4 lnRNA (AC026462.3, COMETT, EMX2OS, HAGLR)	1	TIMER; ESTIMATE	B cell, CD4 T cell, CD8 T cell, DC, macrophage, neutrophil positively correlated with risk score Higher immune and stromal scores		(233)
33	**RCC#**	8 genes (*BCL2*, *KDELR3*, *KLF6*, *PCK1*, *PLAUR*, *PPARGC1A*, *RORA*, *WSB1*)	1	CIBERSORT	Treg, CD8 T cell, follicular helper T cell, plasma cell, M0 macrophage, activated NK cell	Resting CD4 memory, monocyte, M1 macrophage, resting mast cell, resting NK cell	(234)

‡*Number of independent patient cohorts analyzed with indicated method to investigate immune tumor microenvironment.*

#*Studies reporting higher immune checkpoint inhibitors or immunosuppressive cytokines or both in High-risk group.*

**Studies reporting higher immune checkpoint inhibitors or immunosuppressive cytokines or both in Low-risk group.*

§*Immune score calculated based on an eighteen gene tumor inflammation signature. The cytolytic index calculated based on the geometric mean of the GZMA (granzyme A) and PRF1 (perforin-1) produced by activated cytolytic CD8+ T cells (197).*

s.n, serial number; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BC, breast cancer; CRC, colorectal cancer; GC, gastric cancer; HNSCC, head and neck squamous cell carcinoma; HCC, hepatocellular carcinoma; NSCLC, non-small cell lung cancer; SKCM, skin cutaneous melanoma; OSCC, oral squamous cell carcinoma; OS, osteosarcoma; OVC, ovarian carcinoma; PDAC, pancreatic ductal adenocarcinoma; RCC, renal cell carcinoma; lnRNA, long non-coding RNA; CIBERSORT, Cell-type Identification By Estimating Relative Subsets Of RNA Transcripts; ESTIMATE, Estimation of STromal and Immune cells in MAlignant Tumours using Expression data; GSEA, gene set enrichment analysis; ssGSEA, single-sample GSEA; MCP-counter, Microenvironment Cell Populations-counter; TIMER, Tumor IMmune Estimation Resource; ImmuCellAI, Immune Cell Abundance Identifier; DC, dendritic cell; aDC, activated DC; iDC, immature DC; pDC, plasmacytoid DC; Th1, type 1 T helper cell; Th2, type 2 T helper cell; Th17, T helper 17 cell; Treg, regulatory T cell; iTreg, induced Treg; nTreg, natural Treg; HLA, human leukocyte antigen; TIL, tumor infiltrating leukocytes; IFN, interferon; MHC, major histocompatibility complex.

admitted. Therefore, controlling hypoxic stress to avoid tumor resistance and to reshape the hypoxic immunosuppressive TME in order to improve cancer immunotherapy remains a relevant challenge. Developing pharmacological agents to modulate HIF-1α signaling pathway is still attracting significant interest in the field of oncoimmunology. Several sub-types of drugs have been reported to inhibit HIF-1α activity including inhibitors of HIF-1α/HIF-1β dimerization (for example, acriflavine) (239, 240). Very recently, we demonstrated that suppression of the transcriptional activity of HIF-1α resulted in an increased infiltration of NK cells and CD8+ T cells in the tumor microenvironment of melanoma (13). Hypoxia could therefore be a potential immunometabolic checkpoint with prognostic value by regulating the TME and affecting the interaction between tumor cells and immune cells.

It is now well established that high expression of clonal tumor neoantigens correlates with an upregulation of lymphocyte infiltration within a tumor, enhanced patient survival and a prolonged response to immunotherapy. Recently, others and we have demonstrated that hypoxia interferes with genetic instability by inducing DNA damages, inducing DNA repair alteration (186) and presumably the emergence of tumor neoantigens. While the main predictive biomarkers for immunotherapy involve microsatellite instability/defective mismatch repair (MSI/dMMR), and tumor mutational burden, based on our previous reports and those of other teams, tumor hypoxia should be also be exploited as a potential biomarker to predict immunotherapy outcomes.

A deeper understanding of the role of hypoxia in killer cell induction and migration, immune suppression and EMT could enable the creation of more highly refined, innovative and integrative immunotherapies, targeting tumor plasticity and heterogeneity and aiding in overcoming the inherent constraints of currently applied anticancer therapies. In addition to the known hypoxic signatures reported, we believe that the design of minimally- or even non-invasive techniques able to predict treatment efficacy and tumor recurrence through algorithm-based modeling of network dynamics and by generating models based on artificial intelligence, or through the integration of "omics", must be considered in the field of oncoimmunology.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

FUNDING

This work was funded by the Gulf Medical University.

ACKNOWLEDGMENTS

SC and GV would like to acknowledge the support received from the Sheikh Hamdan Bin Rashid Al Maktoum Award for Medical Sciences: MRG-266/2017-2018 and MRG-230/2017-2018. ST would like to acknowledge the support received by Association pour la Recherche sur les Tumeurs de la Prostate (ARTP), Institut National du Cancer (INCa) and Cancéropôle Ile-de-France (2021-1-EMERG63). KB would like to acknowledge Military Institute of Medicine statutory grant no 1/8974 (519).

REFERENCES

1. Galon J, Bruni D. Tumor Immunology and Tumor Evolution: Intertwined Histories. *Immunity* (2020) 52(1):55–81. doi: 10.1016/j.immuni.2019.12.018
2. Chouaib S, Umansky V, Kieda C. The Role of Hypoxia in Shaping the Recruitment of Proangiogenic and Immunosuppressive Cells in the Tumor Microenvironment. *Contemp Oncol (Pozn)* (2018) 22(1A):7–13. doi: 10.5114/wo.2018.73874
3. You L, Wu W, Wang X, Fang L, Adam V, Nepovimova E, et al. The Role of Hypoxia-Inducible Factor 1 in Tumor Immune Evasion. *Med Res Rev* (2021) 41(3):1622–43. doi: 10.1002/med.21771
4. Semenza GL. Intratumoral Hypoxia and Mechanisms of Immune Evasion Mediated by Hypoxia-Inducible Factors. *Physiol (Bethesda)* (2021) 36 (2):73–83. doi: 10.1152/physiol.00034.2020
5. Samanta D, Semenza GL. Metabolic Adaptation of Cancer and Immune Cells Mediated by Hypoxia-Inducible Factors. *Biochim Biophys Acta Rev Cancer* (2018) 1870(1):15–22. doi: 10.1016/j.bbcan.2018.07.002
6. Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, et al. Mitochondrial Autophagy Is an HIF-1-Dependent Adaptive Metabolic Response to Hypoxia. *J Biol Chem* (2008) 283(16):10892–903. doi: 10.1074/jbc.M800102200
7. Lequeux A, Noman MZ, Xiao M, Sauvage D, Van Moer K, Viry E, et al. Impact of Hypoxic Tumor Microenvironment and Tumor Cell Plasticity on the Expression of Immune Checkpoints. *Cancer Lett* (2019) 458:13–20. doi: 10.1016/j.canlet.2019.05.021
8. Tang M, Bolderson E, O'Byrne KJ, Richard DJ. Tumor Hypoxia Drives Genomic Instability. *Front Cell Dev Biol* (2021) 9:626229. doi: 10.3389/fcell.2021.626229
9. Abou Khouzam R, Brodaczewska K, Filipiak A, Zeinelabdin NA, Buart S, Szczylik C, et al. Tumor Hypoxia Regulates Immune Escape/Invasion: Influence on Angiogenesis and Potential Impact of Hypoxic Biomarkers on Cancer Therapies. *Front Immunol* (2020) 11:613114. doi: 10.3389/fimmu.2020.613114
10. Semenza GL. Defining the Role of Hypoxia-Inducible Factor 1 in Cancer Biology and Therapeutics. *Oncogene* (2010) 29(5):625–34. doi: 10.1038/onc.2009.441
11. Noman MZ, Hasmim M, Lequeux A, Xiao M, Duhem C, Chouaib S, et al. Improving Cancer Immunotherapy by Targeting the Hypoxic Tumor Microenvironment: New Opportunities and Challenges. *Cells* (2019) 8 (9):1083. doi: 10.3390/cells8091083
12. Jayaprakash P, Ai M, Liu A, Budhani P, Bartkowiak T, Sheng J, et al. Targeted Hypoxia Reduction Restores T Cell Infiltration and Sensitizes Prostate Cancer to Immunotherapy. *J Clin Invest* (2018) 128(11):5137–49. doi: 10.1172/JCI96268
13. Lequeux A, Noman MZ, Xiao M, Van Moer K, Hasmim M, Benoit A, et al. Targeting HIF-1 Alpha Transcriptional Activity Drives Cytotoxic Immune Effector Cells Into Melanoma and Improves Combination Immunotherapy. *Oncogene* (2021) 40(28):4725–35. doi: 10.1038/s41388-021-01846-x
14. Gropper Y, Feferman T, Shalit T, Salame TM, Porat Z, Shakhar G. Culturing CTLs Under Hypoxic Conditions Enhances Their Cytolysis and Improves Their Anti-Tumor Function. *Cell Rep* (2017) 20(11):2547–55. doi: 10.1016/j.celrep.2017.08.071
15. Palazon A, Tyrakis PA, Macias D, Velica P, Rundqvist H, Fitzpatrick S, et al. An HIF-1alpha/VEGF-A Axis in Cytotoxic T Cells Regulates Tumor Progression. *Cancer Cell* (2017) 32(5):669–83 e5. doi: 10.1016/j.ccell.2017.10.003
16. Cho SH, Raybuck AL, Blagih J, Kemboi E, Haase VH, Jones RG, et al. Hypoxia-Inducible Factors in CD4(+) T Cells Promote Metabolism, Switch Cytokine Secretion, and T Cell Help in Humoral Immunity. *Proc Natl Acad Sci USA* (2019) 116(18):8975–84. doi: 10.1073/pnas.1811702116
17. Hayakawa Y, Kelly JM, Westwood JA, Darcy PK, Diefenbach A, Raulet D, et al. Cutting Edge: Tumor Rejection Mediated by NKG2D Receptor-Ligand Interaction Is Dependent Upon Perforin. *J Immunol* (2002) 169(10):5377–81. doi: 10.4049/jimmunol.169.10.5377
18. Cheon H, Borden EC, Stark GR. Interferons and Their Stimulated Genes in the Tumor Microenvironment. *Semin Oncol* (2014) 41(2):156–73. doi: 10.1053/j.seminoncol.2014.02.002
19. Rouette A, Trofimov A, Haberl D, Boucher G, Lavallée VP, D'Angelo G, et al. Expression of Immunoproteasome Genes Is Regulated by Cell-Intrinsic and -Extrinsic Factors in Human Cancers. *Sci Rep* (2016) 6:34019. doi: 10.1038/srep34019
20. Aras S, Zaidi MR. TAMeless Traitors: Macrophages in Cancer Progression and Metastasis. *Br J Cancer* (2017) 117(11):1583–91. doi: 10.1038/bjc.2017.356
21. Orecchioni M, Ghosheh Y, Pramod AB, Ley K. Macrophage Polarization: Different Gene Signatures in M1(LPS+) vs. Classically and M2(LPS-) vs. Alternatively Activated Macrophages. *Front Immunol* (2019) 10:1084. doi: 10.3389/fimmu.2019.01084
22. Chen DS, Mellman I. Oncology Meets Immunology: The Cancer-Immunity Cycle. *Immunity* (2013) 39(1):1–10. doi: 10.1016/j.immuni.2013.07.012
23. Gardner A, Ruffell B. Dendritic Cells and Cancer Immunity. *Trends Immunol* (2016) 37(12):855–65. doi: 10.1016/j.it.2016.09.006
24. Leone RD, Powell JD. Metabolism of Immune Cells in Cancer. *Nat Rev Cancer* (2020) 20(9):516–31. doi: 10.1038/s41568-020-0273-y
25. Chiche J, Brahimi-Horn MC, Pouysségur J. Tumour Hypoxia Induces a Metabolic Shift Causing Acidosis: A Common Feature in Cancer. *J Cell Mol Med* (2010) 14(4):771–94. doi: 10.1111/j.1582-4934.2009.00994.x
26. Chouaib S, Noman MZ, Kosmatopoulos K, Curran MA. Hypoxic Stress: Obstacles and Opportunities for Innovative Immunotherapy of Cancer. *Oncogene* (2017) 36(4):439–45. doi: 10.1038/onc.2016.225
27. Pietrobon V, Marincola FM. Hypoxia and the Phenomenon of Immune Exclusion. *J Transl Med* (2021) 19(1):9. doi: 10.1186/s12967-020-02667-4
28. Chang CH, Curtis JD, Maggi LB, Faubert B, Villarino AV, O'Sullivan D, et al. Posttranscriptional Control of T Cell Effector Function by Aerobic Glycolysis. *Cell* (2013) 153(6):1239–51. doi: 10.1016/j.cell.2013.05.016
29. Chang CH, Qiu J, O'Sullivan D, Buck MD, Noguchi T, Curtis JD, et al. Metabolic Competition in the Tumor Microenvironment Is a Driver of Cancer Progression. *Cell* (2015) 162(6):1229–41. doi: 10.1016/j.cell.2015.08.016
30. Sheppard S, Santosa EK, Lau CM, Violante S, Giovanelli P, Kim H, et al. Lactate Dehydrogenase A-Dependent Aerobic Glycolysis Promotes Natural Killer Cell Anti-Viral and Anti-Tumor Function. *Cell Rep* (2021) 35 (9):109210. doi: 10.1016/j.celrep.2021.109210
31. Angelin A, Gil-de-Gómez L, Dahiya S, Jiao J, Guo L, Levine MH, et al. Foxp3 Reprograms T Cell Metabolism to Function in Low-Glucose, High-Lactate Environments. *Cell Metab* (2017) 25(6):1282–93.e7. doi: 10.1016/j.cmet.2016.12.018
32. Husain Z, Huang Y, Seth P, Sukhatme VP. Tumor-Derived Lactate Modifies Antitumor Immune Response: Effect on Myeloid-Derived Suppressor Cells and NK Cells. *J Immunol* (2013) 191(3):1486–95. doi: 10.4049/jimmunol.1202702
33. Brand A, Singer K, Koehl GE, Kolitzus M, Schoenhammer G, Thiel A, et al. LDHA-Associated Lactic Acid Production Blunts Tumor Immunosurveillance by T and NK Cells. *Cell Metab* (2016) 24(5):657–71. doi: 10.1016/j.cmet.2016.08.011
34. Gottfried E, Kunz-Schughart LA, Ebner S, Mueller-Klieser W, Hoves S, Andreesen R, et al. Tumor-Derived Lactic Acid Modulates Dendritic Cell Activation and Antigen Expression. *Blood* (2006) 107(5):2013–21. doi: 10.1182/blood-2005-05-1795
35. Comito G, Iscaro A, Bacci M, Morandi A, Ippolito L, Parri M, et al. Lactate Modulates CD4. *Oncogene* (2019) 38(19):3681–95. doi: 10.1038/s41388-019-0688-7
36. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, et al. Functional Polarization of Tumour-Associated Macrophages by Tumour-Derived Lactic Acid. *Nature* (2014) 513(7519):559–63. doi: 10.1038/nature13490
37. Shan T, Chen S, Chen X, Wu T, Yang Y, Li S, et al. M2–TAM Subsets Altered by Lactic Acid Promote T–Cell Apoptosis Through the PD–L1/PD–1 Pathway. *Oncol Rep* (2020) 44(5):1885–94. doi: 10.3892/or.2020.7767
38. Hao X, Ren Y, Feng M, Wang Q, Wang Y. Metabolic Reprogramming Due to Hypoxia in Pancreatic Cancer: Implications for Tumor Formation, Immunity, and More. *BioMed Pharmacother* (2021) 141:111798. doi: 10.1016/j.biopha.2021.111798
39. Klysz D, Tai X, Robert PA, Craveiro M, Cretenet G, Oburoglu L, et al. Glutamine-Dependent α-Ketoglutarate Production Regulates the Balance Between T Helper 1 Cell and Regulatory T Cell Generation. *Sci Signal* (2015) 8(396):ra97. doi: 10.1126/scisignal.aab2610

40. Leone RD, Zhao L, Englert JM, Sun IM, Oh MH, Sun IH, et al. Glutamine Blockade Induces Divergent Metabolic Programs to Overcome Tumor Immune Evasion. *Science* (2019) 366(6468):1013–21. doi: 10.1126/science.aav2588
41. Ye LY, Chen W, Bai XL, Xu XY, Zhang Q, Xia XF, et al. Hypoxia-Induced Epithelial-To-Mesenchymal Transition in Hepatocellular Carcinoma Induces an Immunosuppressive Tumor Microenvironment to Promote Metastasis. *Cancer Res* (2016) 76(4):818–30. doi: 10.1158/0008-5472.CAN-15-0977
42. Munn DH, Sharma MD, Baban B, Harding HP, Zhang Y, Ron D, et al. GCN2 Kinase in T Cells Mediates Proliferative Arrest and Anergy Induction in Response to Indoleamine 2,3-Dioxygenase. *Immunity* (2005) 22(5):633–42. doi: 10.1016/j.immuni.2005.03.013
43. Song X, Zhang Y, Zhang L, Song W, Shi L. Hypoxia Enhances Indoleamine 2,3-Dioxygenase Production in Dendritic Cells. *Oncotarget* (2018) 9 (14):11572–80. doi: 10.18632/oncotarget.24098
44. Liu J, Zhang H, Jia L, Sun H. Effects of Treg Cells and IDO on Human Epithelial Ovarian Cancer Cells Under Hypoxic Conditions. *Mol Med Rep* (2015) 11(3):1708–14. doi: 10.3892/mmr.2014.2893
45. Schmidt SK, Ebel S, Keil E, Woite C, Ernst JF, Benzin AE, et al. Regulation of IDO Activity by Oxygen Supply: Inhibitory Effects on Antimicrobial and Immunoregulatory Functions. *PloS One* (2013) 8(5):e6301:3. doi: 10.1371/journal.pone.0063301
46. Mohapatra SR, Sadik A, Tykocinski LO, Dietze J, Poschet G, Heiland I, et al. Hypoxia Inducible Factor 1α Inhibits the Expression of Immunosuppressive Tryptophan-2,3-Dioxygenase in Glioblastoma. *Front Immunol* (2019) 10:2762. doi: 10.3389/fimmu.2019.02762
47. Labadie BW, Bao R, Luke JJ. Reimagining IDO Pathway Inhibition in Cancer Immunotherapy *via* Downstream Focus on the Tryptophan-Kynurenine-Aryl Hydrocarbon Axis. *Clin Cancer Res* (2019) 25(5):1462–71. doi: 10.1158/1078-0432.CCR-18-2882
48. Geiger R, Rieckmann JC, Wolf T, Basso C, Feng Y, Fuhrer T, et al. L-Arginine Modulates T Cell Metabolism and Enhances Survival and Anti-Tumor Activity. *Cell* (2016) 167(3):829–42.e13. doi: 10.1016/j.cell.2016.09.031
49. Corzo CA, Condamine T, Lu L, Cotter MJ, Youn JI, Cheng P, et al. HIF-1α Regulates Function and Differentiation of Myeloid-Derived Suppressor Cells in the Tumor Microenvironment. *J Exp Med* (2010) 207(11):2439–53. doi: 10.1084/jem.20100587
50. Rodriguez PC, Quiceno DG, Ochoa AC. L-Arginine Availability Regulates T-Lymphocyte Cell-Cycle Progression. *Blood* (2007) 109(4):1568–73. doi: 10.1182/blood-2006-06-031856
51. Doedens AL, Stockmann C, Rubinstein MP, Liao D, Zhang N, DeNardo DG, et al. Macrophage Expression of Hypoxia-Inducible Factor-1 Alpha Suppresses T-Cell Function and Promotes Tumor Progression. *Cancer Res* (2010) 70(19):7465–75. doi: 10.1158/0008-5472.CAN-10-1439
52. Rodriguez PC, Hernandez CP, Quiceno D, Dubinett SM, Zabaleta J, Ochoa JB, et al. Arginase I in Myeloid Suppressor Cells Is Induced by COX-2 in Lung Carcinoma. *J Exp Med* (2005) 202(7):931–9. doi: 10.1084/jem.20050715
53. Barsoum IB, Koti M, Siemens DR, Graham CH. Mechanisms of Hypoxia-Mediated Immune Escape in Cancer. *Cancer Res* (2014) 74(24):7185–90. doi: 10.1158/0008-5472.CAN-14-2598
54. Stagg J, Smyth MJ. Extracellular Adenosine Triphosphate and Adenosine in Cancer. *Oncogene* (2010) 29(39):5346–58. doi: 10.1038/onc.2010.292
55. Giatromanolaki A, Kouroupi M, Pouliliou S, Mitrakas A, Hasan F, Pappa A, et al. Ectonucleotidase CD73 and CD39 Expression in Non-Small Cell Lung Cancer Relates to Hypoxia and Immunosuppressive Pathways. *Life Sci* (2020) 259:118389. doi: 10.1016/j.lfs.2020.118389
56. Cekic C, Linden J. Purinergic Regulation of the Immune System. *Nat Rev Immunol* (2016) 16(3):177–92. doi: 10.1038/nri.2016.4
57. Leone RD, Horton MR, Powell JD. Something in the Air: Hyperoxic Conditioning of the Tumor Microenvironment for Enhanced Immunotherapy. *Cancer Cell* (2015) 27(4):435–6. doi: 10.1016/j.ccell.2015.03.014
58. Sitkovsky MV. T Regulatory Cells: Hypoxia-Adenosinergic Suppression and Re-Direction of the Immune Response. *Trends Immunol* (2009) 30(3):102–8. doi: 10.1016/j.it.2008.12.002
59. Steingold JM, Hatfield SM. Targeting Hypoxia-A2A Adenosinergic Immunosuppression of Antitumor T Cells During Cancer Immunotherapy. *Front Immunol* (2020) 11:570041. doi: 10.3389/fimmu.2020.570041
60. Zhang R, Zhu F, Ren J, Huang L, Liu P, Wu G. Beclin1/PI3K-Mediated Autophagy Prevents Hypoxia-Induced Apoptosis in EAhy926 Cell Line. *Cancer Biother Radiopharm* (2011) 26(3):335–43. doi: 10.1089/cbr.2010.0814
61. Sivridis E, Koukourakis MI, Mendrinos SE, Karpouzis A, Fiska A, Kouskoukis C, et al. Beclin-1 and LC3A Expression in Cutaneous Malignant Melanomas: A Biphasic Survival Pattern for Beclin-1. *Melanoma Res* (2011) 21(3):188–95. doi: 10.1097/CMR.0b013e328346612c
62. Menrad H, Werno C, Schmid T, Copanaki E, Deller T, Dehne N, et al. Roles of Hypoxia-Inducible Factor-1alpha (HIF-1alpha) Versus HIF-2alpha in the Survival of Hepatocellular Tumor Spheroids. *Hepatology* (2010) 51(6):2183–92. doi: 10.1002/hep.23597
63. Tasdemir E, Maiuri MC, Galluzzi L, Vitale I, Djavaheri-Mergny M, D'Amelio M, et al. Regulation of Autophagy by Cytoplasmic P53. *Nat Cell Biol* (2008) 10(6):676–87. doi: 10.1038/ncb1730
64. Papandreou I, Lim AL, Laderoute K, Denko NC. Hypoxia Signals Autophagy in Tumor Cells *via* AMPK Activity, Independent of HIF-1, BNIP3, and BNIP3L. *Cell Death Differ* (2008) 15(10):1572–81. doi: 10.1038/cdd.2008.84
65. Wan XB, Fan XJ, Chen MY, Xiang J, Huang PY, Guo L, et al. Elevated Beclin 1 Expression Is Correlated With HIF-1alpha in Predicting Poor Prognosis of Nasopharyngeal Carcinoma. *Autophagy* (2010) 6(3):395–404. doi: 10.4161/auto.6.3.11303
66. Koukourakis MI, Giatromanolaki A, Sivridis E, Pitiakoudis M, Gatter KC, Harris AL. Beclin 1 Over- and Underexpression in Colorectal Cancer: Distinct Patterns Relate to Prognosis and Tumour Hypoxia. *Br J Cancer* (2010) 103(8):1209–14. doi: 10.1038/sj.bjc.6605904
67. Ma XH, Piao S, Wang D, McAfee QW, Nathanson KL, Lum JJ, et al. Measurements of Tumor Cell Autophagy Predict Invasiveness, Resistance to Chemotherapy, and Survival in Melanoma. *Clin Cancer Res* (2011) 17 (10):3478–89. doi: 10.1158/1078-0432.CCR-10-2372
68. Wilson WR, Hay MP. Targeting Hypoxia in Cancer Therapy. *Nat Rev Cancer* (2011) 11(6):393–410. doi: 10.1038/nrc3064
69. Liu XW, Su Y, Zhu H, Cao J, Ding WJ, Zhao YC, et al. HIF-1α-Dependent Autophagy Protects HeLa Cells From Fenretinide (4-HPR)-Induced Apoptosis in Hypoxia. *Pharmacol Res* (2010) 62(5):416–25. doi: 10.1016/j.phrs.2010.07.002
70. Healy SJ, Gorman AM, Mousavi-Shafaei P, Gupta S, Samali A. Targeting the Endoplasmic Reticulum-Stress Response as an Anticancer Strategy. *Eur J Pharmacol* (2009) 625(1-3):234–46. doi: 10.1016/j.ejphar.2009.06.064
71. Salmon H, Remark R, Gnjatic S, Merad M. Host Tissue Determinants of Tumour Immunity. *Nat Rev Cancer* (2019) 19(4):215–27. doi: 10.1038/s41568-019-0125-9
72. Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural Innate and Adaptive Immunity to Cancer. *Annu Rev Immunol* (2011) 29:235–71. doi: 10.1146/annurev-immunol-031210-101324
73. Amaravadi R, Kimmelman AC, White E. Recent Insights Into the Function of Autophagy in Cancer. *Genes Dev* (2016) 30(17):1913–30. doi: 10.1101/gad.287524.116
74. Arensman MD, Yang XS, Zhong W, Bisulco S, Upeslacis E, Rosfjord EC, et al. Anti-Tumor Immunity Influences Cancer Cell Reliance Upon ATG7. *Oncoimmunology* (2020) 9(1):1800162. doi: 10.1080/2162402X.2020.1800162
75. Michaud M, Martins I, Sukkurwala AQ, Adjemian S, Ma Y, Pellegatti P, et al. Autophagy-Dependent Anticancer Immune Responses Induced by Chemotherapeutic Agents in Mice. *Science* (2011) 334(6062):1573–7. doi: 10.1126/science.1208347
76. Le Noci V, Sommariva M, Bianchi F, Triulzi T, Tagliabue E, Balsari A, et al. Local Administration of Caloric Restriction Mimetics to Promote the Immune Control of Lung Metastases. *J Immunol Res* (2019) 2019:2015892. doi: 10.1155/2019/2015892
77. Simone BA, Palagani A, Strickland K, Ko K, Jin L, Lim MK, et al. Caloric Restriction Counteracts Chemotherapy-Induced Inflammation and Increases Response to Therapy in a Triple Negative Breast Cancer Model. *Cell Cycle* (2018) 17(13):1536–44. doi: 10.1080/15384101.2018.1471314

78. Lo Re O, Panebianco C, Porto S, Cervi C, Rappa F, Di Biase S, et al. Fasting Inhibits Hepatic Stellate Cells Activation and Potentiates Anti-Cancer Activity of Sorafenib in Hepatocellular Cancer Cells. *J Cell Physiol* (2018) 233(2):1202–12. doi: 10.1002/jcp.25987
79. Di Tano M, Raucci F, Vernieri C, Caffa I, Buono R, Fanti M, et al. Synergistic Effect of Fasting-Mimicking Diet and Vitamin C Against KRAS Mutated Cancers. *Nat Commun* (2020) 11(1):2332. doi: 10.1038/s41467-020-16243-3
80. Guerriero JL. Macrophages: Their Untold Story in T Cell Activation and Function. *Int Rev Cell Mol Biol* (2019) 342:73–93. doi: 10.1016/bs.ircmb.2018.07.001
81. Vodnala SK, Eil R, Kishton RJ, Sukumar M, Yamamoto TN, Ha NH, et al. T Cell Stemness and Dysfunction in Tumors Are Triggered by a Common Mechanism. *Science* (2019) 363(6434):eaau0135. doi: 10.1126/science.aau0135
82. Pan H, Chen L, Xu Y, Han W, Lou F, Fei W, et al. Autophagy-Associated Immune Responses and Cancer Immunotherapy. *Oncotarget* (2016) 7 (16):21235–46. doi: 10.18632/oncotarget.6908
83. Wei J, Long L, Yang K, Guy C, Shrestha S, Chen Z, et al. Autophagy Enforces Functional Integrity of Regulatory T Cells by Coupling Environmental Cues and Metabolic Homeostasis. *Nat Immunol* (2016) 17(3):277–85. doi: 10.1038/ni.3365
84. Chen P, Cescon M, Bonaldo P. Autophagy-Mediated Regulation of Macrophages and Its Applications for Cancer. *Autophagy* (2014) 10 (2):192–200. doi: 10.4161/auto.26927
85. Xu X, Araki K, Li S, Han JH, Ye L, Tan WG, et al. Autophagy Is Essential for Effector CD8(+) T Cell Survival and Memory Formation. *Nat Immunol* (2014) 15(12):1152–61. doi: 10.1038/ni.3025
86. Botbol Y, Guerrero-Ros I, Macian F. Key Roles of Autophagy in Regulating T-Cell Function. *Eur J Immunol* (2016) 46(6):1326–34. doi: 10.1002/eji.201545955
87. Murera D, Arbogast F, Arnold J, Bouis D, Muller S, Gros F. CD4 T Cell Autophagy Is Integral to Memory Maintenance. *Sci Rep* (2018) 8(1):5951. doi: 10.1038/s41598-018-23993-0
88. Rožman S, Yousefi S, Oberson K, Kaufmann T, Benarafa C, Simon HU. The Generation of Neutrophils in the Bone Marrow Is Controlled by Autophagy. *Cell Death Differ* (2015) 22(3):445–56. doi: 10.1038/cdd.2014.169
89. Krzywinska E, Kantari-Mimoun C, Kerdiles Y, Sobecki M, Isagawa T, Gotthardt D, et al. Loss of HIF-1α in Natural Killer Cells Inhibits Tumour Growth by Stimulating Non-Productive Angiogenesis. *Nat Commun* (2017) 8(1):1597. doi: 10.1038/s41467-017-01599-w
90. Emily R, Cluff JN, Collins C, Varadaraj A, Rajasekaran N. Hypoxia-Inducible Factor-1α Is Upregulated in Natural Killer Cells by Interleukin-2 and Hypoxia *via* PI3K/mTOR Signaling Pathway. *J Immunol* (2019) 202 (1):194.37.
91. Parodi M, Raggi F, Cangelosi D, Manzini C, Balsamo M, Blengio F, et al. Hypoxia Modifies the Transcriptome of Human NK Cells, Modulates Their Immunoregulatory Profile, and Influences NK Cell Subset Migration. *Front Immunol* (2018) 9:2358. doi: 10.3389/fimmu.2018.02358
92. Solocinski K, Padget MR, Fabian KP, Wolfson B, Cecchi F, Hembrough T, et al. Overcoming Hypoxia-Induced Functional Suppression of NK Cells. *J Immunother Cancer* (2020) 8(1):e000246. doi: 10.1136/jitc-2019-000246
93. Teng R, Wang Y, Lv N, Zhang D, Williamson RA, Lei L, et al. Hypoxia Impairs NK Cell Cytotoxicity Through SHP-1-Mediated Attenuation of STAT3 and ERK Signaling Pathways. *J Immunol Res* (2020) 2020:4598476. doi: 10.1155/2020/4598476
94. Lim SA, Moon Y, Shin MH, Kim TJ, Chae S, Yee C, et al. Hypoxia-Driven HIF-1α Activation Reprograms Pre-Activated NK Cells Towards Highly Potent Effector Phenotypes *via* ERK/STAT3 Pathways. *Cancers (Basel)* (2021) 13(8):1904. doi: 10.3390/cancers13081904
95. Noman MZ, Buart S, Van Pelt J, Richon C, Hasmim M, Leleu N, et al. The Cooperative Induction of Hypoxia-Inducible Factor-1 Alpha and STAT3 During Hypoxia Induced an Impairment of Tumor Susceptibility to CTL-Mediated Cell Lysis. *J Immunol* (2009) 182(6):3510–21. doi: 10.4049/jimmunol.0800854
96. Amaravadi RK, Yu D, Lum JJ, Bui T, Christophorou MA, Evan GI, et al. Autophagy Inhibition Enhances Therapy-Induced Apoptosis in a Myc-Induced Model of Lymphoma. *J Clin Invest* (2007) 117(2):326–36. doi: 10.1172/JCI28833
97. Noman MZ, Janji B, Kaminska B, Van Moer K, Pierson S, Przanowski P, et al. Blocking Hypoxia-Induced Autophagy in Tumors Restores Cytotoxic T-Cell Activity and Promotes Regression. *Cancer Res* (2011) 71(18):5976–86. doi: 10.1158/0008-5472.CAN-11-1094
98. Noman MZ, Janji B, Berchem G, Mami-Chouaib F, Chouaib S. Hypoxia-Induced Autophagy: A New Player in Cancer Immunotherapy? *Autophagy* (2012) 8(4):704–6. doi: 10.4161/auto.19572
99. Cui Y, Li YY, Li J, Zhang HY, Wang F, Bai X, et al. STAT3 Regulates Hypoxia-Induced Epithelial Mesenchymal Transition in Oesophageal Squamous Cell Cancer. *Oncol Rep* (2016) 36(1):108–16. doi: 10.3892/or.2016.4822
100. Viry E, Baginska J, Berchem G, Noman MZ, Medves S, Chouaib S, et al. Autophagic Degradation of GZMB/granzyme B: A New Mechanism of Hypoxic Tumor Cell Escape From Natural Killer Cell-Mediated Lysis. *Autophagy* (2014) 10(1):173–5. doi: 10.4161/auto.26924
101. Tittarelli A, Janji B, Van Moer K, Noman MZ, Chouaib S. The Selective Degradation of Synaptic Connexin 43 Protein by Hypoxia-Induced Autophagy Impairs Natural Killer Cell-Mediated Tumor Cell Killing. *J Biol Chem* (2015) 290(39):23670–9. doi: 10.1074/jbc.M115.651547
102. Gleisner MA, Navarrete M, Hofmann F, Salazar-Onfray F, Tittarelli A. Mind the Gaps in Tumor Immunity: Impact of Connexin-Mediated Intercellular Connections. *Front Immunol* (2017) 8:1067. doi: 10.3389/fimmu.2017.01067
103. Das V, Bhattacharya S, Chikkaputtaiah C, Hazra S, Pal M. The Basics of Epithelial-Mesenchymal Transition (EMT): A Study From a Structure, Dynamics, and Functional Perspective. *J Cell Physiol* (2019) 234(9):14535–55. doi: 10.1002/jcp.28160
104. Tam SY, Wu VWC, Law HKW. Hypoxia-Induced Epithelial-Mesenchymal Transition in Cancers: HIF-1α and Beyond. *Front Oncol* (2020) 10:486. doi: 10.3389/fonc.2020.00486
105. Luo M, Li JF, Yang Q, Zhang K, Wang ZW, Zheng S, et al. Stem Cell Quiescence and Its Clinical Relevance. *World J Stem Cells* (2020) 12 (11):1307–26. doi: 10.4252/wjsc.v12.i11.1307
106. Abou Khouzam R, Goutham HV, Zaarour RF, Chamseddine AN, Francis A, Buart S, et al. Integrating Tumor Hypoxic Stress in Novel and More Adaptable Strategies for Cancer Immunotherapy. *Semin Cancer Biol* (2020) 65:140–54. doi: 10.1016/j.semcancer.2020.01.003
107. Hadjimichael C, Chanoumidou K, Papadopoulou N, Arampatzi P, Papamatheakis J, Kretsovali A. Common Stemness Regulators of Embryonic and Cancer Stem Cells. *World J Stem Cells* (2015) 7(9):1150–84. doi: 10.4252/wjsc.v7.i9.1150
108. Sahlgren C, Gustafsson MV, Jin S, Poellinger L, Lendahl U. Notch Signaling Mediates Hypoxia-Induced Tumor Cell Migration and Invasion. *Proc Natl Acad Sci USA* (2008) 105(17):6392–7. doi: 10.1073/pnas.0802047105
109. Zhang Q, Bai X, Chen W, Ma T, Hu Q, Liang C, et al. Wnt/β-Catenin Signaling Enhances Hypoxia-Induced Epithelial-Mesenchymal Transition in Hepatocellular Carcinoma *via* Crosstalk With Hif-1α Signaling. *Carcinogenesis* (2013) 34(5):962–73. doi: 10.1093/carcin/bgt027
110. Bijlsma MF, Groot AP, Oduro JP, Franken RJ, Schoenmakers SHHF, Peppelenbosch MP, et al. Hypoxia Induces a Hedgehog Response Mediated by HIF-1alpha. *J Cell Mol Med* (2009) 13(8B):2053–60. doi: 10.1111/j.1582-4934.2008.00491.x
111. Shi YH, Bingle L, Gong LH, Wang YX, Corke KP, Fang WG. Basic FGF Augments Hypoxia Induced HIF-1-Alpha Expression and VEGF Release in T47D Breast Cancer Cells. *Pathology* (2007) 39(4):396–400. doi: 10.1080/00313020701444549
112. Zhao W, Li Y, Zhang X. Stemness-Related Markers in Cancer. *Cancer Transl Med* (2017) 3(3):87–95. doi: 10.4103/ctm.ctm_69_16
113. Guo Q, Yang C, Gao F. The State of CD44 Activation in Cancer Progression and Therapeutic Targeting. *FEBS J* (2021). doi: 10.1111/febs.16179
114. Natesh J, Chandola C, Meeran SM, Neerathilingam M. Targeted Delivery of Doxorubicin Through CD44 Aptamer to Cancer Cells. *Ther Delivery* (2021) 12(10):693–703. doi: 10.4155/tde-2021-0038
115. Glumac PM, LeBeau AM. The Role of CD133 in Cancer: A Concise Review. *Clin Transl Med* (2018) 7(1):18. doi: 10.1186/s40169-018-0198-1
116. Terry S, Engelsen AST, Buart S, Elsayed WS, Venkatesh GH, Chouaib S. Hypoxia-Driven Intratumor Heterogeneity and Immune Evasion. *Cancer Lett* (2020) 492:1–10. doi: 10.1016/j.canlet.2020.07.004

117. Hasmim M, Noman MZ, Messai Y, Bordereaux D, Gros G, Baud V, et al. Cutting Edge: Hypoxia-Induced Nanog Favors the Intratumoral Infiltration of Regulatory T Cells and Macrophages *via* Direct Regulation of TGF-β1. *J Immunol* (2013) 191(12):5802–6. doi: 10.4049/jimmunol.1302140
118. Terry S, Buart S, Tan TZ, Gros G, Noman MZ, Lorens JB, et al. Acquisition of Tumor Cell Phenotypic Diversity Along the EMT Spectrum Under Hypoxic Pressure: Consequences on Susceptibility to Cell-Mediated Cytotoxicity. *Oncoimmunology* (2017) 6(2):e1271858. doi: 10.1080/2162402X.2016.1271858
119. Terry S, Abdou A, Engelsen AST, Buart S, Dessen P, Corgnac S, et al. AXL Targeting Overcomes Human Lung Cancer Cell Resistance to NK- and CTL-Mediated Cytotoxicity. *Cancer Immunol Res* (2019) 7(11):1789–802. doi: 10.1158/2326-6066.CIR-18-0903
120. Axelrod H, Pienta KJ. Axl as a Mediator of Cellular Growth and Survival. *Oncotarget* (2014) 5(19):8818–52. doi: 10.18632/oncotarget.2422
121. Schoumacher M, Burbridge M. Key Roles of AXL and MER Receptor Tyrosine Kinases in Resistance to Multiple Anticancer Therapies. *Curr Oncol Rep* (2017) 19(3):19. doi: 10.1007/s11912-017-0579-4
122. Goyette MA, Elkholi IE, Apcher C, Kuasne H, Rothlin CV, Muller WJ, et al. Targeting Axl Favors an Antitumorigenic Microenvironment That Enhances Immunotherapy Responses by Decreasing Hif-1α Levels. *Proc Natl Acad Sci USA* (2021) 118(29):e2023868118. doi: 10.1073/pnas.2023868118
123. Samanta D, Park Y, Ni X, Li H, Zahnow CA, Gabrielson E, et al. Chemotherapy Induces Enrichment of CD47. *Proc Natl Acad Sci USA* (2018) 115(6):E1239–E48. doi: 10.1073/pnas.1718197115
124. Noman MZ, Van Moer K, Marani V, Gemmill RM, Tranchevent LC, Azuaje F, et al. CD47 Is a Direct Target of SNAI1 and ZEB1 and Its Blockade Activates the Phagocytosis of Breast Cancer Cells Undergoing EMT. *Oncoimmunology* (2018) 7(4):e1345415. doi: 10.1080/2162402X.2017.1345415
125. Zhang D, Tang DG, Rycaj K. Cancer Stem Cells: Regulation Programs, Immunological Properties and Immunotherapy. *Semin Cancer Biol* (2018) 52(Pt 2):94–106. doi: 10.1016/j.semcancer.2018.05.001
126. Faget J, Groeneveld S, Boivin G, Sankar M, Zangger N, Garcia M, et al. Neutrophils and Snail Orchestrate the Establishment of a Pro-Tumor Microenvironment in Lung Cancer. *Cell Rep* (2017) 21(11):3190–204. doi: 10.1016/j.celrep.2017.11.052
127. Silva-Filho AF, Sena WLB, Lima LRA, Carvalho LVN, Pereira MC, Santos LGS, et al. Glycobiology Modifications in Intratumoral Hypoxia: The Breathless Side of Glycans Interaction. *Cell Physiol Biochem* (2017) 41 (5):1801–29. doi: 10.1159/000471912
128. Arriagada C, Silva P, Torres VA. Role of Glycosylation in Hypoxia-Driven Cell Migration and Invasion. *Cell Adh Migr* (2019) 13(1):13–22. doi: 10.1080/19336918.2018.1491234
129. Pereira MS, Alves I, Vicente M, Campar A, Silva MC, Padrao NA, et al. Glycans as Key Checkpoints of T Cell Activity and Function. *Front Immunol* (2018) 9:2754. doi: 10.3389/fimmu.2018.02754
130. Madsen CB, Petersen C, Lavrsen K, Harndahl M, Buus S, Clausen H, et al. Cancer Associated Aberrant Protein O-Glycosylation Can Modify Antigen Processing and Immune Response. *PloS One* (2012) 7(11):e50139. doi: 10.1371/journal.pone.0050139
131. Silva MC, Fernandes A, Oliveira M, Resende C, Correia A, de-Freitas-Junior JC, et al. Glycans as Immune Checkpoints: Removal of Branched N-Glycans Enhances Immune Recognition Preventing Cancer Progression. *Cancer Immunol Res* (2020) 8(11):1407–25. doi: 10.1158/2326-6066.CIR-20-0264
132. Huang Y, Zhang HL, Li ZL, Du T, Chen YH, Wang Y, et al. FUT8-Mediated Aberrant N-Glycosylation of B7H3 Suppresses the Immune Response in Triple-Negative Breast Cancer. *Nat Commun* (2021) 12(1):2672. doi: 10.1038/s41467-021-22618-x
133. Peixoto A, Fernandes E, Gaiteiro C, Lima L, Azevedo R, Soares J, et al. Hypoxia Enhances the Malignant Nature of Bladder Cancer Cells and Concomitantly Antagonizes Protein O-Glycosylation Extension. *Oncotarget* (2016) 7(39):63138–57. doi: 10.18632/oncotarget.11257
134. Peixoto A, Ferreira D, Azevedo R, Freitas R, Fernandes E, Relvas-Santos M, et al. Glycoproteomics Identifies HOMER3 as a Potentially Targetable Biomarker Triggered by Hypoxia and Glucose Deprivation in Bladder Cancer. *J Exp Clin Cancer Res* (2021) 40(1):191. doi: 10.1186/s13046-021-01988-6
135. Labrada M, Dorvignit D, Hevia G, Rodriguez-Zhurbenko N, Hernandez AM, Vazquez AM, et al. GM3(Neu5Gc) Ganglioside: An Evolution Fixed Neoantigen for Cancer Immunotherapy. *Semin Oncol* (2018) 45(1-2):41–51. doi: 10.1053/j.seminoncol.2018.04.003
136. Dorvignit D, Boligan KF, Relova-Hernandez E, Clavell M, Lopez A, Labrada M, et al. Antitumor Effects of the GM3(Neu5Gc) Ganglioside-Specific Humanized Antibody 14f7ht Against Cmah-Transfected Cancer Cells. *Sci Rep* (2019) 9(1):9921. doi: 10.1038/s41598-019-46148-1
137. Yin J, Hashimoto A, Izawa M, Miyazaki K, Chen GY, Takematsu H, et al. Hypoxic Culture Induces Expression of Sialin, a Sialic Acid Transporter, and Cancer-Associated Gangliosides Containing Non-Human Sialic Acid on Human Cancer Cells. *Cancer Res* (2006) 66(6):2937–45. doi: 10.1158/0008-5472.CAN-05-2615
138. Bousquet PA, Sandvik JA, Jeppesen Edin NF, Krengel U. Hypothesis: Hypoxia Induces *De Novo* Synthesis of NeuGc Gangliosides in Humans Through CMAH Domain Substitute. *Biochem Biophys Res Commun* (2018) 495(1):1562–6. doi: 10.1016/j.bbrc.2017.11.183
139. Groux-Degroote S, Delannoy P. Cancer-Associated Glycosphingolipids as Tumor Markers and Targets for Cancer Immunotherapy. *Int J Mol Sci* (2021) 22(11):6145. doi: 10.3390/ijms22116145
140. Perfahl H, Byrne HM, Chen T, Estrella V, Alarcon T, Lapin A, et al. Multiscale Modelling of Vascular Tumour Growth in 3D: The Roles of Domain Size and Boundary Conditions. *PloS One* (2011) 6(4):e14790. doi: 10.1371/journal.pone.0014790
141. Ribatti D, Nico B, Crivellato E, Roccaro AM, Vacca A. The History of the Angiogenic Switch Concept. *Leukemia* (2007) 21(1):44–52. doi: 10.1038/sj.leu.2404402
142. Nikitenko LL. Vascular Endothelium in Cancer. *Cell Tissue Res* (2009) 335 (1):223–40. doi: 10.1007/s00441-008-0707-4
143. Krock BL, Skuli N, Simon MC. Hypoxia-Induced Angiogenesis: Good and Evil. *Genes Cancer* (2011) 2(12):1117–33. doi: 10.1177/1947601911423654
144. Zuazo-Gaztelu I, Casanovas O. Unraveling the Role of Angiogenesis in Cancer Ecosystems. *Front Oncol* (2018) 8:248. doi: 10.3389/fonc.2018.00248
145. Carmona-Rodriguez L, Martinez-Rey D, Mira E, Manes S. SOD3 Boosts T Cell Infiltration by Normalizing the Tumor Endothelium and Inducing Laminin-Alpha4. *Oncoimmunology* (2020) 9(1):1794163. doi: 10.1080/2162402X.2020.1794163
146. Griffioen AW, Damen CA, Blijham GH, Groenewegen G. Tumor Angiogenesis Is Accompanied by a Decreased Inflammatory Response of Tumor-Associated Endothelium. *Blood* (1996) 88(2):667–73. doi: 10.1182/blood.V88.2.667.bloodjournal882667
147. Peeters CF, Ruers TJ, Westphal JR, de Waal RM. Progressive Loss of Endothelial P-Selectin Expression With Increasing Malignancy in Colorectal Cancer. *Lab Invest* (2005) 85(2):248–56. doi: 10.1038/labinvest.3700217
148. Wu X, Giobbie-Hurder A, Liao X, Lawrence D, McDermott D, Zhou J, et al. VEGF Neutralization Plus CTLA-4 Blockade Alters Soluble and Cellular Factors Associated With Enhancing Lymphocyte Infiltration and Humoral Recognition in Melanoma. *Cancer Immunol Res* (2016) 4(10):858–68. doi: 10.1158/2326-6066.CIR-16-0084
149. Morita Y, Zhang R, Leslie M, Adhikari S, Hasan N, Chervoneva I, et al. Pathologic Evaluation of Tumor-Associated Macrophage Density and Vessel Inflammation in Invasive Breast Carcinomas. *Oncol Lett* (2017) 14(2):2111–8. doi: 10.3892/ol.2017.6466
150. Nummer D, Suri-Payer E, Schmitz-Winnenthal H, Bonertz A, Galindo L, Antolovich D, et al. Role of Tumor Endothelium in CD4+ CD25+ Regulatory T Cell Infiltration of Human Pancreatic Carcinoma. *J Natl Cancer Inst* (2007) 99(15):1188–99. doi: 10.1093/jnci/djm064
151. Morita Y, Leslie M, Kameyama H, Lokesh GLR, Ichimura N, Davis R, et al. Functional Blockade of E-Selectin in Tumor-Associated Vessels Enhances Anti-Tumor Effect of Doxorubicin in Breast Cancer. *Cancers (Basel)* (2020) 12(3):725. doi: 10.3390/cancers12030725
152. Lambrechts D, Wauters E, Boeckx B, Aibar S, Nittner D, Burton O, et al. Phenotype Molding of Stromal Cells in the Lung Tumor Microenvironment. *Nat Med* (2018) 24(8):1277–89. doi: 10.1038/s41591-018-0096-5
153. Platel V, Faure S, Corre I, Clere N. Endothelial-To-Mesenchymal Transition (EndoMT): Roles in Tumorigenesis, Metastatic Extravasation and Therapy Resistance. *J Oncol* (2019) 2019:8361945. doi: 10.1155/2019/8361945

154. Liu S, Qin T, Liu Z, Wang J, Jia Y, Feng Y, et al. Anlotinib Alters Tumor Immune Microenvironment by Downregulating PD-L1 Expression on Vascular Endothelial Cells. *Cell Death Dis* (2020) 11(5):309. doi: 10.1038/s41419-020-2511-3
155. Hirsch L, Flippot R, Escudier B, Albiges L. Immunomodulatory Roles of VEGF Pathway Inhibitors in Renal Cell Carcinoma. *Drugs* (2020) 80 (12):1169–81. doi: 10.1007/s40265-020-01327-7
156. Suzuki H, Onishi H, Wada J, Yamasaki A, Tanaka H, Nakano K, et al. VEGFR2 Is Selectively Expressed by FOXP3high CD4+ Treg. *Eur J Immunol* (2010) 40(1):197–203. doi: 10.1002/eji.200939887
157. Voron T, Colussi O, Marcheteau E, Pernot S, Nizard M, Pointet AL, et al. VEGF-A Modulates Expression of Inhibitory Checkpoints on CD8+ T Cells in Tumors. *J Exp Med* (2015) 212(2):139–48. doi: 10.1084/jem.20140559
158. Murakami M, Simons M. Fibroblast Growth Factor Regulation of Neovascularization. *Curr Opin Hematol* (2008) 15(3):215–20. doi: 10.1097/MOH.0b013e3282f97d98
159. Im JH, Buzzelli JN, Jones K, Franchini F, Gordon-Weeks A, Markelc B, et al. FGF2 Alters Macrophage Polarization, Tumour Immunity and Growth and can be Targeted During Radiotherapy. *Nat Commun* (2020) 11(1):4064. doi: 10.1038/s41467-020-17914-x
160. Auletta JJ, Zale EA, Welter JF, Solchaga LA. Fibroblast Growth Factor-2 Enhances Expansion of Human Bone Marrow-Derived Mesenchymal Stromal Cells Without Diminishing Their Immunosuppressive Potential. *Stem Cells Int* (2011) 2011:235176. doi: 10.4061/2011/235176
161. Palakurthi S, Kuraguchi M, Zacharek SJ, Zudaire E, Huang W, Bonal DM, et al. The Combined Effect of FGFR Inhibition and PD-1 Blockade Promotes Tumor-Intrinsic Induction of Antitumor Immunity. *Cancer Immunol Res* (2019) 7(9):1457–71. doi: 10.1158/2326-6066.CIR-18-0595
162. Raica M, Cimpean AM. Platelet-Derived Growth Factor (PDGF)/PDGF Receptors (PDGFR) Axis as Target for Antitumor and Antiangiogenic Therapy. *Pharm (Basel)* (2010) 3(3):572–99. doi: 10.3390/ph3030572
163. Heldin CH. Targeting the PDGF Signaling Pathway in Tumor Treatment. *Cell Commun Signal* (2013) 11:97. doi: 10.1186/1478-811X-11-97
164. Agrawal S, Ganguly S, Hajian P, Cao JN, Agrawal A. PDGF Upregulates CLEC-2 to Induce T Regulatory Cells. *Oncotarget* (2015) 6(30):28621–32. doi: 10.18632/oncotarget.5765
165. Shi J, Wei PK. Interleukin-8: A Potent Promoter of Angiogenesis in Gastric Cancer. *Oncol Lett* (2016) 11(2):1043–50. doi: 10.3892/ol.2015.4035
166. David JM, Dominguez C, Hamilton DH, Palena C. The IL-8/IL-8r Axis: A Double Agent in Tumor Immune Resistance. *Vaccines (Basel)* (2016) 4(3):22. doi: 10.3390/vaccines4030022
167. Luoto KR, Kumareswaran R, Bristow RG. Tumor Hypoxia as a Driving Force in Genetic Instability. *Genome Integr* (2013) 4(1):5. doi: 10.1186/2041-9414-4-5
168. Scanlon SE, Glazer PM. Multifaceted Control of DNA Repair Pathways by the Hypoxic Tumor Microenvironment. *DNA Repair (Amst)* (2015) 32:180–9. doi: 10.1016/j.dnarep.2015.04.030
169. Francis A, Venkatesh GH, Zaarour RF, Zeinelabdin NA, Nawafleh HH, Prasad P, et al. Tumor Hypoxia: A Key Determinant of Microenvironment Hostility and a Major Checkpoint During the Antitumor Response. *Crit Rev Immunol* (2018) 38(6):505–24. doi: 10.1615/CritRevImmunol.2019030168
170. Ng N, Purshouse K, Foskolou IP, Olcina MM, Hammond EM. Challenges to DNA Replication in Hypoxic Conditions. *FEBS J* (2018) 285(9):1563–71. doi: 10.1111/febs.14377
171. Bhandari V, Li CH, Bristow RG, Boutros PC, Consortium P. Divergent Mutational Processes Distinguish Hypoxic and Normoxic Tumours. *Nat Commun* (2020) 11(1):737. doi: 10.1038/s41467-019-14052-x
172. Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J, Endesfelder D, et al. Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing. *N Engl J Med* (2012) 366(10):883–92. doi: 10.1056/NEJMoa1113205
173. Dentro SC, Leshchiner I, Haase K, Tarabichi M, Wintersinger J, Deshwar AG, et al. Characterizing Genetic Intra-Tumor Heterogeneity Across 2,658 Human Cancer Genomes. *Cell* (2021) 184(8):2239–54.e39. doi: 10.1016/j.cell.2021.03.009
174. Zhang J, Wu T, Simon J, Takada M, Saito R, Fan C, et al. VHL Substrate Transcription Factor ZHX2 as an Oncogenic Driver in Clear Cell Renal Cell Carcinoma. *Science* (2018) 361(6399):290–5. doi: 10.1126/science.aap8411
175. Bratslavsky G, Sudarshan S, Neckers L, Linehan WM. Pseudohypoxic Pathways in Renal Cell Carcinoma. *Clin Cancer Res* (2007) 13(16):4667–71. doi: 10.1158/1078-0432.CCR-06-2510
176. Gerlinger M, Horswell S, Larkin J, Rowan AJ, Salm MP, Varela I, et al. Genomic Architecture and Evolution of Clear Cell Renal Cell Carcinomas Defined by Multiregion Sequencing. *Nat Genet* (2014) 46(3):225–33. doi: 10.1038/ng.2891
177. Sansregret L, Vanhaesebroeck B, Swanton C. Determinants and Clinical Implications of Chromosomal Instability in Cancer. *Nat Rev Clin Oncol* (2018) 15(3):139–50. doi: 10.1038/nrclinonc.2017.198
178. Madan E, Peixoto ML, Dimitrion P, Eubank TD, Yekelchyk M, Talukdar S, et al. Cell Competition Boosts Clonal Evolution and Hypoxic Selection in Cancer. *Trends Cell Biol* (2020) 30(12):967–78. doi: 10.1016/j.tcb.2020.10.002
179. Graeber TG, Osmanian C, Jacks T, Housman DE, Koch CJ, Lowe SW, et al. Hypoxia-Mediated Selection of Cells With Diminished Apoptotic Potential in Solid Tumours. *Nature* (1996) 379(6560):88–91. doi: 10.1038/379088a0
180. Vito A, El-Sayes N, Mossman K. Hypoxia-Driven Immune Escape in the Tumor Microenvironment. *Cells* (2020) 9(4):992. doi: 10.3390/cells9040992
181. Rofstad EK, Johnsen NM, Lyng H. Hypoxia-Induced Tetraploidisation of a Diploid Human Melanoma Cell Line *In Vitro*. *Br J Cancer Suppl* (1996) 27: S136–9.
182. Caracciolo D, Riillo C, Arbitrio M, Di Martino MT, Tagliaferri P, Tassone P. Error-Prone DNA Repair Pathways as Determinants of Immunotherapy Activity: An Emerging Scenario for Cancer Treatment. *Int J Cancer* (2020) 147(10):2658–68. doi: 10.1002/ijc.33038
183. Kakoti S, Sato H, Laskar S, Yasuhara T, Shibata A. DNA Repair and Signaling in Immune-Related Cancer Therapy. *Front Mol Biosci* (2020) 7:205. doi: 10.3389/fmolb.2020.00205
184. Bader SB, Ma TS, Simpson CJ, Liang J, Maezono SEB, Olcina MM, et al. Replication Catastrophe Induced by Cyclic Hypoxia Leads to Increased APOBEC3B Activity. *Nucleic Acids Res* (2021) 49(13):7492–506. doi: 10.1093/nar/gkab551
185. Wang P, Chen Y, Wang C. Beyond Tumor Mutation Burden: Tumor Neoantigen Burden as a Biomarker for Immunotherapy and Other Types of Therapy. *Front Oncol* (2021) 11:672677. doi: 10.3389/fonc.2021.672677
186. Hassan Venkatesh G, Bravo P, Shaaban Moustafa Elsayed W, Amirtharaj F, Wojtas B, Abou Khouzam R, et al. Hypoxia Increases Mutational Load of Breast Cancer Cells Through Frameshift Mutations. *Oncoimmunology* (2020) 9(1):1750750. doi: 10.1080/2162402X.2020.1750750
187. Hassan Venkatesh G, Abou Khouzam R, Shaaban Moustafa Elsayed W, Ahmed Zeinelabdin N, Terry S, Chouaib S. Tumor Hypoxia: An Important Regulator of Tumor Progression or a Potential Modulator of Tumor Immunogenicity? *Oncoimmunology* (2021) 10(1):1974233. doi: 10.1080/2162402X.2021.1974233
188. Liu CC, Steen CB, Newman AM. Computational Approaches for Characterizing the Tumor Immune Microenvironment. *Immunology* (2019) 158(2):70–84. doi: 10.1111/imm.13101
189. Zhang F, Wang X, Bai Y, Hu H, Yang Y, Wang J, et al. Development and Validation of a Hypoxia-Related Signature for Predicting Survival Outcomes in Patients With Bladder Cancer. *Front Genet* (2021) 12:670384. doi: 10.3389/fgene.2021.670384
190. Liu Z, Tang Q, Qi T, Othmane B, Yang Z, Chen J, et al. A Robust Hypoxia Risk Score Predicts the Clinical Outcomes and Tumor Microenvironment Immune Characters in Bladder Cancer. *Front Immunol* (2021) 12:725223. doi: 10.3389/fimmu.2021.725223
191. Qi L, Chen J, Yang Y, Hu W. Hypoxia Correlates With Poor Survival and M2 Macrophage Infiltration in Colorectal Cancer. *Front Oncol* (2020) 10:566430. doi: 10.3389/fonc.2020.566430
192. Pei JP, Zhang CD, Yusupu M, Zhang C, Dai DQ. Screening and Validation of the Hypoxia-Related Signature of Evaluating Tumor Immune Microenvironment and Predicting Prognosis in Gastric Cancer. *Front Immunol* (2021) 12:705511. doi: 10.3389/fimmu.2021.705511
193. Lin W, Wu S, Chen X, Ye Y, Weng Y, Pan Y, et al. Characterization of Hypoxia Signature to Evaluate the Tumor Immune Microenvironment and Predict Prognosis in Glioma Groups. *Front Oncol* (2020) 10:796. doi: 10.3389/fonc.2020.00796
194. Zhang Q, Qiao L, Liao J, Liu Q, Liu P, Liu L. A Novel Hypoxia Gene Signature Indicates Prognosis and Immune Microenvironments Characters

in Patients With Hepatocellular Carcinoma. *J Cell Mol Med* (2021) 25 (8):3772–84. doi: 10.1111/jcmm.16249
195. Mo Z, Yu L, Cao Z, Hu H, Luo S, Zhang S. Identification of a Hypoxia-Associated Signature for Lung Adenocarcinoma. *Front Genet* (2020) 11:647. doi: 10.3389/fgene.2020.00647
196. Shou Y, Yang L, Yang Y, Zhu X, Li F, Xu J. Determination of Hypoxia Signature to Predict Prognosis and the Tumor Immune Microenvironment in Melanoma. *Mol Omics* (2021) 17(2):307–16. doi: 10.1039/D0MO00159G
197. Abou Khouzam R, Rao SP, Venkatesh GH, Zeinelabdin NA, Buart S, Meylan M, et al. An Eight-Gene Hypoxia Signature Predicts Survival in Pancreatic Cancer and Is Associated With an Immunosuppressed Tumor Microenvironment. *Front Immunol* (2021) 12:680435. doi: 10.3389/fimmu.2021.680435
198. Finotello F, Trajanoski Z. Quantifying Tumor-Infiltrating Immune Cells From Transcriptomics Data. *Cancer Immunol Immunother* (2018) 67 (7):1031–40. doi: 10.1007/s00262-018-2150-z
199. Jiménez-Sánchez A, Cast O, Miller ML. Comprehensive Benchmarking and Integration of Tumor Microenvironment Cell Estimation Methods. *Cancer Res* (2019) 79(24):6238–46. doi: 10.1158/0008-5472.CAN-18-3560
200. Barbie DA, Tamayo P, Boehm JS, Kim SY, Moody SE, Dunn IF, et al. Systematic RNA Interference Reveals That Oncogenic KRAS-Driven Cancers Require TBK1. *Nature* (2009) 462(7269):108–12. doi: 10.1038/nature08460
201. Vallania F, Tam A, Lofgren S, Schaffert S, Azad TD, Bongen E, et al. Leveraging Heterogeneity Across Multiple Datasets Increases Cell-Mixture Deconvolution Accuracy and Reduces Biological and Technical Biases. *Nat Commun* (2018) 9(1):4735. doi: 10.1038/s41467-018-07242-6
202. Bolis M, Vallerga A, Fratelli M. Computational Deconvolution of Transcriptomic Data for the Study of Tumor-Infiltrating Immune Cells. *Int J Biol Markers* (2020) 35(1_suppl):20–2. doi: 10.1177/1724600820903317
203. Yoshihara K, Shahmoradgoli M, Martínez E, Vegesna R, Kim H, Torres-Garcia W, et al. Inferring Tumour Purity and Stromal and Immune Cell Admixture From Expression Data. *Nat Commun* (2013) 4:2612. doi: 10.1038/ncomms3612
204. Abbas AR, Baldwin D, Ma Y, Ouyang W, Gurney A, Martin F, et al. Immune Response *In Silico* (IRIS): Immune-Specific Genes Identified From a Compendium of Microarray Expression Data. *Genes Immun* (2005) 6 (4):319–31. doi: 10.1038/sj.gene.6364173
205. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, et al. Robust Enumeration of Cell Subsets From Tissue Expression Profiles. *Nat Methods* (2015) 12(5):453–7. doi: 10.1038/nmeth.3337
206. Newman AM, Steen CB, Liu CL, Gentles AJ, Chaudhuri AA, Scherer F, et al. Determining Cell Type Abundance and Expression From Bulk Tissues With Digital Cytometry. *Nat Biotechnol* (2019) 37(7):773–82. doi: 10.1038/s41587-019-0114-2
207. Becht E, Giraldo NA, Lacroix L, Buttard B, Elarouci N, Petitprez F, et al. Estimating the Population Abundance of Tissue-Infiltrating Immune and Stromal Cell Populations Using Gene Expression. *Genome Biol* (2016) 17 (1):218. doi: 10.1186/s13059-016-1070-5
208. Avila Cobos F, Vandesompele J, Mestdagh P, De Preter K. Computational Deconvolution of Transcriptomics Data From Mixed Cell Populations. *Bioinformatics* (2018) 34(11):1969–79. doi: 10.1093/bioinformatics/bty019
209. Miao YR, Zhang Q, Lei Q, Luo M, Xie GY, Wang H, et al. ImmuCellAI: A Unique Method for Comprehensive T-Cell Subsets Abundance Prediction and Its Application in Cancer Immunotherapy. *Adv Sci (Weinh)* (2020) 7 (7):1902880. doi: 10.1002/advs.201902880
210. Jiang P, Gu S, Pan D, Fu J, Sahu A, Hu X, et al. Signatures of T Cell Dysfunction and Exclusion Predict Cancer Immunotherapy Response. *Nat Med* (2018) 24(10):1550–8. doi: 10.1038/s41591-018-0136-1
211. Chen X, Yan L, Lu Y, Jiang F, Zeng N, Yang S, et al. A Hypoxia Signature for Predicting Prognosis and Tumor Immune Microenvironment in Adrenocortical Carcinoma. *J Oncol* (2021) 2021:2298973. doi: 10.1155/2021/2298973
212. Jiang M, Ren L, Chen Y, Wang H, Wu H, Cheng S, et al. Identification of a Hypoxia-Related Signature for Predicting Prognosis and the Immune Microenvironment in Bladder Cancer. *Front Mol Biosci* (2021) 8:613359. doi: 10.3389/fmolb.2021.613359
213. Sun X, Zhou Z, Zhang Y, Wang J, Zhao X, Jin L, et al. Identification and Validation of a Hypoxia-Related Prognostic and Immune Microenvironment Signature in Bladder Cancer. *Cancer Cell Int* (2021) 21(1):251. doi: 10.1186/s12935-021-01954-4
214. Gong PJ, Shao YC, Huang SR, Zeng YF, Yuan XN, Xu JJ, et al. Corrigendum: Hypoxia-Associated Prognostic Markers and Competing Endogenous RNA Co-Expression Networks in Breast Cancer. *Front Oncol* (2020) 10:637481. doi: 10.3389/fonc.2020.579868
215. Zhang Y, Yang F, Peng X, Li X, Luo N, Zhu W, et al. Hypoxia Constructing the Prognostic Model of Colorectal Adenocarcinoma and Related to the Immune Microenvironment. *Front Cell Dev Biol* (2021) 9:665364. doi: 10.3389/fcell.2021.665364
216. Chen YF, Yu ZL, Lv MY, Zheng B, Tan YX, Ke J, et al. Genome-Wide Analysis Reveals Hypoxic Microenvironment Is Associated With Immunosuppression in Poor Survival of Stage II/III Colorectal Cancer Patients. *Front Med (Lausanne)* (2021) 8:686885. doi: 10.3389/fmed.2021.686885
217. Zhang L, Wang S, Wang Y, Zhao W, Zhang Y, Zhang N, et al. Effects of Hypoxia in Intestinal Tumors on Immune Cell Behavior in the Tumor Microenvironment. *Front Immunol* (2021) 12:645320. doi: 10.3389/fimmu.2021.645320
218. Ding Z, Li H, Yu D. Development and Validation of a Hypoxia-Related Gene Pair Signature to Predict Overall Survival in Head and Neck Squamous Cell Carcinoma. *Eur Arch Otorhinolaryngol* (2021) 278(10):3973–83. doi: 10.1007/s00405-020-06580-w
219. Deng F, Chen D, Wei X, Lu S, Luo X, He J, et al. Development and Validation of a Prognostic Classifier Based on HIF-1 Signaling for Hepatocellular Carcinoma. *Aging (Albany NY)* (2020) 12(4):3431–50. doi: 10.18632/aging.102820
220. Liu Z, Liu L, Lu T, Wang L, Li Z, Jiao D, et al. Hypoxia Molecular Characterization in Hepatocellular Carcinoma Identifies One Risk Signature and Two Nomograms for Clinical Management. *J Oncol* (2021) 2021:6664386. doi: 10.1155/2021/6664386
221. Zhang B, Tang B, Gao J, Li J, Kong L, Qin L. A Hypoxia-Related Signature for Clinically Predicting Diagnosis, Prognosis and Immune Microenvironment of Hepatocellular Carcinoma Patients. *J Transl Med* (2020) 18(1):342. doi: 10.1186/s12967-020-02492-9
222. Jiang HY, Ning G, Wang YS, Lv WB. Ahypoxia-Related Signature Enhances the Prediction of the Prognosis in Hepatocellular Carcinoma Patients and Correlates With Sorafenib Treatment Response. *Am J Transl Res* (2020) 12 (12):7762–81.
223. Zeng F, Zhang Y, Han X, Zeng M, Gao Y, Weng J. Employing Hypoxia Characterization to Predict Tumour Immune Microenvironment, Treatment Sensitivity and Prognosis in Hepatocellular Carcinoma. *Comput Struct Biotechnol J* (2021) 19:2775–89. doi: 10.1016/j.csbj.2021.03.033
224. Xu Z, Wei J, Qin F, Sun Y, Xiang W, Yuan L, et al. Hypoxia-Associated Alternative Splicing Signature in Lung Adenocarcinoma. *Epigenomics* (2021) 13(1):47–63. doi: 10.2217/epi-2020-0399
225. Huang Z, Wang S, Zhang HJ, Zhou YL, Tang X, Shi JH. Characteristics of Hypoxic Tumor Microenvironment in Non-Small Cell Lung Cancer, Involving Molecular Patterns and Prognostic Signature. *Transl Lung Cancer Res* (2021) 10(5):2132–47. doi: 10.21037/tlcr-20-1314
226. Shao J, Zhang B, Kuai L, Li Q. Integrated Analysis of Hypoxia-Associated lncRNA Signature to Predict Prognosis and Immune Microenvironment of Lung Adenocarcinoma Patients. *Bioengineered* (2021) 12(1):6186–200. doi: 10.1080/21655979.2021.1973874
227. Zhao C, Zhou Y, Ma H, Wang J, Guo H, Liu H. A Four-Hypoxia-Genes-Based Prognostic Signature for Oral Squamous Cell Carcinoma. *BMC Oral Health* (2021) 21(1):232. doi: 10.1186/s12903-021-01587-z
228. Fu Y, Bao Q, Liu Z, He G, Wen J, Liu Q, et al. Development and Validation of a Hypoxia-Associated Prognostic Signature Related to Osteosarcoma Metastasis and Immune Infiltration. *Front Cell Dev Biol* (2021) 9:633607. doi: 10.3389/fcell.2021.633607
229. Jiang F, Miao XL, Zhang XT, Yan F, Mao Y, Wu CY, et al. A Hypoxia Gene-Based Signature to Predict the Survival and Affect the Tumor Immune Microenvironment of Osteosarcoma in Children. *J Immunol Res* (2021) 2021:5523832. doi: 10.1155/2021/5523832

230. Chen X, Lan H, He D, Xu R, Zhang Y, Cheng Y, et al. Multi-Omics Profiling Identifies Risk Hypoxia-Related Signatures for Ovarian Cancer Prognosis. *Front Immunol* (2021) 12:645839. doi: 10.3389/fimmu.2021.645839
231. Ding J, He X, Cheng X, Cao G, Chen B, Chen S, et al. A 4-Gene-Based Hypoxia Signature Is Associated With Tumor Immune Microenvironment and Predicts the Prognosis of Pancreatic Cancer Patients. *World J Surg Oncol* (2021) 19(1):123. doi: 10.1186/s12957-021-02204-7
232. Zhang H, Qin C, Liu HW, Guo X, Gan H. An Effective Hypoxia-Related Long Non-Coding RNAs Assessment Model for Prognosis of Clear Cell Renal Carcinoma. *Front Oncol* (2021) 11:616722. doi: 10.3389/fonc.2021.616722
233. Chen H, Pan Y, Jin X, Chen G. Identification of a Four Hypoxia-Associated Long Non-Coding RNA Signature and Establishment of a Nomogram Predicting Prognosis of Clear Cell Renal Cell Carcinoma. *Front Oncol* (2021) 11:713346. doi: 10.3389/fonc.2021.713346
234. Zhang Z, Li Q, Wang F, Ma B, Meng Y, Zhang Q. Identifying Hypoxia Characteristics to Stratify Prognosis and Assess the Tumor Immune Microenvironment in Renal Cell Carcinoma. *Front Genet* (2021) 12:606816. doi: 10.3389/fgene.2021.606816
235. Stickels RR, Murray E, Kumar P, Li J, Marshall JL, Di Bella DJ, et al. Highly Sensitive Spatial Transcriptomics at Near-Cellular Resolution With Slide-Seqv2. *Nat Biotechnol* (2021) 39(3):313–9. doi: 10.1038/s41587-020-0739-1
236. Phillips D, Schürch CM, Khodadoust MS, Kim YH, Nolan GP, Jiang S. Highly Multiplexed Phenotyping of Immunoregulatory Proteins in the Tumor Microenvironment by CODEX Tissue Imaging. *Front Immunol* (2021) 12:687673. doi: 10.3389/fimmu.2021.687673
237. Baginska J, Viry E, Berchem G, Poli A, Noman MZ, van Moer K, et al. Granzyme B Degradation by Autophagy Decreases Tumor Cell Susceptibility to Natural Killer-Mediated Lysis Under Hypoxia. *Proc Natl Acad Sci USA* (2013) 110(43):17450–5. doi: 10.1073/pnas.1304790110
238. Noman MZ, Buart S, Romero P, Ketari S, Janji B, Mari B, et al. Hypoxia-Inducible miR-210 Regulates the Susceptibility of Tumor Cells to Lysis by Cytotoxic T Cells. *Cancer Res* (2012) 72(18):4629–41. doi: 10.1158/0008-5472.CAN-12-1383
239. Semenza GL. Hypoxia-Inducible Factors: Mediators of Cancer Progression and Targets for Cancer Therapy. *Trends Pharmacol Sci* (2012) 33(4):207–14. doi: 10.1016/j.tips.2012.01.005
240. Scholz CC, Taylor CT. Targeting the HIF Pathway in Inflammation and Immunity. *Curr Opin Pharmacol* (2013) 13(4):646–53. doi: 10.1016/j.coph.2013.04.009

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frontiers in Immunology

MINI REVIEW
published: 10 February 2022
doi: 10.3389/fimmu.2022.842038

Angiogenesis as Therapeutic Target in Metastatic Prostate Cancer – Narrowing the Gap Between Bench and Bedside

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Angiogenesis in metastatic castration-resistant prostate cancer (mCRPC) has been extensively investigated as a promising druggable biological process. Nonetheless, targeting angiogenesis has failed to impact overall survival (OS) in patients with mCRPC despite promising preclinical and early clinical data. This discrepancy prompted a literature review highlighting the tumor heterogeneity and biological context of Prostate Cancer (PCa). Narrowing the gap between the bench and bedside appears critical for developing novel therapeutic strategies. Searching clinicaltrials.gov for studies examining angiogenesis inhibition in patients with PCa resulted in n=20 trials with specific angiogenesis inhibitors currently recruiting (as of September 2021). Moreover, several other compounds with known anti-angiogenic properties – such as Metformin or Curcumin – are currently investigated. In general, angiogenesis-targeting strategies in PCa include biomarker-guided treatment stratification – as well as combinatorial approaches. Beyond established angiogenesis inhibitors, PCa therapies aiming at PSMA (Prostate Specific Membrane Antigen) hold the promise to have a substantial anti-angiogenic effect – due to PSMA´s abundant expression in tumor vasculature.

Keywords: Prostate adenocarcinoma, PCa, angiogenesis inhibitors, TKI, immunotherapy, tumor microenvironment, clinical trials, PSMA

OPEN ACCESS

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Specialty section:
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

Received: *23 December 2021*
Accepted: *21 January 2022*
Published: *10 February 2022*

Citation:
Solimando AG, Kalogirou C and Krebs M (2022) Angiogenesis as Therapeutic Target in Metastatic Prostate Cancer – Narrowing the Gap Between Bench and Bedside. Front. Immunol. 13:842038. doi: 10.3389/fimmu.2022.842038

INTRODUCTION

The biological context of angiogenesis and prostate cancer (PCa) inspired a plethora of research, specifically in metastatic PCa and more specifically in castration-resistant disease (CRPC), the clinical stage in which the majority of clinical trials on angiogenesis inhibition was performed (1). Metastatic PCa is an androgen-driven and -dependent cancer (2), with androgen deprivation therapy (ADT) being the primary treatment. Despite high response rates – practically 90% of patients initially respond to hormone therapy – the vast majority will end up relapsing (3) in a predictable and irreversible manner. There has been a fair amount of research to try to analyze the mechanisms of progression to CRPC, which is the lethal phenotype of metastatic PCa – and current evidence suggest a function of

clonal selection and adaptation by androgen receptor (AR)-dependent and independent mechanisms (4).

Indeed, ADT together with next generation hormonal agents such as Abiraterone (5) and Enzalutamide (6) still represent the foundation of systemic PCa treatment. Beyond hormone therapy, approved chemotherapy regimens mainly consist of Docetaxel and Cabazitaxel as microtubule inhibitors (7–9). Regarding bone as a favorite localization of PCa metastasis (10–12), therapeutic (combination) approaches include Radium-223 (13). In recent years, PCa treatment has rapidly developed towards precision oncology by addressing two novel target pathways: DNA repair and Prostate-specific membrane antigen (PSMA)-related signaling. Regarding DNA repair, cancers with mutations in BRCA1/2 (Breast Cancer Associated Genes 1 and 2) can be treated with PARP (Poly-ADP-Ribose-Polymerase) inhibitors originally established in Ovarian Cancer (14, 15). For PSMA, strategies include radioligand therapy as a theragnostic approach performed by nuclear medicine specialists (16).

Beyond these established and approved cancer therapies, this review aims to address an obvious treatment gap – given the crucial role of angiogenesis for PCa development and progression. Despite this fundamental promise reflected by *in vitro* and preclinical evidence, phase III trials with angiogenesis inhibitors failed to meet clinical endpoints.

PROSTATE CANCER AND VEGF-MEDIATED ANGIOGENESIS – PROMISES AND CHALLENGES

About 50 years ago, Folkman and colleagues highlighted the importance of angiogenesis and neovascularization for tumor growth – reasoning that targeting tumor blood vessels might prove beneficial for patients with cancer (17). Meanwhile, state-of-the-art techniques highlighted the crucial but not completely understood link between angiogenesis (endothelial cells) and tumor immunity (18). For PCa, histopathology pinpoints high micro-vessel density and increased VEGF (Vascular Endothelial Growth Factor) expression compared to non-neoplastic conditions. Moreover, VEGF levels are associated with higher tumor stages as well as advanced grading and plasma VEGF is increased in metastatic PCa versus localized disease (19–21). Higher VEGF expression evaluated by immunohistochemistry has also been associated with reduced disease-specific survival in patients with PCa (22). In addition, levels of urinary VEGF were associated with worse survival (23) and elevated plasma VEGF/sVCAM-1, a vascular cell adhesion molecule, correlated with worse outcome (24).

In principle, many drugs and angiogenic target structures known from other solid and hematological malignancies are available for PCa (25–30). As a consequence, clinical trials combined antiangiogenic agents with Taxanes in mCRPC (31); however, not a single drug combined with Docetaxel showed a statistically significant success in terms of outcome (32). Therefore, clinicians started trials in less symptomatic patients, investigating compounds as single agents. Unfortunately, all of these phase III trials with thousands of patients were collectively negative for OS – despite promising biological preclinical as well as promising phase II trials. Despite efforts studying more than 1,000 patients, the combination of Bevacizumab or Aflibercept with chemotherapy showed no improvement compared to chemotherapy alone (33, 34). Sunitinib as a single agent compared to prednisone showed no improvement, either (35).

Making it even worse, Lenalidomide treatment resulted in a sobering scenario (36): While effective in several hematologic conditions (37–40), combination treatment of patients with PCa (Lenalidomide + Docetaxel + Prednisone) led to a significantly worse OS compared to treatment with Docetaxel and Prednisone (36). Another surprising and quite sobering example is Cabozantinib, an oral inhibitor of Tyrosine Kinases including MET and VEGFR2, two major drivers of malignant progression in several neoplasia (41–47), which did not guarantee an OS advantage in patients with PCa (48). Indeed, Cabozantinib showed anti-angiogenic and antitumor effects in a wide range of preclinical tumor models (49–51), also blocking progression of PCa xenografts in soft tissue and bone (52–54). Additionally, Cabozantinib affected key actors of the bone niche – with reduction in osteoclasts and biphasic effects osteoblasts, while altering bone remodeling with increased volume in mice (55). MET and VEGFR2 cooperate to promote tumor survival, thereby boosting angiogenesis *via* improved tumor blood flow and improved oxygenation. Moreover, MET promotes migration and invasion, also facilitating the escape from hypoxic areas. Consequently, bone metastases are associated with high levels of MET expression. In specific, MET expression increased with androgen deprivation in preclinical models and with progression and metastasis in bone and lymph nodes (56). Promising early phase II trial results from bone scans upon combined Docetaxel and Cabozantinib treatment showed activity in 300 patients (48, 57). Soft tissue effects were also present, with objective response and significant progression-free survival (PFS) benefit (48). Improvement in pain and reduction of narcotics corroborated these initial results (58). These data were paralleled by a reduction of circulating tumor cells (57), while keeping activity in subjects heavily pretreated with Docetaxel, Abiraterone and/or Enzalutamide (48, 57). The lowest effective dose of these studies was 40 mg/day (59). Nevertheless, within phase III trial, Cabozantinib did not perform better than Prednisone (60). The dose and the stage of disease could have been the cause for this failure.

CURRENT CLINICAL TRIALS ON ANTI-ANGIOGENESIS IN PROSTATE CANCER

To determine the *status quo* of clinical trials investigating anti-angiogenesis in PCa, we performed a database research on clinicaltrials.gov. As of September 2021, a total sum of 866 actively recruiting interventional trials were registered for patients suffering from PCa. As outlined in **Table 1**, only a minority of clinical trials investigated the effects of angiogenesis inhibitors/Tyrosine kinase inhibitors. Specifically, we identified 20 clinical trials addressing angiogenesis inhibition. While some

TABLE 1 | Recruiting interventional trials examining anti-angiogenesis in prostate cancer (PCa) registered within clinicaltrials.gov database (December 2021).

Trial Identifier	Stage/Entity	Title/characteristics	Treatment	Comment
NCT01567800	PCa	Prostate Hypoxia FAZA	18F-FAZA	Hypoxia-specific PET tracer
NCT02465060	Advanced Cancer	MATCH screening trial; Phase II	(…), Sunitinib, (…)	Biomarker-driven Basket trial for various compounds
NCT02484404	Advanced solid tumors	Phase I/II	Combinations of Cediranib, Durvalumab and Olaparib	Cediranib: pan-VEGFR inhibitor
NCT02643667	Localized PCa	Phase I/II	Ibrutinib before Radical Prostatectomy	Ibrutinib: BTK inhibitor; Neoadjuvant setting
NCT03170960	Advanced solid tumors	Phase I/II	Cabozantinib ± Atezolizumab	
NCT03385655	PCa	Phase II	(…), Savolitinib, (…)	Biomarker-driven therapy stratification
NCT03556228	PCa and other malignancies	Phase I	VMD-928	VMD-928: TrkA inhibitor
NCT03845166	Advanced solid tumors	Phase I	XL092 AND Atezolizumab OR XL092 AND Avelumab	XL092: Tyrosine Kinase inhibitor (incl. VEGFR2)
NCT03866382	Rare genitourinary tumors	Phase II	Cabozantinib AND Nivolumab AND Ipilimumab	Metastatic Prostate Small Cell Neuroendocrine CA
NCT03878524	Advanced Cancer	SMMART; Phase I	(…), Bevacizumab, Cabozantinib, Sorafenib, Sunitinib, (…)	Biomarker-driven Basket trial for various compounds
NCT03964337	PCa before surgery	SPARC; Phase II	Neoadjuvant Cabozantinib	
NCT04159896	mCRPC	Phase II	ESK981 AND Nivolumab	ESK981: Pan-VEGFR/TIE2 inhibitor
NCT04446117	mCRPC	CONTACT-02; Phase III	Cabozantinib AND Atezolizumab	
NCT04477512	mCSPC	CABIOS; Phase I	Cabozantinib AND Abiraterone/Prednisone AND Nivolumab	
NCT04514484	Advanced Cancer AND HIV infection	Phase I	Cabozantinib AND Nivolumab	
NCT04521686	Advanced solid tumors with IDH1 mutations	Phase I	LY3410738	LY3410738: IDH1 inhibitor
NCT04631744	mCRPC	Phase II	Cabozantinib	
NCT04635059	PCa: biochemical recurrence	BLAST; Phase II	Pacritinib	Pacritinib: JAK/FLT3 inhibitor
NCT04742959	Advanced solid tumors	Phase I/II	TT-00420 ± Nab-Paclitaxel	TT-00420: Tyrosine Kinase inhibitor (incl. VEGFRs)
NCT04848337	Advanced/metastatic neuroendocrine PCa	PLANE-PC; Phase II	Lenvatinib AND Pembrolizumab	Lenvatinib: VEGFR inhibitor
Further compounds with known anti-angiogenic properties				
NCT02935205	CRPC	Phase I/II	Indomethacin AND Enzalutamide	
NCT00268476	mCSPC	STAMPEDE; Phase II/III	(…), Metformin, (…)	
NCT01864096	low-risk PCa under Active Surveillance	MAST; Phase III	Metformin	
NCT02064673	PCa after Radical Prostatectomy	Phase III	Curcumin	
NCT02176161	PCa after therapy and a high-risk setting	Phase II	Metformin	
NCT02804815	PCa and other malignancies after curative therapy	Phase III	Aspirin	
NCT03031821	PCa with indication for ADT	PRIME; Phase III	Metformin AND ADT	
NCT03535675	PCa: PSA recurrence after definitive treatment	Phase III	Muscadine Grape extract	Patient pre-selection according to genotype
NCT03769766	low-risk PC under Active Surveillance	Phase III	Curcumin	
NCT03819101	CRPC	PEACE-4; Phase III	Acetylsalicylic acid ± Atorvastatin	
NCT03899987	PCa before Radical Prostatectomy	Phase II	Aspirin AND Rintatolimod ± interferon-alpha 2b	
NCT04300855	PCa under Active Surveillance	Phase II	Green Tea Catechins (Sunphenon)	
NCT04519879	PCa: recurrent/therapy-naive	Phase III	White Button Mushroom extract	
NCT04536805	PCa: relapse in previously irradiated Prostate bed	REPAIRGETUGP16; Phase I/II	Metformin AND Radiation	
NCT04597359	PCa under Active Surveillance	Phase II	Green Tea Catechins	

Ctr, Control; CRPC, castration-resistant Prostate Cancer; CSPC, castration-sensitive Prostate Cancer; mCRPC, metastatic castration-resistant Prostate Cancer; mCSPC, metastatic castration-sensitive Prostate Cancer; ADT, Androgen deprivation therapy.

studies aim to identify predictive biomarkers for future clinical stratification in entity-independent trials (NCT02465060, NCT03878524), others combine angiogenesis inhibition with immune checkpoint blockade – e. g. CONTACT-02 trial investigating Cabozantinib in combination with Atezolizumab in patients with mCRPC (NCT04446117). Of note, other studies include patients in different stages, such as metastatic castration sensitive disease (CABIOS phase I trial, NCT04477512) and even localized disease in a neoadjuvant setting before Radical Prostatectomy (SPARC phase II trial, NCT03964337).

Beyond this relatively small number of trials directly aiming at tumor vessels, we found several studies investigating

compounds known to have additional anti-angiogenic effects (bottom part of **Table 1**). Curcumin, Green Tea Catechins and Metformin were among the substances identified. For Metformin, a tumor suppressive role was shown in several cancer entities (61). Moreover, adjuvant Metformin intake was associated with improved outcome in Clear Cell Renal Cell Carcinoma patients treated with Tyrosine Kinase inhibitors in two independent cohorts (62, 63). One reason for this protective effect could be the role of Metformin as a mitochondrial inhibitor. Interestingly, recent evidence implies a prominent role for mitochondrial signaling not only in Clear Cell Renal Cell Carcinoma (64), but also in high-grade PCa (65). Potentially, angiogenesis inhibition could be more effective in patients suffering from PC when combined with adjuvants such as Metformin.

DISCUSSION

From a histopathological and preclinical perspective, there is convincing evidence for a significant role of angiogenesis in PCa development and progression. For example, VEGFR2 was shown to mark PCa cases with a high risk of progression (30, 66). In addition, angiogenesis-related microRNAs such as let-7, miR-195 and miR-205 (67) are also deregulated and play prominent roles in PCa (68–70). However, no angiogenesis-specific inhibitor has met its clinical endpoint in phase III trials (see **Figure 1A**). Consequently, angiogenesis inhibitors currently do not play a role in PCa treatment guidelines. As shown by our database search on clinicaltrials.gov, several clinical trials are currently recruiting patients with PCa to address the discrepancy between promising preclinical findings and sobering clinical trial results.

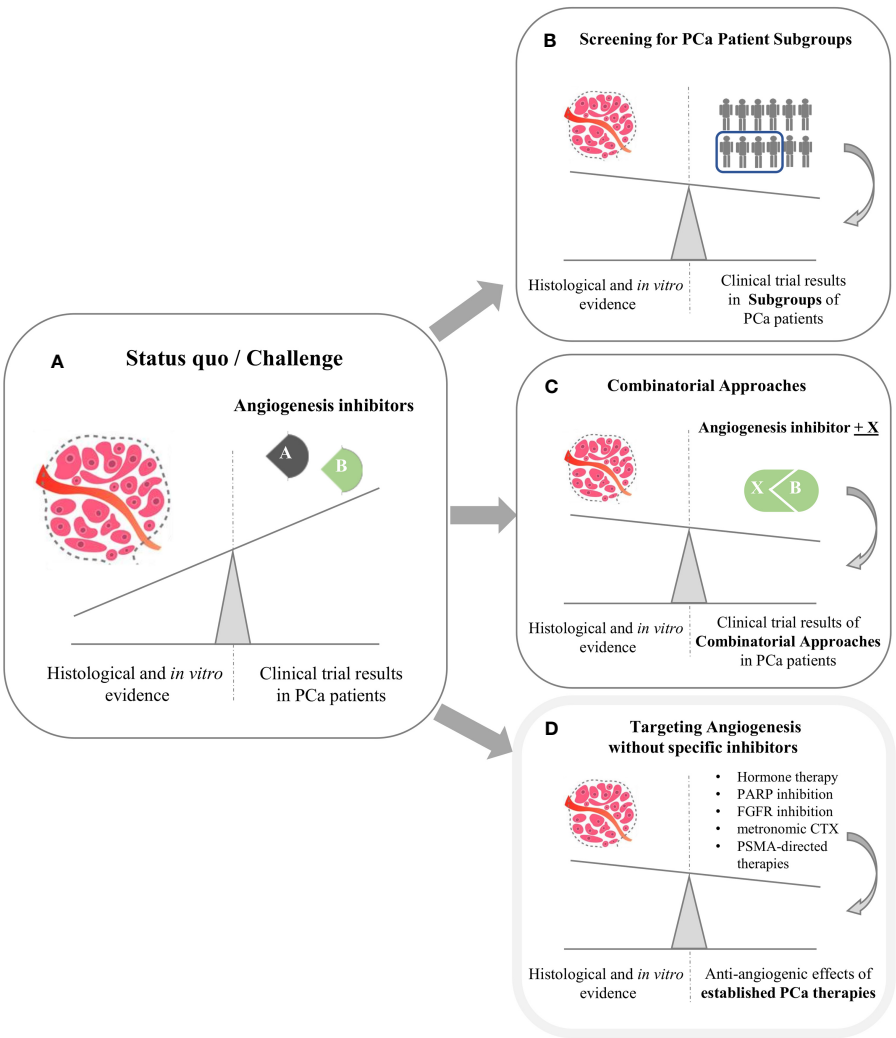

FIGURE 1 | The clinical challenge of angiogenesis inhibition in Prostate Cancer (PCa). **(A)** Despite promising preclinical evidence from histopathological and *in vitro* analyses, phase III clinical trials with angiogenesis inhibitors failed to meet clinical endpoints. **(B, C)** Main strategies aiming to leverage the impact of angiogenesis inhibition are biomarker-aided identification of PC patient subgroups most susceptible towards anti-angiogenesis **(B)** and combinatorial approaches **(C)**. Moreover, several established PCa therapies partly exhibit anti-angiogenic effects as mode of action **(D)**.

Current Therapeutic Strategies to Narrow the Gap Between Bench and Bedside

As illustrated in **Figure 1**, two main strategies aim to establish therapeutic anti-angiogenesis in patients with PCa. Within the first strategic approach, clinicians are searching for PCa subgroups most susceptible towards angiogenesis inhibition (**Figure 1B**). It is tempting to assume that targeting tumor neovascularization could be more efficient when used early in the course of disease (71) in order to prevent metastases (44, 72). In line with this assumption, clinicians examine effects in PCa subgroups other than mCRPC. Specifically, SPARC investigates Cabozantinib in a neoadjuvant setting. PCa patients suffering from biochemical recurrence are currently recruited for the BLAST trial, which investigates the JAK/FLT3 inhibitor Pacritinib. Moreover, the CABIOS trial recruits CSPC patients receiving Cabozantinib, Abiraterone and Nivolumab (thereby also representing the second strategic approach of combinatorial therapies). Up to now, neither predictive nor response biomarkers have been established to stratify PCa patients regarding anti-angiogenic therapy (18, 26). Of note, most biomarker-driven trials trying to meet the needs are not PCa-specific. Recruiting patients suffering from advanced cancer, the MATCH screening trial constitutes a biomarker-driven basket study for various compounds including Sunitinib. In a similar setting, SMMART investigates compounds such as Bevacizumab, Cabozantinib, Sorafenib and Sunitinib.

As a second strategic approach to narrow the gap between bench and bedside (**Figure 1C**), clinicians and researchers combine angiogenesis inhibitors with other established cancer compounds. Most of the respective trials identified by our search teamed angiogenesis inhibitors with immune checkpoint inhibitors (ICI) – e. g. Cabozantinib and Atezolizumab (CONTACT-02 trial). However, the primary rationale of these approaches is not to establish anti-angiogenesis as a treatment option for PCa, but to break therapy resistance towards ICI (73–75).

BRCA in Metastatic Prostate Cancer - Recommendations and Perspectives

As a second bullet point to envision next steps narrowing the gap between the bench and bedside, it is important to highlight that genetic alterations of BRCA2 and BRCA1 occur in metastatic PCa with a frequency of 13% and 5.3% for the somatic component, and 0.3% and 0.9% for the germline component, respectively (76, 77). Germline mutations in BRCA2 are associated with pathways also related to VEGF signaling (78). Thus, phase II and III studies investigating effect on PFS and ORR in mCRPC hold promise to further elucidate the complex relationship of disease biology, since genomic alterations and several genes are screened (**Table 2**). TRITON2 and GALAHAD studies showed objectives and PSA responses in patients with BRCA1/2 alterations employing Rucaparib and Niraparib, respectively (79, 80). Nonetheless, the Profound trial testing Olaparib, confirmed that BRCA2 is the most frequently altered gene and with BRCA1 and ATM genes allowed to reach a radiographic PFS improvement of Olaparib treated over control (HR.34 P<.0001, CI.25-.47). Those results are remarkable since checkpoint inhibitors may have limited efficacy in PCa as single agents; thus, combination approaches are being examined to potentially improve their efficacy in this as in other urological diseases (30, 44). The hypothetical synergism between PARP inhibitors and ICI is centered on evidence that DNA damage resulting from PARP inhibition triggers the cGAS-STING pathway (81), which consequently boosts the interferon signaling, leading to enhanced immunogenicity (82). There is also rationale for an additive effect in cancers with high microsatellite instability (MSI) and BRCA mutations (83). Moreover, cancers with CDK12 mutations are often sensitive to PARP inhibitors - and preclinical and biological data from patients with PCa showed that CDK12 inactivation is related to increased burden of neoantigens, which can in turn enhance the immunogenicity (84). ICI hold anti-mCRPC activity potential in high degree of MSI. Indeed, the KEYNOTE-365 trial comparing Pembrolizumab plus Olaparib in biomarker-unstratified mCRPC subjects after prior taxane-based regimen uncovered that 36.6% of

TABLE 2 | Trials screening genes involved in prostate cancer (PCa) registered within clinicaltrials.gov database (December 2021). See text for details.

	PROFOUND	TRITON 2	GALAHAD
Drug	Olaparib 300 mg bid	Rucaparib 600 mg bid	Niraparib 300 mg qd
Study design	Phase III	Phase II	Phase II
Population	mCRPC progression to ARSI	mCRPC progression to ARSI and taxane	mCRPC progression to ARSI and taxane
Primary objective	rPFS in pts with alterations in ATM, BRCA1, BRCA2	ORR and PSA response (≥50% decline) in pts with DDR alterations	ORR in patients with bi-allelic BRCA1/2 alterations
Specimen tested	Tumor tissue central	Plasma or tumor tissue central/local	Plasma central
Test used	FoundationOne®	FoundationOne® FoundationACT® Local	Resolution-HRD®
Genes screened	*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, PPP2RA, RAD51B, RAD51C, RAD51D, RAD54L*	*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK2, FANCA, NBN, PALB2, RAD51, RAD51B, RAD51C, RAD51D, RAD54L*	*ATM, BRCA1, BRCA2, BRIP1, CHEK2, FANCA, HDAC2, PALB2*
Genomic alteration required	Mono- and Bi- allelic alterations in DDR genes	Mono- and Bi- allelic alterations in DDR genes	Bi- allelic alterations in DDR genes

individuals obtained a PSA response (85). The KEYLYNK-010 phase III study has been designed to deeper elucidate the combination of Pembrolizumab plus Olaparib in patients with mCRPC in a biomarker-unselected population after progression on androgen-deprivation therapy and androgen receptor signaling inhibitor (86). In line with this, Nivolumab plus Rucaparib in the phase II CheckMate 9KD trial focusing on mCRPC revealed that best response rates were among BRCA2 mutated cases and that the combination was not efficient in individuals without homologous recombination mutations (87). Statistically powered studies aiming to corroborate these hypothesis-generating results are needed. Nonetheless, based on the available data, the FDA approved both Niraparib and Rucaparib as well as Olaparib in May 2020 (88). Nonetheless, EMA approved Olaparib for the treatment of patients with mCRPC and BRCA1/2 mutations, either germline or somatic after progression following a prior line including a hormonal agent, based on the results published by Hussain M. et al. (89). Collectively, the BRCA mutational status assessment in mCRPC is not merely a predictor of response to PARP inhibition, but is rather a biomarker of aggressiveness and therefore can sketch a disease phenotype for whom additional biomarker might be added (90). Indeed, BRCA status might also predict a decreased taxane sensitivity compared to Abiraterone and Enzalutamide, nonetheless confirmatory trials are also needed.

Targeting Angiogenesis Without Specific Inhibitors – Established and Evolving Therapies

While our database search on clinicaltrials.gov revealed a limited number of studies with specific inhibitors of angiogenesis, a plethora of trials investigated compounds such as antiandrogens, PARP inhibitors and PSMA-directed agents. At first sight, these approaches might not appear tightly related to tumor angiogenesis. Yet, recent findings imply that all these strategies obtain a significant anti-angiogenic component. Regarding AR-related signaling, a growing amount of literature investigates the complex crosstalk with VEGF-mediated pathways in cancer (91). As mentioned, for PARP inhibitors such as Olaparib, an anti-angiogenic effect besides an anti-mCRPC is widely accepted (14, 92, 93). Moreover, FGF (Fibroblast Growth Factor) and its receptors (FGFRs) play prominent pro-angiogenic roles in several malignancies, including PCa (94, 95). Consequently, the FGFR inhibitor Erdafitinib is currently investigated in patients with CRPC as a single drug (NCT04754425) and combined with Abiraterone or Enzalutamide in patients with CRPC (NCT03999515).

Metronomic (low-dose) chemotherapy is another well-described therapeutic strategy to target tumor-associated neo-vasculature in various cancer entities. Frequent and regular administration of chemotherapeutic agents at doses constituting a fraction of the MTD (maximum tolerated dose) was shown to have substantial therapeutic effects – especially on tumor endothelium. Moreover, these regimens frequently exhibited favorable toxicity profiles (96, 97). For PCa, clinical evidence highlights the potential of metronomic therapies especially in mCRPC: studies investigated metronomic Cyclophosphamide in combination with Docetaxel (98) or in heavily pretreated patients after Docetaxel or Abiraterone/Enzalutamide (99–102) – showing effectiveness and good tolerability. In addition, researchers examined the efficacy of metronomic application of Vinorelbine (103) and metronomic Cyclophosphamide, Celecoxib and Dexamethasone in patients suffering from mCRPC (104). Interestingly, metronomic Cyclophosphamide application also induced an immune reaction (in terms of T cell reactivation) in patients with biochemical recurrence (105). Although the mode of action of metronomic therapies is not completely understood, a recent study identified key genes which were associated with (metronomic) Topotecan dosing in PCa cell lines (106).

Regarding PSMA, receptor expression not only exists on the surface of PCa cells. Instead, tumor-associated endothelium frequently displays robust levels of PSMA in various cancer entities (107–109). Future research must show the impact of targeting PSMA in terms of anti-angiogenic activity – for PCa but also for other entities with PSMA-positive tumor endothelium. Given the rationale of adding angiogenesis inhibitors to ICI in order to break resistance towards immune-based approaches (73–75), it also appears tempting to assume that targeting PSMA could have an impact on the immunogenicity of PCa.

In a nutshell: While specific angiogenesis inhibitors currently do not have an established role in PCa, targeting tumor angiogenesis and tumor-associated blood vessels probably is part of established PCa therapies – especially regarding PSMA-directed approaches.

CONCLUSION

Targeting angiogenesis with specific inhibitors unfortunately has failed to impact OS in patients with mCRPC despite promising early data – and despite convincing clinical activity in several other malignancies. This discrepancy highlights the importance of the microenvironment niche, as PCa is characterized by substantial inter- and intra-patient heterogeneity and adaptive biology. Therapeutic strategies to overcome this challenge include biomarker-guided screening for patient subgroups most likely to benefit from anti-angiogenesis. Moreover, several trials investigate combinatorial approaches. Beyond specific angiogenesis inhibitors, approved compounds such as antiandrogens, PARP inhibitors and PSMA-targeting approaches probably also have a substantial anti-angiogenic impact in PCa biology.

AUTHOR CONTRIBUTIONS

Conceptualization: AS and MK. Methodology: AS and MK. Writing – draft preparation: AS, CK, and MK. Writing – review and editing: AS, CK, and MK. All authors contributed to the article and approved the submitted version.

FUNDING

This project was supported in part by the Apulian Regional Project Medicina di Precisione to A.G.S. Moreover, M.K. was funded by a personal grant from Else-Kröner-Foundation (Else Kröner Integrative Clinician Scientist College for Translational Immunology, University Hospital Würzburg, Germany). This publication was supported by the Open Access Publication Fund of the University of Würzburg.

REFERENCES

1. Nicholson B, Theodorescu D. Angiogenesis and Prostate Cancer Tumor Growth. *J Cell Biochem* (2004) 91:125–50. doi: 10.1002/jcb.10772
2. Noble RL. Hormonal Control of Growth and Progression in Tumors of Nb Rats and a Theory of Action. *Cancer Res* (1977) 37:82–94.
3. Isaacs JT, Coffey DS. Adaptation Versus Selection as the Mechanism Responsible for the Relapse of Prostatic Cancer to Androgen Ablation Therapy as Studied in the Dunning R-3327-H Adenocarcinoma. *Cancer Res* (1981) 41:5070–5.
4. Debes JD, Tindall DJ. Mechanisms of Androgen-Refractory Prostate Cancer. *N Engl J Med* (2004) 351:1488–90. doi: 10.1056/NEJMp048178
5. Fizazi K, Tran N, Fein L, Matsubara N, Rodriguez-Antolin A, Alekseev BY, et al. Abiraterone Plus Prednisone in Metastatic, Castration-Sensitive Prostate Cancer. *N Engl J Med* (2017) 377:352–60. doi: 10.1056/NEJMoa1704174
6. Scher HI, Fizazi K, Saad F, Taplin M-E, Sternberg CN, Miller K, et al. Increased Survival With Enzalutamide in Prostate Cancer After Chemotherapy. *N Engl J Med* (2012) 367:1187–97. doi: 10.1056/NEJMoa1207506
7. Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, et al. Docetaxel Plus Prednisone or Mitoxantrone Plus Prednisone for Advanced Prostate Cancer. *N Engl J Med* (2004) 351:1502–12. doi: 10.1056/NEJMoa040720
8. Tucci M, Bertaglia V, Vignani F, Buttigliero C, Fiori C, Porpiglia F, et al. Addition of Docetaxel to Androgen Deprivation Therapy for Patients With Hormone-Sensitive Metastatic Prostate Cancer: A Systematic Review and Meta-Analysis. *Eur Urol* (2016) 69:563–73. doi: 10.1016/j.eururo.2015.09.013
9. de Wit R, de Bono J, Sternberg CN, Fizazi K, Tombal B, Wülfing C, et al. Cabazitaxel Versus Abiraterone or Enzalutamide in Metastatic Prostate Cancer. *N Engl J Med* (2019) 381:2506–18. doi: 10.1056/NEJMoa1911206
10. Antonio G, Oronzo B, Vito L, Angela C, Antonel-la A, Roberto C, et al. Immune System and Bone Microenvironment: Rationale for Targeted Cancer Therapies. *Oncotarget* (2020) 11:480–7. doi: 10.18632/oncotarget.27439
11. Argentiero A, Solimando AG, Brunetti O, Calabrese A, Pantano F, Iuliani M, et al. Skeletal Metastases of Unknown Primary: Biological Landscape and Clinical Overview. *Cancers* (2019) 11:1270. doi: 10.3390/cancers11091270
12. Body J-J, Casimiro S, Costa L. Targeting Bone Metastases in Prostate Cancer: Improving Clinical Outcome. *Nat Rev Urol* (2015) 12:340–56. doi: 10.1038/nrurol.2015.90
13. Cursano MC, Iuliani M, Casadei C, Stellato M, Tonini G, Paganelli G, et al. Combination Radium-223 Therapies in Patients With Bone Metastases From Castration-Resistant Prostate Cancer: A Review. *Crit Rev Oncol Hematol* (2020) 146:102864. doi: 10.1016/j.critrevonc.2020.102864
14. Konstantinopoulos PA, Matulonis UA. PARP Inhibitors in Ovarian Cancer: A Trailblazing and Transformative Journey. *Clin Cancer Res* (2018) 24:4062–5. doi: 10.1158/1078-0432.CCR-18-1314
15. Ratta R, Guida A, Scotté F, Neuzillet Y, Teillet AB, Lebret T, et al. PARP Inhibitors as a New Therapeutic Option in Metastatic Prostate Cancer: A Systematic Review. *Prostate Cancer Prostatic Dis* (2020) 23:549–60. doi: 10.1038/s41391-020-0233-3
16. Sartor O, de Bono J, Chi KN, Fizazi K, Herrmann K, Rahbar K, et al. Lutetium-177–PSMA-617 for Metastatic Castration-Resistant Prostate Cancer. *N Engl J Med* (2021) 385:1091–103. doi: 10.1056/NEJMoa2107322
17. Sherwood LM, Parris EE, Folkman J. Tumor Angiogenesis: Therapeutic Implications. *N Engl J Med* (1971) 285:1182–6. doi: 10.1056/NEJM197111182852108
18. Solimando AG, Summa SD, Vacca A, Ribatti D. Cancer-Associated Angiogenesis: The Endothelial Cell as a Checkpoint for Immunological Patrolling. *Cancers* (2020) 12:3380. doi: 10.3390/cancers12113380
19. Strohmeyer D, Rössing C, Bauerfeind A, Kaufmann O, Schlechte H, Bartsch G, et al. Vascular Endothelial Growth Factor and Its Correlation With Angiogenesis and P53 Expression in Prostate Cancer. *Prostate* (2000) 45:216–24. doi: 10.1002/1097-0045(20001101)45:3<216::aid-pros3>3.0.co;2-c
20. Duque JLF, Loughlin KR, Adam RM, Kantoff PW, Zurakowski D, Freeman MR. Plasma Levels of Vascular Endothelial Growth Factor Are Increased in Patients With Metastatic Prostate Cancer. *Urology* (1999) 54:523–7. doi: 10.1016/S0090-4295(99)00167-3
21. Doll JA, Reiher FK, Crawford SE, Pins MR, Campbell SC, Bouck NP. Thrombospondin-1, Vascular Endothelial Growth Factor and Fibroblast Growth Factor-2 Are Key Functional Regulators of Angiogenesis in the Prostate. *Prostate* (2001) 49:293–305. doi: 10.1002/pros.10025
22. Borre M, Nerstrøm B, Overgaard J. Association Between Immunohistochemical Expression of Vascular Endothelial Growth Factor (VEGF), VEGF-Expressing Neuroendocrine-Differentiated Tumor Cells, and Outcome in Prostate Cancer Patients Subjected to Watchful Waiting. *Clin Cancer Res* (2000) 6:1882–90.
23. Bok RA, Halabi S, Fei DT, Rodriquez CR, Hayes DF, Vogelzang NJ, et al. Vascular Endothelial Growth Factor and Basic Fibroblast Growth Factor Urine Levels as Predictors of Outcome in Hormone-Refractory Prostate Cancer Patients: A Cancer and Leukemia Group B Study. *Cancer Res* (2001) 61:2533–6.
24. Shariat SF, Anwuri VA, Lamb DJ, Shah NV, Wheeler TM, Slawin KM. Association of Preoperative Plasma Levels of Vascular Endothelial Growth Factor and Soluble Vascular Cell Adhesion Molecule-1 With Lymph Node Status and Biochemical Progression After Radical Prostatectomy. *JCO* (2004) 22:1655–63. doi: 10.1200/JCO.2004.09.142
25. Mukherji D, Temraz S, Wehbe D, Shamseddine A. Angiogenesis and Anti-Angiogenic Therapy in Prostate Cancer. *Crit Rev Oncol/Hematol* (2013) 87:122–31. doi: 10.1016/j.critrevonc.2013.01.002
26. Ribatti D, Solimando AG, Pezzella F. The Anti-VEGF(R) Drug Discovery Legacy: Improving Attrition Rates by Breaking the Vicious Cycle of Angiogenesis in Cancer. *Cancers* (2021) 13:3433. doi: 10.3390/cancers13143433
27. Solimando AG, Da Vià MC, Leone P, Borrelli P, Croci GA, Tabares P, et al. Halting the Vicious Cycle Within the Multiple Myeloma Ecosystem: Blocking JAM-A on Bone Marrow Endothelial Cells Restores Angiogenic Homeostasis and Suppresses Tumor Progression. *Haematol* (2020) 106:1943–56. doi: 10.3324/haematol.2019.239913
28. Rao L, Giannico D, Leone P, Solimando AG, Maiorano E, Caporusso C, et al. HB-EGF–EGFR Signaling in Bone Marrow Endothelial Cells Mediates Angiogenesis Associated With Multiple Myeloma. *Cancers* (2020) 12:173. doi: 10.3390/cancers12010173
29. Solimando AG, Annese T, Tamma R, Ingravallo G, Maiorano E, Vacca A, et al. New Insights Into Diffuse Large B-Cell Lymphoma Pathobiology. *Cancers* (2020) 12:1869. doi: 10.3390/cancers12071869
30. Krebs M, Solimando AG, Kalogirou C, Marquardt A, Frank T, Sokolakis I, et al. MiR-221-3p Regulates VEGFR2 Expression in High-Risk Prostate Cancer and Represents an Escape Mechanism From Sunitinib *In Vitro*. *JCM* (2020) 9:670. doi: 10.3390/jcm9030670
31. Melegh Z, Oltean S. Targeting Angiogenesis in Prostate Cancer. *IJMS* (2019) 20:2676. doi: 10.3390/ijms20112676
32. Sarkar C, Goswami S, Basu S, Chakroborty D. Angiogenesis Inhibition in Prostate Cancer: An Update. *Cancers* (2020) 12:2382. doi: 10.3390/cancers12092382
33. Kelly WK, Halabi S, Carducci M, George D, Mahoney JF, Stadler WM, et al. Randomized, Double-Blind, Placebo-Controlled Phase III Trial Comparing Docetaxel and Prednisone With or Without Bevacizumab in Men With Metastatic Castration-Resistant Prostate Cancer: CALGB 90401. *JCO* (2012) 30:1534–40. doi: 10.1200/JCO.2011.39.4767
34. Tannock IF, Fizazi K, Ivanov S, Karlsson CT, Fléchon A, Skoneczna I, et al. Aflibercept Versus Placebo in Combination With Docetaxel and Prednisone for Treatment of Men With Metastatic Castration-Resistant Prostate Cancer (VENICE): A Phase 3, Double-Blind Randomised Trial. *Lancet Oncol* (2013) 14:760–8. doi: 10.1016/S1470-2045(13)70184-0
35. Michaelson MD, Oudard S, Ou Y-C, Sengeløv L, Saad F, Houede N, et al. Randomized, Placebo-Controlled, Phase III Trial of Sunitinib Plus Prednisone Versus Prednisone Alone in Progressive, Metastatic, Castration-Resistant Prostate Cancer. *JCO* (2014) 32:76–82. doi: 10.1200/JCO.2012.48.5268
36. Petrylak DP, Vogelzang NJ, Budnik N, Wiechno PJ, Sternberg CN, Doner K, et al. Docetaxel and Prednisone With or Without Lenalidomide in Chemotherapy-Naive Patients With Metastatic Castration-Resistant Prostate Cancer (MAINSAIL): A Randomised, Double-Blind, Placebo-Controlled Phase 3 Trial. *Lancet Oncol* (2015) 16:417–25. doi: 10.1016/S1470-2045(15)70025-2
37. Solimando AG, Vacca A, Ribatti DA. Comprehensive Biological and Clinical Perspective Can Drive a Patient-Tailored Approach to Multiple Myeloma: Bridging the Gaps Between the Plasma Cell and the Neoplastic Niche. *J Oncol* (2020) 2020:1–16. doi: 10.1155/2020/6820241
38. Lamanuzzi A, Saltarella I, Desantis V, Frassanito MA, Leone P, Racanelli V, et al. Inhibition of MTOR Complex 2 Restrains Tumor Angiogenesis in

Multiple Myeloma. *Oncotarget* (2018) 9:20563–77. doi: 10.18632/oncotarget.25003
39. Solimando AG, Da Vià MC, Cicco S, Leone P, Di Lernia G, Giannico D, et al. High-Risk Multiple Myeloma: Integrated Clinical and Omics Approach Dissects the Neoplastic Clone and the Tumor Microenvironment. *JCM* (2019) 8:997. doi: 10.3390/jcm8070997
40. Stahl M, Zeidan AM. Lenalidomide Use in Myelodysplastic Syndromes: Insights Into the Biologic Mechanisms and Clinical Applications: Use of Lenalidomide in MDS: Biology and Efficacy. *Cancer* (2017) 123:1703–13. doi: 10.1002/cncr.30585
41. Moschetta M, Basile A, Ferrucci A, Frassanito MA, Rao L, Ria R, et al. Novel Targeting of Phospho-CMET Overcomes Drug Resistance and Induces Antitumor Activity in Multiple Myeloma. *Clin Cancer Res* (2013) 19:4371–82. doi: 10.1158/1078-0432.CCR-13-0039
42. Ferrucci A, Moschetta M, Frassanito MA, Berardi S, Catacchio I, Ria R, et al. A HGF/CMET Autocrine Loop Is Operative in Multiple Myeloma Bone Marrow Endothelial Cells and May Represent a Novel Therapeutic Target. *Clin Cancer Res* (2014) 20:5796–807. doi: 10.1158/1078-0432.CCR-14-0847
43. Gnoni A, Licchetta A, Memeo R, Argentiero A, Solimando AG, Longo V, et al. Role of BRAF in Hepatocellular Carcinoma: A Rationale for Future Targeted Cancer Therapies. *Medicina* (2019) 55:754. doi: 10.3390/medicina55120754
44. Argentiero A, Solimando AG, Krebs M, Leone P, Susca N, Brunetti O, et al. Anti-Angiogenesis and Immunotherapy: Novel Paradigms to Envision Tailored Approaches in Renal Cell-Carcinoma. *JCM* (2020) 9:1594. doi: 10.3390/jcm9051594
45. Solimando AG, Susca N, Argentiero A, Brunetti O, Leone P, De Re V, et al. Second-Line Treatments for Advanced Hepatocellular Carcinoma: A Systematic Review and Bayesian Network Meta-Analysis. *Clin Exp Med* (2021) 1–10. doi: 10.1007/s10238-021-00727-7
46. Gherardi E, Birchmeier W, Birchmeier C, Woude GV. Targeting MET in Cancer: Rationale and Progress. *Nat Rev Cancer* (2012) 12:89–103. doi: 10.1038/nrc3205
47. Chatterjee S, Heukamp LC, Siobal M, Schöttle J, Wieczorek C, Peifer M, et al. Tumor VEGF : VEGFR2 Autocrine Feed-Forward Loop Triggers Angiogenesis in Lung Cancer. *J Clin Invest* (2013) 123:1732–40. doi: 10.1172/JCI65385
48. Smith DC, Smith MR, Sweeney C, Elfiky AA, Logothetis C, Corn PG, et al. Cabozantinib in Patients With Advanced Prostate Cancer: Results of a Phase II Randomized Discontinuation Trial. *JCO* (2013) 31:412–9. doi: 10.1200/JCO.2012.45.0494
49. Schimmoller F, Zayzafoon M, Chung LWK, Zhau HE, Fagerlund KM, Aftab DT. Abstract A233: Cabozantinib (XL184), a Dual MET-VEGFR2 Inhibitor, Blocks Osteoblastic and Osteolytic Progression of Human Prostate Cancer Xenografts in Mouse Bone. In: *Proceedings of the Therapeutic Agents: Small Molecule Kinase Inhibitors*. San Francisco, CA: American Association for Cancer Research (2011). p. A233–3.
50. Yakes FM, Chen J, Tan J, Yamaguchi K, Shi Y, Yu P, et al. Cabozantinib (XL184), a Novel MET and VEGFR2 Inhibitor, Simultaneously Suppresses Metastasis, Angiogenesis, and Tumor Growth. *Mol Cancer Ther* (2011) 10:2298–308. doi: 10.1158/1535-7163.MCT-11-0264
51. Sennino B, Ishiguro-Oonuma T, Wei Y, Naylor RM, Williamson CW, Bhagwandin V, et al. Suppression of Tumor Invasion and Metastasis by Concurrent Inhibition of C-Met and VEGF Signaling in Pancreatic Neuroendocrine Tumors. *Cancer Discov* (2012) 2:270–87. doi: 10.1158/2159-8290.CD-11-0240
52. Nguyen HM, Ruppender N, Zhang X, Brown LG, Gross TS, Morrissey C, et al. Cabozantinib Inhibits Growth of Androgen-Sensitive and Castration-Resistant Prostate Cancer and Affects Bone Remodeling. *PloS One* (2013) 8: e78881. doi: 10.1371/journal.pone.0078881
53. Dai J, Zhang H, Karatsinides A, Keller JM, Kozloff KM, Aftab DT, et al. Cabozantinib Inhibits Prostate Cancer Growth and Prevents Tumor-Induced Bone Lesions. *Clin Cancer Res* (2014) 20:617–30. doi: 10.1158/1078-0432.CCR-13-0839
54. Graham TJ, Box G, Tunariu N, Crespo M, Spinks TJ, Miranda S, et al. Preclinical Evaluation of Imaging Biomarkers for Prostate Cancer Bone Metastasis and Response to Cabozantinib. *JNCI: J Natl Cancer Institute* (2014) 106(4):dju033. doi: 10.1093/jnci/dju033
55. Stern PH, Alvares K. Antitumor Agent Cabozantinib Decreases RANKL Expression in Osteoblastic Cells and Inhibits Osteoclastogenesis and PTHrP-Stimulated Bone Resorption: Cabozantinib and the Bone Microenvironment. *J Cell Biochem* (2014) 2033–8. doi: 10.1002/jcb.24879. n/a-n/a.
56. Zhang S, Zhau HE, Osunkoya AO, Iqbal S, Yang X, Fan S, et al. Vascular Endothelial Growth Factor Regulates Myeloid Cell Leukemia-1 Expression Through Neuropilin-1-Dependent Activation of C-MET Signaling in Human Prostate Cancer Cells. *Mol Cancer* (2010) 9:9. doi: 10.1186/1476-4598-9-9
57. Smith MR, Sweeney CJ, Corn PG, Rathkopf DE, Smith DC, Hussain M, et al. Cabozantinib in Chemotherapy-Pretreated Metastatic Castration-Resistant Prostate Cancer: Results of a Phase II Nonrandomized Expansion Study. *JCO* (2014) 32:3391–9. doi: 10.1200/JCO.2013.54.5954
58. Basch E, Autio KA, Smith MR, Bennett AV, Weitzman AL, Scheffold C, et al. Effects of Cabozantinib on Pain and Narcotic Use in Patients With Castration-Resistant Prostate Cancer: Results From a Phase 2 Nonrandomized Expansion Cohort. *Eur Urol* (2015) 67:310–8. doi: 10.1016/j.eururo.2014.02.013
59. Lee RJ, Saylor PJ, Dror Michaelson M, Michael Rothenberg S, Smas ME, Miyamoto DT, et al. A Dose-Ranging Study of Cabozantinib in Men With Castration-Resistant Prostate Cancer and Bone Metastases. *Clin Cancer Res* (2013) 19:3088–94. doi: 10.1158/1078-0432.CCR-13-0319
60. Smith M, De Bono J, Sternberg C, Le Moulec S, Oudard S, De Giorgi U, et al. Phase III Study of Cabozantinib in Previously Treated Metastatic Castration-Resistant Prostate Cancer: COMET-1. *JCO* (2016) 34:3005–13. doi: 10.1200/JCO.2015.65.5597
61. Schulten H-J. Pleiotropic Effects of Metformin on Cancer. *Int J Mol Sci* (2018) 19(10):2850. doi: 10.3390/ijms19102850
62. Keizman D, Ish-Shalom M, Sella A, Gottfried M, Maimon N, Peer A, et al. Metformin Use and Outcome of Sunitinib Treatment in Patients With Diabetes and Metastatic Renal Cell Carcinoma. *Clin Genitourin Cancer* (2016) 14:420–5. doi: 10.1016/j.clgc.2016.04.012
63. Hamieh L, McKay RR, Lin X, Moreira RB, Simantov R, Choueiri TK. Effect of Metformin Use on Survival Outcomes in Patients With Metastatic Renal Cell Carcinoma. *Clin Genitourin Cancer* (2017) 15:221–9. doi: 10.1016/j.clgc.2016.06.017
64. Marquardt A, Solimando AG, Kerscher A, Bittrich M, Kalogirou C, Kübler H, et al. Subgroup-Independent Mapping of Renal Cell Carcinoma—Machine Learning Reveals Prognostic Mitochondrial Gene Signature Beyond Histopathologic Boundaries. *Front Oncol* (2021) 11:621278. doi: 10.3389/fonc.2021.621278
65. Schöpf B, Weissensteiner H, Schäfer G, Fazzini F, Charoentong P, Naschberger A, et al. OXPHOS Remodeling in High-Grade Prostate Cancer Involves MtDNA Mutations and Increased Succinate Oxidation. *Nat Commun* (2020) 11:1487. doi: 10.1038/s41467-020-15237-5
66. Huss WJ, Hanrahan CF, Barrios RJ, Simons JW, Greenberg NM. Angiogenesis and Prostate Cancer: Identification of a Molecular Progression Switch. *Cancer Res* (2001) 61:2736–43.
67. Annese T, Tamma R, De Giorgis M, Ribatti D. MicroRNAs Biogenesis, Functions and Role in Tumor Angiogenesis. *Front Oncol* (2020) 10:581007. doi: 10.3389/fonc.2020.581007
68. Liu C, Kelnar K, Vlassov AV, Brown D, Wang J, Tang DG. Distinct MicroRNA Expression Profiles in Prostate Cancer Stem/Progenitor Cells and Tumor-Suppressive Functions of Let-7. *Cancer Res* (2012) 72:3393–404. doi: 10.1158/0008-5472.CAN-11-3864
69. Xie P, Liu M, Chen F, Wu S, Shao T, Wang W, et al. Long Non-Coding RNA AGAP2-AS1 Silencing Inhibits PDLIM5 Expression Impeding Prostate Cancer Progression *via* Up-Regulation of MicroRNA-195-5p. *Front Genet* (2020) 11:1030. doi: 10.3389/fgene.2020.01030
70. Kalogirou C, Linxweiler J, Schmucker P, Snaebjornsson MT, Schmitz W, Wach S, et al. MiR-205-Driven Downregulation of Cholesterol Biosynthesis Through SQLE-Inhibition Identifies Therapeutic Vulnerability in Aggressive Prostate Cancer. *Nat Commun* (2021) 12:5066. doi: 10.1038/s41467-021-25325-9
71. Moschetta M, Mishima Y, Kawano Y, Manier S, Paiva B, Palomera L, et al. Targeting Vasculogenesis to Prevent Progression in Multiple Myeloma. *Leukemia* (2016) 30:1103–15. doi: 10.1038/leu.2016.3

72. Solimando AG, Da Via' MC, Leone P, Croci G, Borrelli P, Tabares Gaviria P, et al. Adhesion-Mediated Multiple Myeloma (MM) Disease Progression: Junctional Adhesion Molecule a Enhances Angiogenesis and Multiple Myeloma Dissemination and Predicts Poor Survival. *Blood* (2019) 134:855–5. doi: 10.1182/blood-2019-126674
73. Huinen ZR, Huijbers EJM, van Beijnum JR, Nowak-Sliwinska P, Griffioen AW. Anti-Angiogenic Agents — Overcoming Tumour Endothelial Cell Anergy and Improving Immunotherapy Outcomes. *Nat Rev Clin Oncol* (2021) 18:527–40. doi: 10.1038/s41571-021-00496-y
74. Chouaib S, Messai Y, Couve S, Escudier B, Hasmim M, Noman MZ. Hypoxia Promotes Tumor Growth in Linking Angiogenesis to Immune Escape. *Front Immun* (2012) 3:21. doi: 10.3389/fimmu.2012.00021
75. Song Y, Fu Y, Xie Q, Zhu B, Wang J, Zhang B. Anti-Angiogenic Agents in Combination With Immune Checkpoint Inhibitors: A Promising Strategy for Cancer Treatment. *Front Immunol* (2020) 11:1956. doi: 10.3389/fimmu.2020.01956
76. Mateo J, Carreira S, Sandhu S, Miranda S, Mossop H, Perez-Lopez R, et al. DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer. *N Engl J Med* (2015) 373:1697–708. doi: 10.1056/NEJMoa1506859
77. Pritchard CC, Mateo J, Walsh MF, De Sarkar N, Abida W, Beltran H, et al. Inherited DNA-Repair Gene Mutations in Men With Metastatic Prostate Cancer. *N Engl J Med* (2016) 375:443–53. doi: 10.1056/NEJMoa1603144
78. Taylor RA, Fraser M, Livingstone J, Espiritu SMG, Thorne H, Huang V, et al. Germline BRCA2 Mutations Drive Prostate Cancers With Distinct Evolutionary Trajectories. *Nat Commun* (2017) 8:13671. doi: 10.1038/ncomms13671
79. Abida W, Campbell D, Patnaik A, Sautois B, Shapiro J, Vogelzang NJ, et al. Preliminary Results From the TRITON2 Study of Rucaparib in Patients (Pts) With DNA Damage Repair (DDR)-Deficient Metastatic Castration-Resistant Prostate Cancer (MCRPC): Updated Analyses. *Ann Oncol* (2019) 30:v327–8. doi: 10.1093/annonc/mdz248.003
80. Smith MR, Sandhu SK, Kelly WK, Scher HI, Efstathiou E, Lara PN, et al. Pre-Specified Interim Analysis of GALAHAD: A Phase II Study of Niraparib in Patients (Pts) With Metastatic Castration-Resistant Prostate Cancer (MCRPC) and Biallelic DNA-Repair Gene Defects (DRD). *Ann Oncol* (2019) 30:v884–5. doi: 10.1093/annonc/mdz394.043
81. Kwon J, Bakhoum SF. The Cytosolic DNA-Sensing CGAS-STING Pathway in Cancer. *Cancer Discovery* (2020) 10:26–39. doi: 10.1158/2159-8290.CD-19-0761
82. Hoong BYD, Gan YH, Liu H, Chen ES. CGAS-STING Pathway in Oncogenesis and Cancer Therapeutics. *Oncotarget* (2020) 11:2930–55. doi: 10.18632/oncotarget.27673
83. Teply BA, Antonarakis ES. Treatment Strategies for DNA Repair-Deficient Prostate Cancer. *Expert Rev Clin Pharmacol* (2017) 10:889–98. doi: 10.1080/17512433.2017.1338138
84. Melo CM, Vidotto T, Chaves LP, Lautert-Dutra W, Reis RBD, Squire JA. The Role of Somatic Mutations on the Immune Response of the Tumor Microenvironment in Prostate Cancer. *Int J Mol Sci* (2021) 22:9550. doi: 10.3390/ijms22179550
85. Arranz Arija JA, Yu EY, Piulats JM, Gravis G, Laguerre B, Oudard S, et al. 621p Pembrolizumab (Pembro) Plus Olaparib in Patients (Pts) With Docetaxel-Pretreated Metastatic Castration-Resistant Prostate Cancer (MCRPC): KEYNOTE-365 Cohort A Update. *Ann Oncol* (2020) 31:S513–4. doi: 10.1016/j.annonc.2020.08.880
86. Yu E, Xu L, Kim J, Antonarakis ES. KEYLYNK-010: Phase III Study of Pembrolizumab (Pembro) Plus Olaparib (OLA) vs Enzalutamide (ENZA) or Abiraterone (ABI) in ENZA- or ABI-Pretreated Patients (Pts) With Metastatic Castration-Resistant Prostate Cancer (MCRPC) Who Had Progression on Chemotherapy (CTx). *Ann Oncol* (2019) 30:v351–2. doi: 10.1093/annonc/mdz248.050
87. Nizialek E, Antonarakis ES. PARP Inhibitors in Metastatic Prostate Cancer: Evidence to Date. *Cancer Manag Res* (2020) 12:8105–14. doi: 10.2147/CMAR.S227033
88. Antonarakis ES, Gomella LG, Petrylak DP. When and How to Use PARP Inhibitors in Prostate Cancer: A Systematic Review of the Literature With an Update on On-Going Trials. *Eur Urol Oncol* (2020) 3:594–611. doi: 10.1016/j.euo.2020.07.005
89. Hussain M, Mateo J, Fizazi K, Saad F, Shore N, Sandhu S, et al. Survival With Olaparib in Metastatic Castration-Resistant Prostate Cancer. *N Engl J Med* (2020) 383:2345–57. doi: 10.1056/NEJMoa2022485
90. Castro E, Romero-Laorden N, Del Pozo A, Lozano R, Medina A, Puente J, et al. PROREPAIR-B: A Prospective Cohort Study of the Impact of Germline DNA Repair Mutations on the Outcomes of Patients With Metastatic Castration-Resistant Prostate Cancer. *J Clin Oncol* (2019) 37:490–503. doi: 10.1200/JCO.18.00358
91. Eisermann K, Fraizer G. The Androgen Receptor and VEGF: Mechanisms of Androgen-Regulated Angiogenesis in Prostate Cancer. *Cancers* (2017) 9:32. doi: 10.3390/cancers9040032
92. Tentori L, Lacal PM, Muzi A, Dorio AS, Leonetti C, Scarsella M, et al. Poly(ADP-Ribose) Polymerase (PARP) Inhibition or PARP-1 Gene Deletion Reduces Angiogenesis. *Eur J Cancer* (2007) 43:2124–33. doi: 10.1016/j.ejca.2007.07.010
93. Rajesh M, Mukhopadhyay P, Godlewski G, Bátkai S, Haskó G, Liaudet L, et al. Poly(ADP-Ribose)Polymerase Inhibition Decreases Angiogenesis. *Biochem Biophys Res Commun* (2006) 350:1056–62. doi: 10.1016/j.bbrc.2006.09.160
94. Touat M, Ileana E, Postel-Vinay S, André F, Soria J-C. Targeting FGFR Signaling in Cancer. *Clin Cancer Res* (2015) 21:2684–94. doi: 10.1158/1078-0432.CCR-14-2329
95. Giacomini A, Grillo E, Rezzola S, Ribatti D, Rusnati M, Ronca R, et al. The FGF/FGFR System in the Physiopathology of the Prostate Gland. *Physiol Rev* (2021) 101:569–610. doi: 10.1152/physrev.00005.2020
96. Hanahan D, Bergers G, Bergsland E. Less Is More, Regularly: Metronomic Dosing of Cytotoxic Drugs Can Target Tumor Angiogenesis in Mice. *J Clin Invest* (2000) 105:1045–7. doi: 10.1172/JCI9872
97. Kerbel RS, Kamen BA. The Anti-Angiogenic Basis of Metronomic Chemotherapy. *Nat Rev Cancer* (2004) 4:423–36. doi: 10.1038/nrc1369
98. Derosa L, Galli L, Orlandi P, Fioravanti A, Di Desidero T, Fontana A, et al. Docetaxel Plus Oral Metronomic Cyclophosphamide: A Phase II Study With Pharmacodynamic and Pharmacogenetic Analyses in Castration-Resistant Prostate Cancer Patients: Docetaxel and Metronomic Chemotherapy. *Cancer* (2014) 120:3923–31. doi: 10.1002/cncr.28953
99. Barroso-Sousa R, da Fonseca LG, Souza KT, Chaves ACR, Kann AG, de Castro G, et al. Metronomic Oral Cyclophosphamide Plus Prednisone in Docetaxel-Pretreated Patients With Metastatic Castration-Resistant Prostate Cancer. *Med Oncol* (2015) 32:443. doi: 10.1007/s12032-014-0443-4
100. Calvani N, Morelli F, Naglieri E, Gnoni A, Chiuri VE, Orlando L, et al. Metronomic Chemotherapy With Cyclophosphamide Plus Low Dose of Corticosteroids in Advanced Castration-Resistant Prostate Cancer Across the Era of Taxanes and New Hormonal Drugs. *Med Oncol* (2019) 36:80. doi: 10.1007/s12032-019-1304-y
101. Caffo O, Facchini G, Biasco E, Ferraù F, Morelli F, Donini M, et al. Activity and Safety of Metronomic Cyclophosphamide in the Modern Era of Metastatic Castration-Resistant Prostate Cancer. *Future Oncol* (2019) 15:1115–23. doi: 10.2217/fon-2018-0715
102. Ladoire S, Eymard JC, Zanetta S, Mignot G, Martin E, Kermarrec I, et al. Metronomic Oral Cyclophosphamide Prednisolone Chemotherapy Is an Effective Treatment for Metastatic Hormone-Refractory Prostate Cancer After Docetaxel Failure. *Anticancer Res* (2010) 30:4317–23.
103. Di Desidero T, Derosa L, Galli L, Orlandi P, Fontana A, Fioravanti A, et al. Clinical, Pharmacodynamic and Pharmacokinetic Results of a Prospective Phase II Study on Oral Metronomic Vinorelbine and Dexamethasone in Castration-Resistant Prostate Cancer Patients. *Invest N Drugs* (2016) 34:760–70. doi: 10.1007/s10637-016-0385-0
104. Fontana A, Galli L, Fioravanti A, Orlandi P, Galli C, Landi L, et al. Clinical and Pharmacodynamic Evaluation of Metronomic Cyclophosphamide, Celecoxib, and Dexamethasone in Advanced Hormone-Refractory Prostate Cancer. *Clin Cancer Res* (2009) 15:4954–62. doi: 10.1158/1078-0432.CCR-08-3317
105. Laheurte C, Thiery-Vuillemin A, Calcagno F, Legros A, Simonin H, Boullerot L, et al. Metronomic Cyclophosphamide Induces Regulatory T Cells Depletion and PSA-Specific T Cells Reactivation in Patients With Biochemical Recurrent Prostate Cancer. *Int J Cancer* (2020) 147:1199–205. doi: 10.1002/ijc.32803
106. Mitra Ghosh T, White J, Davis J, Mazumder S, Kansom T, Skarupa E, et al. Identification and Characterization of Key Differentially Expressed Genes Associated With Metronomic Dosing of Topotecan in Human Prostate Cancer. *Front Pharmacol* (2021) 12:736951. doi: 10.3389/fphar.2021.736951
107. Chang SS, Reuter VE, Heston WD, Bander NH, Grauer LS, Gaudin PB. Five Different Anti-Prostate-Specific Membrane Antigen (PSMA) Antibodies

Confirm PSMA Expression in Tumor-Associated Neovasculature. *Cancer Res* (1999) 59:3192–8.
108. Nguyen DP, Xiong PL, Liu H, Pan S, Leconet W, Navarro V, et al. Induction of PSMA and Internalization of an Anti-PSMA MAb in the Vascular Compartment. *Mol Cancer Res* (2016) 14:1045–53. doi: 10.1158/1541-7786.MCR-16-0193
109. Derlin T, Kreipe H-H, Schumacher U, Soudah B. PSMA Expression in Tumor Neovasculature Endothelial Cells of Follicular Thyroid Adenoma as Identified by Molecular Imaging Using 68ga-PSMA Ligand PET/CT. *Clin Nucl Med* (2017) 42:e173–4. doi: 10.1097/RLU.0000000000001487

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frontiers in Immunology

ORIGINAL RESEARCH
published: 22 February 2022
doi: 10.3389/fimmu.2022.843077

Molecular Characteristics, Clinical Significance, and Cancer Immune Interactions of Angiogenesis-Associated Genes in Gastric Cancer

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Specialty section:
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

Received: 24 December 2021
Accepted: 03 February 2022
Published: 22 February 2022

Citation:
Qing X, Xu W, Liu S, Chen Z, Ye C and Zhang Y (2022) Molecular Characteristics, Clinical Significance, and Cancer Immune Interactions of Angiogenesis-Associated Genes in Gastric Cancer. Front. Immunol. 13:843077. doi: 10.3389/fimmu.2022.843077

Background: Immunotherapy has evolved as a critical option to treat diverse cancers. The active response to immunotherapy relies on the unique interaction between cancer and the tumor microenvironment (TME). Angiogenesis is one of the hallmarks of cancer. However, the association between angiogenesis and clinical outcome, immune cell infiltration, and immunotherapy remains unknown in gastric cancer (GC).

Methods: We systematically assessed 36 angiogenesis-associated genes (AAGs) and comprehensively identified the correlation between angiogenesis and transcriptional patterns, prognosis, and immune cell infiltration. The AAG_score was applied to quantify the angiogenesis subtypes of each patient. We then evaluated their values in prognostic prediction and therapeutic responses in GC.

Results: We discussed the mutations of AAGs in GC specimens from genetic levels and identified their expression patterns from TCGA and GEO cohorts. We determined two different molecular subtypes and observed that AAG mutations were related to patients' clinicopathological characteristics, prognosis, and infiltrating TME. Next, an AAG_score for predicting overall survival (OS) was established and its reliable predictive ability in GC patients was confirmed. Furthermore, we created a highly reliable nomogram to facilitate the clinical viability of the AAG_score. A low AAG_score, characterized by elevated microsatellite instability-high, mutation burden, and immune activation, demonstrated a superior OS. Additionally, the AAG_score was remarkedly correlated with the cancer stem cell index and drug susceptibility.

Conclusion: Collectively, we identified a prognostic AAG signature for GC patients. This signature may contribute to clarifying the characteristics of TME and enable the exploration of more potent immunotherapy strategies.

Keywords: gastric cancer, angiogenesis, prognosis, tumor microenvironment, immunotherapy

INTRODUCTION

Immunotherapy is a blooming treatment modality for diverse tumors, and its effectiveness against tumors is being confirmed by a growing body of clinical studies (1–3). Common immunotherapeutic strategies include ICP inhibitors (ICIs), therapeutic antibodies, and cell therapy. The studies of ICIs for PD-1, PD-L1, and CTLA-4 are emerging and clinical reports have proven their safety and effectiveness (4, 5). However, persistent benefits were only realized in a minority of patients. Accumulative studies demonstrate that the tumor microenvironment (TME) is responsible for the aggressive behaviors of tumors and affects the tumor response for immunotherapy (6). The TME consists of various factors, including tumor cells, blood vessels, infiltrating immune cells, stromal cells, tissue fluid, and cytokines (7). The formation of new blood vessels is a hallmark of TME and is characterized by continuous and disordered. Typically, tumor cells promote angiogenesis and inflammation, thus evading the surveillance and clearance of the immune system (8). Therefore, global analysis of the relationship between angiogenesis and TME can discover different neoplastic immunophenotypes and boost the predictive power of immunotherapy.

Gastric cancer (GC), a prevalent malignancy, has a rapid increase in incidence annually (9). Despite advances in chemotherapeutic regimens for advanced GC, such as 5-FU-based regimen and platinum-based regimen, chemotherapy effects remain unsatisfactory, with overall survival (OS) struggling to exceed 2 years (10, 11). Accordingly, targeted therapy is a future development direction to target GC. In recent years, various targeted drugs have been developed, however, overall results remain disappointing (12). Immunotherapy offers additional options for GC patients and brings hope for the treatment of GC. Although immunotherapy has brought huge benefits to GC patients, it has also been found that specific types of patients benefit from immunotherapy (13). It is necessary to develop valuable biomarkers that can classify patients with different characteristics into diverse groups and predict the effect of immunotherapy.

Angiogenesis is one of the crucial elements to support tumor growth and development, and various angiogenic factors tend to be overexpressed (14). Recently, the inhibition of angiogenesis has emerged as an encouraging therapeutic option, particularly for tumors where conventional treatment is unavailable (15). However, the majority of the present studies are focused on identifying the role of individual angiogenesis-associated genes (AAGs) on the progression and prognosis of GC. In addition, Expression proteins of AAGs are often used as therapeutic targets for tumors, and exploring the relationship between AAGs and tumor innate immune may contribute to further combining targeted therapy and immunotherapy (16, 17).

We systematically analyzed the expression of AAGs and their impact on the development, prognosis, TME, and therapeutic response of GC patients. We identified three distinct angiogenesis subgroups in GC with the TCGA database and GEO database. Next, we assessed the molecular characteristics, prognostic significance, and infiltrating immune cell intensities of the identifying angiogenesis clusters. Furthermore, we obtained an AAG_score that accurately predicted the clinical outcome of GC patients and immunotherapeutic effect. We expect that this study will contribute to the development of viable immunotherapies for GC.

MATERIALS AND METHODS

Data Collection

The RNA expression data, somatic mutation data, CNV files, and corresponding clinicopathological information of GC were retrieved from the TCGA-STAD program, and GSE84337 from the GEO repository was utilized to acquire clinical parameters and normalized gene expression data (18). Samples lacking significant clinicopathological or survival information were excluded from further analysis. 36 AAGs were obtained from the MSigDB Team (Hallmark Gene set) (**Table S1**).

Consensus Clustering Analysis of AAGs

Consensus clustering was employed to define distinct angiogenesis-related patterns by the k-means algorithms (19). The quantity, as well as consistency of clusters, were built by the consensus clustering algorithm, which is available in the "ConsensuClusterPlus" package (20). 1000 iterations were performed to ensure the stability of these categories. To identify the biological functional differences in AAGs, gene set variation analysis (GSVA) was conducted with the KEGG gene set (c2.cp.kegg.v7.4) (21).

Association Between Molecular Patterns With the Clinical Characteristics and Prognosis of GC

To determine the clinical significance of the clusters generated by consensus clustering, we investigated the association between molecular patterns, clinical features, and survival outcomes. The clinical variables included age, gender, T-stage, and N-stage. Moreover, the differences in OS between different patterns were evaluated with Kaplan–Meier analysis obtained by the "survival" and "survminer" packages (22).

Relationship of Molecular Patterns With TME in GC

We assess the immune and stromal scores of GC patients with the ESTIMATE algorithm (23). Next, the levels of 22 immune cell subtypes of each patient were computed with the CIBERSORT algorithm (24). The infiltrating fractions of immune cells were also identified with a single-sample gene set enrichment analysis (ssGSEA) algorithm (25). We then evaluated the association between the two subsets on PD-1, PD-L1, and CTLA-4 expression.

Identification of DEGs and Functional Enrichment Analysis

To identify DEGs in the distinct angiogenesis subgroups, we used the "limma" package with criteria of $|\log2\text{-fold change (FC)}| \geq 1$ and p-value < 0.05. On the basis of these DEGs, GO and KEGG analysis was carried out with the "clusterProfiler" package (26).

Development of the Angiogenesis-Associated Prognostic AAG_Score

An AAG_score was constructed to quantitatively assess angiogenesis in individual GC patients. The expression data of DEGs from distinct angiogenesis clusters were standardized across GC specimens and the intersect genes were selected. The differential assessment demonstrated that there are 234 DEGs between the two angiogenesis clusters. Next, we conducted univariate Cox regression (uniCox) analysis for DEGs. Survival-related genes were retained for further analysis. We carried on principal component analysis (PCA) to generate angiogenesis-associated gene scores with the following algorithm: AAG_score = expression of a gene [1] × corresponding coefficient [1] + expression of a gene [2] × corresponding coefficient [2] + expression of gene [n] × corresponding coefficient [n].

Clinical Significance and Classification Analysis of the Prognostic AAG_Score

The relevance of the AAG_score to clinical variables was investigated. To identify whether AAG_score was an independent prognostic predictor, we conducted uniCox and multivariate Cox regression (multiCox) analysis for all cohorts. Then, we conducted a classification analysis to explore whether the AAG_score remains its predictive reliable in distinct subgroups based on multiple clinical variables. Furthermore, the infiltrating levels of immune cells and immune checkpoint (ICP) were compared in the different risk score subgroups. Additionally, we examined the correlations between AAG_score and tumor mutation burden (TMB) score, microsatellite instability (MSI) score, and cancer stem cells (CSC) score.

Establishment of a Predictive Nomogram

A nomogram was depicted to provide valuable clinical predictions for HCC patients with their risk scores and other clinicopathological characteristics, particularly about 1-, 3-, and 5-year OS. Next, we performed calibration curve analysis and decision curve analysis (DCA) to verify the clinical reliability of the established nomogram.

Mutation and Drug Sensitivity Analysis

To identify the mutational profiles of GC patients between different risk groups, the mutation annotation format (MAF) from the TCGA database was created with the "maftools" package (27). We also assessed tumor immune dysfunction and exclusion (TIDE) and immunophenotype score (IPS) for GC patients in the two groups. To investigate the clinic performance of chemotherapy agents in patients, we computed the semi-inhibitory concentration (IC50) values of common drugs with the "pRRophetic" package (28).

Statistical Analysis

R software (version 4.1.2) and its relevant packages are applied to process, analyze and present the data. A two-sided P <0.05 was deemed valuable.

RESULTS

Genetic Mutation Landscape of AAGs in GC

We first identified the expression levels of the 36 AAGs in tumor specimens and normal specimens with the TCGA-STAD dataset. A total of 26 DEGs were found, and most of the DEGs were abundant in the tumor samples (**Figure 1A**). A protein-protein interaction (PPI) analysis through the string website was established to reveal the interactivity of DEGs, which indicated that VEGFA, SPP1, POSTN, VTN, COL3A1, and TIMP1 were hub genes (**Figure 1B**). Next, we determined the incidence of CNVs and somatic mutations of 36 AAGs in GC. As depicted in **Figure 1C**, 147 of 433 (33.95%) GC samples presented genetic mutations, and the findings suggested VCAN as the gene with the highest mutation incidence, followed by ITGAV and COL5A2, among the 36 AAGs. Furthermore, we explore CNV mutational incidence, which indicated that 36 AAGs demonstrated evident CNV alterations (**Figure 1D**). **Figure 1E** displays the site of CNV alterations of 36 AAGs on chromosomes. We summarized that CNV may serve a regulative role in the expression of AAGs. The findings indicated a substantial difference in the genomic background and expression levels of AAGs between GC and normal specimens, suggesting the potential role of AAGs in GC tumorigenesis.

Generation of Angiogenesis Subgroups in GC

The detailed flowchart of this work is shown in **Figure S1**. 804 GC patients from TCGA-STAD and GSE84437 were enrolled in this study to reveal the relationship between angiogenesis and tumorigenesis. Complete information of these patients was listed in **Table S2**. The prognostic values of 36 AAGs in GC patients were identified with uniCox and Kaplan–Meier analysis (**Table S3**). Next, the correlation network of AAG interactions, regulator relationships, and their survival significance in GC patients was presented in **Figure 2A**, and **Table S4**.

To further determine the relationship between expression patterns of AAGs and GC subtypes, we performed a consensus clustering analysis to classify GC patients according to the expression levels of these AAGs. Our findings indicated that the optimal clustering variable was 2 (**Figure 2B**), and GC patients in the entire cohort were well dispersed in cluster A (n=430) and cluster B (n=378). The result of PCA analysis also confirmed the excellent intergroup distribution (**Figure 2C**). Furthermore, the OS time of the two clusters was discussed, and a significant survival difference was observed (**Figure 2D**). Additionally, as displayed in **Figure 2E**, the genomic expression and clinicopathological variables of both clusters were compared, and a substantial difference of AAGs expression and clinical features were identified.

Characteristics of the TME in Different Subgroups

According to the findings of GSVA analysis, cluster A was abundant in cancer-associated pathways (multiple cancer such as renal cell carcinoma, glioma, prostate cancer, and melanoma)

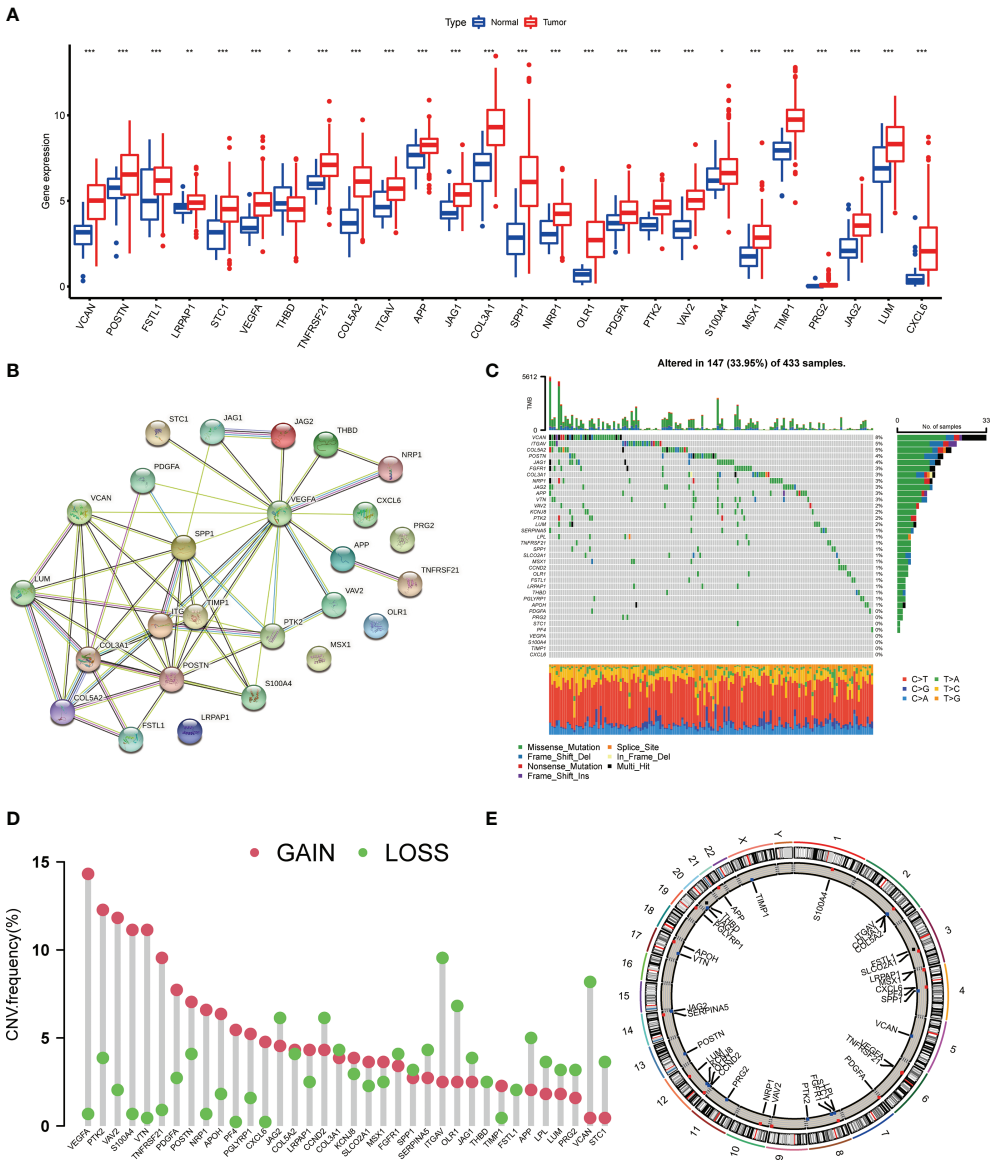

FIGURE 1 | Genetic mutational landscape of AAGs in GC. **(A)** Expression distributions of DEGs between GC and normal tissues. **(B)** The PPI network acquired from the STRING database among the DEGs. **(C)** Genetic alteration on a query of AAGs. **(D)** Frequencies of CNV gain, loss, and non-CNV among AAGs. **(E)** Circus plots of chromosome distributions of AAGs. ($p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***).

and metastasis-associated pathways (regulation of cell adhesion molecules cams, ECM receptor interaction, and focal adhesion) (**Figure 3A** and **Table S5**). To identify the relationship between AAGs and the TME of GC, we explore the infiltrating levels of 23 human immune cell subpopulations in the two clusters with the CIBERSORT algorithm (**Table S6**). As shown in **Figure 3B**, a substantial enrichment difference of most immune cells between both clusters was noticed. The enrichment levels of activated B cell, activated CD8 T cell, activated DC cell, CD56bright NK cell, gd T cell, immature B cell, immature DC cell, MDSC, macrophage, mast cell, NK T cell, NK cell, plasmacytoid DC cell, regulatory T cell, T follicular helper cell, and type 1 T helper cell were markedly higher in the cluster A than cluster B, while the opposite performance of neutrophil was observed. Moreover, the expression of three critical ICPs (PD-1, PD-L1, and CTLA-4) was notably greater of cluster A than cluster B (**Figures 3C–E**). And TME scores could evaluate the abundance of immune and stromal elements in TME, we further executed the ESTIMATE algorithm to obtain the TME scores in the different clusters, including stromal score, immune score, and estimate score. The findings indicated patients in cluster A had higher TME scores (**Figure 3F**).

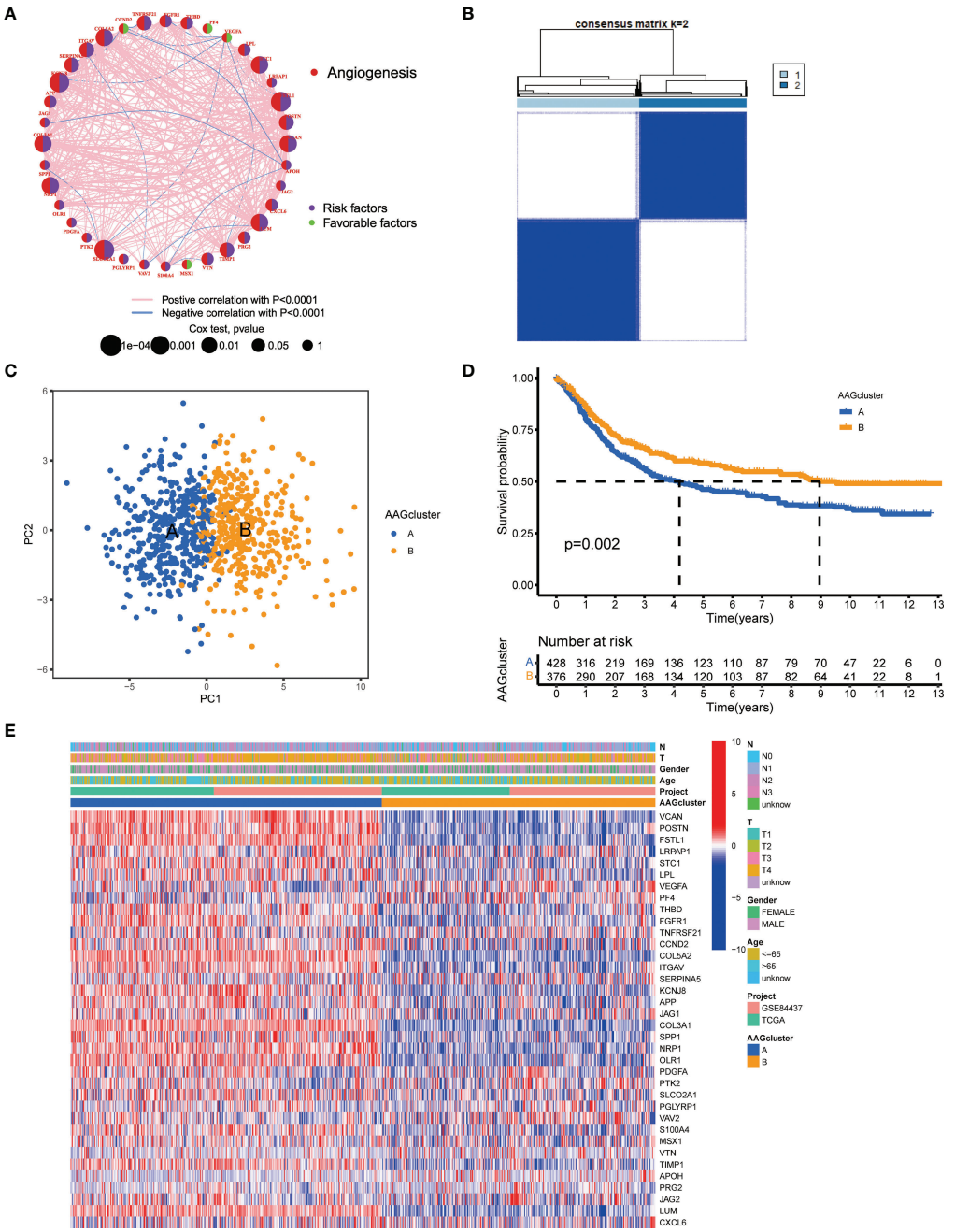

FIGURE 2 | AAG subgroups and clinicopathological and biological characteristics of two distinct subtypes of samples divided by consistent clustering. **(A)** A network of correlations including AAGs in the TCGA cohort. **(B)** Consensus matrix heatmap defining two clusters (k = 2) and their correlation area. **(C)** PCA analysis indicating an obvious difference in transcriptomes between the two subgroups. **(D)** Univariate analysis showing 36 AAGs correlated with OS. **(E)** Differences in clinicopathologic characteristics and expression levels of AAGs between the two distinct subgroups.

Identification of Gene Subgroups Based on DEGs

To investigate the underlying biological activity of angiogenesis subgroups, we obtained 234 angiogenesis clusters-associated DEGs with the "limma" package and conducted functional enrichment analysis (**Table S7**). These angiogenesis subgroups-associated DEGs were mainly enriched in metastasis-associated biological processes (**Figure 4A**). KEGG analysis demonstrated the abundance of cancer- and metastasis-associated pathways (**Figure 4B**), implying that angiogenesis serves as a crucial factor in the modulation of tumor metastasis. Then, we performed uniCox analysis to determine the survival significance of these

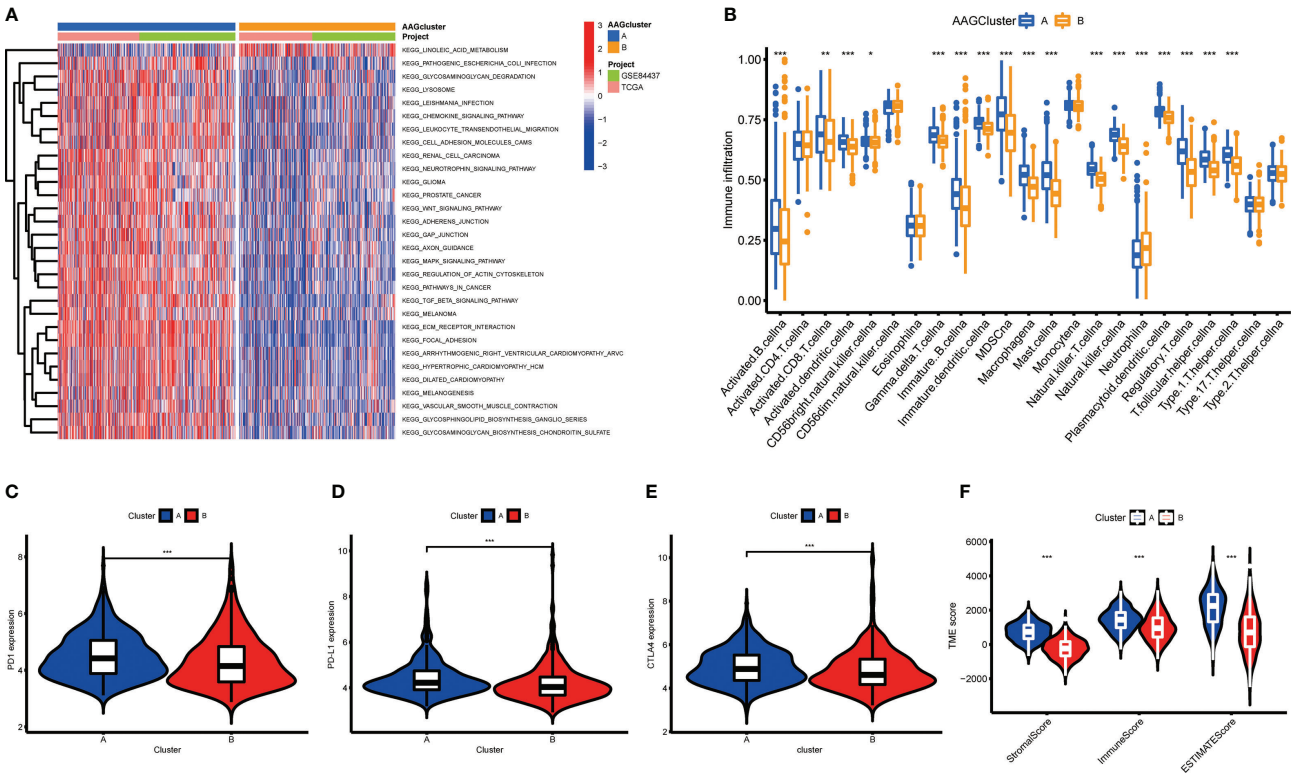

FIGURE 3 | Correlations of tumor immune microenvironments and two GC subgroups. **(A)** GSVA of biological pathways between two distinct subgroups. **(B)** Abundance of 23 infiltrating immune cell types in the two GC subgroups. **(C–E)** Expression levels of PD-1, PD-L1, and CTLA-4 in the two GC subgroups. **(F)** Correlations between the two GC subgroups and TME score. ($p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***).

genes, and 204 genes were extracted with a criterion of $p < 0.05$ (**Table S8**). To investigate specific adjustment mechanisms, a consensus clustering method was utilized to separate patients into different gene clusters (Clusters A-C) on the basis of prognostic genes (**Figure S2**). Kaplan-Meier analysis demonstrated that patients in cluster A had the shortest OS time, whereas patients in cluster C had the superior OS time (**Figure 4C**). Additionally, angiogenesis gene cluster A patterns were related to advanced T- and N-stage (**Figure 4D**). The angiogenesis gene clusters demonstrated substantial discrepancies in AAGs expression, as expected from the angiogenesis subgroups (**Figure 4E**).

Development and Validation of the Prognostic AAG_Score

The AAG_score was created on the basis of cluster-associated DEGs. The GC patients were randomly assigned into a training cohort (n=402) or a test cohort (n=402) at a ratio of 1:1. LASSO and multivariate Cox (multiCox) analysis for 204 angiogenesis cluster-associated prognostic DEGs were conducted to establish an optimal predictive model (**Figure S3**). Ultimately, we acquired two genes (MMP11 and APOD), and the AAG_score was accessed as described: Risk score = (0.1347* expression of MMP11) + (0.1099* expression of APOD). **Figure 5A** displayed the patients' distribution in the two angiogenesis clusters, three gene clusters, and two AAG_score groups.

We discovered a substantial difference in the AAG_score of the angiogenesis clusters and gene clusters (**Figures 5B, C**). We observed the highest AAG_score in gene cluster A and the lowest AAG_score in gene cluster C, implying a low AAG_score may be correlated with immune activation-associated characteristics. Based on the abovementioned survival analysis, we identified that higher risk scores of both classifications were correlated with worse survival. Furthermore, Kaplan-Meier analysis in the training cohort indicated that low-risk patients had a better OS over high-risk patients (**Figure 5D**), and the AUCs of 1-, 3-, and 5-years OS were 0.611, 0.627, and 0.622, respectively (**Figure 5E**). PCA analysis revealed a clear distribution between the two risk groups (**Figure 5F**). The risk plot of AAG_score indicated that as AAG_score increased, OS time decreased while mortality rise (**Figures 5G, H**). Additionally, a heatmap of selected genes was presented in **Figure 5I**.

To evaluate the predictive robustness of AAG_score, we obtained AAG_score of the test cohort and entire cohort (**Figures S4, S5**). The patients were also assigned into different risk subgroups depending on the median score of the training cohort. Similarly, survival analysis presented a superior OS of low-risk patients compared to high-risk patients. Prediction of the 1-, 3-, and 5-year survival probability suggested that the AAG_score still had excellent AUC values, implying that the AAG_score had a great performance to assess the prognosis of GC patients.

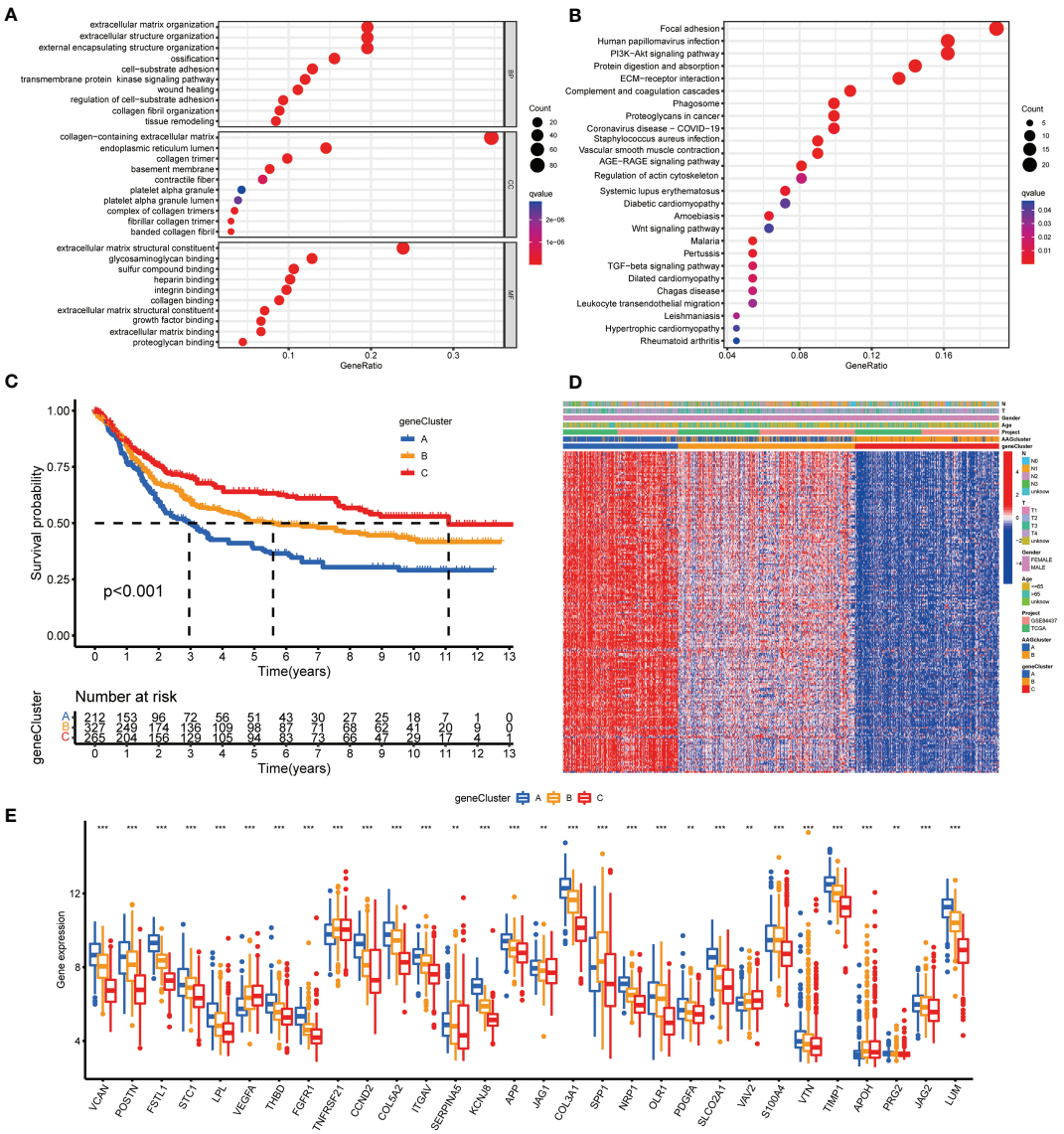

FIGURE 4 | Identification of gene subgroups based on DEGs. **(A, B)** GO and KEGG enrichment analyses of DEGs among two angiogenesis subgroups. **(C)** Kaplan–Meier curves for OS of the three gene clusters. **(D)** Relationships between clinicopathologic features and the three gene clusters. **(E)** Differences in the expression of 36 AAGs among the three gene clusters. ($p < 0.01$ **; $p < 0.001$ ***).

Clinical Correlation Analysis of the Prognostic AAG_Score

To determine the relationship of the AAG_score with clinicopathological features, we discussed the interaction between AAG_score and diverse clinical parameters (age, gender, T-stage, N-stage, and survival status). We found increased risk scores in the higher T- and N-stage (**Figure S6**). Furthermore, the independent prognostic value of AAG_score for GC patients was evaluated. We performed uniCox and multiCox analyses to explore prognostic independence of multiple clinical factors. As presented in **Figure S7**, age, T-stage, N-stage, and risk score in the training cohort demonstrated significant differences, which were concordant with the findings available in the test cohort and entire cohort (**Figure S7**). Moreover, to further explore the prognostic significance of AAG_scores in GC patients, the patients were assigned into different subgroups based on clinical parameters. Overall, the high-risk patient's survival was generally poorer compared to low-risk patients (**Figure S8**).

Construction of a Nomogram to Predict Patients' Prognosis

Due to the high correlation between risk scores and patients' prognosis, we incorporated clinical parameters to establish a nomogram. This nomogram was utilized to estimate 1-, 3-, and 5-year OS for GC patients (**Figure 6A**). The calibration

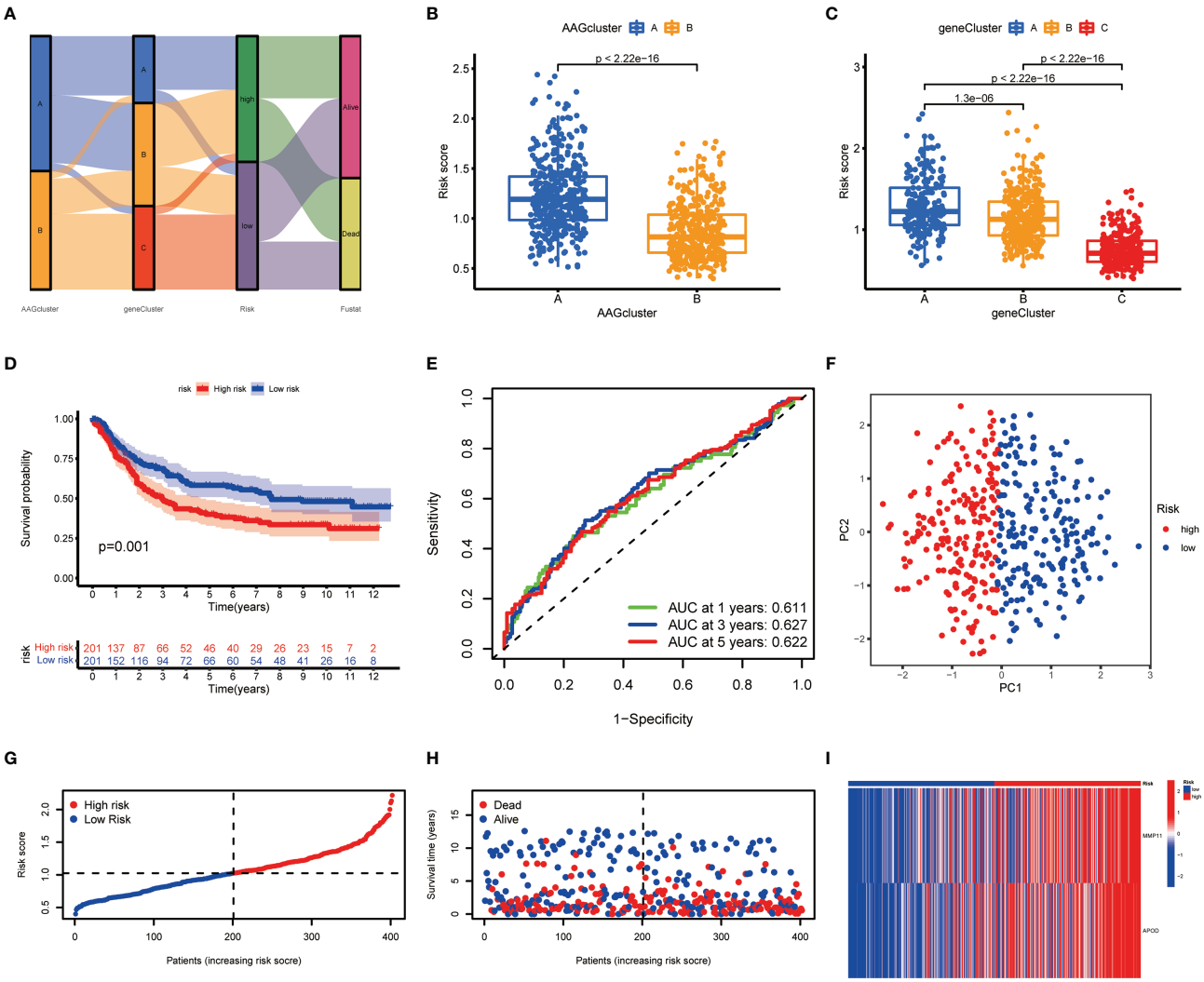

FIGURE 5 | Construction of the AAG_score in the training cohort. **(A)** Alluvial diagram of subgroup distributions in groups with different AAG_scores and clinical outcomes. **(B)** Differences in AAG_score between the two angiogenesis clusters. **(C)** Differences in AAG_score between the three gene clusters. **(D)** Kaplan–Meier analysis of the OS between the two groups. **(E)** ROC curves to predict the sensitivity and specificity of 1-, 3-, and 5-year survival according to the AAG_score. **(F)** PCA analysis based on the prognostic signature. **(G, H)** Ranked dot and scatter plots showing the AAG_score distribution and patient survival status. **(I)** Expression patterns of 2 selected prognostic genes in high- and low-risk groups.

curves of this established nomogram presented great accuracy between actual observations and predicted values (**Figure 6B**). Furthermore, we estimated the AUC values of these clinical factors for predicting OS at 1-, 3-, and 5-year, respectively. As shown in **Figures 6C–E**, the AUC values were as expected, implying this nomogram had an excellent predictive ability for prognosis. Moreover, we also found that this prognostic model with diverse clinical factors presented more net benefits for predicting the prognosis (**Figures 6F–H**). Additionally, we also compared AAG_scores and previously reported prognostic prediction models (29, 30), and the results showed AAG_scores had a superior predictive performance (**Figure S9**).

Assessment of TME and Checkpoints in Distinct Groups

The CIBERSORT algorithm was utilized to evaluate the correlation between AAG_score and immune cells abundance. As depicted in **Figure 7A**, the AAG_score was positively associated with the infiltration of regulatory T cells, resting mast cells, M0 macrophages, M2 macrophages, and resting dendritic cells, while the opposite performance was observed in relationship with AAG_score and follicular helper T cells, CD8 + T cells, activated memory CD4 + T cells, plasma cells, resting NK cells, neutrophils, and activated dendritic cells. Moreover, the AAG_score was positively linked to stromal score, and immune score (**Figure 7B**). We then

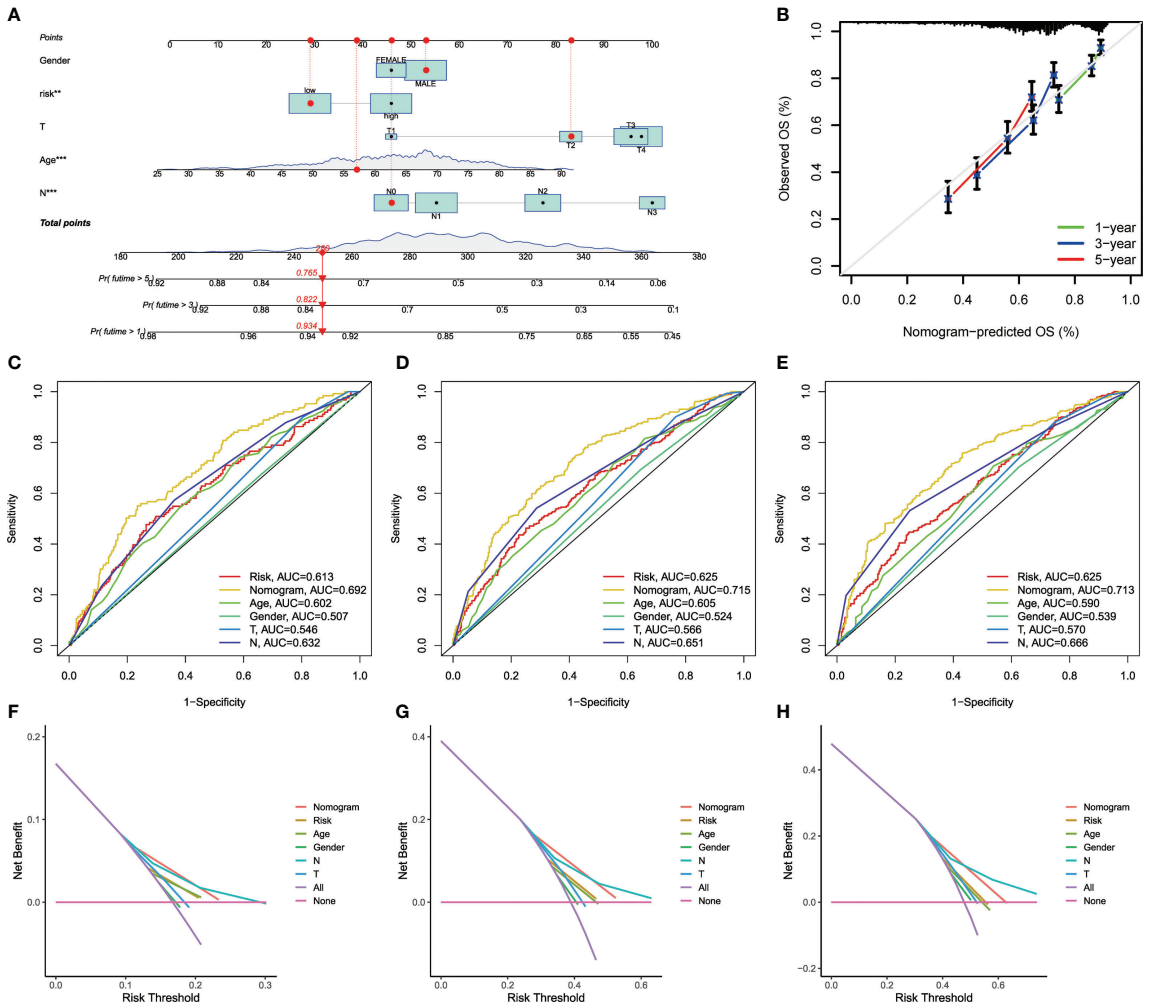

FIGURE 6 | Construction and validation of a nomogram. **(A)** Nomogram for predicting the 1-, 3-, and 5-year OS of GC patients in the entire cohort. **(B)** ROC curves for predicting the 1-, 3-, and 5-year ROC curves in the entire cohort. **(C–E)** The time–dependent ROC curves of the nomograms compared for 1–, 3–, and 5–year OS in GC, respectively. **(F–H)** The DCA curves of the nomograms compared for 1–, 3–, and 5–year OS in HCC, respective.

explore the correlation between the selected genes in the prognostic signature and the enrichments of immune cells. We concluded that the majority of immune cells were closely related to the selected genes (**Figure 7C**). Additionally, we assessed the relationship between ICPs and this prognostic signature. **Figure 7D** demonstrates that 24 ICPs were discrepantly represented in the two risk subgroups, such as PD-1, LAIR1, and VTCN1.

Association of AAG_Score With TMB, MSI, and CSC Score

Numerous studies revealed that TMB and MSI were valuable predictive indicators for tumor immune response, and patients with high TMB or high MSI can benefit from ICP inhibitors (31–33). Our findings demonstrated a higher TMB in the low-risk groups over high-risk groups (**Figure 8A**), suggesting that low-risk patients may benefit more from immunotherapy. A negative correlation of AAG_score and TMB was also observed with Spearman correlation analysis (**Figure 8B**). To explore the impact of TMB status on prognosis in GC patients, we also conducted survival analysis across different TMB subgroups. High-TMB patients had a superior prognosis than low-TMB patients (**Figure 8C**). Subsequently, we combined TMB and AAG_score for survival analysis of GC patients, and the prognostic benefit in the high-TMB group was eliminated by the AAG_score (**Figure 8D**). Similarly, correlation evaluation demonstrated that a low AAG_score was linked to MSI-H pattern, while a high AAG_score was related to the microsatellite stable (MSS) pattern (**Figures 8E, F**). These results also suggested that low-risk patients may be more sensitive to immunotherapy. Furthermore, we integrated the AAG_score and CSC score to evaluate their latent relevance in GC. The relationship between AAG_score and CSC score was presented in **Figure 8G**. We summarized that AAG_score was

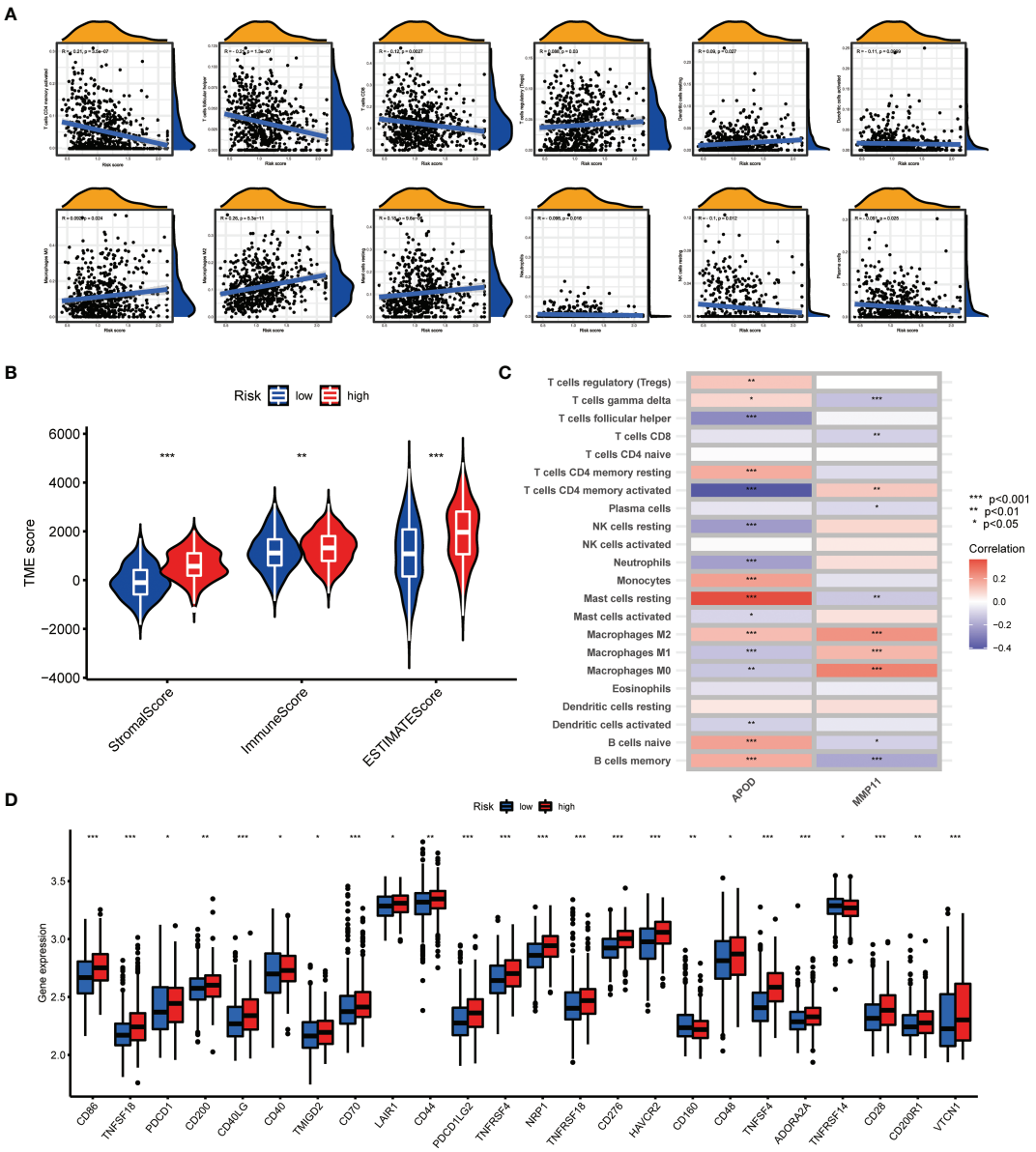

FIGURE 7 | Evaluation of the TME and checkpoints between the two groups. **(A)** Correlations between AAG_score and immune cell types. **(B)** Correlations between AAG_score and both immune and stromal scores. **(C)** Correlations between the abundance of immune cells and selected genes in the prognostic model. **(D)** Expression of immune checkpoints in the high and low-risk groups. ($p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***).

negatively related to the CSC score, suggesting that GC cells with lower AAG_score had more prominent stem cell characteristics and a lower level of cell differentiation. Additionally, we investigated the distribution differences of the somatic mutations between AAG_score patterns in the TCGA-STAD dataset. As presented in **Figures 8H, I**, the mutation incidences of TP53, TTN, MUC16, ARID1A, LRP1B, and SYNE1 were higher than or equal to 20% in GC patients in two risk groups. Interestingly, these genes were mutated at a greater possibility in the low-risk group versus the high-risk group.

Drug Sensitivity Analysis

For unresectable GC patients, chemotherapy, targeted therapy, and immunotherapy may limit tumor progression and improve patients' prognoses (34). To assess the immune response of GC patients, we calculated TIDE scores and IPS scores to predict patients' response-ability. As shown in **Figures 9A–E**, low-risk groups had a lower TIDE score and a higher IPS score, implying that low-risk patients may be more sensitive to immunotherapy (35, 36). Next, to identify the efficacy of AAG_score as a biomarker to predict therapeutic response in GC patients, we estimated the IC50 values

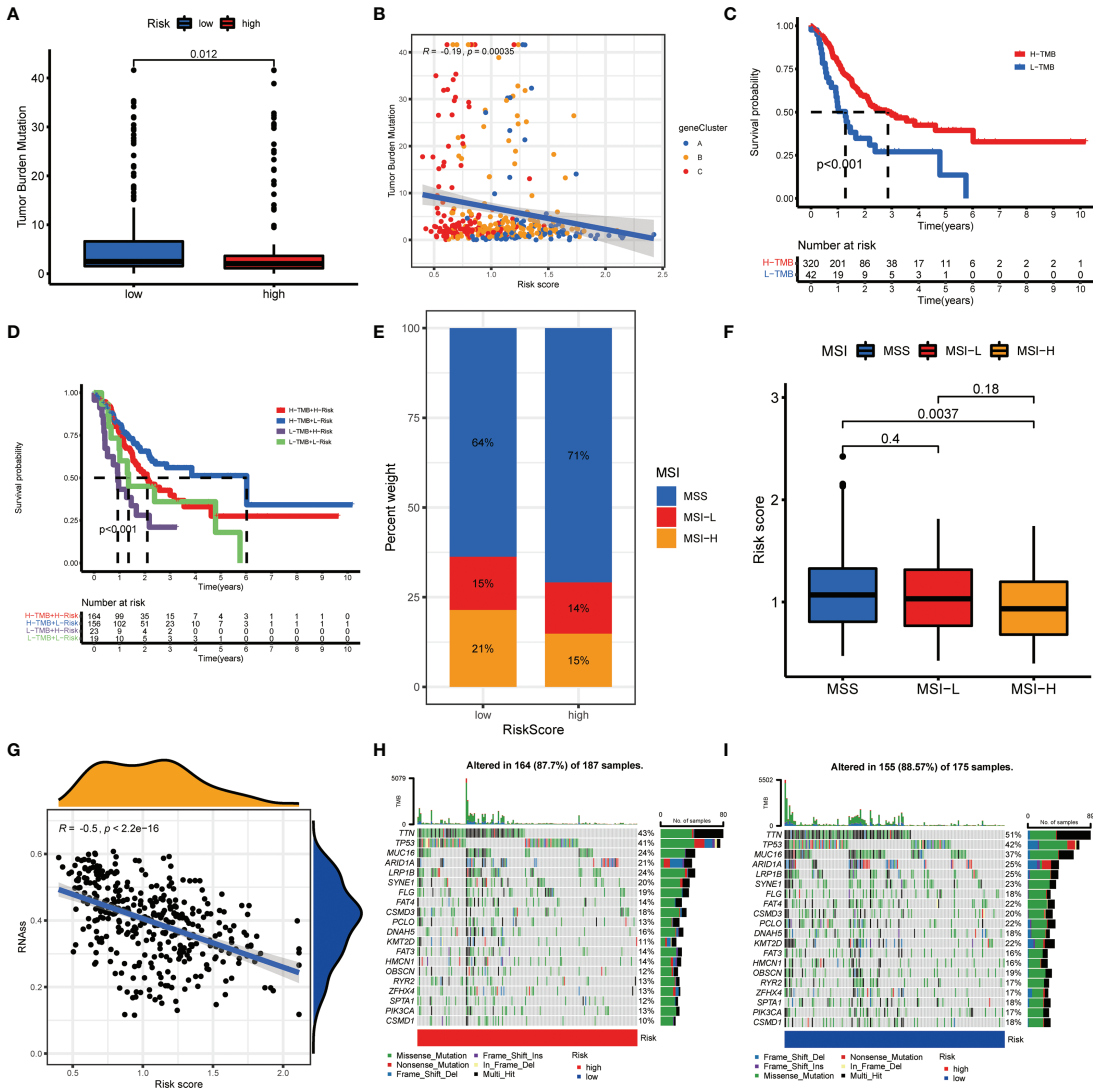

FIGURE 8 | Comprehensive analysis of the AAG_score in GC. **(A, B)** Relationships between AAG_score and TMB. **(C)** Kaplan–Meier analysis of the OS between the low- and high-TMB groups. **(D)** Survival analysis among four patient groups stratified by both TMB and AAG_score. **(E, F)** Relationships between AAG_score and MSI. **(G)** Relationships between AAG_score and CSC index. **(H, I)** The waterfall plot of somatic mutation features established with high and low AAG_scores.

of 138 drugs in TCGA-STAD patients. We discovered that patients with low AAG_scores may positively react to ATRA, gefitinib, gemcitabine, obatoclax.Mesylate, paclitaxel, sorafenib, and bosutinib, while patients with high AAG_scores maybe respond better to docetaxel, shikonin, KU.55933, and multiple targeted therapy agents, including axitinib, dasatinib, erlotinib, imatinib, lapatinib, and nilotinib (**Figures 9F–L**). Overall, these findings indicated that AAGs were correlated with drug sensitivity.

DISCUSSION

Angiogenic cytokines are critical pro-angiogenesis drivers, as well as important immune regulators. Angiogenic cytokines can regulate angiogenic switches as activators or inhibitors during tumor progression in GC (37). And angiogenic cytokines secreted by GC cells activate endothelial cells and autocrine loops to modulate tumor development (38). Additionally, angiogenic cytokines contribute to immune suppression by inhibiting antigen-presenting cells and immune effector cells, or by activating suppressing immune cells (such as Treg and tumor-associated macrophages). These suppressive immune cells can in turn stimulate angiogenesis, resulting in a vicious pattern of impaired immune activation (39). Accumulative evidence has demonstrated the inevitable association between angiogenesis and intrinsic immunity, and angiogenesis targeting may serve a critical role in enhancing cancer immunotherapy (40, 41). However, numerous reports have only emphasized a single AAG or a specific immune cell subtype. Therefore, it is necessary to further clarify the holistic impact and TME

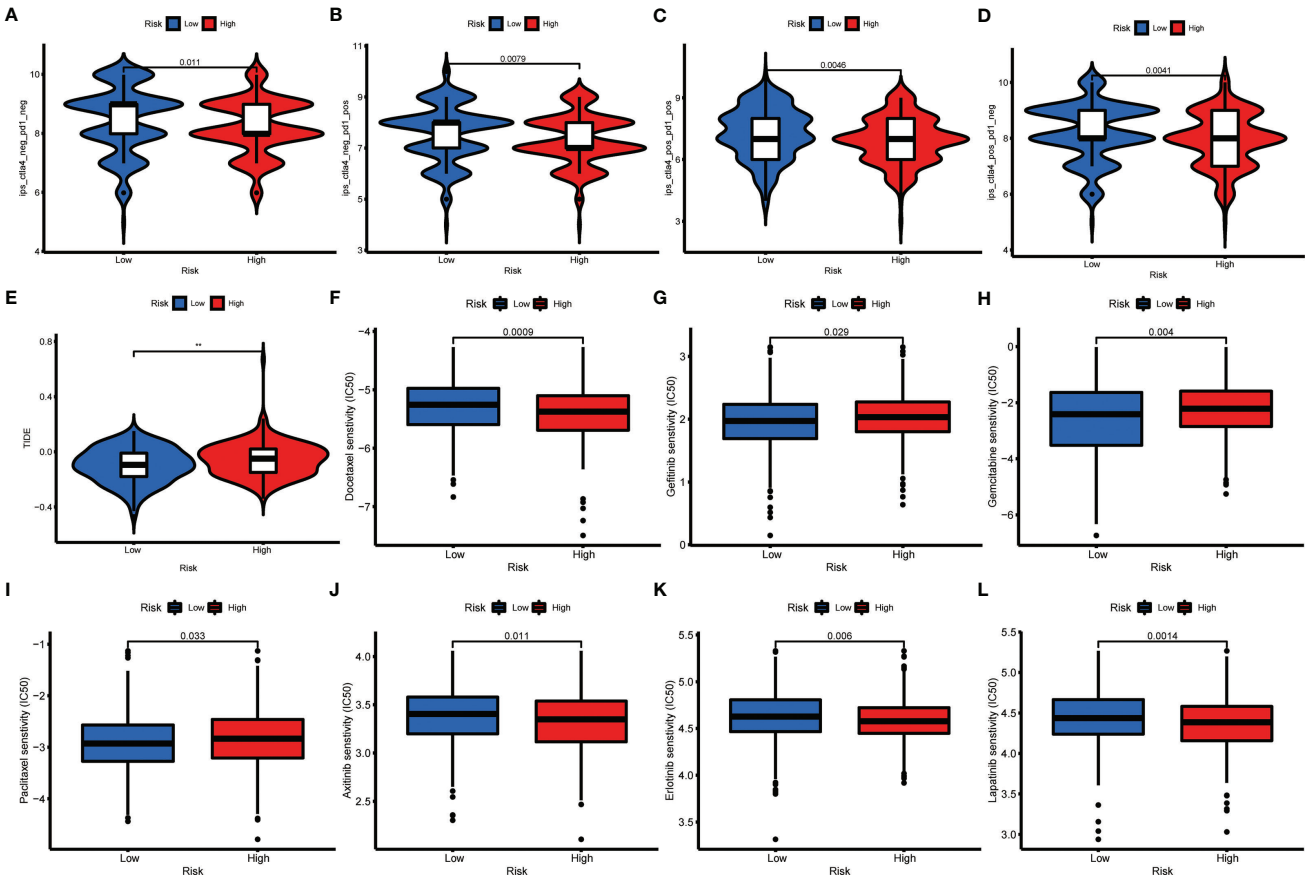

FIGURE 9 | Relationships between AAG_score and therapeutic sensitivity. **(A–D)** IPS in different AAG_score groups. **(E)** TIDE in different AAG_score groups. **(F–L)** Relationships between AAG_score and chemotherapeutic sensitivity. ($p < 0.01$ **).

infiltration features regulated by the combinatorial action of diverse AAGs.

In this research, we identified the transcriptional alterations and expression of AAGs on the basis of the TCGA–STAD cohort. Despite the low mutational intensity of AAGs, most of them are up-regulated in GC patients and associated with prognosis. We then divided GC patients into two angiogenesis subgroups (Cluster A and B) with the unsupervised clustering approach. There were obvious discrepancies in clinical outcomes, immune infiltrations, and functions between the two subgroups. Gene mutations in GC may serve a leading role in the response to immunotherapy. Based on the DEGs related to the subgroups signature, three gene clusters with different clinical features, immune activities, and functions were created for GC. By LASSO Cox regression, AAG_score was established to quantify the angiogenesis subgroups. The cluster A and gene cluster A with the poorest clinical outcomes had the greatest AAG_score among AAG_clusters and three gene clusters. Interestingly, patients with a high AAG_score had unfavorable OS, suggesting that a high AAG_score could predict an unfavorable prognosis. Angiogenesis is involved in the malignant behavior of diverse tumors, including GC (42, 43). Consistently, our GSEA findings demonstrated that cancer- and metastasis-associated pathways were markedly enriched, confirming the existing conclusions.

AAG_score was remarkedly relevant to clinicopathological features of GC. After controlling confounding parameters, the results indicated that AAG_score was an independent predictor for GC patients' survival outcomes. ROCs validated its predictive robustness for 1-, 3-, and 5-year OS. Recently, an angiogenesis-associated risk score has been established for the clinical outcomes of GC patients. Accordingly, AAG_score may have a reliable predictive capacity for patients' prognoses. The aggregation of gene mutations results in carcinogenesis, which is associated with neo-angiogenesis. Our results proved that there was a significant discrepancy in genomic alterations between low and high AAG_scores. Higher TMB has been validated to be related to a better prognosis for GC patients, consistent with our findings (44). The clinical outcomes in the low AAG_score group were evidently superior to those in the low TMB groups, suggesting AAG_score could be utilized to independently predict the responsiveness of immunotherapy.

Immune interactions are critical characteristics of tumorigenesis and therapeutic target for GC. Stromal cells and immune cells are

the primary elements of the TME, and immune and stromal scores are related to clinic characteristics and prognosis in GC (45, 46). We calculated these scores with the ESTIMATE algorithm and found that a high AAG_score group obviously presented higher immune and stromal scores than a low AAG_score group. This suggested that angiogenesis could be associated with the involvement of the TME, thus regulating neoplastic occurrence and development. We identified that higher enrichment of T cells (T helper, CD 4+ and CD 8+T cells) and DCs were correlated with low AAG_score. The enrichment of Tregs, inhibiting the anti-tumor immunoreactivity, was related to poor survival (47). This is concordant with our findings of abundant Tregs in the TME of patients with high AAG_scores. Previous reports also demonstrated that angiogenesis factors may serve as immune modulators, and the immune system could participate in carcinogenesis by inducing pathological vascularization (48, 49). Therefore, targeting angiogenesis may be a valuable regulative strategy for immunotherapy of GC.

At present, GC is gradually resistant to chemotherapy (50). This study identified the potential sensitive drugs for patients in different AAG_socre groups, and the combination of these drugs and targeting angiogenesis may contribute to alleviating drug resistance and improving clinical outcomes. Furthermore, the effectiveness of immunotherapy requires specific biomarkers as a predictive pattern. TIDE and IPS signatures have been created to evaluate ICIs response. Accordingly, we observed that GC patients with low AAG_scores displayed low TIDE scores and positive responsiveness for anti-PD1 and anti-CTLA-4 therapy. Elevated levels of diverse immune cell infiltration were also found in low AAG_scores. This demonstrates that AAG_score has the potential to determine patients who have a better response for ICB.

This study has several limitations. Data from public databases are obtained retrospectively, and inherent selection bias may affect their robustness. And additional clinical variables should be introduced into the study to fully explore the clinical value of AAG_scores. Furthermore, extensive prospective studies and complementary *in vivo* and *in vitro* experimental studies are necessary to gain insight into the relationship between risk scores and TME, thus confirming our findings.

CONCLUSION

Briefly, our systematic analysis of AAGs demonstrates a comprehensive regulatory strategy, and thus influences TME, prognosis, and clinical characteristics of GC patients. We also clarify the potency of AAGs as a biomarker of therapeutic response. Our study reveals the critical clinical significance of AAGs and offers a valuable basis for further researches on personalized therapy in GC patients.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

All authors contributed to the study's conception and design. XQ, WX, and SL performed data collection and analysis. XQ and WX wrote the manuscript. YZ polished and revised the manuscript. All authors commented on previous versions of the manuscript and read and approved the final manuscript.

FUNDING

This study was supported by the National Natural Science Foundation of China (No. 81872255, 62041101), Jiangsu Provincial Maternal and child health scientific research project (No. F202005) and the Key Medical Talents Foundation of Jiangsu Province (No. 2016KJQWZDRC-03).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.843077/full#supplementary-material

REFERENCES

1. Wang F, Wei XL, Wang FH, Xu N, Shen L, Dai GH, et al. Safety, Efficacy and Tumor Mutational Burden as a Biomarker of Overall Survival Benefit in Chemo-Refractory Gastric Cancer Treated With Toripalimab, a PD-1 Antibody in Phase Ib/II Clinical Trial NCT02915432. *Ann Oncol* (2019) 30 (9):1479–86. doi: 10.1093/annonc/mdz197
2. Chalabi M, Fanchi LF, Dijkstra KK, Van den Berg JG, Aalbers AG, Sikorska K, et al. Neoadjuvant Immunotherapy Leads to Pathological Responses in MMR-Proficient and MMR-Deficient Early-Stage Colon Cancers. *Nat Med* (2020) 26(4):566–76. doi: 10.1038/s41591-020-0805-8
3. Sheih A, Voillet V, Hanafi LA, DeBerg HA, Yajima M, Hawkins R, et al. Clonal Kinetics and Single-Cell Transcriptional Profiling of CAR-T Cells in Patients Undergoing CD19 CAR-T Immunotherapy. *Nat Commun* (2020) 11 (1):219. doi: 10.1038/s41467-019-13880-1
4. Jahanafrooz Z, Mosafer J, Akbari M, Hashemzaei M, Mokhtarzadeh A, Baradaran B. Colon Cancer Therapy by Focusing on Colon Cancer Stem Cells and Their Tumor Microenvironment. *J Cell Physiol* (2020) 235(5):4153–66. doi: 10.1002/jcp.29337
5. Saleh R, Taha RZ, Toor SM, Sasidharan Nair V, Murshed K, Khawar M, et al. Expression of Immune Checkpoints and T Cell Exhaustion Markers in Early and Advanced Stages of Colorectal Cancer. *Cancer Immunol Immunother* (2020) 69(10):1989–99. doi: 10.1007/s00262-020-02593-w
6. Bader JE, Voss K, Rathmell JC. Targeting Metabolism to Improve the Tumor Microenvironment for Cancer Immunotherapy. *Mol Cell* (2020) 78(6):1019–33. doi: 10.1016/j.molcel.2020.05.034
7. Kaymak I, Williams KS, Cantor JR, Jones RG. Immunometabolic Interplay in the Tumor Microenvironment. *Cancer Cell* (2021) 39(1):28–37. doi: 10.1016/j.ccell.2020.09.004
8. Jin MZ, Jin WL. The Updated Landscape of Tumor Microenvironment and Drug Repurposing. *Signal Transduct Target Ther* (2020) 5(1):166. doi: 10.1038/s41392-020-00280-x
9. Thrift AP, El-Serag HB. Burden of Gastric Cancer. *Clin Gastroenterol Hepatol* (2020) 18(3):534–42. doi: 10.1016/j.cgh.2019.07.045
10. Biondi A, Lirosi MC, D'Ugo D, Fico V, Ricci R, Santullo F, et al. Neo-Adjuvant Chemo(Radio)Therapy in Gastric Cancer: Current Status and Future Perspectives. *World J Gastrointest Oncol* (2015) 7(12):389–400. doi: 10.4251/wjgo.v7.i12.389

11. Russo AE, Strong VE. Gastric Cancer Etiology and Management in Asia and the West. *Annu Rev Med* (2019) 70:353–67. doi: 10.1146/annurev-med-081117-043436
12. Patel TH, Cecchini M. Targeted Therapies in Advanced Gastric Cancer. *Curr Treat Options Oncol* (2020) 21(9):70. doi: 10.1007/s11864-020-00774-4
13. Zhao Q, Cao L, Guan L, Bie L, Wang S, Xie B, et al. Immunotherapy for Gastric Cancer: Dilemmas and Prospect. *Brief Funct Genomics* (2019) 18 (2):107–12. doi: 10.1093/bfgp/ely019
14. Viallard C, Larrivee B. Tumor Angiogenesis and Vascular Normalization: Alternative Therapeutic Targets. *Angiogenesis* (2017) 20(4):409–26. doi: 10.1007/s10456-017-9562-9
15. El-Kenawi AE, El-Remessy AB. Angiogenesis Inhibitors in Cancer Therapy: Mechanistic Perspective on Classification and Treatment Rationales. *Br J Pharmacol* (2013) 170(4):712–29. doi: 10.1111/bph.12344
16. Ramjiawan RR, Griffioen AW, Duda DG. Anti-Angiogenesis for Cancer Revisited: Is There a Role for Combinations With Immunotherapy? *Angiogenesis* (2017) 20(2):185–204. doi: 10.1007/s10456-017-9552-y
17. Yu WD, Sun G, Li J, Xu J, Wang X. Mechanisms and Therapeutic Potentials of Cancer Immunotherapy in Combination With Radiotherapy and/or Chemotherapy. *Cancer Lett* (2019) 452:66–70. doi: 10.1016/j.canlet.2019.02.048
18. Conesa A, Madrigal P, Tarazona S, Gomez-Cabrero D, Cervera A, McPherson A, et al. A Survey of Best Practices for RNA-Seq Data Analysis. *Genome Biol* (2016) 17:13. doi: 10.1186/s13059-016-0881-8
19. Sabah A, Tiun S, Sani NS, Ayob M, Taha AY. Enhancing Web Search Result Clustering Model Based on Multiview Multirepresentation Consensus Cluster Ensemble (Mmcc) Approach. *PLoS One* (2021) 16(1):e0245264. doi: 10.1371/journal.pone.0245264
20. Seiler M, Huang CC, Szalma S, Bhanot G. ConsensusCluster: A Software Tool for Unsupervised Cluster Discovery in Numerical Data. *OMICS* (2010) 14 (1):109–13. doi: 10.1089/omi.2009.0083
21. Hanzelmann S, Castelo R, Guinney J. GSVA: Gene Set Variation Analysis for Microarray and RNA-Seq Data. *BMC Bioinf* (2013) 14:7. doi: 10.1186/1471-2105-14-7
22. Rich JT, Neely JG, Paniello RC, Voelker CC, Nussenbaum B, Wang EW. A Practical Guide to Understanding Kaplan-Meier Curves. *Otolaryngol Head Neck Surg* (2010) 143(3):331–6. doi: 10.1016/j.otohns.2010.05.007
23. Meng Z, Ren D, Zhang K, Zhao J, Jin X, Wu H. Using ESTIMATE Algorithm to Establish an 8-mRNA Signature Prognosis Prediction System and Identify Immunocyte Infiltration-Related Genes in Pancreatic Adenocarcinoma. *Aging (Albany NY)* (2020) 12(6):5048–70. doi: 10.18632/aging.102931
24. Chen B, Khodadoust MS, Liu CL, Newman AM, Alizadeh AA. Profiling Tumor Infiltrating Immune Cells With CIBERSORT. *Methods Mol Biol* (2018) 1711:243–59. doi: 10.1007/978-1-4939-7493-1_12
25. Huang L, Wu C, Xu D, Cui Y, Tang J. Screening of Important Factors in the Early Sepsis Stage Based on the Evaluation of ssGSEA Algorithm and ceRNA Regulatory Network. *Evol Bioinform Online* (2021) 17:11769343211058463. doi: 10.1177/11769343211058463
26. Yu G, Wang LG, Han Y, He QY. Clusterprofiler: An R Package for Comparing Biological Themes Among Gene Clusters. *OMICS* (2012) 16(5):284–7. doi: 10.1089/omi.2011.0118
27. Mayakonda A, Lin DC, Assenov Y, Plass C, Koeffler HP. Maftools: Efficient and Comprehensive Analysis of Somatic Variants in Cancer. *Genome Res* (2018) 28(11):1747–56. doi: 10.1101/gr.239244.118
28. Geeleher P, Cox N, Huang RS. Prrophetic: An R Package for Prediction of Clinical Chemotherapeutic Response From Tumor Gene Expression Levels. *PLoS One* (2014) 9(9):e107468. doi: 10.1371/journal.pone.0107468
29. Ren H, Zhu J, Yu H, Bazhin AV, Westphalen CB, Renz BW, et al. Angiogenesis-Related Gene Expression Signatures Predicting Prognosis in Gastric Cancer Patients. *Cancers (Basel)* (2020) 12(12):3685. doi: 10.3390/cancers12123685
30. Zheng S, Zhang Z, Ding N, Sun J, Lin Y, Chen J, et al. Identification of the Angiogenesis Related Genes for Predicting Prognosis of Patients With Gastric Cancer. *BMC Gastroenterol* (2021) 21(1):146. doi: 10.1186/s12876-021-01734-4
31. Cristescu R, Mogg R, Ayers M, Albright A, Murphy E, Yearley J, et al. Pan-Tumor Genomic Biomarkers for PD-1 Checkpoint Blockade-Based Immunotherapy. *Science* (2018) 362(6411):eaar3593. doi: 10.1126/science.aar3593
32. Liu L, Bai X, Wang J, Tang XR, Wu DH, Du SS, et al. Combination of TMB and CNA Stratifies Prognostic and Predictive Responses to Immunotherapy Across Metastatic Cancer. *Clin Cancer Res* (2019) 25(24):7413–23. doi: 10.1158/1078-0432.CCR-19-0558
33. Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, et al. Tumor Mutational Load Predicts Survival After Immunotherapy Across Multiple Cancer Types. *Nat Genet* (2019) 51(2):202–6. doi: 10.1038/s41588-018-0312-8
34. Digklia A, Wagner AD. Advanced Gastric Cancer: Current Treatment Landscape and Future Perspectives. *World J Gastroenterol* (2016) 22 (8):2403–14. doi: 10.3748/wjg.v22.i8.2403
35. Jiang P, Gu S, Pan D, Fu J, Sahu A, Hu X, et al. Signatures of T Cell Dysfunction and Exclusion Predict Cancer Immunotherapy Response. *Nat Med* (2018) 24(10):1550–8. doi: 10.1038/s41591-018-0136-1
36. Mei J, Xing Y, Lv J, Gu D, Pan J, Zhang Y, et al. Construction of an Immune-Related Gene Signature for Prediction of Prognosis in Patients With Cervical Cancer. *Int Immunopharmacol* (2020) 88:106882. doi: 10.1016/j.intimp.2020.106882
37. Hsieh HL, Tsai MM. Tumor Progression-Dependent Angiogenesis in Gastric Cancer and Its Potential Application. *World J Gastrointest Oncol* (2019) 11 (9):686–704. doi: 10.4251/wjgo.v11.i9.686
38. Nienhuser H, Schmidt T. Angiogenesis and Anti-Angiogenic Therapy in Gastric Cancer. *Int J Mol Sci* (2017) 19(1):43. doi: 10.3390/ijms19010043
39. Rahma OE, Hodi FS. The Intersection Between Tumor Angiogenesis and Immune Suppression. *Clin Cancer Res* (2019) 25(18):5449–57. doi: 10.1158/1078-0432.CCR-18-1543
40. Rivera LB, Bergers G. Intertwined Regulation of Angiogenesis and Immunity by Myeloid Cells. *Trends Immunol* (2015) 36(4):240–9. doi: 10.1016/j.it.2015.02.005
41. Trenti A, Tedesco S, Boscaro C, Trevisi L, Bolego C, Cignarella A. Estrogen, Angiogenesis, Immunity and Cell Metabolism: Solving the Puzzle. *Int J Mol Sci* (2018) 19(3):859. doi: 10.3390/ijms19030859
42. Sajib S, Zahra FT, Lionakis MS, German NA, Mikelis CM. Mechanisms of Angiogenesis in Microbe-Regulated Inflammatory and Neoplastic Conditions. *Angiogenesis* (2018) 21(1):1–14. doi: 10.1007/s10456-017-9583-4
43. Annese T, Tamma R, De Giorgis M, Ribatti D. microRNAs Biogenesis, Functions and Role in Tumor Angiogenesis. *Front Oncol* (2020) 10:581007. doi: 10.3389/fonc.2020.581007
44. Cai H, Jing C, Chang X, Ding D, Han T, Yang J, et al. Mutational Landscape of Gastric Cancer and Clinical Application of Genomic Profiling Based on Target Next-Generation Sequencing. *J Transl Med* (2019) 17(1):189. doi: 10.1186/s12967-019-1941-0
45. Quail DF, Joyce JA. Microenvironmental Regulation of Tumor Progression and Metastasis. *Nat Med* (2013) 19(11):1423–37. doi: 10.1038/nm.3394
46. Pitt JM, Marabelle A, Eggermont A, Soria JC, Kroemer G, Zitvogel L. Targeting the Tumor Microenvironment: Removing Obstruction to Anticancer Immune Responses and Immunotherapy. *Ann Oncol* (2016) 27 (8):1482–92. doi: 10.1093/annonc/mdw168
47. Goschl L, Scheinecker C, Bonelli M. Treg Cells in Autoimmunity: From Identification to Treg-Based Therapies. *Semin Immunopathol* (2019) 41 (3):301–14. doi: 10.1007/s00281-019-00741-8
48. Ribatti D, Crivellato E. Immune Cells and Angiogenesis. *J Cell Mol Med* (2009) 13(9A):2822–33. doi: 10.1111/j.1582-4934.2009.00810.x
49. Minton K. Connecting Angiogenesis and Autoimmunity. *Nat Rev Immunol* (2019) 19(10):596–7. doi: 10.1038/s41577-019-0217-5
50. Garrido M, Fonseca PJ, Vieitez JM, Frunza M, Lacave AJ. Challenges in First Line Chemotherapy and Targeted Therapy in Advanced Gastric Cancer. *Expert Rev Anticancer Ther* (2014) 14(8):887–900. doi: 10.1586/14737140.2014.915194

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frontiers in Oncology

CASE REPORT
published: 03 March 2022
doi: 10.3389/fonc.2022.809068

Case Report: Complete Remission With Anti–PD–1 and Anti–VEGF Combined Therapy of a Patient With Metastatic Primary Splenic Angiosarcoma

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Primary splenic angiosarcoma (PSA) is a rare malignancy with poor prognosis. At present, little study is available on immunotherapy in PSA. Here, we report a case of a patient with metastatic PSA who was treated with programmed death-1 (PD-1) inhibitors and vascular endothelial growth factor (VEGF) tyrosine kinase inhibitors combined therapy and achieved complete response (CR). The patient was a 57–year–old woman with three liver metastases. She was treated with seven cycles of toripalimab plus anlotinib. Programmed death-ligand 1 (PD-L1) immunohistochemistry and next-generation sequencing was performed, and the PD-L1 tumor proportion score was 75%. Finally, she achieved CR after six cycles of the combined therapy regimen. No serious adverse events were detected. To the best of our knowledge, this is the first clinical evidence that anti-PD-1 plus anti-VEGF therapy might be a promising option for patients with metastatic PSA. However, more clinical trials are needed to verify this conclusion.

Keywords: primary splenic angiosarcoma, PD-1 inhibitor, immunotherapy, anti-VEGF therapy, complete remission

OPEN ACCESS

Edited by:
Salem Chouaib, Institut Gustave Roussy, France

Reviewed by:
Luigi Cerbone, Gustave Roussy Cancer Campus, France
Carolina Alves Costa Silva, Gustave Roussy Cancer Campus, France

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Specialty section:
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Oncology

Received: *04 November 2021*
Accepted: *01 February 2022*
Published: *03 March 2022*

Citation:
Xu W, Wang K, Gu W, Nie X, Zhang H, Tang C, Lin L and Liang J (2022) Case Report: Complete Remission With Anti–PD–1 and Anti–VEGF Combined Therapy of a Patient With Metastatic Primary Splenic Angiosarcoma. Front. Oncol. 12:809068. doi: 10.3389/fonc.2022.809068

INTRODUCTION

Primary splenic angiosarcoma (PSA) is a rare and aggressive tumor with poor prognosis and a high rate of liver metastasis. The typical symptom of PSA is left upper abdomen pain (1–3); other symptoms include weakness or fatigue, fever, chest pain, weight loss, and bleeding (1, 4). Splenectomy is the only potentially curative treatment for patients with early-stage PSA. In addition, some patients with distant metastasis may receive emergency splenectomy due to splenic rupture.

Although some case reports have reported the potential benefit of systemic therapy in PSA, traditional chemotherapy have limited efficacy in metastatic PSA (1, 5). In recent years, programmed death-1 (PD-1) inhibitors have significantly improved the long-term survival of patients with various tumors (6, 7). Moreover, vascular endothelial growth factor (VEGF) tyrosine kinase inhibitors (TKIs) have shown promising effects in patients with angiosarcoma (8).

Here, we report a case of a metastatic PSA patient with high expression of programmed death-ligand 1 (PD-L1) who reached complete response (CR) after anti–PD–1 and anti–VEGF combination therapy.

CASE REPORT

The patient was a 57–year–old woman who was diagnosed with PSA in 2020. She presented to a local hospital with left-sided upper abdominal pain for three hours.

There was no bloating, nausea, or vomiting. No family cancer history was noted. Abdominal contrast-enhanced computed tomography (CT) showed spontaneous rupture of a spleen neoplasm and abdominal hemorrhage, three suspicious lesions in the liver were also detected. One day later, an emergency splenectomy was performed. The postoperative immunohistochemical staining results were as follows: CD31 (+), CD34(+), EGFR (+), CK (−), P63(−), CK20(−), CK5/6(−), Syn (−), and CK7(−). The final pathologic results confirmed the diagnosis of angiosarcoma (**Figure 1**). Positron emission tomography-CT (PET-CT) was performed 1 month after surgery, demonstrating three hypermetabolic foci in the liver, which were diagnosed as hepatic metastases and correspond to the same lesions initially found in CT. No other distant metastases were identified.

One month later, she was referred to our hospital for further care. PD-L1 staining with a 22C3 antibody was performed, and the results indicated high PD-L1 expression [tumor proportion score (TPS)=75%]. Moreover, we performed next-generation sequencing of 1021 cancer-related genes using tumor tissue and matched lymphocyte samples. The results suggested microsatellite-stable (MSS). No germline pathogenic or likely pathogenic variants were identified, but eight somatic mutations (MYC, TP53, TSC2, BR*D2, MAP2K4, NCOR1, PTEN, FAS*) were found in this patient. She finally received combined anti-VEGF and anti-PD-1 inhibitor treatment in 3-week cycles, with 240 mg toripalimab admitted intravenously on day 1 of each cycle and 10 mg anlotinib given daily on days 1 to 14 of each cycle. Adverse reactions, including grade 1 myelosuppression and grade 2 diarrhea, were noted. However, no serious adverse events were observed in this patient.

The efficacy of the combination treatment was assessed using CT and magnetic resonance imaging scans. Tumor load was evaluated at baseline and after every two cycles of treatment using response evaluation criteria in solid tumors (RECIST) guidelines (version1.1). In total, seven cycles of the combined treatment were delivered. The patient exhibited a favorable response to our combined regimen. The evaluation of efficacy after two and four cycles was partial response. The tumor shrank approximately 70% after four cycles of treatment. Surprisingly, our patient finally achieved CR after six cycles of treatment (**Figure 1**). PET-CT was performed to confirm CR after the seventh cycle, and no 18F-fluorodeoxyglucose elevation was detected in the original metastatic sites. The patient declined further treatment for personal reasons. Three months after the final cycle of treatment, imaging examinations revealed no evidence of tumor relapse. As the influence of COVID-19, the patient did not come to our hospital regularly for re-examination since then.

DISCUSSION

PSA is a kind of malignancy that derived from the splenic vascular endothelium with an extremely low incidence. Fewer

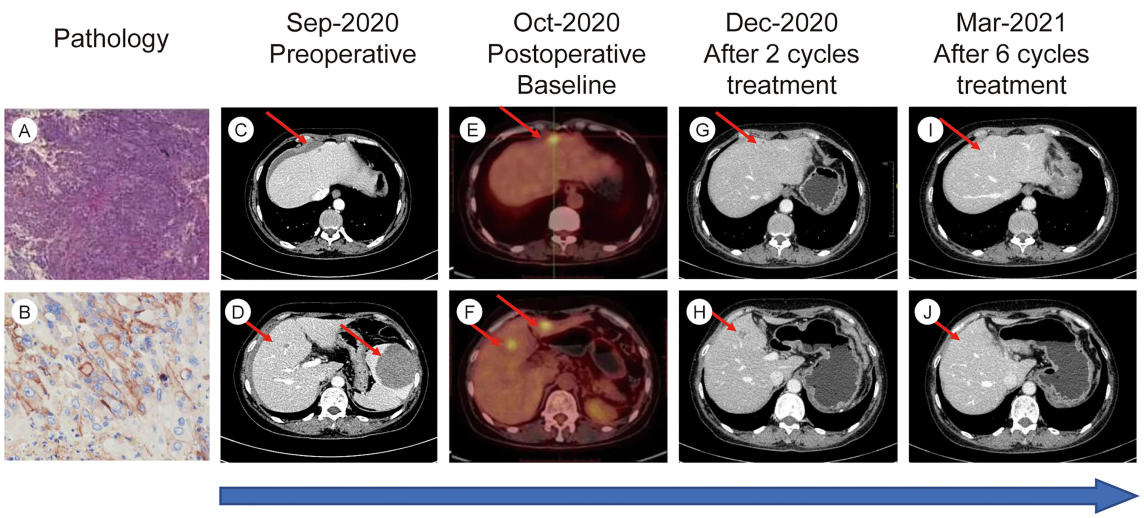

FIGURE 1 | **(A)** H&E stain from postoperative tumor tissue (10X magnification). **(B)** Immunohistochemical staining for PD-L1 in postoperative tumor tissue. **(C–J)** Timelines of imaging changes.

than 300 cases of PSA have been reported to date. The average age at diagnosis is 50–60 years (9, 10). As previously reported, the median survival time of metastatic PSA is approximately one year. Rupture of the spleen and distant metastasis are considered poor prognostic factors (11).

Because of the rarity of the disease, there is no standard treatment protocol. Furthermore, there are no randomized clinical trial data to support a systemic treatment regimen for metastatic PSA. Based on the literature of other sarcomas, some researchers have attempted several first-line chemotherapy regimens, including paclitaxel, anthracycline, doxorubicin, and ifosfamide (12–15). Unfortunately, the overall response rate was relatively low.

Combination treatment with antiangiogenic drugs and immune checkpoint inhibitors has been demonstrated to be effective in multiple malignancies (16). The underlying mechanism involves normalization of the tumor vessels through anti-VEGF therapy, which might improve the infiltration of tumors by activating effector T cells and subsequently convert the immunosuppressive tumor microenvironment (TME) into an immune-active TME (17, 18).

There have been several case reports regarding the combination therapy of antiangiogenic drugs and immune checkpoint inhibitors in sarcomas. A patient with metastatic undifferentiated pleomorphic sarcoma received pembrolizumab and pazopanib after multiple lines of therapy and had a partial response for 9 months (19). In addition, a patient with recurrent intestinal follicular dendritic cell sarcoma received sintilimab plus lenvatinib as third-line treatment and achieved a progression-free survival of 7 months (20). Several clinical trials have also explored the combination therapy in sarcoma. A phase II single-arm study of pembrolizumab plus lenvatinib in previously treated classic Kaposi sarcoma is in progress (https://www.clinicaltrialsregister.eu/ctr-search/search?query=2020-004426-36).Toripalimab is a newly developed monoclonal antibody that blocks PD-1. Clinical trial data have exhibited a promising antitumor activity of toripalimab in metastatic sarcoma and other malignancies (21, 22). Anlotinib is a novel TKI targeting VEGF1-3 and has shown encouraging effects in sarcoma (23, 24). A Phase II clinical trial (NCT04172805) is aimed to test the safety and effectiveness of anlotinib and toripalimab in soft tissue sarcoma. However, the results have not been published (https://clinicaltrials.gov/ct2/show/NCT04172805). In our patient, we innovatively attempted toripalimab and anlotinib combined therapy for metastatic PSA, and the regimen was surprisingly effective and well-tolerated.

High expression of PD-L1 predicts better efficacy of immunotherapy in several cancers (25, 26). A previous study found that the positive rate of PD-L1 was approximately 60% in angiosarcoma, and the differentiation level of the tumor was significantly associated with the PD-L1 status (27). The TPS of our patient was as high as 75% and the tumor rapidly decreased in size after several cycles of combined treatment.

Of note, we performed next-generation sequencing of tumor tissue and further analyzed angiosarcoma datasets from The Cancer Genome Atlas (TCGA). TCGA data showed the mutation frequencies of these genes in angiosarcoma patients (**Figure 2**). We further analyzed the correlation between the expression of these genes and the tumor mutational burden (TMB) in angiosarcoma cases from the TCGA database. Finally, we observed that *PTEN* mutation was negatively correlated with TMB (**Figure 3**).

We also found that some gene mutations of our patients were correlated with the efficacy of immunotherapy. *TSC2* was associated with T cell exhaustion inhibition, which upregulated PD-L1 on tumors (28). The *TP53* mutation status was related to the survival benefit of PD-1 inhibitors in non-small cell lung cancer (29). *NCOR1* mutation was reported as a potential positive biomarker to predict the efficacy of immunotherapy in bladder cancer (30). However, we also identified negative predictors of immunotherapy in our patient, such as *PTEN* mutation (31). This gene alteration reduces CD8-positive T cells in the immune microenvironment, leading to an inadequate response to PD-1 inhibitors.

Surprisingly, our patient quickly achieved CR during toripalimab plus anlotinib treatment and had no serious adverse events. Our case suggests that anti-PD-1 plus anti-VEGF therapy might be a promising option for metastatic PSA

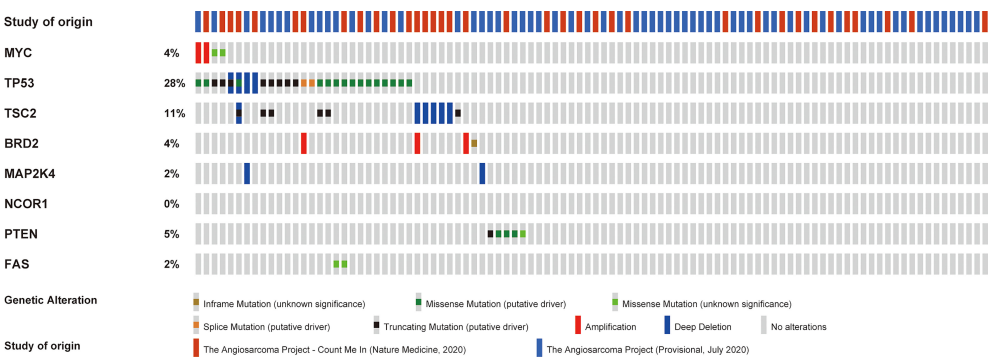

FIGURE 2 | Analysis of the patients' mutation genes in TCGA sarcoma data.

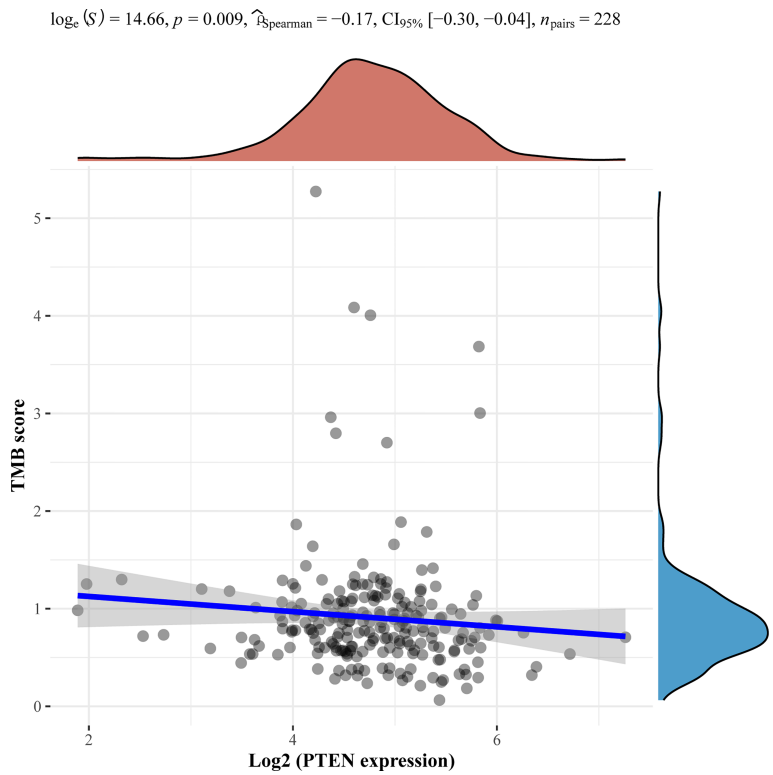

FIGURE 3 | Correlation analysis of PTEN gene expression and TMB. The horizontal axis in the figure represents the expression distribution of the gene, and the ordinate is the expression distribution of the TMB score. The density curve on the right represents the distribution trend of the TMB score; the upper density curve represents the distribution trend of the gene; the top side represents the correlation p value, correlation coefficient and correlation calculation method.

patients with high expression of PD-L1. However, clinical trials are warranted to confirm the efficacy of this regimen for these patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

Ethical approval was obtained from the Peking University International Hospital Research Ethics Committee. The patient provided her written informed consent to participate in this study. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

WX drafted the manuscript. XN revised the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

We thank Melissa Crawford, PhD, from Liwen Bianji (Edanz) (www.liwenbianji.cn/) for editing the English text of a draft of this manuscript.

REFERENCES

1. Neuhauser TS, Derringer GA, Thompson LD, Fanburg-Smith JC, Miettinen M, Saaristo A, et al. Splenic Angiosarcoma: A Clinicopathologic and Immunophenotypic Study of 28 Cases. *Modern Pathol* (2000) 13(9):978–87. doi: 10.1038/modpathol.3880178
2. Falk S, Krishnan J, Meis J. Primary Angiosarcoma of the Spleen. A Clinicopathologic Study of 40 Cases. *Am J Surg Pathol* (1993) 17(10):959–70. doi: 10.1097/00000478-199310000-00001
3. Valbuena JR, Levenback C, Mansfield P, Liu J. Angiosarcoma of the Spleen Clinically Presenting as Metastatic Ovarian Cancer. A Case Report and Review of the Literature. *Ann Diagn Pathol* (2005) 9(5):289–92. doi: 10.1016/j.anndiagpath.2005.03.007

4. Peckova K, Michal M, Hadravsky L, Damjanov I, Miesbauerova M, et al. Littoral Cell Angioma of the Spleen: A Study of 25 Cases With Confirmation of Frequent Association With Visceral Malignancies. *Histopathology* (2016) 69(5):762–74. doi: 10.1111/his.13026
5. Sordillo EM, Sordillo PP, Hajdu SI. Primary Hemangiosarcoma of the Spleen: Report of Four Cases. *Med Pediatr Oncol* (1981) 9(4):319–24. doi: 10.1002/mpo.2950090403
6. Mahoney KM, Freeman GJ, McDermott DF. The Next Immune-Checkpoint Inhibitors: PD-1/PD-L1 Blockade in Melanoma. *Clin Ther* (2015) 37(4):764–82. doi: 10.1016/j.clinthera.2015.02.018
7. Sui H, Ma N, Wang Y, Li H, Liu X, Su Y, et al. Anti-PD-1/PD-L1 Therapy for Non-Small-Cell Lung Cancer: Toward Personalized Medicine and Combination Strategies. *J Immunol Res* (2018) 2018:6984948. doi: 10.1155/2018/6984948
8. Young RJ, Woll P, Staton C, Reed M, Brown N. Vascular-Targeted Agents for the Treatment of Angiosarcoma. *Cancer Chemother Pharmacol* (2014) 73 (2):259–70. doi: 10.1007/s00280-013-2345-0
9. Despoina M, Dionysios D, Georgios A, Konstantinos S, Efstratios K, Adamantia Z-S. Primary Angiosarcoma of the Spleen: An Oncological Enigma. *Case Rep Oncol Med* (2014) 2014:193036. doi: 10.1155/2014/193036
10. Fotiadis C, Georgopoulos I, Stoidis C, Patapis P. Primary Tumors of the Spleen. *Int J Biomed Sci: IJBS* (2009) 5(2):85.
11. Chen X, Li H, Wang F, Liu H. Early Detection and Integral Resection Are Keys to Extend Survival in Patients Suffered From Primary Angiosarcoma of the Spleen: A Care-Compliant Case Report and Literature Review. *Medicine* (2018) 97(5):e9718. doi: 10.1097/MD.0000000000009718
12. Ferreira BP, Rodler ET, Loggers ET, Pollack SM, Jones RL. Systemic Therapy in Primary Angiosarcoma of the Spleen. *Rare Tumors* (2012) 4(4):178–80. doi: 10.4081/rt.2012.e55
13. Vakkalanka B, Milhem M. Paclitaxel as Neoadjuvant Therapy for High Grade Angiosarcoma of the Spleen: A Brief Report and Literature Review. *Clin Med Insights: Oncol* (2010) 4:107–10. CMO. S5329. doi: 10.4137/CMO.S5329
14. Skubitz KM, Haddad PA. Paclitaxel and Pegylated-Liposomal Doxorubicin Are Both Active in Angiosarcoma. *Cancer: Interdiscip Int J Am Cancer Soc* (2005) 104(2):361–6. doi: 10.1002/cncr.21140
15. Lewcun JA, Pameijer C, Kass R, Cream L, Hershock D, Brooks AJ, et al. Doxorubicin, Paclitaxel, and Cisplatin Based Chemotherapy for the Treatment of Angiosarcoma: Two Case Reports. *Int J Surg Case Rep* (2020) 68:83–7. doi: 10.1016/j.ijscr.2020.02.036
16. Huang Y, Goel S, Duda DG, Fukumura D, Jain RK. Vascular Normalization as an Emerging Strategy to Enhance Cancer Immunotherapy. *Cancer Res* (2013) 73(10):2943–8. doi: 10.1158/0008-5472.CAN-12-4354
17. Pitt JM, Vétizou M, Daillère R, Roberti MP, Yamazaki T, Routy B, et al. Resistance Mechanisms to Immune-Checkpoint Blockade in Cancer: Tumor-Intrinsic and-Extrinsic Factors. *Immunity* (2016) 44(6):1255–69. doi: 10.1016/j.immuni.2016.06.001
18. Fukumura D, Kloepper J, Amoozgar Z, Duda DG, Jain RK. Enhancing Cancer Immunotherapy Using Antiangiogenics: Opportunities and Challenges. *Nat Rev Clin Oncol* (2018) 15(5):325–40. doi: 10.1038/nrclinonc.2018.29
19. Arora S, Rastogi S, Shamim SA, Barwad A, Sethi M. Good and Sustained Response to Pembrolizumab and Pazopanib in Advanced Undifferentiated Pleomorphic Sarcoma: A Case Report. *Clin Sarcoma Res* (2020) 10(1):1–6. doi: 10.1186/s13569-020-00133-9
20. Jiang M, Lei Y, Zhao S. Unexpected Favorable Outcome to PD-1 Antibody Plus Lenvatinib in a Patient With Recurrent Intestinal Follicular Dendritic Cell Sarcoma: A Case Report and Literature Review. *Front Immunol* (2021) 12:653319. doi: 10.3389/fimmu.2021.653319
21. Yang J, Dong L, Yang S, Han X, Han Y, Jiang S, et al. Safety and Clinical Efficacy of Toripalimab, a PD-1 mAb, in Patients With Advanced or Recurrent Malignancies in a Phase I Study. *Eur J Cancer* (2020) 130:182–92. doi: 10.1016/j.ejca.2020.01.028
22. Tang B, Chi Z, Chen Y, Liu X, Wu D, Chen J, et al. Safety, Efficacy, and Biomarker Analysis of Toripalimab in Previously Treated Advanced Melanoma: Results of the POLARIS-01 Multicenter Phase II Trial. *Clin Cancer Res* (2020) 26(16):4250–9. doi: 10.1158/1078-0432.CCR-19-3922
23. Wang H-y, Chu J-f, Zhang P, Wang J-q, Yan Z, Yao S-n, et al. Safety and Efficacy of Chemotherapy Combined With Anlotinib Plus Anlotinib Maintenance in Chinese Patients With Advanced/Metastatic Soft Tissue Sarcoma. *OncoTargets Ther* (2020) 13:1561. doi: 10.2147/OTT.S235349
24. Liu Z, Yao W, Zhao Y, Liu O, Zhang P, Ge H. Efficacy and Safety of Anlotinib Combined With Liposomal Doxorubicin Followed by Anlotinib Maintenance in Metastatic Soft Tissue Sarcomas. *Cancer Manage Res* (2021) 13:1009. doi: 10.2147/CMAR.S286322
25. Gong J, Chehrazi-Raffle A, Reddi S, Salgia R. Development of PD-1 and PD-L1 Inhibitors as a Form of Cancer Immunotherapy: A Comprehensive Review of Registration Trials and Future Considerations. *J Immunother Cancer* (2018) 6(1):1–18. doi: 10.1186/s40425-018-0316-z
26. Jiang Y, Chen M, Nie H, Yuan Y. PD-1 and PD-L1 in Cancer Immunotherapy: Clinical Implications and Future Considerations. *Hum Vaccines Immunother* (2019) 15(5):1111–22. doi: 10.1080/21645515.2019.1571892
27. Botti G, Scognamiglio G, Marra L, Pizzolorusso A, Di Bonito M, De Cecio R, et al. Programmed Death Ligand 1 (PD-L1) Expression in Primary Angiosarcoma. *J Cancer* (2017) 8(16):3166. doi: 10.7150/jca.19060
28. Liu H-J, Lizotte PH, Du H, Speranza MC, Lam HC, Vaughan S, et al. TSC2-Deficient Tumors Have Evidence of T Cell Exhaustion and Respond to Anti–PD-1/Anti–CTLA-4 Immunotherapy. *JCI Insight* (2018) 3(8):e98674. doi: 10.1172/jci.insight.98674
29. Assoun S, Theou-Anton N, Nguenang M, Cazes A, Danel C, Abbar B, et al. Association of TP53 Mutations With Response and Longer Survival Under Immune Checkpoint Inhibitors in Advanced Non-Small-Cell Lung Cancer. *Lung Cancer* (2019) 132:65–71. doi: 10.1016/j.lungcan.2019.04.005
30. Lin A, Qiu Z, Zhang J, Luo P. Effect of NCOR1 Mutations on Immune Microenvironment and Efficacy of Immune Checkpoint Inhibitors in Patient With Bladder Cancer. *Front Immunol* (2021) 12:248. doi: 10.3389/fimmu.2021.630773
31. Cretella D, Digiacomo G, Giovannetti E, Cavazzoni A. PTEN Alterations as a Potential Mechanism for Tumor Cell Escape From PD-1/PD-L1 Inhibition. *Cancers* (2019) 11(9):1318. doi: 10.3390/cancers11091318

Conflict of Interest: Author WG was employed by Geneplus-Beijing. Co-author XN and HZ were full-time employee of Shanghai Junshi Biosciences Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Immunology

REVIEW
published: 20 June 2022
doi: 10.3389/fimmu.2022.880810

The Promise of Targeting Hypoxia to Improve Cancer Immunotherapy: Mirage or Reality?

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OPEN ACCESS

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Specialty section:
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

Received: *21 February 2022*
Accepted: *10 May 2022*
Published: *20 June 2022*

Citation:
Janji B and Chouaib S (2022) The Promise of Targeting Hypoxia to Improve Cancer Immunotherapy: Mirage or Reality? Front. Immunol. 13:880810. doi: 10.3389/fimmu.2022.880810

Almost all solid tumors display hypoxic areas in the tumor microenvironment associated with therapeutic failure. It is now well established that the abnormal growth of malignant solid tumors exacerbates their susceptibility to hypoxia. Therefore, targeting hypoxia remains an attractive strategy to sensitize tumors to various therapies. Tumor cell adaptions to hypoxia are primarily mediated by hypoxia-inducible factor-1 alpha (HIF-1α). Sensing hypoxia by HIF-1α impairs the apoptotic potential of tumor cells, thus increasing their proliferative capacity and contributing to the development of a chaotic vasculature in the tumor microenvironment. Therefore, in addition to the negative impact of hypoxia on tumor response to chemo- and radio-therapies, hypoxia has also been described as a major hijacker of the tumor response by impairing the tumor cell susceptibility to immune cell killing. This review is not intended to provide a comprehensive overview of the work published by several groups on the multiple mechanisms by which hypoxia impairs the anti-tumor immunity and establishes the immunosuppressive tumor microenvironment. There are several excellent reviews highlighting the value of targeting hypoxia to improve the benefit of immunotherapy. Here, we first provide a brief overview of the mechanisms involved in the establishment of hypoxic stress in the tumor microenvironment. We then discuss our recently published data on how targeting hypoxia, by deleting a critical domain in HIF-1α, contributes to the improvement of the anti-tumor immune response. Our aim is to support the current dogma about the relevance of targeting hypoxia in cancer immunotherapy.

Keywords: hypoxia, immune checkpoints, immune landscape, innate and adaptive immune response, pro-inflammatory chemokines, cancer immunotherapy, cold and hot tumor

INTRODUCTION

In solid tumors, the establishment of hypoxia in the tumor microenvironment relies on the failure of abnormal vasculature to meet increasing oxygen demands from rapidly proliferating cancer cells. Therefore, within the same tumor, the O_2 level varies depending on the quality and the integrity of blood vessels. Several areas in the tumor microenvironment can be identified according to the oxygenation level of tumor tissue: well oxygenated, poorly oxygenated, and non-oxygenated or necrotic areas (1) **(Figure 1)**. In addition to the tumor size and the quality of the tumor vascularization, the different levels of O_2 in the microenvironment of different tumors rely on the initial physiological oxygenation levels observed in the corresponding healthy tissue and on the degree of the tumor heterogeneity. **Figure 2** shows the oxygen levels (reported as a percentage) in several tumors and corresponding healthy tissues. The percentage of O_2 in healthy tissues range from 9.5% (observed in kidney healthy tissue) to 3.5% (reported in healthy prostate tissue). Hence, the average of O_2 in the healthy tissues reported in **Figure 2** is 5.9%. The oxygen levels in the corresponding tumors range from 2.5% (observed in rectal tumor) to 0.3% (reported in liver and prostate tumors). Therefore, the average of O_2 in the tumors reported in **Figure 2** is 1.3%. Based on these values, most tumors exhibit median oxygen levels below 2%. The term of normoxia should not be used to describe the oxygenation level in healthy tissues, however, it can defines the O2 level in tissue culture flasks where the oxygenation is about 20-21%. The term of physioxia is more appropriate to describe the oxygenation status in healthy tissues as previously reported (2). Therefore, it is important to control the O_2 in cell culture settings to mimic as far as possible the O2 levels found in healthy and tumor tissues.

The mechanism of cell adaptation to hypoxia is currently well described. William G. Kaelin Jr., Sir Peter J. Ratcliffe, and Gregg L. Semenza were awarded the Nobel Prize in Medicine 2019 in recognition of their seminal discovery on the molecular mechanisms and signaling pathways by which cells sense and adapt to hypoxia.

While the negative impact of hypoxia on tumor response to conventional chemo- and radiotherapy is now well recognized (3, 4), an accumulating new body of data highlights its involvement in tumor resistance to immunotherapy (5). Here, we describe recent evidence on how hypoxia plays a role as a culprit of immunotherapy failure. We will mainly discuss our recent experimental and preclinical evidence data showing that strategies targeting hypoxia can provide the basis for innovative combination therapies that may improve the immunotherapeutic efficacy. Hypoxia-inducible factors (HIFs) are essential transcription factors mediating cell adaptation to hypoxia, and thus we will first briefly describe how HIFs expression and stability are regulated under hypoxia in tumor cells.

HYPOXIA INDUCIBLE FACTORS - MECHANISMS OF REGULATION AND STABILITY

HIFs are heterodimer complexes consistent of an O_2-inducible alpha subunit and constitutively expressed beta subunit (HIF-1β/ARNT). Three alpha subunits have been identified: HIF-1α, HIF-2α, and HIF-3α. The well-studied alpha subunit is HIF-1α and contains N-terminal basic-helix-loop-helix (bHLH) required for DNA interaction. There are also two Per-Arnt-Sim (PAS) domains (PASa and PASb) essential for heterodimerization with HIF-1β. Two oxygen-dependent degradation domains (ODDD) have been identified in the N-terminal (N-ODDD) and C-terminal (C-ODDD) parts of the protein in addition to two transactivation domains (TADs). One overlaps with the C-ODDD, and the second is found in the C-terminal part (6).

Under normoxic conditions, HIF-1α is continuously synthesized, but it is rapidly degraded by the ubiquitin–proteasome system (UPS). The short half-life of HIF-1α under normoxia is less than five minutes (7). The basal expression level of HIF-1α under normoxia is low, but varies in different cells. Such variations depend on the rate of HIF-1α synthesis (O_2-

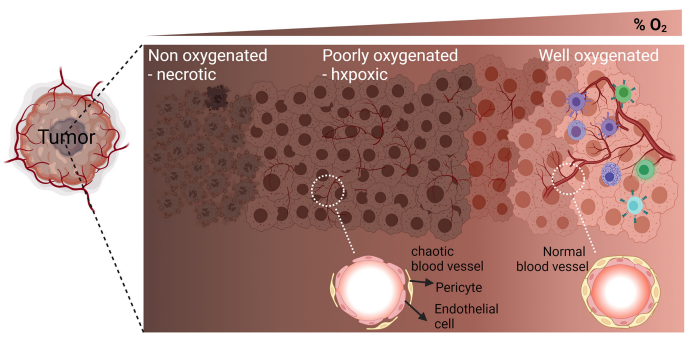

FIGURE 1 | Graphic representation of the different areas in the tumor microenvironment according to the oxygenation level (percent of O_2): Well oxygenated, poorly oxygenated, and non-oxygenated or necrotic areas. Enlargement of a blood vessel section in the poorly oxygenated hypoxic area shows defect in the organization of endothelial cells and pericytes' coverage. Enlargement of a blood vessel section in the well oxygenated area shows well-structured endothelial cells and pericytes' coverage.

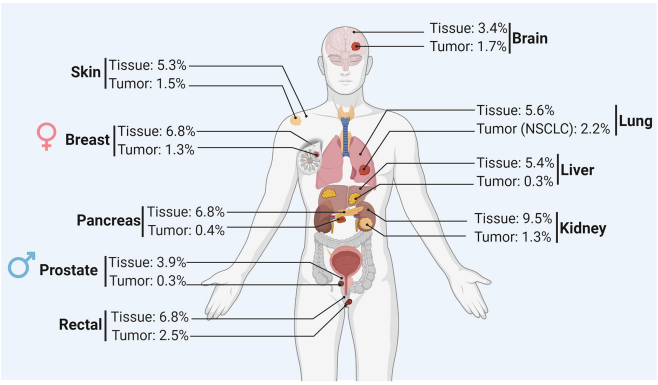

FIGURE 2 | Summary of the oxygen level (reported as a percentage) in the healthy tissue and corresponding tumor of different organs.

independent mechanism) and the rate of HIF-1α degradation (O_2-dependent mechanism).

The degradation of HIF-1α under normoxia depends on its hydroxylation on proline residues located at positions 402 and/or 564 in the ODDD by prolyl hydroxylase domain protein 2 (PHD2). Thus, hydroxylated HIF-1α binds to von Hippel-Lindau (pVHL) protein, which is part of the E3 ubiquitin-protein ligase complex. It is subsequently subjected to degradation by the UPS [reviewed in (8)].

The enzymatic activity of PHD2 requires O_2 as a substrate, and thus the protein becomes inactive in hypoxic cells (9). Therefore, HIF-1α is no longer hydroxylated under low O_2 pressure; as a result, its interaction with pVHL and subsequent degradation by UPS are blocked. Thus, the failure of the mechanism involved in HIF-1α degradation under hypoxia leads to its accumulation in the cytoplasm, translocation to the nucleus, and interaction with HIF-1β. The heterodimer HIF-1α/HIF-1β binds to the hypoxia-responsive element (HRE) motif found in the promoter of several genes involved in several biological processes that tolerate cellular adaptation to hypoxia and confer a survival benefit to tumor cells.

HIF-2α displays similar DNA binding and dimerization domains as HIF-1α, but these differs in the transactivation domains (10). Therefore, the hydroxylation of HIF-2α is also regulated in an oxygen-dependent manner (11). Both HIF-1α and HIF-2α regulate common downstream target genes, but each can also regulate specific genes (12). Unlike HIF-1α and HIF-2α, HIF-3α lacks the transactivation domain. It can inhibit the activity of HIF-1α and HIF-2α (13), and HIFs are involved in the regulation of several microRNAs (HRM) (14) and chromatin-modifying enzymes (15). HIFs can directly regulate more than 800 genes involved in several biological functions as revealed by ChIP-seq analysis and genome-wide chromatin immunoprecipitation combined with DNA microarrays (ChIP-on-chip) (16, 17). The expression of downstream target genes is achieved by binding HIF-1α to 50-base pair cis-acting hypoxia responsive element (HRE) motifs found in their enhancer and promoter regions (18). The HRE motif contains the core sequence 5'-[A/G]CGT-3', which is usually ACGTG (19). Considering the preferential binding of the heterodimer complex HIF-1α/HIF-1β to specific bases in the 5' and 3' ends of the HRE motif, the following HRE consensus sequence [T/G/C][A/G]**CGTG**[CGA][GTC][GTC][CTG] has been described (19).

STRATEGIES FOR TARGETING HYPOXIA - CHALLENGES AND OPPORTUNITIES

Inhibiting hypoxia has inspired significant interest because it can improve therapeutic outcomes. Strategies used to inhibit hypoxia rely on bio-reductive prodrugs (20) or inhibitors targeting pathways upon which the survival of hypoxic cells depends (21). However, targeting HIF-dependent pathways is extremely challenging because various signaling pathways converge on—and emerge from—HIFs (22). Additional approaches have been proposed consisting of targeting HIFs directly. Although considerable efforts have been undertaken to identify selective inhibitors of HIFs, enthusiasm has been tempered by the reality that transcription factors, including HIFs, seem to be "undruggable" or at least no selective drugs inhibiting HIFs have been identified.

Considering the well-described molecular mechanism of HIF-1α protein activity, various strategies have been proposed to impair such activity. Such mechanisms inhibit HIF-1α protein synthesis or stabilization; they can also prevent HIF-1α/β heterodimerization or HIFs/DNA binding (23).

INHIBITING HYPOXIA BY PREVENTING HIF-1α/β, HETERODIMERIZATION REGULATES PRO-INFLAMMATORY CHEMOKINES AND IMPROVES THE BENEFIT OF IMMUNOTHERAPIES

In a highly hypoxic and PD-1-resistant B16-F10 melanoma mouse model (24, 25), we recently reported that inhibiting

hypoxia by preventing HIF-1α/β heterodimerization in a mouse melanoma model drives immune cells into the tumor microenvironment and improves anti-PD-1- and vaccine-based immunotherapies (26). Using CRISPR/Cas9 technology, we showed that the deletion (in HIF-1α) of the domain responsible for the interaction with HIF-1β still leads to the accumulation of the protein in hypoxic cells; however, this remarkably inhibits its transcription activity as demonstrated by suppressing the expression of well-known HIF-1α downstream target genes CAIX, VEGF, and Glut1. Similar to the full-length HIF-1α (HIF-1α^{FL}), the deleted HIF-1α (hereafter reported to as HIF-1α^{Del}) accumulated in the cytoplasm of hypoxic cells. However, unlike HIF-1α^{FL}, HIF-1α^{Del} displayed a defect in the nuclear translocation as seen *via* confocal microscopy analysis. By assessing the tumor growth *in vivo*, we showed a significant decrease in the growth and weight of B16-F10 tumors expressing HIF-1α^{Del} versus those expressing HIF-1α^{FL}. Such effects were observed in immunocompetent but not in immunocompromised NOD scid gamma (NSG) mice lacking mature B, T, and NK cells (26). These data emphasize that targeting hypoxia in tumors inhibits tumor growth *via* the immune system. Indeed, we revealed a significant increase in the infiltration of CD45+, NK, CD4+, and CD8+ cells into HIF-1α^{Del} versus HIF-1α^{FL} **(Figure 3)**. These data strongly suggest that targeting the transcription activity of HIFs can switch the microenvironment of tumors from cold non-inflamed/not-infiltrated into hot inflamed and infiltrated by cytotoxic immune cells.

The infiltration and trafficking of immune cells to the tumor microenvironment relies on the establishment of a chemokine network. The recruitment of T cells and natural killer (NK) cells into the tumor can be achieved by chemokines CXCL9, 10, 11, 16 as well as CX3CL1. CCL19 and 21 can promote the recruitment of DCs into T-cell priming sites, thus leading to T-cell activation (27). CXCL16 has been associated with the infiltration of tumor-infiltrating lymphocytes (TILs) and better prognosis in colorectal cancer (28). We previously reported that driving NK cells to melanoma tumors depends on the release of CCL5 to the tumor microenvironment by tumor cells (29). Other studies showed that the chemokines CCL2, 3, 4, and 5 as well as CXCL9 and 10 were involved in T-cell migration into a melanoma tumor microenvironment (30). By assessing the chemokine network in HIF-1α^{Del} tumors, we see that the increased infiltration of major cytotoxic immune cells described above was associated with the release of proinflammatory chemokines in the tumor microenvironment—notably CCL5 and CCL2. Therefore, we believe that targeting the transcriptional activity of HIF-1α in tumor cells contributes to the establishment of an inflammatory microenvironment, which helps recruit cytotoxic immune effector cells.

The translational value of our study is underlined by the data generated in preclinical mouse model and using a cohort of melanoma patients. Treatment of melanoma-bearing mice with acriflavine, reported to prevent HIF-1α/HIF-1β heterodimerization, improved immunotherapy strategies based on TRP-2 peptide vaccination and anti-PD-1 antibody. We

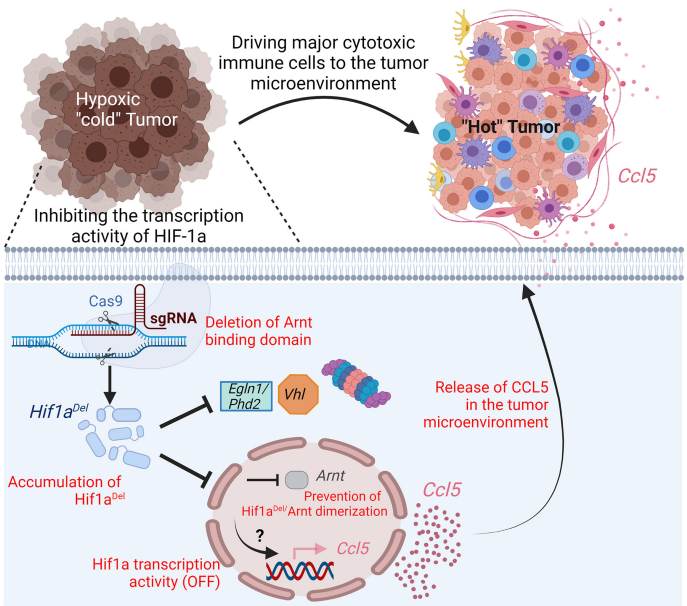

FIGURE 3 | Impact of targeting the transcription activity of Hif1a on driving immune cells into melanoma tumor microenvironment. Hypoxic melanoma are "cold" poorly infiltrated by immune cells. Deletion, in Hif1a, of the domain responsible for the formation of a heterodimer with Arnt by CRISPR/Cas9 gene-editing technology, prevents its transcription activity. In hypoxic cells expressing deleted Hif1a^{Del}, the pro-inflammatory (C-C motif) ligand 5 chemokine (Ccl5) is overexpressed by a mechanism which is not fully understood. The release of Ccl5 by tumor cells in the tumor microenvironment drives major cytotoxic immune cells and contributes to the establishment of pro-inflammatory "hot" tumor.

further showed that melanoma patients having low Winter hypoxia score survive better and show increased CCL5 as well as high tumor infiltration by NK and CD8 T-cells versus those having a high hypoxia score.

HIF-1α INDUCES TUMOR ESCAPE FROM IMMUNE SURVEILLANCE BY UPREGULATING THE EXPRESSION OF IMMUNE CHECKPOINTS AND ACTIVATING VARIOUS SURVIVAL PATHWAYS IN TUMOR CELLS

Accumulating evidence points to a critical role of HIFs in regulating various immune checkpoints [reviewed in (31)]. Briefly, HIF-1α binds directly to the HRE motif in the promoter of PD-L1 gene and induces its expression in various cancer cells such as melanoma, lung, breast, and prostate cancer. Such overexpression resulted in tumor escape from immune surveillance (32, 33) **(Figure 4)**. Similarly, the constitutive accumulation of HIF-2α in clear cell renal cell carcinoma (ccRCC), due to the mutation status of VHL, facilitates PD-L1 upregulation (34). In addition to tumor cells, HIF-1α also operates in the immune suppressive cells present in hypoxic tumor microenvironment. In MDSCs, HIF-1α directly upregulates PD-L1 expression resulting in impaired cytotoxic T lymphocytes (CTL) activity (32).

VISTA is an additional immune checkpoint regulated by HIF-1α. VISTA is expressed on several myeloid cells infiltrating hypoxic tumors including CD11b^{high}Gr1+ MDSCs. The recruitment of MDSCs to the tumor microenvironment is mediated by hypoxia-dependent upregulation of stromal-derived factor 1 (SDF1, CXCL12) (35). HIF-1α, but not HIF-2α, binds to VISTA and induces its expression—this process in turn suppresses T-cell proliferation and activity (36) **(Figure 4).**

CD47 is an inhibitory immune checkpoint expressed on the cell surface of tumor cells and involved in blocking the phagocytosis following the interaction with its ligands: signal regulatory protein α (SIRPα) and thrombospondin-1 (TSP-1). These two proteins are expressed on the surface of macrophages and dendritic cells (37). CD47/SIRPa or TSP-1 interaction delivers a strong "don't eat me" signal to block phagocytosis (38). Upregulation of CD47 is associated with the expression of HIF-1α downstream target genes. The expression of CD47 is upregulated by HIF-1α in triple-negative breast cancer cells resulting in a stem cell phenotypic switch through which cancer cells escape from phagocytosis (39). The upregulation of CD47 by hypoxia has also been reported in pancreatic adenocarcinoma (40, 41) **(Figure 4).**

In addition to regulating the expression of immune checkpoints and the establishing immunosuppressive tumor

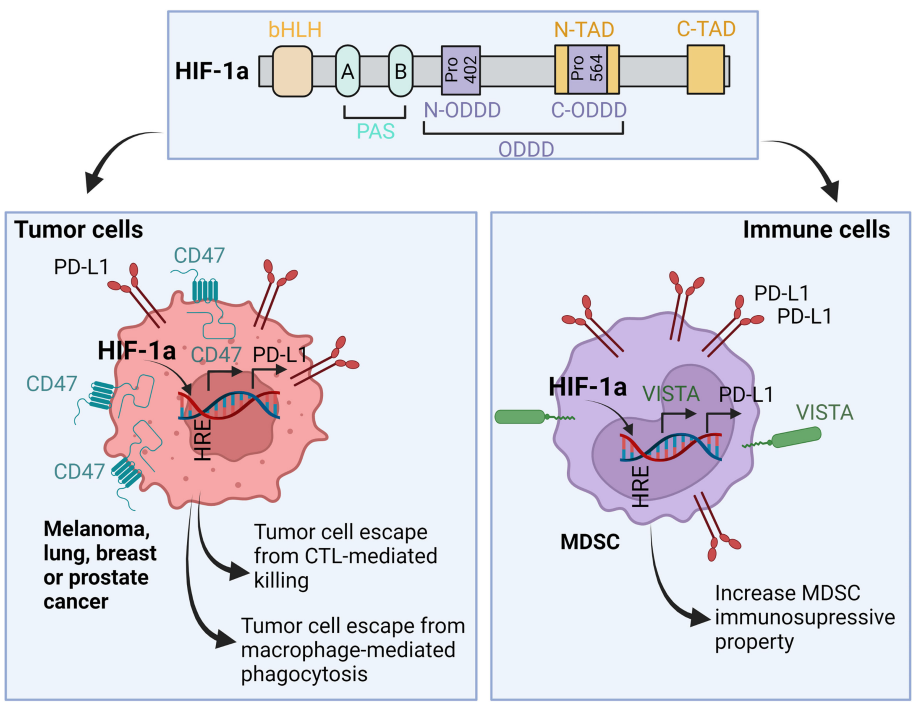

FIGURE 4 | Role of HIF-1α in the regulation of immune checkpoints expression in both tumor and immune cells. In hypoxic microenvironment, HIF-1α binds to the HRE motifs found in the promoters of PD-L1, CD47 and VISTA. As a result, HIF-1α-depenedent overexpression of PD-L1 and CD47 in tumor cells leads to tumor escape from CTL-mediated killing and macrophage-mediated phagocytosis, respectively. In MDSC, HIF-1α-dependent upregulation of PD-L1 and VISTA increases their immunosuppressive properties in the tumor microenvironment. bHLH, basic-helix-loop-helix; PAS, Per-Arnt-Sim domains; Pro, Proline residue; N- and C-ODDD, NH_2-terminal and COOH-terminal Oxygen-Dependent Degradation Domains; N- and C-TAD, NH_2-terminal and COOH-terminal transactivation domain.

microenvironment, the accumulation of HIF-1α in tumor cells decreases tumor cell susceptibility to CTL-mediated lysis through several mechanisms [reviewed in (31)]. Briefly, these mechanisms include the activation of autophagy (24, 42), the upregulation of stem cell self-renewal transcription factor Nanog (43, 44), and the induction of microRNA (miR)-210 involved in repressing the non-receptor protein tyrosine phosphatase type 1 (PTPN1), homeobox A1 (HOXA1), and tumor protein p53-inducible protein 11 (TP53I11) (45).

Hypoxia also impairs NK-mediated killing of tumor cells by downregulating and/or shedding the major histocompatibility complex (MHC) class I polypeptide-related sequence A (MICA) on the surface of cancer cells (46, 47). In hypoxic tumor cells, the activation of autophagy leads to the degradation of the serine protease granzyme B (GZMB) released by NK cells. This in turn led to tumor escape from NK-mediated killing (48, 49).

In addition of NK cells, hypoxia also impacts the activity of T cells. Briefly, under hypoxia, activated T cells are able to adapt changes in energy supplies by switching their metabolism to glycolysis and regulating extracellular-adenosine receptor signaling. Such adaptation alter the balance between T helper 1 cells and T helper 2 cells and results in impairing the anti-tumor immune response [reviewed in (50)]. In this context, it should be highlighted that hypoxia-dependent regulation of A2A adenosine receptor (A2AR)–mediated signaling is considered as one of the major mechanisms of the establishment of immunosuppressive tumor microenvironment [reviewed in (51)]

TARGETING HYPOXIA: A TRICKY APPROACH

Several reports indicate that the increased tumor aggressiveness is partially associated from hypoxia-induced genomic instability. It is currently well established that tumor cells exposed to hypoxic stress are able to acquire genetic instability through altered translation of DNA repair proteins. Therefore, hypoxic tumor cells display defective repair as well as an increased mutation rate. It is widely admitted that PD-L1 expression, tumor mutation burden (TMB) development, immune cell infiltration at the tumor site and neoantigen load are all thought to be influenced by tumor genomic instability (52). Clearly a more holistic approach that considers the complexity of hypoxia effects to better discriminate between the beneficial roles of hypoxic stress from the hostile ones is crucial. Given the dual effect of hypoxia, a clear understanding of how hypoxic stress induces tumor resistance and genomic instability resulting in an increased tumor immunogenicity is of paramount importance for identifying the time window of hypoxia targeting to improve cancer immunotherapy. Nevertheless, there is currently a Phase III clinical trial (NCT04195750) aiming to compare the efficacy and safety of HIF-2α inhibitor MK-6482 (also known as WELIREG) with the mTOR inhibitor everolimus in previously treated advanced ccRCC patients. Among the patients enrolled in the trial are those treated with anti–PD-1/PD-L1 or VEGF-targeted therapy which are randomly assigned to MK-6482 or everolimus arm. The estimated study completion will be in 2025. WELIREG or MK-6482 is the first inhibitor approved in U.S. which reduces the transcription and expression of HIF-2α target genes associated with cellular proliferation, angiogenesis and tumor growth.

CONCLUDING REMARKS

This review provides an additional clue supporting the role of targeting hypoxia in improving the benefit of cancer immunotherapy. Hypoxia has long been considered an attractive target to overcome resistance and improve the benefits to various therapies including immunotherapy. Numerous strategies have been proposed to inhibit hypoxia and target the transcription activity of HIF-1α such as the development of hypoxia-activated prodrugs or small molecules interfering with the transcription activity of HIFs (53–55). Several experimental studies offer preclinical proof-of-concept that strategies targeting hypoxia can improve the therapeutic benefits of current cancer therapies. However, there are still no approved drugs that selectively target hypoxia or HIF-dependent pathways despite they have clear anticancer effects. Obviously, such lack of selectivity does not disqualify these drugs as anticancer agents, but it becomes challenging to attribute the potential effect observed in patients to their anti-hypoxic properties. Nevertheless, the failure of developing selective drugs could be attributed to the biological complexity of HIF-1α pathways. Indeed, HIF-1α controls a highly complex network connecting several signaling pathways and various overlapping mechanisms in tumor cells and other cells in the tumor microenvironment. Such properties make HIF-1α undruggable. Therefore, we strongly believe that better dissecting hypoxia-inducible responses and understanding HIF-dependent signaling would lead to novel targets and new treatment opportunities.

The key role of hypoxia in hijacking the anti-tumor immune response is now firmly grounded in a substantial body of research. Therefore, the use of hypoxia modulators—especially those interfering with the transcription activity of HIF-1α—holds much promise for improving the therapeutic benefit of cancer immunotherapies. There is no doubt that combining hypoxia modulators with cancer immunotherapy approaches provide a unique opportunity for innovative combination strategies. Additional efforts are needed for highly selective hypoxia inhibitors, which remain an unmet need and are among the greatest challenges in cancer therapy.

AUTHOR CONTRIBUTIONS

BJ and SC contributed to writing the manuscript and preparing the figures. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by grants from Luxembourg National Research Fund (BRIDGES2020/BM/15412275/SMART COMBO and BRIDGES2021/BM/16358198/TRICK-ALDH INTER/EUROSTARS21/16896480/C2I), FNRS-Televie (7.4560.21 INCITE21, 7.4579.20 CD73 and 7.4559.2 IMPACT21), Roche Pharma, "Fondation Recherche Cancer et Sang", Luxembourg (INCOM BIOM) and Sheik Hamdan Bin Rashid Al Maktoum Foundation, United Arab Emirates.

REFERENCES

1. Singleton DC, Macann A, Wilson WR. Therapeutic Targeting of the Hypoxic Tumour Microenvironment. *Nat Rev Clin Oncol* (2021) 18(12):751–72. doi: 10.1038/s41571-021-00539-4
2. McKeown SR. Defining Normoxia, Physoxia and Hypoxia in Tumours-Implications for Treatment Response. *Br J Radiol* (2014) 87 (1035):20130676. doi: 10.1259/bjr.20130676
3. McAleese CE, Choudhury C, Butcher NJ, Minchin RF. Hypoxia-Mediated Drug Resistance in Breast Cancers. *Cancer Lett* (2021) 502:189–99. doi: 10.1016/j.canlet.2020.11.045
4. Chedeville AL, Madureira PA. The Role of Hypoxia in Glioblastoma Radiotherapy Resistance. *Cancers (Basel)* (2021) 13(3):542. doi: 10.3390/cancers13030542
5. Kopecka J, Salaroglio IC, Perez-Ruiz E, Sarmento-Ribeiro AB, Saponara S, De Las Rivas J, et al. Hypoxia as a Driver of Resistance to Immunotherapy. *Drug Resist Update* (2021) 59:100787. doi: 10.1016/j.drup.2021.100787
6. Semenza GL. Evaluation of Hif-1 Inhibitors as Anticancer Agents. *Drug Discovery Today* (2007) 12(19-20):853–9. doi: 10.1016/j.drudis.2007.08.006
7. Lisy K, Peet DJ. Turn Me On: Regulating Hif Transcriptional Activity. *Cell Death Differ* (2008) 15(4):642–9. doi: 10.1038/sj.cdd.4402315
8. Masoud GN, Li W. Hif-1alpha Pathway: Role, Regulation and Intervention for Cancer Therapy. *Acta Pharm Sin B* (2015) 5(5):378–89. doi: 10.1016/j.apsb.2015.05.007
9. Yang M, Su H, Soga T, Kranc KR, Pollard PJ. Prolyl Hydroxylase Domain Enzymes: Important Regulators of Cancer Metabolism. *Hypoxia (Auckl)* (2014) 2:127–42. doi: 10.2147/HP.S47968
10. Infantino V, Santarsiero A, Convertini P, Todisco S, Iacobazzi V. Cancer Cell Metabolism in Hypoxia: Role of Hif-1 as Key Regulator and Therapeutic Target. *Int J Mol Sci* (2021) 22(11):5703. doi: 10.3390/ijms22115703
11. Patel SA, Simon MC. Biology of Hypoxia-Inducible Factor-2alpha in Development and Disease. *Cell Death Differ* (2008) 15(4):628–34. doi: 10.1038/cdd.2008.17
12. Lau KW, Tian YM, Raval RR, Ratcliffe PJ, Pugh CW. Target Gene Selectivity of Hypoxia-Inducible Factor-Alpha in Renal Cancer Cells Is Conveyed by Post-DNA-Binding Mechanisms. *Br J Cancer* (2007) 96(8):1284–92. doi: 10.1038/sj.bjc.6603675
13. Albadari N, Deng S, Li W. The Transcriptional Factors Hif-1 and Hif-2 and Their Novel Inhibitors in Cancer Therapy. *Expert Opin Drug Discov* (2019) 14 (7):667–82. doi: 10.1080/17460441.2019.1613370
14. Kulshreshtha R, Davuluri RV, Calin GA, Ivan M. A Microrna Component of the Hypoxic Response. *Cell Death Differ* (2008) 15(4):667–71. doi: 10.1038/sj.cdd.4402310
15. Wu MZ, Tsai YP, Yang MH, Huang CH, Chang SY, Chang CC, et al. Interplay Between Hdac3 and Wdr5 Is Essential for Hypoxia-Induced Epithelial-Mesenchymal Transition. *Mol Cell* (2011) 43(5):811–22. doi: 10.1016/j.molcel.2011.07.012
16. Xia X, Lemieux ME, Li W, Carroll JS, Brown M, Liu XS, et al. Integrative Analysis of Hif Binding and Transactivation Reveals Its Role in Maintaining Histone Methylation Homeostasis. *Proc Natl Acad Sci USA* (2009) 106 (11):4260–5. doi: 10.1073/pnas.0810067106
17. Schodel J, Oikonomopoulos S, Ragoussis J, Pugh CW, Ratcliffe PJ, Mole DR. High-Resolution Genome-Wide Mapping of Hif-Binding Sites by Chip-Seq. *Blood* (2011) 117(23):e207–17. doi: 10.1182/blood-2010-10-314427
18. Semenza GL, Nejfelt MK, Chi SM, Antonarakis SE. Hypoxia-Inducible Nuclear Factors Bind to an Enhancer Element Located 3' to the Human Erythropoietin Gene. *Proc Natl Acad Sci USA* (1991) 88(13):5680–4. doi: 10.1073/pnas.88.13.5680
19. Wenger RH, Gassmann M. Oxygen(Es) and the Hypoxia-Inducible Factor-1. *Biol Chem* (1997) 378(7):609–16.
20. Anduran E, Dubois LJ, Lambin P, Winum JY. Hypoxia-Activated Prodrug Derivatives of Anti-Cancer Drugs: A Patent Review 2006 - 2021. *Expert Opin Ther Pat* (2022) 32(1):1–12. doi: 10.1080/13543776.2021.1954617
21. Wilson WR, Hay MP. Targeting Hypoxia in Cancer Therapy. *Nat Rev Cancer* (2011) 11(6):393–410. doi: 10.1038/nrc3064
22. Ratcliffe P, Koivunen P, Myllyharju J, Ragoussis J, Bovee JV, Batinic-Haberle I, et al. Update on Hypoxia-Inducible Factors and Hydroxylases in Oxygen Regulatory Pathways: From Physiology to Therapeutics. *Hypoxia (Auckl)* (2017) 5:11–20. doi: 10.2147/HP.S127042
23. Xia Y, Choi HK, Lee K. Recent Advances in Hypoxia-Inducible Factor (Hif)-1 Inhibitors. *Eur J Med Chem* (2012) 49:24–40. doi: 10.1016/j.ejmech.2012.01.033
24. Noman MZ, Janji B, Kaminska B, Van Moer K, Pierson S, Przanowski P, et al. Blocking Hypoxia-Induced Autophagy in Tumors Restores Cytotoxic T-Cell Activity and Promotes Regression. *Cancer Res* (2011) 71(18):5976–86. doi: 10.1158/0008-5472.CAN-11-1094
25. Noman MZ, Parpal S, Van Moer K, Xiao M, Yu Y, Viklund J, et al. Inhibition of Vps34 Reprograms Cold Into Hot Inflamed Tumors and Improves Anti-Pd-1/Pd-L1 Immunotherapy. *Sci Adv* (2020) 6(18):eaax7881. doi: 10.1126/sciadv.aax7881
26. Lequeux A, Noman MZ, Xiao M, Van Moer K, Hasmim M, Benoit A, et al. Targeting Hif-1 Alpha Transcriptional Activity Drives Cytotoxic Immune Effector Cells Into Melanoma and Improves Combination Immunotherapy. *Oncogene* (2021) 40(28):4725–35. doi: 10.1038/s41388-021-01846-x
27. Gorbachev AV, Fairchild RL. Regulation of Chemokine Expression in the Tumor Microenvironment. *Crit Rev Immunol* (2014) 34(2):103–20. doi: 10.1615/critrevimmunol.2014010062
28. Hojo S, Koizumi K, Tsuneyama K, Arita Y, Cui Z, Shinohara K, et al. High-Level Expression of Chemokine Cxcl16 by Tumor Cells Correlates With a Good Prognosis and Increased Tumor-Infiltrating Lymphocytes in Colorectal Cancer. *Cancer Res* (2007) 67(10):4725–31. doi: 10.1158/0008-5472.CAN-06-3424
29. Mgrditchian T, Arakelian T, Paggetti J, Noman MZ, Viry E, Moussay E, et al. Targeting Autophagy Inhibits Melanoma Growth by Enhancing Nk Cells Infiltration in a Ccl5-Dependent Manner. *Proc Natl Acad Sci USA* (2017) 114 (44):E9271–E9. doi: 10.1073/pnas.1703921114
30. Harlin H, Meng Y, Peterson AC, Zha Y, Tretiakova M, Slingluff C, et al. Chemokine Expression in Melanoma Metastases Associated With Cd8+ T-Cell Recruitment. *Cancer Res* (2009) 69(7):3077–85. doi: 10.1158/0008-5472.CAN-08-2281
31. Noman MZ, Hasmim M, Lequeux A, Xiao M, Duhem C, Chouaib S, et al. Improving Cancer Immunotherapy by Targeting the Hypoxic Tumor Microenvironment: New Opportunities and Challenges. *Cells* (2019) 8 (9):1083. doi: 10.3390/cells8091083
32. Noman MZ, Desantis G, Janji B, Hasmim M, Karray S, Dessen P, et al. Pd-L1 Is a Novel Direct Target of Hif-1alpha, and Its Blockade Under Hypoxia Enhanced Mdsc-Mediated T Cell Activation. *J Exp Med* (2014) 211(5):781–90. doi: 10.1084/jem.20131916
33. Barsoum IB, Smallwood CA, Siemens DR, Graham CH. A Mechanism of Hypoxia-Mediated Escape From Adaptive Immunity in Cancer Cells. *Cancer Res* (2014) 74(3):665–74. doi: 10.1158/0008-5472.CAN-13-0992
34. Messai Y, Gad S, Noman MZ, Le Teuff G, Couve S, Janji B, et al. Renal Cell Carcinoma Programmed Death-Ligand 1, a New Direct Target of Hypoxia-Inducible Factor-2 Alpha, Is Regulated by Von Hippel-Lindau Gene Mutation Status. *Eur Urol* (2016) 70(4):623–32. doi: 10.1016/j.eururo.2015.11.029
35. Zou W, Chen L. Inhibitory B7-Family Molecules in the Tumour Microenvironment. *Nat Rev Immunol* (2008) 8(6):467–77. doi: 10.1038/nri2326
36. Deng J, Li J, Sarde A, Lines JL, Lee YC, Qian DC, et al. Hypoxia-Induced Vista Promotes the Suppressive Function of Myeloid-Derived Suppressor Cells in

the Tumor Microenvironment. *Cancer Immunol Res* (2019) 7(7):1079–90. doi: 10.1158/2326-6066.CIR-18-0507
37. Jaiswal S, Jamieson CH, Pang WW, Park CY, Chao MP, Majeti R, et al. Cd47 Is Upregulated on Circulating Hematopoietic Stem Cells and Leukemia Cells to Avoid Phagocytosis. *Cell* (2009) 138(2):271–85. doi: 10.1016/j.cell.2009.05.046
38. Willingham SB, Volkmer JP, Gentles AJ, Sahoo D, Dalerba P, Mitra SS, et al. The Cd47-Signal Regulatory Protein Alpha (Sirpa) Interaction Is a Therapeutic Target for Human Solid Tumors. *Proc Natl Acad Sci USA* (2012) 109(17):6662–7. doi: 10.1073/pnas.1121623109
39. Zhang H, Lu H, Xiang L, Bullen JW, Zhang C, Samanta D, et al. Hif-1 Regulates Cd47 Expression in Breast Cancer Cells to Promote Evasion of Phagocytosis and Maintenance of Cancer Stem Cells. *Proc Natl Acad Sci USA* (2015) 112(45):E6215–23. doi: 10.1073/pnas.1520032112
40. Michaels AD, Newhook TE, Adair SJ, Morioka S, Goudreau BJ, Nagdas S, et al. Cd47 Blockade as an Adjuvant Immunotherapy for Resectable Pancreatic Cancer. *Clin Cancer Res* (2018) 24(6):1415–25. doi: 10.1158/1078-0432.CCR-17-2283
41. Soto-Pantoja DR, Terabe M, Ghosh A, Ridnour LA, DeGraff WG, Wink DA, et al. Cd47 in the Tumor Microenvironment Limits Cooperation Between Antitumor T-Cell Immunity and Radiotherapy. *Cancer Res* (2014) 74(23):6771–83. doi: 10.1158/0008-5472.CAN-14-0037-T
42. Noman MZ, Janji B, Berchem G, Mami-Chouaib F, Chouaib S. Hypoxia-Induced Autophagy: A New Player in Cancer Immunotherapy? *Autophagy* (2012) 8(4):704–6. doi: 10.4161/auto.19572
43. Hasmim M, Janji B, Khaled M, Noman MZ, Louache F, Bordereaux D, et al. Cutting Edge: Nanog Activates Autophagy Under Hypoxic Stress by Binding to Bnip3l Promoter. *J Immunol* (2017) 198(4):1423–8. doi: 10.4049/jimmunol.1600981
44. Hasmim M, Noman MZ, Messai Y, Bordereaux D, Gros G, Baud V, et al. Cutting Edge: Hypoxia-Induced Nanog Favors the Intratumoral Infiltration of Regulatory T Cells and Macrophages *Via* Direct Regulation of Tgf-Beta1. *J Immunol* (2013) 191(12):5802–6. doi: 10.4049/jimmunol.1302140
45. Noman MZ, Buart S, Romero P, Ketari S, Janji B, Mari B, et al. Hypoxia-Inducible Mir-210 Regulates the Susceptibility of Tumor Cells to Lysis by Cytotoxic T Cells. *Cancer Res* (2012) 72(18):4629–41. doi: 10.1158/0008-5472.CAN-12-1383
46. Barsoum IB, Hamilton TK, Li X, Cotechini T, Miles EA, Siemens DR, et al. Hypoxia Induces Escape From Innate Immunity in Cancer Cells *Via* Increased Expression of Adam10: Role of Nitric Oxide. *Cancer Res* (2011) 71(24):7433–41. doi: 10.1158/0008-5472.CAN-11-2104
47. Yamada N, Yamanegi K, Ohyama H, Hata M, Nakasho K, Futani H, et al. Hypoxia Downregulates the Expression of Cell Surface Mica Without Increasing Soluble Mica in Osteosarcoma Cells in a Hif-1alpha-Dependent Manner. *Int J Oncol* (2012) 41(6):2005–12. doi: 10.3892/ijo.2012.1630
48. Baginska J, Viry E, Berchem G, Poli A, Noman MZ, van Moer K, et al. Granzyme B Degradation by Autophagy Decreases Tumor Cell Susceptibility to Natural Killer-Mediated Lysis Under Hypoxia. *Proc Natl Acad Sci USA* (2013) 110(43):17450–5. doi: 10.1073/pnas.1304790110
49. Messai Y, Noman MZ, Hasmim M, Janji B, Tittarelli A, Boutet M, et al. Itpr1 Protects Renal Cancer Cells Against Natural Killer Cells by Inducing Autophagy. *Cancer Res* (2014) 74(23):6820–32. doi: 10.1158/0008-5472.CAN-14-0303
50. Sitkovsky M, Lukashev D. Regulation of Immune Cells by Local-Tissue Oxygen Tension: Hif1 Alpha and Adenosine Receptors. *Nat Rev Immunol* (2005) 5(9):712–21. doi: 10.1038/nri1685
51. Sitkovsky MV, Hatfield S, Abbott R, Belikoff B, Lukashev D, Ohta A. Hostile, Hypoxia-A2-Adenosinergic Tumor Biology as the Next Barrier to Overcome for Tumor Immunologists. *Cancer Immunol Res* (2014) 2(7):598–605. doi: 10.1158/2326-6066.CIR-14-0075
52. Terry S, Engelsen AST, Buart S, Elsayed WS, Venkatesh GH, Chouaib S. Hypoxia-Driven Intratumor Heterogeneity and Immune Evasion. *Cancer Lett* (2020) 492:1–10. doi: 10.1016/j.canlet.2020.07.004
53. Wigerup C, Pahlman S, Bexell D. Therapeutic Targeting of Hypoxia and Hypoxia-Inducible Factors in Cancer. *Pharmacol Ther* (2016) 164:152–69. doi: 10.1016/j.pharmthera.2016.04.009
54. Chan MC, Holt-Martyn JP, Schofield CJ, Ratcliffe PJ. Pharmacological Targeting of the Hif Hydroxylases–a New Field in Medicine Development. *Mol Aspects Med* (2016) 47-48:54–75. doi: 10.1016/j.mam.2016.01.001
55. Haase VH. Therapeutic Targeting of the Hif Oxygen-Sensing Pathway: Lessons Learned From Clinical Studies. *Exp Cell Res* (2017) 356(2):160–5. doi: 10.1016/j.yexcr.2017.05.004

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
published: 06 July 2022
doi: 10.3389/fimmu.2022.914890

Host-Related Factors as Targetable Drivers of Immunotherapy Response in Non-Small Cell Lung Cancer Patients

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OPEN ACCESS

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Reviewed by:
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Specialty section:
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

Received: 07 April 2022
Accepted: 13 May 2022
Published: 06 July 2022

Citation:
Baci D, Cekani E, Imperatori A, Ribatti D and Mortara L (2022) Host-Related Factors as Targetable Drivers of Immunotherapy Response in Non-Small Cell Lung Cancer Patients. Front. Immunol. 13:914890. doi: 10.3389/fimmu.2022.914890

Despite some significant therapeutic breakthroughs leading to immunotherapy, a high percentage of patients with non-small cell lung cancer (NSCLC) do not respond to treatment on relapse, thus experiencing poor prognosis and survival. The unsatisfying results could be related to the features of the tumor immune microenvironment and the dynamic interactions between a tumor and immune infiltrate. Host–tumor interactions strongly influence the course of disease and response to therapies. Thus, targeting host-associated factors by restoring their physiologic functions altered by the presence of a tumor represents a new therapeutic approach to control tumor development and progression. In NSCLC, the immunogenic tumor balance is shifted negatively toward immunosuppression due to the release of inhibitory factors as well as the presence of immunosuppressive cells. Among these cells, there are myeloid-derived suppressor cells, regulatory T cells that can generate a tumor-permissive milieu by reprogramming the cells of the hosts such as tumor-associated macrophages, tumor-associated neutrophils, natural killer cells, dendritic cells, and mast cells that acquire tumor-supporting phenotypes and functions. This review highlights the current knowledge of the involvement of host-related factors, including innate and adaptive immunity in orchestrating the tumor cell fate and the primary resistance mechanisms to immunotherapy in NSCLC. Finally, we discuss combinational therapeutic strategies targeting different aspects of the tumor immune microenvironment (TIME) to prime the host response. Further research dissecting the characteristics and dynamic interactions within the interface host–tumor is necessary to improve a patient fitness immune response and provide answers regarding the immunotherapy efficacy, with the aim to develop more successful treatments for NSCLC.

Keywords: non-small cell lung cancer, tumor microenvironment, immunotherapy, immune checkpoint inhibitors, anti-angiogenic therapies

INTRODUCTION

Lung cancer has become a leading cause of cancer death worldwide. The global incidence sees polarized differences according to the economic development of different countries. There is a decrease in the incidence among men in high-income countries due to public health measures and a gradual and progressive increase in both genders in low-income countries where public health initiatives for smoking cessation have lagged and access to healthcare is scarce (1–3). During the first decade of the present century, the outcomes of at least a subset of patients have seen substantial improvements, thanks to a general understanding of disease biology, the application of predictive biomarkers, and refinements in specific treatments (4).

Non-small cell lung cancer (NSCLC) represents approximately 85% of all lung cancer cases. It includes three major histologic classifications: adenocarcinoma (ADC), representing the most common subtype of lung cancer, followed by squamous-cell carcinoma (SCC) and large-cell carcinoma (LCC) (5).

Treatment is highly dependent on many parameters of the patient, particularly their general functional status, comorbidities, the tumor stage, and the molecular features of the disease. The primary treatment for stages I and II and in selected cases for stage III A disease is curative surgery, chemotherapy, radiation therapy (RT), or a combined modality approach. Postoperative adjuvant cisplatin-based chemotherapy is recommended in patients with completely resected stage II–IIIA disease and selected patients with stage IB disease (6). However, this therapy is associated with only a 16% decrease in the risk of disease recurrence or death; for 5 years, it is associated with a 5% decrease in the risk of death (7, 8).

Systemic therapy is pursued in the cases of patients with stage IV disease and in presence of metastases or in the presence of relapse after initial management.

Over a median follow-up of approximately five years, the percentage of patients who have disease recurrence or who die after surgery remains high (ranging from 45% among patients with stage IB disease to 76% among those with stage III disease), regardless of the use of postoperative chemotherapy (8). The opportunity for improving survival is pronounced in early-stage disease and is driving studies integrating targeted therapies and immune checkpoint inhibitors (ICIs). As a result, after the revolutionary data on the metastatic setting of epidermal growth factor receptor (EGFR) inhibitors (9–13), we, nowadays, have the impressive results of the Adaura trial, which led to an important improvement of disease-free survival (DFS) in a subset of patients with EGFR-mutated early-stage lung cancer when osimertinib was added as an adjuvant treatment to the main treatment for the duration of 3 years (14). Unfortunately, this subgroup is limited to only patients with EGFR-targetable mutations.

From the past decade to the present, with additional activating genomic alterations such as those affecting anaplastic lymphoma kinase (ALK), ROS1 proto-oncogene receptor tyrosine kinase, class 1 B-Raf proto-oncogene (BRAF) mutations (V600), mesenchymal-epithelial transition factor (MET), and neurotrophic receptor tyrosine kinase (NTRK) ALK, ROS1, B-Raf V600, MET, and NTRK alterations and the availability of an increasing number of specific tyrosine-kinase inhibitors (TKIs) of various generations, the proportion of patients with an improved prognosis has further increased (15–17).

The second pilar of the modern treatment of metastatic NSCLC is taken by immunotherapy (PD-1 and PD-L1 monoclonal antibodies), which is nowadays in the frontline of treatment in oncogenic driver–negative NSCLC and has produced response and survival rates that were unreachable a few years ago (18, 19).

Patients whose tumors express PD-L1 in at least 50% of the cells are more likely to attain a response and survive longer if treated with these compounds.

After breakthrough immune checkpoint inhibitor data in an advance setting, we now have the first results of immunotherapy in an adjuvant setting. The study IMpower 010 (20) addressed some of the unmet needs for adjuvant treatment oncodriver-negative tumors, adding immunotherapy in the plethora of new approvals in the early setting of NSCLC for patients expressing PD-L1 >1% on tumor cells (20).

In the meantime, immunotherapy continues to demonstrate a significant overall survival (OS) benefit in advanced NSCLC. In particular, pembrolizumab or atezolizumab monotherapies are superior to first-line chemotherapy in tumors with a higher expression of the PD-L1 molecule (21, 22). Interestingly, different chemo-immunotherapy combinations have been shown to be superior to chemotherapy, regardless of PD-L1 expression (23, 24).

Even though immunotherapy can produce great and long-lasting results, not all NSCLC patients seem to benefit from this approach (25). Many attempts have been made to identify predictive biomarkers to select responding patients who would benefit from ICIs. Tumor mutational burden (TMB) is a critical predictive factor for response to immunotherapy, but the available results need further confirmation in prospective randomized trials (26). A critical factor underlying the poor response to immunotherapies is the heterogeneity in the immune cell response to NSCLC and the existence of multiple mechanisms mediating tumor immune suppression (27). Indeed, a limited knowledge of the characteristics of the TME, to a great extent, hinders the development of new targets for immunotherapy. Here, we review the biological functions of immune cells within the tumor immune microenvironment (TIME) and their roles in cancer immunotherapy and discuss the perspectives of the basic and translational studies for improving the effectiveness of the clinical use.

OVERVIEW OF NSCLC TUMOR IMMUNE MICROENVIRONMENT

Taking advantage of new technologies (e.g., single-cell RNA sequencing), multiple ongoing studies are now identifying new subtypes of tumor-associated immune cells to predict the clinical efficacy of different immunotherapy approaches. The study of

immune tumor cell contexture in cancer patients to target the multiple immune-suppressive factors might ameliorate response rates and contribute to develop the era of personalized immune-based therapies. The immune cell populations present in the TIME possess both tumor-killing potentials and may alternatively promote or suppress immune cell activity. Tolerogenic immune cell populations such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) create an immune-suppressive milieu that, in turn, favors the polarization toward a protumor phenotype of other immune cells such as neutrophils, dendritic cells (DCs), and natural killer (NK) cells (**Figure 1** and **Table 1**). Understanding these immunologic states and the mechanisms underpinning them may provide the key to restore an effective anti-tumor immune response and improve the survival rate of NSCLC patients.

Neutrophils

Neutrophils are considered the first line of innate immune defense and are recognized as a critical targetable cellular feature of NSCLC TIME (28). Several preclinical and clinical studies have linked neutrophil trafficking and degranulation with various stages of tumor progression and the attenuation of treatment efficacy (29, 30). A high neutrophil/lymphocyte ratio (NLR) is now considered as a useful predictor associated with a negative clinical outcome, as well as with poor responsiveness to PD-1-/PD-L1 inhibitors (30). In accordance, Gentles et al. discovered that the neutrophil transcript signature was the strongest predictor of mortality and major infiltrating immune cells in adenocarcinoma NSCLC patients (31). In attempts to provide a clear description of the immune cell types present in NSCLC, Kargl et al. implicated neutrophils as the most abundant and dominant immune-suppressive factors associated with the depletion of $CD4^+$ and $CD8^+$ T lymphocytes within TIME (32). Consistent with previous findings, another study demonstrated a positive correlation between an increased tumor burden, high levels of neutrophil-related cytokines, and a dampened T-cell response associated with reduced $CD3^+CD8^+$ T-cell infiltration (33). However, the recruitment of neutrophils to the tumor microenvironment might depend on NSCLC subtypes and the smoking status, and larger studies are needed to define their role and association with survival (34).

Neutrophils in cancer consist of multiple heterogeneous cell populations and retain plasticity. A high expression of lectin-type oxidized LDL receptor 1 (LOX-1) distinguishes PMN-MDSCs

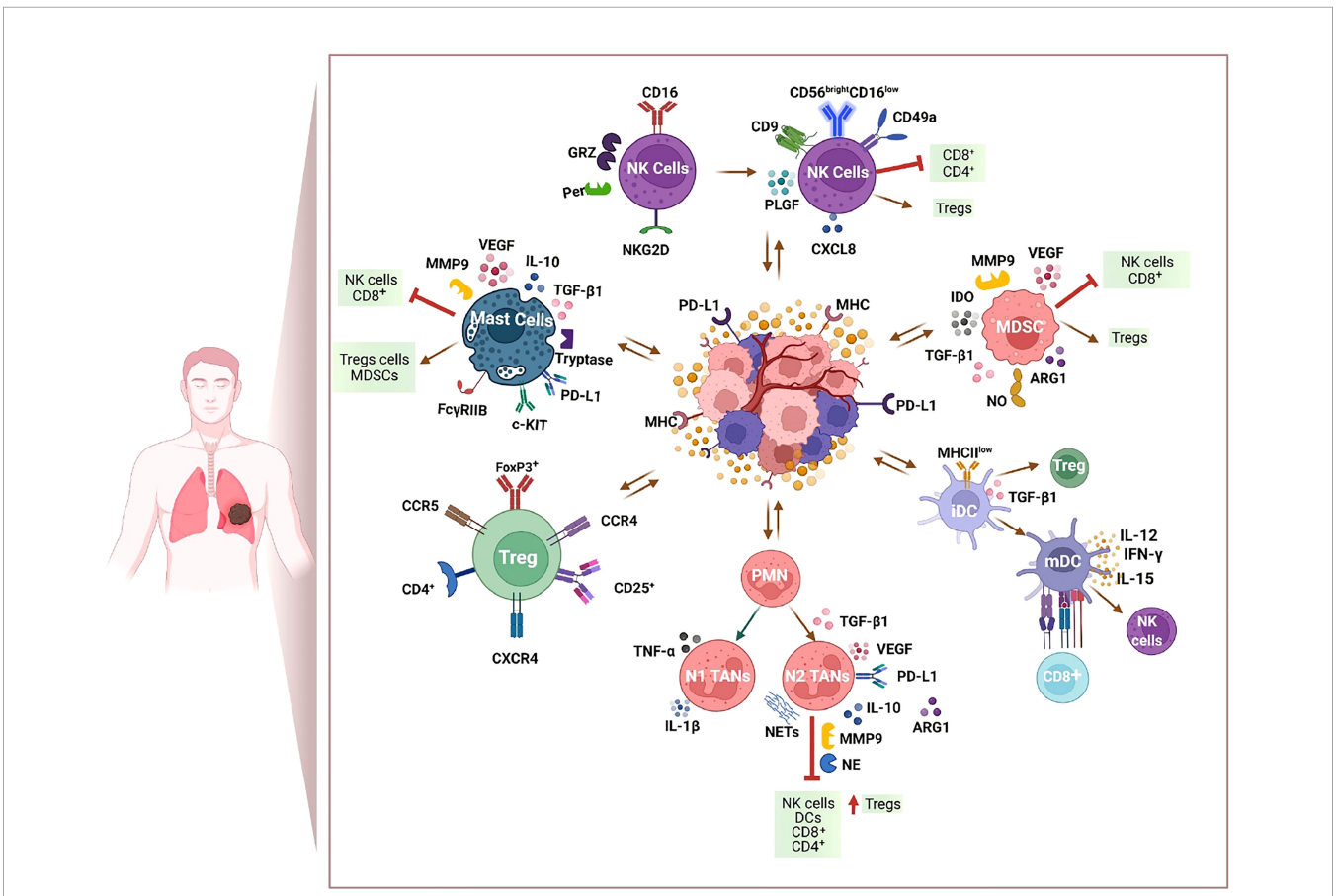

FIGURE 1 | Immunosuppressive milieu within the tumor immune microenvironment (TIME) of NSCLC. Immunotherapy resistance is marked by an immunosuppressive TIME and includes tumor-derived factors, infiltration of T regulatory cells (Tregs), myeloid-derived suppressor cells (MDSCs), and mast cells that, in turn, favor the polarization toward a protumor phenotype of other immune cells such as neutrophils, dendritic cells (DCs), and natural killer (NK) cells. Figure created with http://biorender.com.

TABLE 1 | NSCLC immune landscape: anti- and pro-tumorigenic phenotypes and activities of immune cell populations within the tumor microenvironment.

Tumor immune microenvironment (TIME)	Cell subpopulations	Cell markers	Anti-tumor properties	Pro-tumor properties
Neutrophils	N1 TANs	$CD11b^+$, $CD66b^+$, $CD15^+$, $CD16^+$, HLA-DR^-, TNF-α^{high}, $CXCR2^{low}$, $CXCL8^{low}$	IFN-γ, IL1 and TNF-α-mediated stimulation of immune response; ROS-mediated tumor killing; Promote $CD4^+$ T cell responses	NA
	N2 TANs	$CD11b^+$, $CD66b^+$, $CD15^+$, $CD16^+$, HLA-DR^-, TNF-α^{low}, $CXCR2^{high}$, $CXCL8^{high}$, $ARG1^{high}$	NA	MMP9, NE, VEGF-mediated tumor metastasis and invasion; IL10, TGF-β, ARG1, NETs-mediated immune suppression; Suppression of NK cells and $CD8^+$ T cells immune response
MDSCs	M-MDSCs	$CD11b^+$, $CD15^-$, $CD14^+$, HLA-$DR^{-/low}$, $S100A9^+$, $CD33^+$, $ILT3^{high}$	NA	MMPs, VEGF -mediated angiogenesis, invasiveness and metastasis; IL10, TGF-β, IDO, ARG1, and PGE-mediated immunosuppression; Suppression of NK cells, DCs the functions; Suppression of $CD8^+$ T cells antitumor response; Tregs differentiation and expansion
	PMN-MDSCs	$CD11b^+$, $CD14^-$, $CD15^+$, $CD66b^+$, HLA-$DR^{-/low}$, $Lox\text{-}1^+$		
NK cells	Cytotoxic NK cells	$CD56^{dim}$, $CD16^+$, $Perf^{high}$, GRZ^{high}, TNF-α^{high}, IFN-γ^{high}, $NKG2D^{high}$	Cytotoxic-mediated apoptosis of cancer cells; DCs maturation by releasing IFN-γ;	NA
	Immature/ decidua-like NK Cells	$CD56^{bright}$, $CD16^{low/-}$, $Perf^{low}$, IFN-γ^{low}, TNF-α^{low} $NKG2A^{high}$, $NKG2D^{low}$, CTLA-4^+, PD-1^+, $CD9^+$, $CD49a^+$, $CXCL8^+$	NA	Anergic NK cells-mediated tumor immune evasion; Angiogenesis induction releasing VEGF, PlGF, CXCL8; Suppression of DCs and $CD8^+$ T cells functions
NKT cells	Type I NKT	TCR binding with a α-GalCer, $CD3^+$, $CD4^+$, $CD8^+$, $CD56^+$, $CD161^+$	Killing of $CD1d^+$ tumor cells; IFN-γ-mediated stimulation of $CD8^+$ T cells immune response; Activation of NK cells	NA
	Type II NKT	TCR bindings with sulfatide-loaded CD1d, $CD3^+$	IFN-γ-mediated suppression of tumor growth	IL13-mediated immunosuppression
DCs	mDCs	HLA-DR^+, $CD80^+$, $CD83^+$, $CD86^+$, CD208/DC-$LAMP^+$	Th1 cytotoxic immune response; Stimulation of $CD8^+$ T cells immune response; Antigen presentation to T cells	NA
	iDCs	HLA-DR^{low}, $CD80^{low}$, $CD83^{low}$, $CD86^{low}$, CD208/DC-$LAMP^{low}$		Immunosuppression
Tregs		$CD3^+$, $CD4^+$, $CD25^+$, $FoxP3^+$, $CTLA4^+$, $CD127^{low}$, PD-1^+, CTLA-4^+, $CD39^+$, $CD73^+$	NA	Suppression of $CD8^+$ T cells mediated immune response
Mast cells		$FcεR1α^+$, $FcγRIIb/CD32^+$, CD117/c-kit^{high}, $CD203c^+$, $Tryptase^+$, $CD103^+$	Promote $CD4^+$ T cell responses	MMPs, chymase and tryptase-mediated metastasis; VEGFA-mediated angiogenesis

ARG1, arginase-1; HLA, human leukocyte antigen; iDCs, immature dendritic cells; IDO, indoleamine 2,3-dioxygenase; LOX-1, Lectin-like oxidized low-density lipoprotein (LDL) receptor-1; mDCs, mature dendritic cells; MDSCs, Myeloid-derived suppressor cells; MMP-9, matrix metalloproteinase-9; NA, not applicable; NETs, neutrophil extracellular traps; NK, natural killer; NKT, natural killer T; PMN-MDSCs, Polymorphonuclear-MDSCs; TANs, tumor-associated neutrophils; TGF-β, Transforming growth factor-beta; TIME, Tumor immune microenvironment; TNF-α, tumor necrosis factor-alpha; Treg, Regulatory T; VEGF, vascular endothelial growth factor; α-GalCer, glycolipid α-galactosylceramide.

from neutrophils (**Table 1**) (35). LOX-1$^+$ PMN-MDSC numbers increased with anti-PD-1 therapy in non-responders, suggesting immunosuppressive functions in patients with NSCLC (36). A poor NSCLC prognosis and recurrence after surgery have been associated with increased circulating CD15$^+$ LOX-1$^+$ PMN-MDSCs, thus displaying potential as a diagnostic marker for NSCLC. In patients with advanced stages of lung cancer, there have been reports of the accumulation of low-density neutrophils (LDNs), CD66b$^+$ PMNs, a subset of circulating neutrophils based on their sedimentation properties (37). Following tumor tissue infiltration and under specific tumor microenvironment cues, TANs can acquire a tumor- suppressive (N1) phenotype or become tumor-promoting/tolerogenic (N2) (**Figure 1** and **Table 1**). At the early stages of tumor development, N1 TANs predominate. A cytotoxic action of TANs and tumor regression was reported in a recent work employing a patient-derived xenograft (PDX) mouse model of early-stage NSCLC that received anti-PD-1 ICI, as a monotherapy or with cisplatin (38).

However, tumor growth, together with a shifted balance between IFN-β and TGF-β, can favor N2 neutrophils and/or PMN-MDSC accumulation. The release of tumor recruitment–soluble factors, such as CXCL8, CXCL1, CXCL5, CXCL7, IL-6, and IL-1β, enhances immunosuppressive neutrophil chemotaxis through CXCR2 sensing, found to be highly expressed in NSCLC patients (39, 40). In a murine lung cancer model, CXCR1/2 neutrophil receptor inhibition granted access to CD8$^+$ T cells to the malignant tumor. Notably, the IFN-γ signature was restored, thus overcoming neutrophil-mediated immunosuppression and an associated mitigation of the effectiveness of PD-1-targeted immunotherapy (41). N2 TANS enhances the immunosuppressive milieu by expressing high levels of PD-L1, arginase-1 (ARG1), reactive oxygen species (ROS), nitric oxide (NO), IL-10, and TGF-β1, shaping the tumor landscape and impairing T-cell-mediated cytotoxicity (42) The expression of both CXCL8 and Arg-1 by neutrophils is correlated with ICI therapy failure and poor prognosis in NSCLC (43, 44). N2 TANs also increase angiogenesis by releasing pro-angiogenic factors such as vascular endothelial growth factor (VEGF), enhance extracellular matrix (ECM) remodeling, and foster a pre-metastatic niche formation by directly acting as the primary source of proteolytic enzymes (45, 46). High levels of an MMP9:tissue inhibitor of metalloproteinase-3 (TIMP-3) ratio have been found significantly elevated in NSCLC biopsies. Furthermore, neutrophil elastase (NE) and myeloperoxidase (MPO) high degranulation induce the formation of neutrophil extracellular traps (NETs), directly implicated in metastasis (47, 48) (**Figure 1**). Accordingly, NETs are involved in a vascular endothelium injury mediated by an inflammatory response (48), as well as the wrapping and shielding of tumor cells from cytotoxicity mediated by CD8$^+$ T cells and NK cells (47, 49). The Inhibition of NETosis sensitizes tumors to PD-1 plus CTLA-4 inhibition (47).

Several therapeutic strategies to suppress N2 tumor-promoting phenotypes or reactivate their cytotoxic features toward cancer cells are in preclinical and clinical phases of evaluation (28). Main neutrophil- targeting approaches neutralize tumor-derived chemokines, promoting their influx within the tumor microenvironment and conversion to an MDSC-like phenotype/N2 TANS. Two recent studies suggested that targeting MDSCs *via* the antagonism of GM-CSF and fatty acid transport protein 2 (FATP2) by using lipofermata decreased ROS and PGE2-levels and their immunosuppressive functions in tumor-bearing mice (50, 51). Importantly, FATP2 enhanced anti-PD-L1 tumor immunotherapy and inhibited tumor progression (50, 51). Metformin also targets FATP2, disabling the suppressive capacity of granulocytic myeloid-derived suppressor cells eliciting Th1 and cytotoxic T lymphocytes (CTLs) responses (52). Retrospective studies suggest the synergistic actions of metformin and conventional chemotherapy, improving the survival and outcomes of patients with NSCLC (53, 54). Targeting key immunosuppressive factors in TIME such as TGF-β1 and chemokine receptors CXCR1/2 through pharmacological antagonists represent some of the strategies to block the immunosuppressive milieu, leading to tumor growth and the nonsuccess of immunotherapies.

Myeloid-Derived Suppressor Cells

Myeloid-derived suppressor cells (MDSCs) represent a group of heterogeneous cells derived from immature myeloid progenitors with strong immunosuppressive features and functions. According to their phenotypic and morphological features, MDSCs have been classified into two major subsets: monocytic MDSCs (M-MDSCs) expressing CD14 and granulocytic or polymorphonuclear (PMN-MDSCs) expressing CD15 and CD66b; both types express CD33 in addition to CD11b with the absence of HLA-DR (**Table 1**). Even though PMN-MDSCs and neutrophils share similar phenotypic cell surface markers in humans, they have a distinct unique transcriptomic/phenotypic profile and functions that reflect the different roles within the tumor setting: PMN-MDSC but not neutrophils display immunosuppressive activities (55–57). A specific expression of LOX-1, fewer granules, and a reduced expression of CD16 can distinguish PMN-MDSCs phenotypically from neutrophils (**Table 1**) (35, 57, 58). In addition, neutrophils are high-density cells, whereas PMN-MDSCs are enriched in a low-density mononuclear cell fraction (55, 59). On the other hand, M-MDSCs can be distinguished from monocytes by detecting MHC class II, expressed only on monocytes (HLA-DR$^+$) (60).

M-MDSCs and PMN-MDSCs share similar features; both enable immune response suppression but use different immunosuppressive mechanisms. For instance, PMN-MDSCs express high levels of ROS and low levels of NO, whereas M-MDSCs are the opposite. M-MDSCs preferentially exert their immunosuppressive functions by releasing IL-10, TGF-β, iNOS, and Arg-1 (56, 61).

Several studies have reported an accumulation of M-MDSCs in NSCLC patients (62–64). An increased pool of CD11b$^+$CD14$^-$CD15$^+$CD33$^+$ MDSCs and decreased CD8$^+$ T cytotoxic lymphocytes have been reported in the peripheral blood of NSCLC patients (65). Another study reported a correlation between a subset of MDSC CD14$^+$S100A9$^+$, T-cell

suppression mediated by arginase, iNOS, IL-13/IL-4Rα axis, and poor response to chemotherapy (66). Goeje et al. described increased levels of MDSCs expressing immunoglobulin-like transcript 3 (ILT3), identified as $CD11b^{+}CD14^{-}$ $CD33^{+}CD15^{+}$ $HLA\text{-}DR^{-}ILT3^{high}$, associated with the immunosuppressive function of ILT3 on DCs and with reduced survival (67).

MDSCs are recruited to the tumor site *via* chemokines such as CCL2 and CXCL8 (68, 69). Of notice, CXCL8 is linked to the recruitment and activation of MDSCs and neutrophils. Indeed, the serum levels of CXCL8 may predict the responses to immunotherapies (68, 70, 71). TGF-β signaling has also been reported to promote the recruitment of MDSCs into tumors (72) and directly induce the generation of $CD39^{+}CD73^{+}$ myeloid cells in NSCLC patients *via* the activation of mTOR-HIF-1 signaling (73, 74) (**Figure 1**). $CD39^{+}CD73^{+}$ MDSCs are a distinct immunosuppressive subset, and their frequency in NSCLC patients may be sufficient to predict the chemotherapeutic response (74).

MDSCs are the largest producer of indoleamine 2,3-dioxygenase (IDO), directly acting on the immunosuppressive pathway of anti-tumor $CD8^{+}$ T lymphocytes and the increase of Treg cell activity in the lung tumor microenvironment (75) (**Figure 1**). In a preclinical model of lung cancer, it was demonstrated that MDSC-associated IDO modulates the *in vivo* and *ex vivo* differentiation of B regulatory cells (Bregs), an IL-10 producing subset of B cells, found to be reduced in tumor-bearing IDO deficient mice ($IDO^{-/-}$) (76). The anti-immune functions of MDSCs involve different mechanisms such as the production of NO, ROS, and the elimination of arginine required for T lymphocyte functions. In a $Kras^{G12D}$ GEMM of a lung adenocarcinoma model, the suppression of MDSC arginase activity by an ARG1 inhibitor restored T-cell function by increasing arginine (77). MDSCs could also enhance angiogenesis and metastasis through the production of MMP9 and VEGF (**Figure 1**). A tight association of PMN-MDSC number with a patient response to the ICI anti-PD-1 has been reported (36). An enhanced APC activity and increased frequency of $CD8^{+}$ T or NK intracytoplasmic expression of IFN-γ, perforin, and granzyme were found following MDSC depletion (36). Accordingly, another study demonstrated an increased number and function of NK- and T-cell effectors in the tumor and enhanced therapeutic vaccination responses after the depletion of MDSCs (78). Therefore, the inhibition of MDSC functions represents the key therapeutic solution to restore anti-tumor T lymphocyte effector responses and successful immunotherapy. Until today, only a few preparations endorsed by the U.S. Food and Drug Administration (FDA) have been described to have prominent effects on the recruitment and function of MDSCs (e.g., ATRA, vitamin D, gemcitabine, and bevacizumab). Considering the promising results of targeting MDSCs in murine models of lung cancer, various clinical trials are now ongoing in NSCLC patients (NCT02922764; NCT03846310; NCT03801304; NCT04262388). Breakthroughs in this research area should promote the rational design of new strategies to target MDSCs to improve clinical responses to current immunotherapies.

Natural Killer Cells

NK cells represent innate effector lymphocytes with abilities to counteract or limit both tumor cells and virus-infected cells (79). In humans, the cell surface expression of the CD56 marker is the main phenotype marker for NK cells in association with a negative lineage-defining signature ($CD3^{-}$, $CD14^{-}$, $CD19^{-}$, and TCR^{-}), whereas in mice, it is the NK1.1 marker. NK cells do not need specific antigen stimulation but are activated toward neoplastic or stressed cells through the fine balance between multiple invariant activating and inhibitory receptors. The major inhibitory receptors are represented by the killer cell immunoglobulin–like receptor (KIR) family, which represents 17 distinct genes endowed with a high polymorphism, and the CD94/NKG2A heterodimer. Recognizing MHC Class I (MHC-I) molecules on a target cell, inhibitory receptors block NK cell activation (80).

When MHC-I are lost, or their expression is diminished, and this is the case of most tumor developments, NK cells become more susceptible to activation through the involvement of multiple activating receptors such as NKp30, NKp46, NKp44, CD16, NKG2D, DNAX accessory molecule1 (DNAM1), 2B4, and NKp80. Human peripheral blood NK cells can be classified into two subsets in relation to the expression of CD56 and CD16 markers: $CD56^{dim}CD16^{+}$ NK cells (comprising 90%–95% of total blood NK cells), characterized by their cytotoxic activity exerted by perforin and granzyme release and mediating antibody-dependent cellular cytotoxicity (ADCC) and $CD56^{bright}CD16^{-}$ NK cells (5%–10% of total circulating NK cells), endowed with the capacity of proinflammatory cytokine production, such as IFN-γ and TNF-α and regulatory cytokines like IL-10 (81).

In NSCLC, it has been reported that intratumor NK cells profoundly modify their phenotype and functions, with the expansion of a $CD56^{bright}CD16^{-}$ NK cell subset, impairment of cytotoxicity, inhibition of IFN-γ release, and acquisition of pro-angiogenic features (**Table 1**) (82, 83). This tumor-dependent NK cell subset has similarities with a different NK cell subset termed decidual NK (dNK) cells that was identified within decidua. This dNK cell subset was identified as $CD56^{superbright}CD16^{-}$ NK cell, and it was shown to be an important regulatory cell in the maternal–fetal interface because of its ability to release not only several pro-angiogenic cytokines and growth factors such as VEGF, PlGF, and CXCL8 but also IFN-γ, which are essential to driving the spiral artery formation during the embryo development (84).

Also, in the context of other types of solid cancers, NK cells accumulating within the tumor microenvironment had immature features and a $CD56^{bright}CD16^{low/-}Perf^{low}$ phenotype (**Table 1**).

Several soluble factors derived from tumor cells or neighboring innate immune or stromal cells can inhibit and alter NK-cell functions such as TGF-β, PGE2, IDO, adenosine, and IL-10 (85).

We were the first to characterize the decidual-like $CD56^{bright}CD16^{-}$ of NSCLC patients with the ability to release pro-angiogenic factors: VEGF, PlGF, and CXCL8 (**Figure 1**) (**Table 1**). The NK-cell subset had the *in vitro* ability to trigger human umbilical vein endothelial cell (HUVEC) migration and

the formation of capillary-like structures (86, 87). These peculiar functions are not restricted to the intratumoral NK cells but are also present in their peripheral blood counterpart, suggesting that vascular network induction occurs at a systemic level, too. Moreover, these pro-angiogenic features were detected at a higher intensity in NK cells from patients with squamous cell carcinomas than those with adenocarcinomas. Interestingly, the expansion of pro-angiogenic and decidual-like NK cells was also detected in malignant pleural effusions, colorectal cancer, and prostate cancer patients (88–90).

Recently, Russick et al. analyzed the gene expression profile of intratumoral NK cells and found that in comparison to non-tumorous NK cells, immune cells had a significant decrease of sphingosine-1-phosphate receptor 1 (S1PR1) and CX3CR1 with a concomitant increase of CXCR5 and CXCR6. Intriguingly, they also showed that intratumoral NK cells express inhibitory molecules: CTLA-4 and killer cell lectin like receptor (KLRC1), together with a high expression of CD69 and NKp44, conferring inhibitory capabilities in the context of TIME (91). Indeed, the co-culturing of purified NSCLC NK cells with tumor cells and $CD11c^+$ peripheral blood autologous DC in the presence of LPS resulted in the impairment of DC maturation expressed as a percentage of MHC class II and CD86 on DCs. Interestingly, this phenomenon was partially counteracted by the addition of CTLA-4-blocking antibodies. However, the precise mechanism is still not clear, and beyond CTLA-4 expression, other mechanisms could be involved, such as yet-unidentified secreted molecules from NK-cell-derived tumor cells. However, in a tumor mouse model, another possible mechanism of NK-cell-dependent DC inhibition has been identified *via* PD-L1 with PD-1 expressed on DCs (92).

Moreover, a high intratumor density of NK cells is correlated with an improved clinical outcome only in patients with a low infiltration of $CD8^+$ T cells, while in patients with elevated $CD8^+$ T lymphocyte counts, NK cells conferred a negative impact (91). At later stages, lung tumoral NK cells showed significantly attenuated cytotoxicity, the reduction of levels of granzyme B, perforin, CD107a, IFN-γ, TNF-α, cytotoxic receptor CD27, activating receptor NKG2D, and a higher expression of the inhibitory receptor NKG2A (93).

Natural Killer T Cells

Natural killer T cells (NKT) cells are a subset of heterogeneous innate-like T lymphocytes CD1d-restricted, recognizing lipid antigens and co-expressing both the T-cell receptor and NK-cell markers, such as CD56, CD16, and NKp46 in humans and NKp46 and NK1.1 in mice. NKT cells can be subdivided into two major subsets: type I and type II NKT cells according to TCR rearrangements and glycolipid reactivity (94, 95).

Type I or invariant NKT (iNKT) cells are cytotoxic cells that express an invariant TCRα chain rearrangement, whereas TCRβ chains present a restricted repertoire. These cells include several subsets called NKT1, NKT2, and NKT17, with similarities to Th1, Th2, and Th17 T-cell subsets, respectively. Type II NKT cells, conversely, display a more diverse repertoire of Vα rearrangements (96, 97). Whereas it is well documented that iNKT cells participate in the anti-tumor response (98), type II NKT cells, on the contrary, enhance tumor growth and metastasis, thus indicating a pro-tumor activity.

The predominant anti-tumor feature of iNKT cells mainly resides in their capacity to release large amounts of Th1 cytokines, such as IFN-γ, in addition to their ability to kill CD1d-positive tumor cells (99, 100) (**Table 1**).

Several studies have shown a relationship between the number and activity of iNKT cells and clinical outcomes, making these cells an interesting therapeutic tool against cancer development and metastasis (99, 101).

However, in NSCLCs, it has been shown that iNKT cells were diminished both in blood and in bronchial lavage samples from patients (102). Moreover, the lung CD1d expression is lowered in NSCLC patients and weak CD1d mRNA expression is significantly associated with poor prognosis. Together, this could indicate a role played by these cells in immunity against NSCLC (102). *In vitro* studies using DNA methyltransferase and histone deacetylase inhibitors on two CD1d-negative NSCLC cell lines: A549 and SK-MES-1, showed the induction of CD1d expression and cytotoxicity directed toward them by iNKT cells, making epigenetic manipulation an interesting immunotherapeutic approach against NSCLC.

A study protocol was mentioned in an ongoing exploring phase I/II clinic trial on 30 patients with EGFR mutation–positive stage III/IV NSCLC that will evaluate the efficacy and safety of using allogeneic $CD3^+CD8^+$ iNKT cells in combination with EGFR-TKIs such as gefitinib (103).

Dendritic Cells

Dendritic cells (DCs) are antigen-presenting cells (APCs) and consist of three major subsets: myeloid conventional DC1s (cDC1s), myeloid conventional DC2s (cDC2s), and plasmacytoid DCs (pDCs) (104). Several lines of evidence point out that all DC subsets have the capacity to trigger anti-tumor T- cell responses and that DC1s need cooperativity with the other DC subsets (105). Interestingly, it has been shown that DC1s regulate the response to ICIs in mouse models and correlated with better OS in patients with cancer; however, DC1s can be expanded in tumors that resist checkpoint treatment, suggesting that these cells may be altered in their functions (106). Maier et al., using single-cell RNA sequencing in human and mouse NSCLC specimens identified a type of DCs nominated "mature DCs abundant in immunoregulatory factors" (mregDCs), which possessed both immunoregulatory genes (Cd200, Cd274, and Pdcd1lg2) and maturation genes (Cd40, Ccr7, and Il12b). The mregDC function was detected in both DC1 and DC2 subsets upon interaction with tumor antigens and can exert a dual role, both regulatory and immunogenic. It has been shown that the two key steps crucial for regulatory effects driven by mregDCs were the upregulation of PD-L1 and of IL-12, the first was under the control of the receptor tyrosine kinase AXL while the second under the control of IL-4 signaling (106).

Moreover, immature DCs (imDCs), which are present sometimes in high numbers in the tumor microenvironment, can coordinate an immunosuppressive microenvironment together with other innate cells, such as Tregs, MDSCs, and

TAMs (**Figure 1** and **Table 1**) (107, 108). Several lung patients could present tertiary lymphoid structures in the stroma of NSCLC, representing a well-organized compartment with lymphocytes and a rise in the density of DC-LAMP$^+$ mature DCs, suggesting that these structures might participate in antitumoral immunity. Indeed, several studies showed that these structures were associated with a favorable clinical outcome, together with a Th1 cytotoxic immune response and effective infiltrating CD8$^+$ T cells (109, 110).

Interestingly, Inoshima et al. reported an immunohistochemical study in which they analyzed 132 lung cancer specimens showing that a high expression of VEGF and microvessel density is associated with low intratumoral DC infiltration and worse prognosis, whereas low VEGF and high DC are correlated with a better prognosis (111). VEGF that could be produced not only by tumor cells but also by TAMs and NK cells, in addition to having a role in tumor vascular formation, also has a role as an inhibitory molecule for several classes of immune cells, including DCs. Therefore, the subtle regulatory mechanisms involved in the TIME between NK and DC interactions, not yet fully elucidated, as seen above *via* CTLA-4 or PD-1 on NK cells, could underlie the divergent functions of DCs and, in some cases, therefore lead to negative outcomes for the immune response, that is, the expansion of TAMs and Tregs, with a protumoral effect (91, 92).

Mast Cells

Mast cells are bone marrow–derived immune cells with multiple protective functions against invading microorganisms and harmful agents. These long-lived immune cells exert their regulatory functions in immunity and inflammation by producing key inflammatory mediators, such as tryptase, VEGF, IL-10, TGF-β1, and MMP9, and the relevant data of their anti-tumor or pro-tumor features have been reported (**Figure 1** and **Table 1**). Interestingly, Fontanini et al. investigated the relationship between tumor angiogenesis and survival in 407 NSCLC patients (112). In this study, a worse prognosis was significantly correlated with the increase of the tumor blood vessel network. However, in 2007, a meta-analysis did not confirm an independent prognostic role of vascular density in patients with non-metastatic-treated NSCLC patients (113). The expression of VEGF-A, VEGF-C, and VEGFR-1 was associated with a worse outcome in patients with NSCLC (114). A significant prognostic value of the overexpression of FGF-2 has been reported in patients with operable NSCLC (115). Mast cells are correlated with angiogenesis and a poor outcome in lung adenocarcinoma (116, 117). Angiogenesis assessed by microvessel counts is related with a poor outcome in stage I NSCLC (112, 118–120). Other authors have shown no significant correlations with respect to survival in patients with NSCLC for microvessel density or mast cell infiltration. (121–127). Niczyporuk et al. did not show any correlation between the mast cell count, microvascular count, and survival rate in NSCLC (128). There is no correlation between intratumoral mast cells and angiogenesis in NSCLC (129) and between mast cells and survival in NSCLC (125).

Mast cells present in tumor cell islets are correlated with a marked survival advantage in NSCLC (130). Indeed, whereas mast cell numbers are similar in the tumor stroma of patients with surgically resected NSCLC with no difference to their survival status, there is a substantial survival advantage when mast cells are localized within the clusters of tumor epithelial cells or tumor cell islets (130, 131).

Furthermore, Tomita et al. (132) and Welsh et al. (130) determined a strict correlation between the number of mast cells and a good prognosis in NSCLC. Mast cells have a pro-tumorigenic effect on lung tumor cell lines and an anti-tumorigenic effect *in vivo* (133). Conversely, Stoyanov et al. (133) have reported a significant effect of mast cells and histamine in enhancing NSCLC cell proliferation *in vitro*, whereas in the Lewis lung mouse carcinoma model, they have found that mast cells are crucial negative regulators of cancer development.

Tregs

Regulatory T lymphocytes (Tregs) are involved in the homeostasis of the immune system, inhibiting autoimmune disorders. Moreover, these cells collaborate with other cells and factors in establishing immunosuppressive TIME (**Figure 1**) (134–136).

The transcription factor forkhead box P3 (FoxP3) is crucial for peripheral naïve T cells to become competent Treg cells (**Table 1**). In lung cancer, Foxp3$^+$ Tregs, which suppress auto-reactive T cells to maintain immunological self-tolerance and inhibit autoimmunity, are associated with advanced tumor growth and poor prognosis (137–139). In patients with NSCLC, augmented numbers of blood and intratumoral Tregs are correlated with worse prognosis and a higher risk or recurrence (140).

Several investigations reported significantly higher percentages of CD4$^+$CD25$^+$FoxP3$^+$ Tregs in patients with advanced metastatic NSCLC compared to healthy donors (141–144), whereas the high percentage of CD152$^+$CD4$^+$CD25^{high}FoxP3$^+$ Tregs is correlated with a more advanced stage of disease (141, 145). Moreover, two studies reported a prognostic value of blood CD4$^+$FoxP3$^+$ Tregs in stage I–III NSCLC patients (146, 147).

In NSCLC patients, CD4$^+$CD25$^+$ Treg subtype functions were associated with their FoxP3, CTLA-4, and IL-7Rα expression, and their blood levels were correlated with the clinical outcome of the patients. Conversely, no difference was found in the percentage of CD4$^+$CD25$^+$FoxP3$^+$ Treg between the entire NSCLC patients and healthy donors (148). Interestingly Tao et al. (139) demonstrated that in NSCLC, there was no significant relationship between the Treg number and the tumor Foxp3 status. However, increased numbers of Tregs were associated with worse overall and relapse-free survival, whereas there was no correlation between the tumor FoxP3 status and survival. In the meantime, when FoxP3$^+$ cells were detected within the tumor, the Treg expansion was correlated with the attenuation of worse prognosis. Conversely, the patients in which there was no tumor FoxP3 expression and elevated Treg count had significantly worse overall and relapse-free survival. Collectively, these findings suggest that tumor FoxP3

expression has a better prognostic potential in NSCLC and that, in combination with intratumoral Tregs, the absence of the tumor FoxP3 is correlated with high-risk patients.

THE LUNG TIME AS A TARGET FOR THERAPY

The microenvironment of lung cancers is heterogeneous and plays an important role in determining the outcome. The lungs present a unique milieu in which tumors progress in synergy with the TIME, as evidenced by the regions of aberrant angiogenesis, inflammation, and hypoxia. The altered vasculature seen in lung cancers contributes to hypoxia and makes it difficult to efficiently deliver agents through the bloodstream. Hypoxia is associated with an increased risk of metastases as well as resistance to radiation therapy and perhaps chemotherapy. Neutrophils dominate the tumor microenvironment of NSCLC, suppressing T cells and promoting immunosuppression. The multifaceted microenvironment of lung tumors represents many potential targets for the development of novel anticancer agents. As with other cancers, in NSCLC, chronic inflammation represents a major risk factor for the development and progression of cancer.

Tumor-infiltrating $CD8^+$ T lymphocytes were associated with improved anti-tumor immunity, as well as with better prognosis in the advanced stage of NSCLC patients (149). Other cell types, such as TAMs and TANs and their subtypes, have their own prognostic effects in NSCLC (150). Furthermore, Tuminello et al. demonstrated the positive role of $CD8^+$ T cytotoxic cells, $CD20^+$ B cells, and NK cells with survival in patients with early resectable NSCLC (151).

The assessment of tumor inflammation is also of interest, but again, various approaches are being pursued, including a histological assessment of immune cell infiltrates and the mRNA-based expression signatures of immune-related genes. Increased numbers of antitumor $CD8^+$ and $CD4^+$ T cells have been associated with responding tumors and improved survival, whereas elevated frequencies of Tregs render tumors refractory to immune effector cells (152). The altered vasculature in NSCLC contributes to hypoxia and makes it difficult to efficiently deliver agents through the bloodstream. We have a variety of clinically applicable agents that can modulate the TIME in a way that might improve the response to cytotoxic therapy.

Molecular-targeted therapy represents a fundamental aspect in the treatment of advanced NSCLC. In the past few years, the identification of new molecular subtypes, the search for tumor driver gene mutations, and the development of molecular targeted drugs, such as agents that are able to suppress tumor angiogenesis and regulate tumor immune response, have been the main directions of NSCLC research, clinical diagnosis, and treatment.

In metastatic NSCLC, cytotoxic chemotherapy has been replaced with targeted therapy or immunotherapy. The gene mutation status of EGFR in the tumor tissues of NSCLC is closely related to the efficacy of the TKIs. Getifinib was the first EGFR-TKI tested in patients with advanced NSCLC. The discovery of EGFR mutations provided the biological explanation for the clinical predictors of response to EGFR-TKIs (153). Virtually, all EGFR mutation patients developed acquired resistance to therapy. EMT is implicated in mediating resistance to EGFR inhibitors, chemotherapy, and other targeted drugs in lung cancer (154). In NSCLC, invasive tumor growth is associated with a desmoplastic stroma reaction and the upregulation of EMT markers at the invasive front (155). The inflammatory component of the tumor microenvironment stimulates EMT in lung cancer by contributing to hypoxia, angiogenesis, and the different regulations of miRNAs (156).

Second-generation EGFR TKIs, including afatinib, dacomitinib, and neratinib, have been developed with the intent to delay or overcome acquired resistance (157). Afatinib and dacomitinib resulted in more efficacy than gefitinib in terms of progression-free survival (PFS) and the response rate, whereas gefitinib is associated with fewer side effects (157).

Immunotherapy with anti-PD-1/PD-L1 antibodies has modified the treatment of locally advanced and metastatic NSCLC. The approval of the anti-PD-1 agent pembrolizumab as a standard-of-care first-line treatment in selected patients has made PD-L1 immunohistochemistry a mandatory test in all patients with advanced NSCLC. Immunotherapy alone (pembrolizumab) or in combination with chemotherapy (pembrolizumab or atezolizumab) is the standard of care for first-line therapy in stage IV NSCLC.

In the mouse models of lung cancer, the anti-PD-L1 approach is associated with a rise in exhausted $CD8^+$ T lymphocytes (158). At the same time, enhanced numbers of $PD\text{-}1^+CD8^+$ T lymphocytes were correlated with reduced survival in stage II and III patients (149). The increased expression of CD38 on T cells after PD-1/PD-L1 ICI favors to acquired resistance by inhibiting $CD8^+$ T lymphocyte proliferation and inducing an exhausted phenotype (159). Koh et al. (160) analyzed the correlation between $Foxp3^+$ T cells with clinical outcomes before and after anti-PD-1 immunotherapy in patients with advanced NSCLC and found that a higher frequency of blood Tregs 1 week after immunotherapy was associated with prolonged PFS and OS when compared with patients with a low frequency of Tregs. In the meantime, a high expression of TGF-β was correlated with high levels of Tregs and with a favorable clinical outcome.

ANTI-ANGIOGENIC THERAPIES

Angiogenesis has been strictly related with occurrence, proliferation, and metastasis (161). Targeting the angiogenesis process has been reported to be efficacious in diverse types of cancers, including NSCLC (22). Abnormal vasculature participates in the tumor escape. Anti-angiogenetic agents can normalize blood vessels and thereby reset the TIME from immunosuppressive into immunoreactive. Therefore, combining immunotherapy with anti-angiogenics seems to be a promising strategy for cancer treatments.

The mechanisms appear to be complex and quite a vicious circle where the abnormality of angiogenesis causes an increase in acidity, hypoxia, and interstitial pressure (161, 162), which, later on, are associated with modifications at the molecular and genetic level in blood vessel formation and proliferation, and thus exacerbating and feeding a hostile tumor microenvironment.

In clinical terms, we already have a few monoclonal antibodies approved by the FDA and EMA for the treatment of various cancer types (bevacizumab-binding to VEGF-A, ramucirumab-targeting VEGFR2). By inhibiting the interaction between the VEGF and VEGFR or targeting downstream signaling, these compounds could block tumor angiogenesis. Their efficacy has been proven as a combination therapy with other cytotoxic agents (carboplatin and paclitaxel plus bevacizumab (163), or docetaxel plus ramucirumab (164); meanwhile, as a monotherapy, it showed a limited therapeutic effect in cancer treatment (165).

Ideally, anti-angiogenesis reduces thevascular supply, and thereby impairs tumor cell replication by starving the tumor, but this phenomenon could also decrease the delivery of combination drugs.

Some recent attempts have been taken to solve this paradox. "Vessel normalization" stands at the basis of resetting the perfusion function and structure, enhancing the antitumor immune response by implementing immune cell infiltration (165–168). This procedure gives promises for anti-angiogenesis combined therapies.

Nonetheless, due to the cancer heterogeneity and the multiple aspects of the TIME, the global response rates to ICI therapy are still limited (169). One major factor decreasing the efficacy of ICIs seems to be the elevated recruited numbers of immunosuppressive cells and scarce infiltration of effector cells into the TIME (170).

Latest studies have indicated that pro-angiogenic factors in the tumor microenvironment favor and trigger the development of immunosuppressive cells, and in the meantime, neo vessels impair the infiltration of immune effector cell cancer (171–173).

The use of ICIs in combination with anti-angiogenic agents is hypothesized to be a promising strategy to enhance the global therapeutic efficacy.

There is a progressive and increased understanding on the possible effectivity of anti-angiogenic and IO combination. Nowadays, there are many preclinical and clinical trials suggesting that angiogenesis affects the TIME toward an immunosuppressive state by modifying the recruitment of immune cells (174–178). Later, clinical studies supported that the inhibition of the VEGF/VEGFR signaling can restore the anti-tumor T effector response (172). The use of bevacizumab (avastin) resulted in enhanced cytotoxic T lymphocyte functions in NSCSL as well as in CRC patients (179, 180).

It is well established that TIME is a complex, time-evolving ecosystem consisting not only of tumor cells but also of immune cell blood vessels, stroma cells, and different soluble factors, which turn off antitumor immune responses and favor ineffective immunotherapies (181).

Overstimulation by VEGF signaling in cancer leads to abnormal angiogenesis characterized by increased interstitial fluid pressure, hypoxia, and acidosis. This phenomenon leads to the suppression of the antitumor response through multiple distinct mechanisms (182, 183).

Hypoxia facilitates the infiltration of suppressive immune cells (Tregs, MDSCs, TAMs, and imDCs) by inducing the expression of chemokines (like CSF1, GM-CSF, IL-6, and IL-10) that recruit these immune cells (184); on the other hand, it also inhibits the infiltration of effector T cells through the activation of VEGF (185).

The stimulation and regulation of several key immune cells of TIME such as DCs, MDSCs, Tregs, and TAMs are under the control of VEGF signaling (186, 187). Immunosuppressive factors IL-10, IDO, and TGF-β released by these suppressive immune cells increase even more the immunosuppressive status of TIME (188).

Noteworthy, the inhibition of the VEGF signaling impairs the recruitment of suppressive cells into the tumor microenvironment and, at the same time, increases the infiltration of effector T cells (189). This fact implies that anti-VEGF/VEGFR therapy not only targets the blood vessel function but has the capacity to reactivate antitumor immune responses (173).

In addition to the above negative effects played by VEGF, another effect is related to their capacity to influence an enhanced expression of PD-1, Tim3, and CTLA-4 on activated $CD8^+$ T lymphocytes (190). Moreover, VEGF inhibition could result in enhanced IFN-γ production and consequently the induction of PD-L1 expression on tumor cells. This phenomenon provides a strong promise for the anti-angiogenic and ICI drug combined treatment (172, 173).

Currently, we already have the clinical data of a phase III trial Impower 150 (191), which showed a clinical benefit of the combination of IO and bevacizumab plus chemotherapy in NSCLC; in the meantime, other clinical trials are ongoing to assess the safety and efficacy of this new combination therapy in NSCLC (NCT01454102 (CM 012), NCT03689855 (RamAtezo-1), NCT03836066 (TELMA), and others.

IMMUNOTHERAPEUTIC APPROACHES

Current ICIs directed to CD28-CTLA4/B7 and PD-1/PD-L1 can unleash the power of T cells toward cancer cells by eliminating negative signals that block T-cell functions (192) (193, 194).

Several immune cells such as T cells, NK cells, B cells, and monocytes express PD-1 (195).

Monoclonal antibodies against PD-1, PD-L1, and CTLA-4 are the most used ICIs for NSCLC patients. A number of PD-1, PD-L1, and CTLA-4 inhibitors, including pembrolizumab (196), nivolumab (197), atezolizumab (198), durvalumab (199), avelumab (200), and ipilimumab (201), have been approved for the treatment of advanced NSCLC.

The anti-PD-1 agent pembrolizumab is approved for use as first- and second-line therapy in patients with advanced NSCLC whose tumors express PD-L1 in immunohistochemistry analysis. Nivolumab (anti-PD-1) and atezolizumab (anti-PD-L1) are both indicated for use as second-line therapies regardless of PD-L1

expression. Durvalumab (anti-PD-L1) is approved as a maintenance therapy in patients with unresectable stage III NSCLC whose disease has not progressed following concurrent platinum-based chemoradiotherapy.

Five randomized phase II–III trials testing three ICIs (nivolumab, pembrolizumab, and atezolizumab), all showed a clinically and statistically significant advantage over the same standard comparator docetaxel (21, 22, 197, 202, 203)

ICIs were tested in locoregional NSCLC. A phase III trial demonstrated that adjuvant durvalumab in stage III NSCLC non-progressing after concomitant chemo-radiotherapy improved not only PFS but also OS (204).

Pembrolizumab and nivolumab approval is strictly related with a positive PD-L1 expression.

Checkpoint inhibitors can be used as a combination therapy or as a monotherapy in first- and second-line treatments. The Pacific trial (205) brought immunotherapy in a locally advanced setting and later on, with the publication of IMpower 010 (20), immunotherapy will probably be a practice changing even in early-stage lung cancer.

CHALLENGES AND FUTURE DIRECTIONS

A prognostic role of many TIME biomarkers is not yet part of the current clinical practice, so further investigations that include larger patient cohorts will be necessary.

ICI alone or in combination with chemotherapy or in combination with other ICIs should be the first-line treatment of choice for patients with advanced NSCLC who do not have contraindications to immunotherapy and whose tumors do not harbor actionable driver mutations. Advances with immunotherapy have offered patients with lung cancer substantial improvements in survival and the quality of life. However, better predictive biomarkers are required to ameliorate the benefit of immunotherapy, and further investigations are needed to find out the mechanisms of resistance to ICIs and how to overcome it. Whereas the PD-1 and PD-L1 ICIs have received accelerated FDA approvals, the development of predictive and prognostic biomarkers for these agents have lagged far behind and remains a crucial area for future research.

The ability to increase the clinical benefit for higher numbers of NSCLC patients and preventing drug resistance will be essential prerequisites to achieve in the near future and related to the acquisition of more knowledge of the induced mechanisms underlying effective antitumor effector responses. The next step will be to better identify patients at the risk of primary or acquired resistance and use increasing amounts of translational research data to develop more effective combination therapies, making the promise of ICIs available to all patients with NSCLC. This is the only way to achieve further advances in cancer immunotherapy and succeed in making the promise of ICIs for all patients.

AUTHOR CONTRIBUTIONS

Conceptualization: DB, DR and LM. Text drafting and editing: DB, EC, AI, DR, and LM. Critical revision: DB, EC, AI, and LM. Figure and table preparation: DB. Funds: DB and LM. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the Italian Ministry of Health-Grant Giovani Ricercatori 2019 (GR-019-12370076) to DB, and by Fondi di Ateneo per la Ricerca FAR2019 and FAR2020, University of Insubria to LM.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Raffaella Bombelli and Dr. Marì De Leo for their helpful comments in the preparation of the manuscript.

REFERENCES

1. Jemal A, Center MM, DeSantis C, Ward EM. Global Patterns of Cancer Incidence and Mortality Rates and Trends. *Cancer Epidemiol Biomarkers Prev* (2010) 19(8):1893–907. doi: 10.1158/1055-9965.EPI-10-0437
2. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2020. *CA Cancer J Clin* (2020) 70(1):7–30. doi: 10.3322/caac.21590
3. Giovino GA, Mirza SA, Samet JM, Gupta PC, Jarvis MJ, Bhala N, et al. Tobacco Use in 3 Billion Individuals From 16 Countries: An Analysis of Nationally Representative Cross-Sectional Household Surveys. *Lancet* (2012) 380 (9842):668–79. doi: 10.1016/S0140-6736(12)61085-X
4. Howlader N, Forjaz G, Mooradian MJ, Meza R, Kong CY, Cronin KA, et al. The Effect of Advances in Lung-Cancer Treatment on Population Mortality. *N Engl J Med* (2020) 383(7):640–9. doi: 10.1056/NEJMoa1916623
5. Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin JHM, Beasley MB, et al. The 2015 World Health Organization Classification of Lung Tumors: Impact of Genetic, Clinical and Radiologic Advances Since the 2004 Classification. *J Thorac Oncol* (2015) 10(9):1243–60. doi: 10.1097/JTO.0000000000000630
6. Ghysen K, Vansteenkiste J. Immunotherapy in Patients With Early Stage Resectable Nonsmall Cell Lung Cancer. *Curr Opin Oncol* (2019) 31(1):13–7. doi: 10.1097/CCO.0000000000000497
7. Kris MG, Gaspar LE, Chaft JE, Kennedy EB, Azzoli CG, Ellis PM, et al. Adjuvant Systemic Therapy and Adjuvant Radiation Therapy for Stage I to IIIA Completely Resected Non-Small-Cell Lung Cancers: American Society of Clinical Oncology/Cancer Care Ontario Clinical Practice Guideline Update. *J Clin Oncol* (2017) 35(25):2960–74. doi: 10.1200/JCO.2017.72.4401
8. Pignon JP, Tribodet H, Scagliotti GV, Douillard JY, Shepherd FA, Stephens RJ, et al. Lung Adjuvant Cisplatin Evaluation: A Pooled Analysis by the LACE Collaborative Group. *J Clin Oncol* (2008) 26(21):3552–9. doi: 10.1200/JCO.2007.13.9030
9. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib Versus Standard Chemotherapy as First-Line Treatment for European Patients With Advanced EGFR Mutation-Positive Non-Small-Cell Lung Cancer (EURTAC): A Multicentre, Open-Label, Randomised Phase 3 Trial. *Lancet Oncol* (2012) 13(3):239–46. doi: 10.1016/S1470-2045(11)70393-X

10. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or Carboplatin-Paclitaxel in Pulmonary Adenocarcinoma. *N Engl J Med* (2009) 361(10):947–57. doi: 10.1056/NEJMoa0810699
11. Sequist LV, Yang JC, Yamamoto N, O'Byrne K, Hirsh V, Mok T, et al. Phase III Study of Afatinib or Cisplatin Plus Pemetrexed in Patients With Metastatic Lung Adenocarcinoma With EGFR Mutations. *J Clin Oncol* (2013) 31 (27):3327–34. doi: 10.1200/JCO.2012.44.2806
12. Lee CK, Davies L, Wu YL, Mitsudomi T, Inoue A, Rosell R, et al. Gefitinib or Erlotinib vs Chemotherapy for EGFR Mutation-Positive Lung Cancer: Individual Patient Data Meta-Analysis of Overall Survival. *J Natl Cancer Inst* (2017) 109(6). doi: 10.1093/jnci/djw279
13. Kanitkar AA, Schwartz AG, George J, Soubani AO. Causes of Death in Long-Term Survivors of Non-Small Cell Lung Cancer: A Regional Surveillance, Epidemiology, and End Results Study. *Ann Thorac Med* (2018) 13(2):76–81. doi: 10.4103/atm.ATM_243_17
14. Wu YL, Tsuboi M, He J, John T, Grohe C, Majem M, et al. Osimertinib in Resected EGFR-Mutated Non-Small-Cell Lung Cancer. *N Engl J Med* (2020) 383(18):1711–23. doi: 10.1056/NEJMoa2027071
15. Hida T, Nokihara H, Kondo M, Kim YH, Azuma K, Seto T, et al. Alectinib Versus Crizotinib in Patients With ALK-Positive Non-Small-Cell Lung Cancer (J-ALEX): An Open-Label, Randomised Phase 3 Trial. *Lancet* (2017) 390(10089):29–39. doi: 10.1016/S0140-6736(17)30565-2
16. Shaw AT, Felip E, Bauer TM, Besse B, Navarro A, Postel-Vinay S, et al. Lorlatinib in Non-Small-Cell Lung Cancer With ALK or ROS1 Rearrangement: An International, Multicentre, Open-Label, Single-Arm First-in-Man Phase 1 Trial. *Lancet Oncol* (2017) 18(12):1590–9. doi: 10.1016/S1470-2045(17)30680-0
17. Camidge DR, Kim HR, Ahn MJ, Yang JCH, Han JY, Hochmair MJ, et al. Brigatinib Versus Crizotinib in Advanced ALK Inhibitor-Naive ALK-Positive Non-Small Cell Lung Cancer: Second Interim Analysis of the Phase III ALTA-1l Trial. *J Clin Oncol* (2020) 38(31):3592–603. doi: 10.1200/JCO.20.00505
18. Califano R, Gomes F, Ackermann CJ, Rafee S, Tsakonas G, Ekman S. Immune Checkpoint Blockade for non-Small Cell Lung Cancer: What is the Role in the Special Populations? *Eur J Cancer* (2020) 125:1–11. doi: 10.1016/j.ejca.2019.11.010
19. Xia B, Herbst RS. Immune Checkpoint Therapy for non-Small-Cell Lung Cancer: An Update. *Immunotherapy* (2016) 8(3):279–98. doi: 10.2217/imt.15.123
20. Felip E, Altorki N, Zhou C, Csoszi T, Vynnychenko I, Goloborodko O, et al. Adjuvant Atezolizumab After Adjuvant Chemotherapy in Resected Stage IB-IIIA Non-Small-Cell Lung Cancer (IMpower010): A Randomised, Multicentre, Open-Label, Phase 3 Trial. *Lancet* (2021) 398(10308):1344–57. doi: 10.1016/S0140-6736(21)02098-5
21. Herbst RS, Baas P, Kim DW, Felip E, Perez-Gracia JL, Han JY, et al. Pembrolizumab Versus Docetaxel for Previously Treated, PD-L1-Positive, Advanced Non-Small-Cell Lung Cancer (KEYNOTE-010): A Randomised Controlled Trial. *Lancet* (2016) 387(10027):1540–50. doi: 10.1016/S0140-6736(15)01281-7
22. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csoszi T, Fulop A, et al. Pembrolizumab Versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. *N Engl J Med* (2016) 375(19):1823–33. doi: 10.1056/NEJMoa1606774
23. Gandhi L, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab Plus Chemotherapy in Metastatic Non–Small-Cell Lung Cancer. *New Engl J Med* (2018) 378(22):2078–92. doi: 10.1056/NEJMoa1801005
24. Paz-Ares L, Luft A, Vicente D, Tafreshi A, Gumus M, Mazieres J, et al. Pembrolizumab Plus Chemotherapy for Squamous Non-Small-Cell Lung Cancer. *N Engl J Med* (2018) 379(21):2040–51. doi: 10.1056/NEJMoa1810865
25. Massett HA, Mishkin G, Moscow JA, Gravell A, Steketee M, Kruhm M, et al. Transforming the Early Drug Development Paradigm at the National Cancer Institute: The Formation of NCI's Experimental Therapeutics Clinical Trials Network (ETCTN). *Clin Cancer Res* (2019) 25(23):6925–31. doi: 10.1158/1078-0432.CCR-19-1754
26. Addeo A, Banna GL, Weiss GJ. Tumor Mutation Burden-From Hopes to Doubts. *JAMA Oncol* (2019) 5(7):934–5. doi: 10.1001/jamaoncol.2019.0626
27. Lei X, Lei Y, Li JK, Du WX, Li RG, Yang J, et al. Immune Cells Within the Tumor Microenvironment: Biological Functions and Roles in Cancer Immunotherapy. *Cancer Lett* (2020) 470:126–33. doi: 10.1016/j.canlet.2019.11.009
28. Anderson R, Blidner AG, Rapoport BL. Frontiers in Pharmacology: Review Manuscript Targeting of the Neutrophil as an Adjunctive Strategy in Non-Small Cell Lung Cancer. *Front Pharmacol* (2021) 12. doi: 10.3389/fphar.2021.676399
29. Huang Y, Shen A. The Prediction Potential of Neutrophil-to-Lymphocyte Ratio for the Therapeutic Outcomes of Programmed Death Receptor-1/Programmed Death Ligand 1 Inhibitors in Non-Small Cell Lung Cancer Patients: A Meta-Analysis. *Med (Baltimore)* (2020) 99(34):e21718. doi: 10.1097/MD.0000000000021718
30. Rapoport BL, Theron AJ, Vorobiof DA, Langenhoven L, Hall JM, Van Eeden RI, et al. Prognostic Significance of the Neutrophil/Lymphocyte Ratio in Patients Undergoing Treatment With Nivolumab for Recurrent non-Small-Cell Lung Cancer. *Lung Cancer Manage* (2020) 9(3):LMT37. doi: 10.2217/lmt-2020-0014
31. Gentles AJ, Newman AM, Liu CL, Bratman SV, Feng W, Kim D, et al. The Prognostic Landscape of Genes and Infiltrating Immune Cells Across Human Cancers. *Nat Med* (2015) 21(8):938–45. doi: 10.1038/nm.3909
32. Kargl J, Busch SE, Yang GH, Kim KH, Hanke ML, Metz HE, et al. Neutrophils Dominate the Immune Cell Composition in Non-Small Cell Lung Cancer. *Nat Commun* (2017) 8:14381. doi: 10.1038/ncomms14381
33. Mitchell KG, Diao L, Karpinets T, Negrao MV, Tran HT, Parra ER, et al. Neutrophil Expansion Defines an Immunoinhibitory Peripheral and Intratumoral Inflammatory Milieu in Resected Non-Small Cell Lung Cancer: A Descriptive Analysis of a Prospectively Immunoprofiled Cohort. *J Immunother Cancer* (2020) 8(1). doi: 10.1136/jitc-2019-000405
34. Tamminga M, Hiltermann TJN, Schuuring E, Timens W, Fehrmann RS, Groen HJ. Immune Microenvironment Composition in non-Small Cell Lung Cancer and Its Association With Survival. *Clin Transl Immunol* (2020) 9(6): e1142. doi: 10.1002/cti2.1142
35. Condamine T, Dominguez GA, Youn JI, Kossenkov AV, Mony S, Alicea-Torres K, et al. Lectin-Type Oxidized LDL Receptor-1 Distinguishes Population of Human Polymorphonuclear Myeloid-Derived Suppressor Cells in Cancer Patients. *Sci Immunol* (2016) 1(2). doi: 10.1126/sciimmunol.aaf8943
36. Kim HR, Park SM, Seo SU, Jung I, Yoon HI, Gabrilovich DI, et al. The Ratio of Peripheral Regulatory T Cells to Lox-1(+) Polymorphonuclear Myeloid-Derived Suppressor Cells Predicts the Early Response to Anti-PD-1 Therapy in Patients With Non-Small Cell Lung Cancer. *Am J Respir Crit Care Med* (2019) 199(2):243–6. doi: 10.1164/rccm.201808-1502LE
37. Shaul ME, Eyal O, Guglietta S, Aloni P, Zlotnik A, Forkosh E, et al. Circulating Neutrophil Subsets in Advanced Lung Cancer Patients Exhibit Unique Immune Signature and Relate to Prognosis. *FASEB J* (2020) 34(3):4204–18. doi: 10.1096/fj.201902467R
38. Martin-Ruiz A, Fiuza-Luces C, Martinez-Martinez E, Arias CF, Gutierrez L, Ramirez M, et al. Effects of Anti-PD-1 Immunotherapy on Tumor Regression: Insights From a Patient-Derived Xenograft Model. *Sci Rep* (2020) 10(1):7078. doi: 10.1038/s41598-020-63796-w
39. Saintigny P, Massarelli E, Lin S, Ahn YH, Chen Y, Goswami S, et al. CXCR2 Expression in Tumor Cells Is a Poor Prognostic Factor and Promotes Invasion and Metastasis in Lung Adenocarcinoma. *Cancer Res* (2013) 73(2):571–82. doi: 10.1158/0008-5472.CAN-12-0263
40. Yuan M, Zhu H, Xu J, Zheng Y, Cao X, Liu Q. Tumor-Derived CXCL1 Promotes Lung Cancer Growth *via* Recruitment of Tumor-Associated Neutrophils. *J Immunol Res* (2016) 2016:6530410. doi: 10.1155/2016/6530410
41. Kargl J, Zhu X, Zhang H, Yang GHY, Friesen TJ, Shipley M, et al. Neutrophil Content Predicts Lymphocyte Depletion and Anti-PD1 Treatment Failure in NSCLC. *JCI Insight* (2019) 4(24). doi: 10.1172/jci.insight.130850
42. Jaillon S, Ponzetta A, Di Mitri D, Santoni A, Bonecchi R, Mantovani A. Neutrophil Diversity and Plasticity in Tumour Progression and Therapy. *Nat Rev Cancer* (2020) 20(9):485–503. doi: 10.1038/s41568-020-0281-y
43. Schalper KA, Carleton M, Zhou M, Chen T, Feng Y, Huang SP, et al. Elevated Serum Interleukin-8 Is Associated With Enhanced Intratumor Neutrophils and Reduced Clinical Benefit of Immune-Checkpoint Inhibitors. *Nat Med* (2020) 26(5):688–92. doi: 10.1038/s41591-020-0856-x
44. Chen JJ, Yao PL, Yuan A, Hong TM, Shun CT, Kuo ML, et al. Up-Regulation of Tumor Interleukin-8 Expression by Infiltrating Macrophages: Its

Correlation With Tumor Angiogenesis and Patient Survival in non-Small Cell Lung Cancer. *Clin Cancer Res* (2003) 9(2):729–37.
45. Vannitamby A, Seow HJ, Anderson G, Vlahos R, Thompson M, Steinfort D, et al. Tumour-Associated Neutrophils and Loss of Epithelial PTEN can Promote Corticosteroid-Insensitive MMP-9 Expression in the Chronically Inflamed Lung Microenvironment. *Thorax* (2017) 72(12):1140–3. doi: 10.1136/thoraxjnl-2016-209389
46. Moroy G, Alix AJ, Sapi J, Hornebeck W, Bourguet E. Neutrophil Elastase as a Target in Lung Cancer. *Anticancer Agents Med Chem* (2012) 12(6):565–79. doi: 10.2174/187152012800617696
47. Teijeira A, Garasa S, Gato M, Alfaro C, Migueliz I, Cirella A, et al. CXCR1 and CXCR2 Chemokine Receptor Agonists Produced by Tumors Induce Neutrophil Extracellular Traps That Interfere With Immune Cytotoxicity. *Immunity* (2020) 52(5):856–71.e8. doi: 10.1016/j.immuni.2020.03.001
48. Cools-Lartigue J, Spicer J, McDonald B, Gowing S, Chow S, Giannias B, et al. Neutrophil Extracellular Traps Sequester Circulating Tumor Cells and Promote Metastasis. *J Clin Invest* (2013) (8):3446–58. doi: 10.1158/1538-7445.AM2012-2972
49. Demers M, Wong SL, Martinod K, Gallant M, Cabral JE, Wang Y, et al. Priming of Neutrophils Toward NETosis Promotes Tumor Growth. *Oncoimmunology* (2016) 5(5):e1134073. doi: 10.1080/2162402X.2015.1134073
50. Adeshakin AO, Liu W, Adeshakin FO, Afolabi LO, Zhang M, Zhang G, et al. Regulation of ROS in Myeloid-Derived Suppressor Cells Through Targeting Fatty Acid Transport Protein 2 Enhanced Anti-PD-L1 Tumor Immunotherapy. *Cell Immunol* (2021) 362:104286. doi: 10.1016/j.cellimm.2021.104286
51. Veglia F, Tyurin VA, Blasi M, De Leo A, Kossenkov AV, Donthireddy L, et al. Fatty Acid Transport Protein 2 Reprograms Neutrophils in Cancer. *Nature* (2019) 569(7754):73–8. doi: 10.1038/s41586-019-1118-2
52. Xu P, Yin K, Tang X, Tian J, Zhang Y, Ma J, et al. Metformin Inhibits the Function of Granulocytic Myeloid-Derived Suppressor Cells in Tumor-Bearing Mice. *BioMed Pharmacother* (2019) 120:109458. doi: 10.1016/j.biopha.2019.109458
53. Luo X, Chen X, Wang L, Yang B, Cai S. Metformin Adjunct With Antineoplastic Agents for the Treatment of Lung Cancer: A Meta-Analysis of Randomized Controlled Trials and Observational Cohort Studies. *Front Pharmacol* (2021) 12. doi: 10.3389/fphar.2021.639016
54. Levy A, Doyen J. Metformin for non-Small Cell Lung Cancer Patients: Opportunities and Pitfalls. *Crit Rev Oncol Hematol* (2018) 125:41–7. doi: 10.1016/j.critrevonc.2018.03.001
55. Dumitru CA, Moses K, Trellakis S, Lang S, Brandau S. Neutrophils and Granulocytic Myeloid-Derived Suppressor Cells: Immunophenotyping, Cell Biology and Clinical Relevance in Human Oncology. *Cancer Immunol Immunother* (2012) 61(8):1155–67. doi: 10.1007/s00262-012-1294-5
56. Veglia F, Sanseviero E, Gabrilovich DI. Myeloid-Derived Suppressor Cells in the Era of Increasing Myeloid Cell Diversity. *Nat Rev Immunol* (2021) 21(8):485–98. doi: 10.1038/s41577-020-00490-y
57. Zhou J, Nefedova Y, Lei A, Gabrilovich D. Neutrophils and PMN-MDSC: Their Biological Role and Interaction With Stromal Cells. *Semin Immunol* (2018) 35:19–28. doi: 10.1016/j.smim.2017.12.004
58. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, et al. Recommendations for Myeloid-Derived Suppressor Cell Nomenclature and Characterization Standards. *Nat Commun* (2016) 7:12150. doi: 10.1038/ncomms12150
59. Kotsakis A, Harasymczuk M, Schilling B, Georgoulias V, Argiris A, Whiteside TL. Myeloid-Derived Suppressor Cell Measurements in Fresh and Cryopreserved Blood Samples. *J Immunol Methods* (2012) 381(1-2):14–22. doi: 10.1016/j.jim.2012.04.004
60. Mandruzzato S, Brandau S, Britten CM, Bronte V, Damuzzo V, Gouttefangeas C, et al. Toward Harmonized Phenotyping of Human Myeloid-Derived Suppressor Cells by Flow Cytometry: Results From an Interim Study. *Cancer Immunol Immunother* (2016) 65(2):161–9. doi: 10.1007/s00262-015-1782-5
61. Gabrilovich DI. Myeloid-Derived Suppressor Cells. *Cancer Immunol Res* (2017) 5(1):3–8. doi: 10.1158/2326-6066.CIR-16-0297
62. Zahran AM, Hetta HF, Zahran ZAM, Rashad A, Rayan A, Mohamed DO, et al. Prognostic Role of Monocytic Myeloid-Derived Suppressor Cells in Advanced Non-Small-Cell Lung Cancer: Relation to Different Hematologic Indices. *J Immunol Res* (2021) 2021:3241150. doi: 10.1155/2021/3241150
63. Huang A, Zhang B, Wang B, Zhang F, Fan KX, Guo YJ. Increased CD14(+) HLA-DR (-/Low) Myeloid-Derived Suppressor Cells Correlate With Extrathoracic Metastasis and Poor Response to Chemotherapy in Non-Small Cell Lung Cancer Patients. *Cancer Immunol Immunother* (2013) 62(9):1439–51. doi: 10.1007/s00262-013-1450-6
64. Vetsika EK, Koinis F, Gioulbasani M, Aggouraki D, Koutoulaki A, Skalidaki E, et al. A Circulating Subpopulation of Monocytic Myeloid-Derived Suppressor Cells as an Independent Prognostic/Predictive Factor in Untreated non-Small Lung Cancer Patients. *J Immunol Res* (2014) 2014:659294. doi: 10.1155/2014/659294
65. Liu CY, Wang YM, Wang CL, Feng PH, Ko HW, Liu YH, et al. Population Alterations of L-Arginase- and Inducible Nitric Oxide Synthase-Expressed CD11b+/CD14(-)/CD15+/CD33+ Myeloid-Derived Suppressor Cells and CD8+ T Lymphocytes in Patients With Advanced-Stage Non-Small Cell Lung Cancer. *J Cancer Res Clin Oncol* (2010) 136(1):35–45. doi: 10.1007/s00432-009-0634-0
66. Feng PH, Lee KY, Chang YL, Chan YF, Kuo LW, Lin TY, et al. CD14(+) S100A9(+) Monocytic Myeloid-Derived Suppressor Cells and Their Clinical Relevance in Non-Small Cell Lung Cancer. *Am J Respir Crit Care Med* (2012) 186(10):1025–36. doi: 10.1164/rccm.201204-0636OC
67. de Goeje PL, Bezemer K, Heuvers ME, Dingemans AC, Groen HJ, Smit EF, et al. Immunoglobulin-Like Transcript 3 is Expressed by Myeloid-Derived Suppressor Cells and Correlates With Survival in Patients With non-Small Cell Lung Cancer. *Oncoimmunology* (2015) 4(7):e1014242. doi: 10.1080/2162402X.2015.1014242
68. Alfaro C, Teijeira A, Onate C, Perez G, Sanmamed MF, Andueza MP, et al. Tumor-Produced Interleukin-8 Attracts Human Myeloid-Derived Suppressor Cells and Elicits Extrusion of Neutrophil Extracellular Traps (NETs). *Clin Cancer Res* (2016) 22(15):3924–36. doi: 10.1158/1078-0432.CCR-15-2463
69. Lesokhin AM, Hohl TM, Kitano S, Cortez C, Hirschhorn-Cymerman D, Avogadri F, et al. Monocytic CCR2(+) Myeloid-Derived Suppressor Cells Promote Immune Escape by Limiting Activated CD8 T-Cell Infiltration Into the Tumor Microenvironment. *Cancer Res* (2012) 72(4):876–86. doi: 10.1158/0008-5472.CAN-11-1792
70. Sanmamed MF, Carranza-Rua O, Alfaro C, Onate C, Martin-Algarra S, Perez G, et al. Serum Interleukin-8 Reflects Tumor Burden and Treatment Response Across Malignancies of Multiple Tissue Origins. *Clin Cancer Res* (2014) 20(22):5697–707. doi: 10.1158/1078-0432.CCR-13-3203
71. Sanmamed MF, Perez-Gracia JL, Schalper KA, Fusco JP, Gonzalez A, Rodriguez-Ruiz ME, et al. Changes in Serum Interleukin-8 (IL-8) Levels Reflect and Predict Response to Anti-PD-1 Treatment in Melanoma and Non-Small-Cell Lung Cancer Patients. *Ann Oncol* (2017) 28(8):1988–95. doi: 10.1093/annonc/mdx190
72. Ryzhov S, Novitskiy SV, Goldstein AE, Biktasova A, Blackburn MR, Biaggioni I, et al. Adenosinergic Regulation of the Expansion and Immunosuppressive Activity of CD11b+Gr1+ Cells. *J Immunol* (2011) 187(11):6120–9. doi: 10.4049/jimmunol.1101225
73. Ryzhov SV, Pickup MW, Chytil A, Gorska AE, Zhang Q, Owens P, et al. Role of TGF-Beta Signaling in Generation of CD39+CD73+ Myeloid Cells in Tumors. *J Immunol* (2014) 193(6):3155–64. doi: 10.4049/jimmunol.1400578
74. Li J, Wang L, Chen X, Li L, Li Y, Ping Y, et al. CD39/CD73 Upregulation on Myeloid-Derived Suppressor Cells *via* TGF-beta-mTOR-HIF-1 Signaling in Patients With Non-Small Cell Lung Cancer. *Oncoimmunology* (2017) 6(6):e1320011. doi: 10.1080/2162402X.2017.1320011
75. Sawant A, Schafer CC, Jin TH, Zmijewski J, Tse HM, Roth J, et al. Enhancement of Antitumor Immunity in Lung Cancer by Targeting Myeloid-Derived Suppressor Cell Pathways. *Cancer Res* (2013) 73(22):6609–20. doi: 10.1158/0008-5472.CAN-13-0987
76. Tousif S, Wang Y, Jackson J, Hough KP, Strenkowski JG, Athar M, et al. Indoleamine 2, 3-Dioxygenase Promotes Aryl Hydrocarbon Receptor-Dependent Differentiation Of Regulatory B Cells in Lung Cancer. *Front Immunol* (2021) 12. doi: 10.3389/fimmu.2021.747780
77. Miret JJ, Kirschmeier P, Koyama S, Zhu M, Li YY, Naito Y, et al. Suppression of Myeloid Cell Arginase Activity Leads to Therapeutic Response in a NSCLC Mouse Model by Activating Anti-Tumor Immunity. *J Immunother Cancer* (2019) 7(1):32. doi: 10.1186/s40425-019-0504-5

78. Srivastava MK, Zhu L, Harris-White M, Kar UK, Huang M, Johnson MF, et al. Myeloid Suppressor Cell Depletion Augments Antitumor Activity in Lung Cancer. *PLoS One* (2012) 7(7):e40677. doi: 10.1371/journal.pone.0040677
79. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of Natural Killer Cells. *Nat Immunol* (2008) 9(5):503–10. doi: 10.1038/ni1582
80. Cozar B, Greppi M, Carpentier S, Narni-Mancinelli E, Chiossone L, Vivier E. Tumor-Infiltrating Natural Killer Cells. *Cancer Discov* (2021) 11(1):34–44. doi: 10.1158/2159-8290.CD-20-0655
81. Freud AG, Mundy-Bosse BL, Yu J, Caligiuri MA. The Broad Spectrum of Human Natural Killer Cell Diversity. *Immunity* (2017) 47(5):820–33. doi: 10.1016/j.immuni.2017.10.008
82. Carrega P, Morandi B, Costa R, Frumento G, Forte G, Altavilla G, et al. Natural Killer Cells Infiltrating Human Nonsmall-Cell Lung Cancer are Enriched in CD56 Bright CD16(-) Cells and Display an Impaired Capability to Kill Tumor Cells. *Cancer* (2008) 112(4):863–75. doi: 10.1002/cncr.23239
83. Platonova S, Cherfils-Vicini J, Damotte D, Crozet L, Vieillard V, Validire P, et al. Profound Coordinated Alterations of Intratumoral NK Cell Phenotype and Function in Lung Carcinoma. *Cancer Res* (2011) 71(16):5412–22. doi: 10.1158/0008-5472.CAN-10-4179
84. Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, et al. Decidual NK Cells Regulate Key Developmental Processes at the Human Fetal-Maternal Interface. *Nat Med* (2006) 12(9):1065–74. doi: 10.1038/nm1452
85. Morvan MG, Lanier LL. NK Cells and Cancer: You can Teach Innate Cells New Tricks. *Nat Rev Cancer* (2016) 16(1):7–19. doi: 10.1038/nrc.2015.5
86. Bruno A, Focaccetti C, Pagani A, Imperatori AS, Spagnoletti M, Rotolo N, et al. The Proangiogenic Phenotype of Natural Killer Cells in Patients With non-Small Cell Lung Cancer. *Neoplasia* (2013) 15(2):133–42. doi: 10.1593/neo.121758
87. Bassani B, Baci D, Gallazzi M, Poggi A, Bruno A, Mortara L. Natural Killer Cells as Key Players of Tumor Progression and Angiogenesis: Old and Novel Tools to Divert Their Pro-Tumor Activities Into Potent Anti-Tumor Effects. *Cancers (Basel)* (2019) 11(4). doi: 10.3390/cancers11040461
88. Gallazzi M, Baci D, Mortara L, Bosi A, Buono G, Naselli A, et al. Prostate Cancer Peripheral Blood NK Cells Show Enhanced CD9, CD49a, CXCR4, CXCL8, MMP-9 Production and Secrete Monocyte-Recruiting and Polarizing Factors. *Front Immunol* (2020) 11. doi: 10.3389/fimmu.2020.586126
89. Bosi A, Zanellato S, Bassani B, Albini A, Musco A, Cattoni M, et al. Natural Killer Cells From Malignant Pleural Effusion Are Endowed With a Decidual-Like Proangiogenic Polarization. *J Immunol Res* (2018) 2018:2438598. doi: 10.1155/2018/2438598
90. Bruno A, Bassani B, D'Urso DG, Pitaku I, Cassinotti E, Pelosi G, et al. Angiogenin and the MMP9-TIMP2 Axis are Up-Regulated in Proangiogenic, Decidual NK-Like Cells From Patients With Colorectal Cancer. *FASEB J* (2018) 32(10):5365–77. doi: 10.1096/fj.201701103R
91. Russick J, Joubert PE, Gillard-Bocquet M, Torset C, Meylan M, Petitprez F, et al. Natural Killer Cells in the Human Lung Tumor Microenvironment Display Immune Inhibitory Functions. *J Immunother Cancer* (2020) 8(2). doi: 10.1136/jitc-2020-001054
92. Iraolagoitia XL, Spallanzani RG, Torres NI, Araya RE, Ziblat A, Domaica CI, et al. NK Cells Restrain Spontaneous Antitumor CD8+ T Cell Priming Through PD-1/PD-L1 Interactions With Dendritic Cells. *J Immunol* (2016) 197(3):953–61. doi: 10.4049/jimmunol.1502291
93. Hu Z, Xu X, Wei H. The Adverse Impact of Tumor Microenvironment on NK-Cell. *Front Immunol* (2021) 12. doi: 10.3389/fimmu.2021.633361
94. Taniguchi M, Harada M, Kojo S, Nakayama T, Wakao H. The Regulatory Role of Valpha14 NKT Cells in Innate and Acquired Immune Response. *Annu Rev Immunol* (2003) 21:483–513. doi: 10.1146/annurev.immunol.21.120601.141057
95. Godfrey DI, MacDonald HR, Kronenberg M, Smyth MJ, Van Kaer L. NKT Cells: What's in a Name? *Nat Rev Immunol* (2004) 4(3):231–7. doi: 10.1038/nri1309
96. Smyth MJ, Godfrey DI. NKT Cells and Tumor Immunity–a Double-Edged Sword. *Nat Immunol* (2000) 1(6):459–60. doi: 10.1038/82698
97. Berzofsky JA, Terabe M. NKT Cells in Tumor Immunity: Opposing Subsets Define a New Immunoregulatory Axis. *J Immunol* (2008) 180(6):3627–35. doi: 10.4049/jimmunol.180.6.3627
98. Exley MA, Dellabona P, Casorati G. Exploiting CD1-Restricted T Cells for Clinical Benefit. *Mol Immunol* (2021) 132:126–31. doi: 10.1016/j.molimm.2020.12.015
99. Terabe M, Berzofsky JA. Tissue-Specific Roles of NKT Cells in Tumor Immunity. *Front Immunol* (2018) 9. doi: 10.3389/fimmu.2018.01838
100. Vivier E, Ugolini S, Blaise D, Chabannon C, Brossay L. Targeting Natural Killer Cells and Natural Killer T Cells in Cancer. *Nat Rev Immunol* (2012) 12(4):239–52. doi: 10.1038/nri3174
101. Nelson A, Lukacs JD, Johnston B. The Current Landscape of NKT Cell Immunotherapy and the Hills Ahead. *Cancers (Basel)* (2021) 13(20). doi: 10.3390/cancers13205174
102. Dockry E, O'Leary S, Gleeson LE, Lyons J, Keane J, Gray SG, et al. Epigenetic Induction of CD1d Expression Primes Lung Cancer Cells for Killing by Invariant Natural Killer T Cells. *Oncoimmunology* (2018) 7(6):e1428156. doi: 10.1080/2162402X.2018.1428156
103. Yu W, Ye F, Yuan X, Ma Y, Mao C, Li X, et al. A Phase I/II Clinical Trial on the Efficacy and Safety of NKT Cells Combined With Gefitinib for Advanced EGFR-Mutated Non-Small-Cell Lung Cancer. *BMC Cancer* (2021) 21(1):877. doi: 10.1186/s12885-021-08590-1
104. Collin M, Bigley V. Human Dendritic Cell Subsets: An Update. *Immunology* (2018) 154(1):3–20. doi: 10.1111/imm.12888
105. Del Prete A, Sozio F, Barbazza I, Salvi V, Tiberio L, Laffranchi M, et al. Functional Role of Dendritic Cell Subsets in Cancer Progression and Clinical Implications. *Int J Mol Sci* (2020) 21(11). doi: 10.3390/ijms21113930
106. Maier B, Leader AM, Chen ST, Tung N, Chang C, LeBerichel J, et al. A Conserved Dendritic-Cell Regulatory Program Limits Antitumour Immunity. *Nature* (2020) 580(7802):257–62. doi: 10.1038/s41586-020-2134-y
107. Bell D, Chomarat P, Broyles D, Netto G, Harb GM, Lebecque S, et al. In Breast Carcinoma Tissue, Immature Dendritic Cells Reside Within the Tumor, Whereas Mature Dendritic Cells are Located in Peritumoral Areas. *J Exp Med* (1999) 190(10):1417–26. doi: 10.1084/jem.190.10.1417
108. Perrot I, Blanchard D, Freymond N, Isaac S, Guibert B, Pacheco Y, et al. Dendritic Cells Infiltrating Human Non-Small Cell Lung Cancer are Blocked at Immature Stage. *J Immunol* (2007) 178(5):2763–9. doi: 10.4049/jimmunol.178.5.2763
109. Dieu-Nosjean MC, Antoine M, Danel C, Heudes D, Wislez M, Poulot V, et al. Long-Term Survival for Patients With Non-Small-Cell Lung Cancer With Intratumoral Lymphoid Structures. *J Clin Oncol* (2008) 26(27):4410–7. doi: 10.1200/JCO.2007.15.0284
110. Goc J, Germain C, Vo-Bourgais TK, Lupo A, Klein C, Knockaert S, et al. Dendritic Cells in Tumor-Associated Tertiary Lymphoid Structures Signal a Th1 Cytotoxic Immune Contexture and License the Positive Prognostic Value of Infiltrating CD8+ T Cells. *Cancer Res* (2014) 74(3):705–15. doi: 10.1158/0008-5472.CAN-13-1342
111. Inoshima N, Nakanishi Y, Minami T, Izumi M, Takayama K, Yoshino I, et al. The Influence of Dendritic Cell Infiltration and Vascular Endothelial Growth Factor Expression on the Prognosis of Non-Small Cell Lung Cancer. *Clin Cancer Res* (2002) 8(11):3480–6.
112. Fontanini G, Vignati S, Lucchi M, Mussi A, Calcinai A, Boldrini L, et al. Neoangiogenesis and P53 Protein in Lung Cancer: Their Prognostic Role and Their Relation With Vascular Endothelial Growth Factor (VEGF) Expression. *Br J Cancer* (1997) 75(9):1295–301. doi: 10.1038/bjc.1997.220
113. Trivella M, Pezzella F, Pastorino U, Harris AL, Altman DG. Prognosis In Lung Cancer Collaborative Study G. Microvessel Density as a Prognostic Factor in Non-Small-Cell Lung Carcinoma: A Meta-Analysis of Individual Patient Data. *Lancet Oncol* (2007) 8(6):488–99. doi: 10.1016/S1470-2045(07)70145-6
114. Zheng CL, Qiu C, Shen MX, Qu X, Zhang TH, Zhang JH, et al. Prognostic Impact of Elevation of Vascular Endothelial Growth Factor Family Expression in Patients With Non-Small Cell Lung Cancer: An Updated Meta-Analysis. *Asian Pac J Cancer Prev* (2015) 16(5):1881–95. doi: 10.7314/APJCP.2015.16.5.1881
115. Hu M, Hu Y, He J, Li B. Prognostic Value of Basic Fibroblast Growth Factor (bFGF) in Lung Cancer: A Systematic Review With Meta-Analysis. *PLoS One* (2016) 11(1):e0147374. doi: 10.1371/journal.pone.0147374

116. Imada A, Shijubo N, Kojima H, Abe S. Mast Cells Correlate With Angiogenesis and Poor Outcome in Stage I Lung Adenocarcinoma. *Eur Respir J* (2000) 15(6):1087–93. doi: 10.1034/j.1399-3003.2000.01517.x
117. Takanami I, Takeuchi K, Naruke M. Mast Cell Density Is Associated With Angiogenesis and Poor Prognosis in Pulmonary Adenocarcinoma. *Cancer* (2000) 88(12):2686–92. doi: 10.1002/1097-0142(20000615)88:12<2686::AID-CNCR6>3.0.CO;2-6
118. Duarte IG, Bufkin BL, Pennington MF, Gal AA, Cohen C, Kosinski AS, et al. Angiogenesis as a Predictor of Survival After Surgical Resection for Stage I non-Small-Cell Lung Cancer. *J Thorac Cardiovasc Surg* (1998) 115(3):652–8. doi: 10.1016/S0022-5223(98)70331-9
119. Shibusa T, Shijubo N, Abe S. Tumor Angiogenesis and Vascular Endothelial Growth Factor Expression in Stage I Lung Adenocarcinoma. *Clin Cancer Res* (1998) 4(6):1483–7.
120. Shijubo N, Uede T, Kon S, Maeda M, Segawa T, Imada A, et al. Vascular Endothelial Growth Factor and Osteopontin in Stage I Lung Adenocarcinoma. *Am J Respir Crit Care Med* (1999) 160(4):1269–73. doi: 10.1164/ajrccm.160.4.9807094
121. Brattstrom D, Wester K, Bergqvist M, Hesselius P, Malmstrom PU, Nordgren H, et al. HER-2, EGFR, COX-2 Expression Status Correlated to Microvessel Density and Survival in Resected non-Small Cell Lung Cancer. *Acta Oncol* (2004) 43(1):80–6. doi: 10.1080/02841860310017441
122. Chandrachud LM, Pendleton N, Chisholm DM, Horan MA, Schor AM. Relationship Between Vascularity, Age and Survival in Non-Small-Cell Lung Cancer. *Br J Cancer* (1997) 76(10):1367–75. doi: 10.1038/bjc.1997.562
123. Dazzi C, Cariello A, Maioli P, Solaini L, Scarpi E, Rosti G, et al. Prognostic and Predictive Value of Intratumoral Microvessels Density in Operable non-Small-Cell Lung Cancer. *Lung Cancer* (1999) 24(2):81–8. doi: 10.1016/S0169-5002(99)00036-7
124. Decaussin M, Sartelet H, Robert C, Moro D, Claraz C, Brambilla C, et al. Expression of Vascular Endothelial Growth Factor (VEGF) and its Two Receptors (VEGF-R1-Flt1 and VEGF-R2-Flk1/KDR) in non-Small Cell Lung Carcinomas (NSCLCs): Correlation With Angiogenesis and Survival. *J Pathol* (1999) 188(4):369–77. doi: 10.1002/(SICI)1096-9896(199908)188:4<369::AID-PATH381>3.0.CO;2-X
125. Dundar E, Oner U, Peker BC, Metintas M, Isiksoy S, Ak G. The Significance and Relationship Between Mast Cells and Tumour Angiogenesis in non-Small Cell Lung Carcinoma. *J Int Med Res* (2008) 36(1):88–95. doi: 10.1177/147323000803600112
126. Tanaka F, Otake Y, Yanagihara K, Kawano Y, Miyahara R, Li M, et al. Evaluation of Angiogenesis in Non-Small Cell Lung Cancer: Comparison Between Anti-CD34 Antibody and Anti-CD105 Antibody. *Clin Cancer Res* (2001) 7(11):3410–5.
127. Tsoli E, Zacharatos P, Dasiou-Plakida D, Peros J, Evangelou K, Zavras AI, et al. Growth Index Is Independent of Microvessel Density in non-Small-Cell Lung Carcinomas. *Hum Pathol* (2002) 33(8):812–8. doi: 10.1053/hupa.2002.125379
128. Niczyporuk M, Hermanowicz A, Matuszczak E, Dziadziuszko R, Knas M, Zalewska A, et al. A Lack of Correlation Between Mast Cells, Angiogenesis, and Outcome in Non-Small Cell Lung Cancer. *Exp Lung Res* (2012) 38(6):281–5. doi: 10.3109/01902148.2012.686559
129. Tataroglu C, Kargi A, Ozkal S, Esrefoglu N, Akkoclu A. Association of Macrophages, Mast Cells and Eosinophil Leukocytes With Angiogenesis and Tumor Stage in non-Small Cell Lung Carcinomas (NSCLC). *Lung Cancer* (2004) 43(1):47–54. doi: 10.1016/j.lungcan.2003.08.013
130. Welsh TJ, Green RH, Richardson D, Waller DA, O'Byrne KJ, Bradding P. Macrophage and Mast-Cell Invasion of Tumor Cell Islets Confers a Marked Survival Advantage in Non-Small-Cell Lung Cancer. *J Clin Oncol* (2005) 23(35):8959–67. doi: 10.1200/JCO.2005.01.4910
131. Shikotra A, Ohri CM, Green RH, Waller DA, Bradding P. Mast Cell Phenotype, TNFalpha Expression and Degranulation Status in Non-Small Cell Lung Cancer. *Sci Rep* (2016) 6:38352. doi: 10.1038/srep38352
132. Tomita M, Matsuzaki Y, Onitsuka T. Correlation Between Mast Cells and Survival Rates in Patients With Pulmonary Adenocarcinoma. *Lung Cancer* (1999) 26(2):103–8. doi: 10.1016/S0169-5002(99)00076-8
133. Stoyanov E, Uddin M, Mankuta D, Dubinett SM, Levi-Schaffer F. Mast Cells and Histamine Enhance the Proliferation of Non-Small Cell Lung Cancer Cells. *Lung Cancer* (2012) 75(1):38–44. doi: 10.1016/j.lungcan.2011.05.029
134. Liang J, Tian C, Zeng Y, Yang Q, Liu Y, Liu Y, et al. FOXA1(+) Regulatory T Cells: A Novel T Cell Subset That Suppresses Antitumor Immunity in Lung Cancer. *Biochem Biophys Res Commun* (2019) 514(1):308–15. doi: 10.1016/j.bbrc.2019.04.152
135. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic Self-Tolerance Maintained by Activated T Cells Expressing IL-2 Receptor Alpha-Chains (CD25). Breakdown of a Single Mechanism of Self-Tolerance Causes Various Autoimmune Diseases. *J Immunol* (1995) 155(3):1151–64.
136. Beyer M, Schultze JL. Regulatory T Cells in Cancer. *Blood* (2006) 108(3):804–11. doi: 10.1182/blood-2006-02-002774
137. Petersen RP, Campa MJ, Sperlazza J, Conlon D, Joshi MB, Harpole DHJr., et al. Tumor Infiltrating Foxp3+ Regulatory T-Cells Are Associated With Recurrence in Pathologic Stage I NSCLC Patients. *Cancer* (2006) 107(12):2866–72. doi: 10.1002/cncr.22282
138. Shimizu K, Nakata M, Hirami Y, Yukawa T, Maeda A, Tanemoto K. Tumor-Infiltrating Foxp3+ Regulatory T Cells Are Correlated With Cyclooxygenase-2 Expression and Are Associated With Recurrence in Resected Non-Small Cell Lung Cancer. *J Thorac Oncol* (2010) 5(5):585–90. doi: 10.1097/JTO.0b013e3181d60fd7
139. Tao H, Mimura Y, Aoe K, Kobayashi S, Yamamoto H, Matsuda E, et al. Prognostic Potential of FOXP3 Expression in Non-Small Cell Lung Cancer Cells Combined With Tumor-Infiltrating Regulatory T Cells. *Lung Cancer* (2012) 75(1):95–101. doi: 10.1016/j.lungcan.2011.06.002
140. Woo EY, Chu CS, Goletz TJ, Schlienger K, Yeh H, Coukos G, et al. Regulatory CD4(+)CD25(+) T Cells in Tumors From Patients With Early-Stage non-Small Cell Lung Cancer and Late-Stage Ovarian Cancer. *Cancer Res* (2001) 61(12):4766–72.
141. Chen C, Chen D, Zhang Y, Chen Z, Zhu W, Zhang B, et al. Changes of CD4+CD25+FOXP3+ and CD8+CD28- Regulatory T Cells in Non-Small Cell Lung Cancer Patients Undergoing Surgery. *Int Immunopharmacol* (2014) 18(2):255–61. doi: 10.1016/j.intimp.2013.12.004
142. Li L, Chao QG, Ping LZ, Xue C, Xia ZY, Qian D, et al. The Prevalence of FOXP3+ Regulatory T-Cells in Peripheral Blood of Patients With NSCLC. *Cancer Biother Radiopharm* (2009) 24(3):357–67. doi: 10.1089/cbr.2008.0612
143. Okita R, Saeki T, Takashima S, Yamaguchi Y, Toge T. CD4+CD25+ Regulatory T Cells in the Peripheral Blood of Patients With Breast Cancer and Non-Small Cell Lung Cancer. *Oncol Rep* (2005) 14(5):1269–73. doi: 10.3892/or.14.5.1269
144. Yannelli JR, Tucker JA, Hidalgo G, Perkins S, Kryscio R, Hirschowitz EA. Characteristics of PBMC Obtained From Leukapheresis Products and Tumor Biopsies of Patients With Non-Small Cell Lung Cancer. *Oncol Rep* (2009) 22(6):1459–71. doi: 10.3892/or_00000588
145. Erfani N, Mehrabadi SM, Ghayumi MA, Haghshenas MR, Mojtahedi Z, Ghaderi A, et al. Increase of Regulatory T Cells in Metastatic Stage and CTLA-4 Over Expression in Lymphocytes of Patients With Non-Small Cell Lung Cancer (NSCLC). *Lung Cancer* (2012) 77(2):306–11. doi: 10.1016/j.lungcan.2012.04.011
146. Hanagiri T, Shigematsu Y, Shinohara S, Takenaka M, Oka S, Chikaishi Y, et al. Clinical Significance of the Frequency of Regulatory T Cells in Regional Lymph Node Lymphocytes as a Prognostic Factor for Non-Small-Cell Lung Cancer. *Lung Cancer* (2013) 81(3):475–9. doi: 10.1016/j.lungcan.2013.07.001
147. Hasegawa T, Suzuki H, Yamaura T, Muto S, Okabe N, Osugi J, et al. Prognostic Value of Peripheral and Local Forkhead Box P3(+) Regulatory T Cells in Patients With Non-Small-Cell Lung Cancer. *Mol Clin Oncol* (2014) 2(5):685–94. doi: 10.3892/mco.2014.299
148. Kotsakis A, Koinis F, Katsarou A, Gioulbasani M, Aggouraki D, Kentepozidis N, et al. Prognostic Value of Circulating Regulatory T Cell Subsets in Untreated Non-Small Cell Lung Cancer Patients. *Sci Rep* (2016) 6:39247. doi: 10.1038/srep39247
149. Yang H, Shi J, Lin D, Li X, Zhao C, Wang Q, et al. Prognostic Value of PD-L1 Expression in Combination With CD8(+) TILs Density in Patients With Surgically Resected Non-Small Cell Lung Cancer. *Cancer Med* (2018) 7(1):32–45. doi: 10.1002/cam4.1243
150. Carus A, Ladekarl M, Hager H, Pilegaard H, Nielsen PS, Donskov F. Tumor-Associated Neutrophils and Macrophages in Non-Small Cell Lung Cancer: No Immediate Impact on Patient Outcome. *Lung Cancer* (2013) 81(1):130–7. doi: 10.1016/j.lungcan.2013.03.003

151. Tuminello S, Veluswamy R, Lieberman-Cribbin W, Gnjatic S, Petralia F, Wang P, et al. Prognostic Value of Immune Cells in the Tumor Microenvironment of Early-Stage Lung Cancer: A Meta-Analysis. *Oncotarget* (2019) 10(67):7142–55. doi: 10.18632/oncotarget.27392
152. Geng Y, Shao Y, He W, Hu W, Xu Y, Chen J, et al. Prognostic Role of Tumor-Infiltrating Lymphocytes in Lung Cancer: A Meta-Analysis. *Cell Physiol Biochem* (2015) 37(4):1560–71. doi: 10.1159/000438523
153. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating Mutations in the Epidermal Growth Factor Receptor Underlying Responsiveness of Non-Small-Cell Lung Cancer to Gefitinib. *N Engl J Med* (2004) 350(21):2129–39. doi: 10.1056/NEJMoa040938
154. Garofalo M, Romano G, Di Leva G, Nuovo G, Jeon YJ, Ngankeu A, et al. EGFR and MET Receptor Tyrosine Kinase-Altered microRNA Expression Induces Tumorigenesis and Gefitinib Resistance in Lung Cancers. *Nat Med* (2011) 18(1):74–82. doi: 10.1038/nm.2577
155. Soltermann A. [Epithelial-Mesenchymal Transition in non-Small Cell Lung Cancer]. *Pathologe* (2012) 33 Suppl 2:311–7. doi: 10.1007/s00292-012-1635-3
156. Heinrich EL, Walser TC, Krysan K, Liclican EL, Grant JL, Rodriguez NL, et al. The Inflammatory Tumor Microenvironment, Epithelial Mesenchymal Transition and Lung Carcinogenesis. *Cancer Microenviron* (2012) 5(1):5–18. doi: 10.1007/s12307-011-0089-0
157. Yu HA, Riely GJ. Second-Generation Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Lung Cancers. *J Natl Compr Canc Netw* (2013) 11(2):161–9. doi: 10.6004/jnccn.2013.0024
158. Im SJ, Hashimoto M, Gerner MY, Lee J, Kissick HT, Burger MC, et al. Defining CD8+ T Cells That Provide the Proliferative Burst After PD-1 Therapy. *Nature* (2016) 537(7620):417–21. doi: 10.1038/nature19330
159. Chen L, Diao L, Yang Y, Yi X, Rodriguez BL, Li Y, et al. CD38-Mediated Immunosuppression as a Mechanism of Tumor Cell Escape From PD-1/PD-L1 Blockade. *Cancer Discov* (2018) 8(9):1156–75. doi: 10.1158/2159-8290.CD-17-1033
160. Koh J, Hur JY, Lee KY, Kim MS, Heo JY, Ku BM, et al. Regulatory (FoxP3 (+)) T Cells and TGF-Beta Predict the Response to Anti-PD-1 Immunotherapy in Patients With non-Small Cell Lung Cancer. *Sci Rep* (2020) 10(1):18994. doi: 10.1038/s41598-020-76130-1
161. Aggarwal C, Somaiah N, Simon G. Antiangiogenic Agents in the Management of non-Small Cell Lung Cancer: Where do We Stand Now and Where are We Headed? *Cancer Biol Ther* (2012) 13(5):247–63. doi: 10.4161/cbt.19594
162. Jain RK. Antiangiogenesis Strategies Revisited: From Starving Tumors to Alleviating Hypoxia. *Cancer Cell* (2014) 26(5):605–22. doi: 10.1016/j.ccell.2014.10.006
163. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, et al. Paclitaxel-Carboplatin Alone or With Bevacizumab for non-Small-Cell Lung Cancer. *N Engl J Med* (2006) 355(24):2542–50. doi: 10.1056/NEJMoa061884
164. Garon EB, Ciuleanu TE, Arrieta O, Prabhash K, Syrigos KN, Goksel T, et al. Ramucirumab Plus Docetaxel Versus Placebo Plus Docetaxel for Second-Line Treatment of Stage IV non-Small-Cell Lung Cancer After Disease Progression on Platinum-Based Therapy (REVEL): A Multicentre, Double-Blind, Randomised Phase 3 Trial. *Lancet* (2014) 384(9944):665–73. doi: 10.1016/S0140-6736(14)60845-X
165. Yi M, Jiao D, Qin S, Chu Q, Wu K, Li A. Synergistic Effect of Immune Checkpoint Blockade and Anti-Angiogenesis in Cancer Treatment. *Mol Cancer* (2019) 18(1):60. doi: 10.1186/s12943-019-0974-6
166. Winkler F, Kozin SV, Tong RT, Chae SS, Booth MF, Garkavtsev I, et al. Kinetics of Vascular Normalization by VEGFR2 Blockade Governs Brain Tumor Response to Radiation: Role of Oxygenation, Angiopoietin-1, and Matrix Metalloproteinases. *Cancer Cell* (2004) 6(6):553–63. doi: 10.1016/j.ccr.2004.10.011
167. Mazzone M, Dettori D, de Oliveira RL, Loges S, Schmidt T, Jonckx B, et al. Heterozygous Deficiency of PHD2 Restores Tumor Oxygenation and Inhibits Metastasis *via* Endothelial Normalization. *Cell* (2009) 136(5):839–51. doi: 10.1016/j.cell.2009.01.020
168. Stockmann C, Doedens A, Weidemann A, Zhang N, Takeda N, Greenberg JI, et al. Deletion of Vascular Endothelial Growth Factor in Myeloid Cells Accelerates Tumorigenesis. *Nature* (2008) 456(7223):814–8. doi: 10.1038/nature07445
169. Taube JM, Klein A, Brahmer JR, Xu H, Pan X, Kim JH, et al. Association of PD-1, PD-1 Ligands, and Other Features of the Tumor Immune Microenvironment With Response to Anti-PD-1 Therapy. *Clin Cancer Res* (2014) 20(19):5064–74. doi: 10.1158/1078-0432.CCR-13-3271
170. Guo H, Bai R, Cui J. [Advances in Combination Therapy of Immune Checkpoint Inhibitors for Lung Cancer]. *Zhongguo Fei Ai Za Zhi* (2020) 23(2):101–10. doi: 10.3779/j.issn.1009-3419.2020.02.05
171. Voron T, Marcheteau E, Pernot S, Colussi O, Tartour E, Taieb J, et al. Control of the Immune Response by Pro-Angiogenic Factors. *Front Oncol* (2014) 4. doi: 10.3389/fonc.2014.00070
172. Ren S, Xiong X, You H, Shen J, Zhou P. The Combination of Immune Checkpoint Blockade and Angiogenesis Inhibitors in the Treatment of Advanced Non-Small Cell Lung Cancer. *Front Immunol* (2021) 12. doi: 10.3389/fimmu.2021.689132
173. Mortara L, Benest AV, Bates DO, Noonan DM. Can the Co-Dependence of the Immune System and Angiogenesis Facilitate Pharmacological Targeting of Tumours? *Curr Opin Pharmacol* (2017) 35:66–74. doi: 10.1016/j.coph.2017.05.009
174. Alfaro C, Suarez N, Gonzalez A, Solano S, Erro L, Dubrot J, et al. Influence of Bevacizumab, Sunitinib and Sorafenib as Single Agents or in Combination on the Inhibitory Effects of VEGF on Human Dendritic Cell Differentiation From Monocytes. *Br J Cancer* (2009) 100(7):1111–9. doi: 10.1038/sj.bjc.6604965
175. Oyama T, Ran S, Ishida T, Nadaf S, Kerr L, Carbone DP, et al. Vascular Endothelial Growth Factor Affects Dendritic Cell Maturation Through the Inhibition of Nuclear Factor-Kappa B Activation in Hemopoietic Progenitor Cells. *J Immunol* (1998) 160(3):1224–32.
176. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated Regulation of Myeloid Cells by Tumours. *Nat Rev Immunol* (2012) 12(4):253–68. doi: 10.1038/nri3175
177. Suzuki H, Onishi H, Wada J, Yamasaki A, Tanaka H, Nakano K, et al. VEGFR2 is Selectively Expressed by FOXP3high CD4+ Treg. *Eur J Immunol* (2010) 40(1):197–203. doi: 10.1002/eji.200939887
178. Wada J, Suzuki H, Fuchino R, Yamasaki A, Nagai S, Yanai K, et al. The Contribution of Vascular Endothelial Growth Factor to the Induction of Regulatory T-Cells in Malignant Effusions. *Anticancer Res* (2009) 29(3):881–8.
179. Manzoni M, Rovati B, Ronzoni M, Loupakis F, Mariucci S, Ricci V, et al. Immunological Effects of Bevacizumab-Based Treatment in Metastatic Colorectal Cancer. *Oncology* (2010) 79(3-4):187–96. doi: 10.1159/000320609
180. Martino EC, Misso G, Pastina P, Costantini S, Vanni F, Gandolfo C, et al. Immune-Modulating Effects of Bevacizumab in Metastatic non-Small-Cell Lung Cancer Patients. *Cell Death Discovery* (2016) 2:16025. doi: 10.1038/cddiscovery.2016.25
181. Cheng HS, Lee JXT, Wahli W, Tan NS. Exploiting Vulnerabilities of Cancer by Targeting Nuclear Receptors of Stromal Cells in Tumor Microenvironment. *Mol Cancer* (2019) 18(1):51. doi: 10.1186/s12943-019-0971-9
182. Huang Y, Kim BYS, Chan CK, Hahn SM, Weissman IL, Jiang W. Improving Immune-Vascular Crosstalk for Cancer Immunotherapy. *Nat Rev Immunol* (2018) 18(3):195–203. doi: 10.1038/nri.2017.145
183. Huang Y, Goel S, Duda DG, Fukumura D, Jain RK. Vascular Normalization as an Emerging Strategy to Enhance Cancer Immunotherapy. *Cancer Res* (2013) 73(10):2943–8. doi: 10.1158/0008-5472.CAN-12-4354
184. Movahedi K, Laoui D, Gysemans C, Baeten M, Stange G, Van den Bossche J, et al. Different Tumor Microenvironments Contain Functionally Distinct Subsets of Macrophages Derived From Ly6C(high) Monocytes. *Cancer Res* (2010) 70(14):5728–39. doi: 10.1158/0008-5472.CAN-09-4672
185. Motz GT, Santoro SP, Wang LP, Garrabrant T, Lastra RR, Hagemann IS, et al. Tumor Endothelium FasL Establishes a Selective Immune Barrier Promoting Tolerance in Tumors. *Nat Med* (2014) 20(6):607–15. doi: 10.1038/nm.3541
186. Terme M, Pernot S, Marcheteau E, Sandoval F, Benhamouda N, Colussi O, et al. VEGFA-VEGFR Pathway Blockade Inhibits Tumor-Induced Regulatory T-Cell Proliferation in Colorectal Cancer. *Cancer Res* (2013) 73(2):539–49. doi: 10.1158/0008-5472.CAN-12-2325
187. Huang Y, Chen X, Dikov MM, Novitskiy SV, Mosse CA, Yang L, et al. Distinct Roles of VEGFR-1 and VEGFR-2 in the Aberrant Hematopoiesis

Associated With Elevated Levels of VEGF. *Blood* (2007) 110(2):624–31. doi: 10.1182/blood-2007-01-065714
188. Kudo M. Scientific Rationale for Combined Immunotherapy With PD-1/PD-L1 Antibodies and VEGF Inhibitors in Advanced Hepatocellular Carcinoma. *Cancers (Basel)* (2020) 12(5):1089. doi: 10.3390/cancers12051089
189. Huang Y, Yuan J, Righi E, Kamoun WS, Ancukiewicz M, Nezivar J, et al. Vascular Normalizing Doses of Antiangiogenic Treatment Reprogram the Immunosuppressive Tumor Microenvironment and Enhance Immunotherapy. *Proc Natl Acad Sci USA* (2012) 109(43):17561–6. doi: 10.1073/pnas.1215397109
190. Voron T, Colussi O, Marcheteau E, Pernot S, Nizard M, Pointet A-L, et al. VEGF-A Modulates Expression of Inhibitory Checkpoints on CD8+ T Cells in Tumors. *J Exp Med* (2015) 212(2):139–48. doi: 10.1084/jem.20140559
191. Socinski MA, Nishio M, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, et al. IMpower150 Final Overall Survival Analyses for Atezolizumab Plus Bevacizumab and Chemotherapy in First-Line Metastatic Nonsquamous NSCLC. *J Thorac Oncol* (2021) 16(11):1909–24. doi: 10.1016/j.jtho.2021.07.009
192. Francis DM, Manspeaker MP, Schudel A, Sestito LF, O'Melia MJ, Kissick HT, et al. Blockade of Immune Checkpoints in Lymph Nodes Through Locoregional Delivery Augments Cancer Immunotherapy. *Sci Transl Med* (2020) 12(563). doi: 10.1126/scitranslmed.aay3575
193. Corthay A. Does the Immune System Naturally Protect Against Cancer? *Front Immunol* (2014) 5. doi: 10.3389/fimmu.2014.00197
194. Callahan MK, Wolchok JD. At the Bedside: CTLA-4- and PD-1-Blocking Antibodies in Cancer Immunotherapy. *J Leukoc Biol* (2013) 94(1):41–53. doi: 10.1189/jlb.1212631
195. Dermani FK, Samadi P, Rahmani G, Kohlan AK, Najafi R. PD-1/PD-L1 Immune Checkpoint: Potential Target for Cancer Therapy. *J Cell Physiol* (2019) 234(2):1313–25. doi: 10.1002/jcp.27172
196. Herbst RS, Arkenau HT, Bendell J, Arrowsmith E, Wermke M, Soriano A, et al. Phase 1 Expansion Cohort of Ramucirumab Plus Pembrolizumab in Advanced Treatment-Naive NSCLC. *J Thorac Oncol* (2021) 16(2):289–98. doi: 10.1016/j.jtho.2020.10.004
197. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab Versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. *N Engl J Med* (2015) 373(17):1627–39. doi: 10.1056/NEJMoa1507643
198. Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, et al. Atezolizumab Versus Docetaxel in Patients With Previously Treated Non-Small-Cell Lung Cancer (OAK): A Phase 3, Open-Label, Multicentre Randomised Controlled Trial. *Lancet* (2017) 389(10066):255–65. doi: 10.1016/S0140-6736(16)32517-X
199. Shibata Y, Murakami S. Safety Evaluation of Durvalumab for the Treatment of non-Small-Cell Lung Cancer. *Expert Opin Drug Saf* (2020) 19(6):653–9. doi: 10.1080/14740338.2020.1764936
200. Barlesi F, Vansteenkiste J, Spigel D, Ishii H, Garassino M, de Marinis F, et al. Avelumab Versus Docetaxel in Patients With Platinum-Treated Advanced non-Small-Cell Lung Cancer (JAVELIN Lung 200): An Open-Label, Randomised, Phase 3 Study. *Lancet Oncol* (2018) 19(11):1468–79. doi: 10.1016/S1470-2045(18)30673-9
201. Pinto JA, Raez LE, Oliveres H, Rolfo CC. Current Knowledge of Ipilimumab and its Use in Treating non-Small Cell Lung Cancer. *Expert Opin Biol Ther* (2019) 19(6):509–15. doi: 10.1080/14712598.2019.1610380
202. Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WEE, Poddubskaya E, et al. Nivolumab Versus Docetaxel in Advanced Squamous-Cell Non–Small-Cell Lung Cancer. *New Engl J Med* (2015) 373(2):123–35. doi: 10.1056/NEJMoa1504627
203. Fehrenbacher L, Spira A, Ballinger M, Kowanetz M, Vansteenkiste J, Mazieres J, et al. Atezolizumab Versus Docetaxel for Patients With Previously Treated Non-Small-Cell Lung Cancer (POPLAR): A Multicentre, Open-Label, Phase 2 Randomised Controlled Trial. *Lancet* (2016) 387(10030):1837–46. doi: 10.1016/S0140-6736(16)00587-0
204. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, et al. Overall Survival With Durvalumab After Chemoradiotherapy in Stage III NSCLC. *New Engl J Med* (2018) 379(24):2342–50. doi: 10.1056/NEJMoa1809697
205. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, et al. Durvalumab After Chemoradiotherapy in Stage III Non-Small-Cell Lung Cancer. *N Engl J Med* (2017) 377(20):1919–29. doi: 10.1056/NEJMoa1709937

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 13 September 2022
DOI 10.3389/fonc.2022.896662

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Oncology

RECEIVED 15 March 2022
ACCEPTED 09 August 2022
PUBLISHED 13 September 2022

CITATION
Wang H, Shi F, Zheng S, Zhao M, Pan Z, Xiong L and Zheng L (2022) Feasibility of hepatocellular carcinoma treatment based on the tumor microenvironment.
Front. Oncol. 12:896662.
doi: 10.3389/fonc.2022.896662

Feasibility of hepatocellular carcinoma treatment based on the tumor microenvironment

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The incidence of liver cancer is extremely high worldwide and poses a serious threat to human life and health. But at present, apart from radiotherapy, chemotherapy, liver transplantation, and early resection, sorafenib was the main systemic therapy proven to have clinical efficacy for unresectable liver cancer (HCC) until 2017. Despite the emerging immunotherapy in the past decade with immune inhibitors such as PD - 1 being approved and applied to clinical treatment, there are still some patients with no response. This review aims to elucidate the mechanisms underlying the tumor microenvironment of hepatocellular carcinoma and thus analyze the effectiveness of targeting the tumor microenvironment to improve the therapeutic efficacy of hepatocellular carcinoma, including the effectiveness and feasibility of immunotherapy, tumor oncolytic viruses and anti-vascular proliferation therapy.

KEYWORDS

hepatocellular carcinoma, tumor microenvironment, immunotherapy, intestinal microorganisms, oncolytic viruses, anti-vascular proliferation

Introduction

Liver cancer is one of the most common and deadly malignancies worldwide (1), and hepatocellular carcinoma accounts for 90% of all liver cancers (2), and is an abnormal and malignant proliferation of liver cells, with an estimated one million cases of liver cancer per year by 2025 (3). Hepatocellular carcinoma often develops in the context of underlying liver injury (4), and is closely associated with chronic liver disease. Patients with chronic liver disease are often accompanied by liver inflammation, fibrosis and abnormal hepatocyte regeneration, and these abnormalities may lead to cirrhosis, and cirrhosis increases the risk of hepatocellular carcinoma (1). Risk factors for liver cancer are extensive and include HBV infection, HCV infection, aflatoxin B1 exposure, excessive alcohol intake, non-alcoholic fatty liver, diabetes mellitus, obesity, smoking etc. (5).

Surgery is the most effective treatment (6), ultrasound combined with serum AFP test is sensitive and specific for early stage liver cancer surveillance and specificity is high (7). If detected at an early stage, it can be treated invasively (8),however, most patients are diagnosed only when the tumor is too advanced to be treated by surgical resection, *in situ* liver transplantation or local percutaneous tumor ablation (9), thus leading to a poor prognosis for hepatocellular carcinoma. Local therapy is the most common first-line treatment methods, including percutaneous local ablation, chemoembolization, radioembolization, and external irradiation therapy. Arterial embolization can be used for patients with tumors that are not amenable to radical resection or ablation, without extrahepatic spread and with intact liver function (9). For patients with unresectable hepatocellular carcinoma, The tyrosine kinase inhibitor (TKI) sorafenib is the primary approved systemic therapy as of 2017 (10). Although the clinical treatment of HCC has improved greatly in recent years, the prognosis is relatively poor, due to the lack of efficient treatment for hepatic malignancies and due to the complexity of the tumor microenvironment. For patients with advanced diagnosis of HCC, the survival rate is not high, so further research and analysis are still needed to find a better treatment for hepatocellular carcinoma.

The tumor microenvironment is the site of rapid tumor progression. Various factors in the tumor microenvironment cause abnormal vascular proliferation and immunosuppression, leading to rapid progression of hepatocellular carcinoma. By targeting the tumor microenvironment, and applying immunotherapy alone or in combination with immunoregulation, the state of immunosuppression is transformed into the state of immune stimulation to kill tumor cells. Lysozyme virus directly destroys tumor cells, but also modulates immunity and destroys the tumor vascular system. Anti-vascular endothelial growth factor inhibitors are applied to inhibit abnormal vascular proliferation and block tumor cell nutrient supply, alleviating immunotherapy resistance. These therapies have shown satisfactory efficacy in the treatment of hepatocellular carcinoma and have expanded the idea of hepatocellular carcinoma treatment. This essay searched the PubMed database for the mechanisms of tumor microenvironment generation and the treatment of hepatocellular carcinoma in the past decade, and summarizes the mechanisms and clinical applications of emerging immunotherapies, oncolytic virus therapies and anti-vascular proliferation therapies in recent years.

Tumor microenvironment

The tumor microenvironment is the cellular environment of tumorigenesis, which is involved in regulating the occurrence, development, invasion and metastasis of malignant tumors, and plays a very important role in the development of hepatocellular carcinoma (HCC).

Hypoxia in the tumor microenvironment is thought to be an important driver of hepatocellular carcinoma progression (11). Hypoxia arises from insufficient blood supply due to the combination of excessive proliferation of malignant cells and insufficient vascularization during tumor cell progression (12). Hypoxia can further promote malignant cell proliferation, and experimental results have demonstrated that tumor cells activate PI3K/AKT signaling pathway under hypoxia (13), leading to malignant over proliferation and radiotherapy resistance of cancer cells. Hypoxia also affects immune cells, reconstitutes the tumor immune microenvironment (TIM), suppresses the expression of immune T cells and NK cells, and promotes the expression of immunosuppressive cytokines (12). For example, activation of hypoxia-inducible factor 1α can upregulate PD-L1 expression (14), creating an immunosuppressive environment, thus protecting tumor cells from recognition and clearance by the host immune system, and ultimately leading to tumor escape and immune tolerance.

Abnormal proliferation of blood vessels in the tumor microenvironment is another major risk factor for the progression of hepatocellular carcinoma. HCC is a highly angiogenic cancer (15), angiogenesis plays a large role in tumor growth, early metastasis, and poor survival. The tumor microenvironment (TME) system is complex and consists mainly of cellular and non-cellular components. Cellular components including hepatic stellate cells, fibroblasts, immune cells and endothelial cells (ECs). Non-cellular components include growth factors (such as fibroblast growth factor (FGF), hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF)), protein hydrolases, extracellular matrix (ECM) proteins, and inflammatory factors (16). Activated hepatic stellate cells secrete angiogenic growth factor, which together with vascular endothelial growth factor (VEGF) stimulates angiogenesis, forming a new vascular system within the TME (17) and providing various nutrients for tumor growth.

In addition, hepatic stellate cells are activated in the presence of liver injury and secrete large amounts of transforming growth factor-β (TGF-β), a key immunosuppressive cytokine involved in liver regeneration, inflammation and fibrosis, promoting fibrosis, cirrhosis, and ultimately liver cancer (18). Activated hepatic stellate cells recruit Tregs by suppressing lymphocytes, overexpressing PD-1 cells and promoting immune tolerance, and inhibits the activation of CD8+ T cells by reducing the IL -2/IL-2R T cell signaling pathway and promoting the production of myeloid-derived suppressor cells (MDSC) through the mediation of CD54 (18). Tregs cells as well as myeloid-derived suppressor cells (MDSC) are considered to be immune cells that promote tumor growth in the tumor microenvironment (19),

and thus these are critical for tumor progression, metastasis and invasion.

Another player in TME is exosomes, small vesicular structures that act as communication mediators between cancer and non-cancer cells in the tumor microenvironment (20), containing multiple components such as DNA, RNA and proteins (15). These substances are involved in the growth and metastasis of hepatocellular carcinoma, promote angiogenesis, regulate the inflammatory microenvironment, evade immune surveillance (16), and promote tumor development. For example, Exosome MIRs induce epithelial-mesenchymal transition as well as angiogenesis, which are involved in different processes of hepatocellular carcinoma metastasis (21). And it has been demonstrated that miR-32-5p, delivered by drug-resistant cellular exosomes activates the PI3K/Akt pathway, which leads to multidrug resistance in hepatocellular carcinoma through angiogenesis and EMT, and becomes another obstacle to hepatocellular carcinoma treatment (22). Additional features of TME are low pH and the accumulation of adenosine, which favors tumor cell progression while being inhibitory to immune cells (12), thus participating in the development of an immunosuppressed state. It is worth to mention that exosomes are also considered as therapeutic vectors, and the delivery of miR-150-3p-rich exosomes to HCC cells may have therapeutic applications (23).

To briefly summarize, various factors in the tumor microenvironment cause abnormal vascular proliferation and immunosuppression, resulting in hepatocellular cell carcinoma progressing rapidly in the tumor microenvironment (Figure 1). Therefore, in the treatment of hepatocellular carcinoma, targeted interventions can be made to address the characteristics of the tumor microenvironment.

Immunomodulatory therapy

Because the tumor microenvironment is in a state of immunosuppression and protects tumor cells from escaping and from the attack of immune cells, to control and treat liver cancer, immunity should be regulated and the immunosuppressive environment should be reversed. Immunotherapy is gaining worldwide acceptance as a new standard of care for hepatocellular carcinoma (HCC). Using targeted cytotoxic T Immune checkpoint inhibition of lymphocyte-associated protein-4 (CTLA-4) and anti-programmed cell death protein-1 (PD-1) cancer immunotherapy with pharmaceutical preparations (ICIs) (24), changing the traditional sorafenib treatment mechanism, and as an adjuvant therapy to a certain extent, the recurrence rate has been reduced (25), expanding the treatment ideas for liver cancer and improving the survival rate (26).

PD-1 is an important immunosuppressive checkpoint molecule, mainly expressed on the surface of activated T cells, B cells and NK cells. The binding of PD-1 and its ligand PD-L1 inhibits the activation of T cells (27), decreases autoimmunity and protects tumor escape. PD-1/PD-L1 immune checkpoint blockade enhances the immune function of tumor-specific CD8 + T cells for immune attack on tumors (28). Currently, PD-1 monoclonal antibody nivolumab, Pembrolizumab has been approved by the FDA as a second-line treatment for sorafenib failure (26). Nivolumab also prove the efficacy and safety in the treatment of unresectable HCC (29). In addition, several anti-pd-1 antibodies tislelizumab, camrelizumab and anti-PD-L1 monoclonal antibodies durvalumab, atezolizumab, avelumab have also shown more satisfactory efficacy in clinical trials (30).

CTLA-4 is a protein receptor expressed mainly on T regulatory (Treg) cells. Treg cells, a subset of CD4+ T cells,

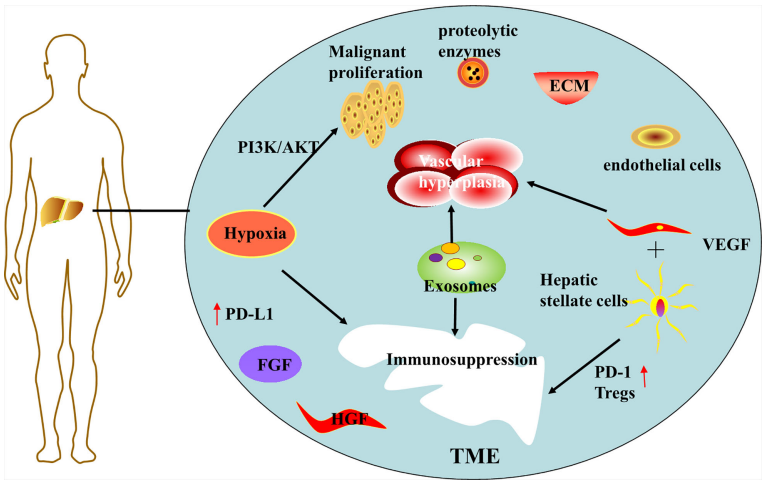

FIGURE 1
Schematic diagram of tumor microenvironment formation mechanism.

can block T cell responses, and blocking CTLA-4 reverses the suppression of T cell activation signaling, making it a potential immunotherapeutic approach (31). The anti-CTLA-4 monoclonal antibodies tremelimumab, ipilimumab is being continuously investigated in the treatment of HCC. A small phase II lead trial (NCT01008358) of the anti-CTLA-4 monoclonal antibody tremelimumab was tested in HCV-infected patients with advanced HCC and showed good partial response (PR) and stable disease (SD) rates and was well tolerated (32).

In addition to PD-1/PD-L1 and CTLA-4, it is essential to explore some new immune checkpoints. LAG3, TIGIT, TIM-3, VISTA, B7-h3, BTLA, have been shown to be promising therapeutic targets that may have opportunities for clinical application in the future (33). Particularly LAG3, as inhibition of LAG3 not only activates CD8+ cytotoxic T cells but also downregulates immunosuppressive regulatory Treg cells (31). PVRL1/TIGIT pathway plays an important role in HCC progression role, and TIGIT is a promising target against PD1 inhibitor resistance (34). TIM-3 is expressed in tumor cells and immune cells. The interaction of TIM-3 with its ligand has been shown to induce T cell suppression. Therefore, blocking TIM-3 expression leads to Tcell proliferation and cytokine production, which triggers immune activation (35). In addition, co-expression of TIM3 and PD1 makes it another attractive target for targeted cancer immunotherapy, and co-blockade of TIM3 and programmed cell death1 (PD1) can lead to a reduction in tumor volume in preclinical models, warranting further study in the clinic (36).

Targeted agents and checkpoint inhibitors are the only drugs approved for systemic treatment of advanced HCC (37). Despite the remarkable clinical success of immune checkpoint therapy, with significant clinical efficacy found for CTLA-4 and PD-1, low response rates and the development of drug resistance in some patients remain issues that need to be addressed. Hypothesized that one of the main reasons for ineffective and resistant PD-1/PD-L1-targeted immunotherapy is that the regulation of PD-L1 is influenced by multiple. For example, in recent studies, USP22 was found to strongly interact with PD-L1 *in vitro* and *in vivo*, inducing PD-L1 deubiquitination, thereby preventing proteasomal degradation of PD-L1 and stabilizing its protein expression levels, counteracting the effects of anti-PD-L1 drugs (38). USP22 is an identified oncoprotein that is highly expressed in hepatocellular carcinoma (HCC) but not in other types of cancer. USP22 can promote multidrug resistance (MDR) in hepatocellular carcinoma cells by activating the SIRT1/AKT/MRP1 pathway, which contributes to tumorigenesis and progression of hepatocellular carcinoma. This gives us a hint that USP22 may be a potential target that could reverse multidrug resistance (MDR) in HCC in the clinic (39). MEF2D promotes tumor growth, metastasis and angiogenesis, affects tumor cells and even the tumor microenvironment, increases PD-L1 expression in HCC cells, and suppresses CD8+ T cell-mediated antitumor immunity. SIRT7 blockade can reduce the dual effect of PD-L1 on hepatocellular carcinoma cell proliferation and decrease anti-tumor immunity through MEF2D regulation, providing a basis for the development of combined SIRT7 inhibitors and anti-pd -(L)1 drugs for the treatment of hepatocellular carcinoma (40). This is a direction worth investigating in the future. It also suggests that immune combination applications are likely to be an effective measure to improve this situation.

Combination of PD-1/PD-L1 inhibitors and CTLA-4 inhibitors

Combination immunotherapy enhances the anti-tumor effects of PD-1/CTLA-4 dual blockers (41). Nivolumab + ipilimumab and durvalumab + tremelimumab are currently approved by the FDA for the treatment of patients with advanced HCC and have achieved better clinical outcomes compared to single agents (26). Nivolumab + ipilimumab is a widely studied combination immunotherapy (42). Data published in ASCO 2019 showed that the anti-Pd-1 antibody nivolumab combined with the anti-CTLA -4 antibody ipilimumab induced complete pathological remission within 6 weeks in 29% of patients with resectable HCC (43).

Immunotherapy combined with MKIs

MKIs such as sorafenib, regorafenib and sunitinib are now used in first and second line treatment of HCC. Their mechanism of action targets multiple kinases by inhibiting various proteins of the VEGF receptor, platelet-derived growth factor, STAT3 and kinase cascades (43). Tyrosine kinase MET is considered an excellent target for hepatocellular carcinoma treatment (44). However, the efficacy of

sorafenib is limited by the development of drug resistance, the major neuronal isoform of RAF, BRAF and MEK pathways play a critical and central role in HCC escape from TKIs activity. A possible strategy could be the combination of RAS/RAF/MEK/ERK pathway inhibitors with other pathways inhibitors, But further clinical studies are needed (45). The growth of HCC cells after sorafenib resistance has been shown to be ameliorated using dual inhibition of Akt and Met, enhancing the effect of sorafenib, but has not been evaluated in patient-derived xenografts (46), and the HGF/MET axis is also considered to be an important pathway for tumor treatment (47). The combination of immunotherapy with tyrosine kinase inhibitors MKIs has been increasingly explored in recent years. Experiments by Li et al. found that MET-mediated phosphorylation and activation of GSK3B resulted in reduced PDL1 expression, and that the combination of anti-PD1 and anti-PD-L1 with MET inhibitors, such as the MET inhibitors

tivantinib and capmatinib, increased PD-L1 expression. And compared with treatment with MET inhibitor or anti-pd1 alone, the duration of both drugs significantly inhibited hepatocellular carcinoma cell growth and prolonged survival time in mice. Treatment of HCC mice with sunitinib in combination with anti-PD-1 resulted in better treatment response and more pronounced tumor regression (43).

Immunotherapy combined with regulation of intestinal microbes

The human intestinal microbiota consists of a complex community of microorganisms, the largest micro-ecosystem in the human body, including archaea, bacteria, viruses, fungi, etc., which work together to regulate nutrition, metabolism and immunity (48). The intestine and liver share a common origin in the foregut, and although the liver has no direct contact with intestinal microorganisms, it has a close relationship through the biliary tract, hepatic portal vein, and bile secretions that coordinate and interact with each other (49), and play a vital role in disease and health status. Growing evidence from experimental and clinical studies suggests that gut microbes play an important role in the development and treatment of liver cancer (50). First, during HCC development and progression, intestinal microorganisms promote the formation of the tumor microenvironment (TME), with the main mechanisms being dysbiosis and leaky gut (51). Dysregulation results in a more permeable intestinal barrier, and a leaky gut allows bacterial metabolites and microbial associated molecular patterns (MAMPs) to translocate and reach the liver (8). It was also found that in China, patients with persistently elevated total serum bile acids had a significantly higher risk of developing HCC, and that bile acids may play an important role in the progression of the underlying liver disease that leads to liver cancer (52). Bound primary bile acids are associated with an increased risk of HBV and HCV-associated HCC, but higher secondary bile acid levels are not associated with an increased risk of HCC (53), corroborating the link between bile acids and hepatocellular carcinoma.

Promisingly, the use of antibiotics, prebiotics and probiotics can be used to regulate intestinal flora and prevent the development of liver cancer (54). Fecal microbiota transplantation (FMT) has been shown in mice to restore intestinal flora diversity and reduce the risk of nonalcoholic steatohepatitis (NASH) developing hepatocellular carcinoma (HCC) (55). Despite the lack of data on the impact of FMT on HCC, fecal microbiota transplantation could be a potential treatment option for NAFLD/NASH progression and could be considered as an augmentation strategy with immune checkpoint inhibitors applied together. Host response to ICIs (PD-1/PD-L1 blockade or CTLA-4 inhibition) may be influenced by the composition of the gut microbiome (48). Stool specimens from immune-responsive patients had higher intestinal flora diversity than specimens from non-responsive patients diversity of intestinal flora (56). Intestinal flora can indirectly affect PD-1 and PD-L1 expression through local or systemic modulation of immune responses, enhancing the antitumor efficacy of PD-1 and PD-L1 blockade therapy (57). The gut microbiota may influence the antitumor immune response through innate and adaptive immunity, but the effect of the gut microbiota on the immune checkpoint inhibitor response has not been validated in HCC and needs to be extensively studied (58).

In addition, combination immunotherapy with CAR-T cells and checkpoint blockade is thought to be the next immunotherapy frontier as it provides the two elements necessary for strong immune responses: CAR-T cells, which provide the infiltrate and PD-1/PD-L1 blockade, which can ensure sustained T cell persistence and function (59). Immunotherapy can also be combined with other local treatments, such as combined local ablation, local radiation therapy, transcatheter arterial chemoembolization (TACE), etc. Local treatment not only destroys the primary tumor, but also stimulates the release of tumor antigens, thus improving the efficiency of immune response in liver cancer (60). A number of clinical trials of immunotherapy and topical treatment clinical trial studies are also underway (61). Although, the clinical efficacy of immunotherapy is very promising, clinical immune-related adverse events (IRAE), and the lack of prognostic markers are still non-negligible issues that need further clinical exploration in the future (62).

Use of oncolytic viruses

Viral therapy was first applied in the 19th century, and was introduced as a treatment for cancer due to the observation that tumors appeared to regress after infection with viruses and the consideration that viruses might have a therapeutic effect on tumors (63). Oncolytic viruses can be divided into two broad categories, those that occur naturally and those that have been genetically modified by humans. Naturally occurring OVs include eutherovirus (Reo), Newcastle disease virus (NDV), enterovirus and measles virus (MV), and microvirus H-1 (H-1PV or Parvoryx), which are used in their native form.On the other hand, human modified viruses, such as herpes simplex virus (HSV), adenovirus (Ad), and cowpox virus (VV), are genetically modified viruses (64).

Targeted regulation of tumor microenvironment by oncolytic viruses

Oncolytic viruses (OVs) are a class of biological agents with tumor-selective and replication capabilities (65). This therapy is

a new and promising treatment for many different types of cancer. Oncolytic viruses is able to selectively replicate and destroy tumor cells, causing tumor cell lysis and subsequent release of viral progeny and tumor cell components, and is able to leave healthy cells unharmed (66). In addition to direct and specific destruction of tumor cells, Oncolytic viruses can also modulate immunity as well as disrupt the tumor vascular system, with multiple effects on the tumor microenvironment (Figure 2).

Induction of immune response

After entering tumor cells, OVs can induce systemic anti-tumor immune responses and induce innate and adaptive immune responses. Upon infection of hepatocellular carcinoma cells by OVs, viral replication leads to endoplasmic reticulum stress and genotoxic stress in cancer cells, releasing tumor-associated antigens TAAs, pathogen-associated molecular pattern molecules PAMPs and damage-associated molecular pattern molecules DAMPs, enhancing the activation of antigen presenting cells (APCs), which leads to the activation of immune cells such as dendritic cells, natural killer cells, macrophages and neutrophils, and inflammatory signaling (67). On the other hand, due to viral replication, activation of antiviral pathways, induction of cytokines and type I IFN, together mediating the activation of immune cells. Activated immune cells, NK cells, in the presence of chemokines such as IL-12, IL-2 and IFN-α/β, metastasize to the tumor area and release IFN-γ, TNF-α and CD107 to exert anti-tumor effects. Mature dendritic cells can initiate T cells in the background of MHC I and II molecules cells, triggering CTL killing of tumor cells through TNF-TNFR signaling, perforin/granzyme pathway. Regarding the regulation of adaptive immunity, according to Twumasi-Boateng et al. it is believed that oncolytic viruses are involved in the entire process of T cell initiation, transport, infiltration, activation and eventual killing of tumors, ultimately reversing immunosuppression and creating a micro-realm of immune stimulation. Therefore, the combination of OVs with tumor immunotherapy can overcome the immune inhibition in TME, thus greatly improving the effect of anti-cancer treatment (68, 69). But there is an important issue, and the number of potential combinations with immunotherapy is enormous, and which combination is most effective requires ongoing research (70).

Disruption of tumor vascular system

There is evidence that poxvirus strains are able to directly destroy infected tumor-associated endothelial cells and replicate within their system, leading to vascular collapse. In a phase II clinical trial, JX-594, a transgenic expression of a recombinant Wyeth poxvirus strain, was used in patients with hepatocellular carcinoma and showed that JX-594 caused acute tumor vascular rupture and reduced tumor perfusion in these patients and was maintained for at least 8 weeks, with no toxicity to normal blood vessels or wound healing noted (71).

In addition to promoting tumor vessel collapse, oncolytic vaccinia virus has recently been found to have antiangiogenic effects. By directly lysing tumor-associated endothelial cells (ECs), oncolytic viruses can reduce the level of vascular

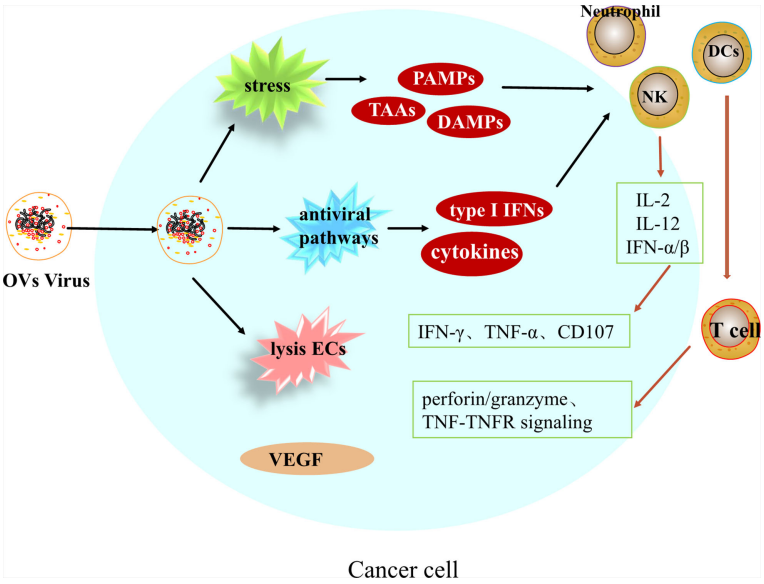

FIGURE 2
Multiple effects of oncolytic viruses on the tumor microenvironment.

endothelial growth factor (VEGF) and thus exert anti-angiogenic effects. Vascular endothelial growth factor (VEGF) levels were significantly reduced in infected tumors after viral treatment, and VEGF production was also reduced in adjacent uninfected cells; therefore, a combination of oncolytic viruses and additional anti-angiogenic therapy may improve treatment outcomes (72).

Clinical application of oncolytic viruses

Reo (73) is a member of the family Reooviridae and is an envelope-free double-stranded RNA virus (64). Induction of interferon (IFN) secretion and innate immune activation in human primary liver tissue in the absence of cytotoxicity and independent of viral genome replication. Meanwhile, Reo-induced cytokine response can effectively inhibit HCV replication and is supported by its clinical potential as a combined antiviral and antitumor therapy in HCC caused by HCV virus infection (74). It is worth noting that some studies have shown that to avoid potential side effects, try to avoid taking oral (75).

Cowpox virus (VV), a double-stranded DNA virus, is currently the most widely studied OVs for the treatment of hepatocellular carcinoma, and its mutant Pexa-Vec, also known as JX-594, is currently being evaluated in a phase III clinical trial in hepatocellular carcinoma (NCT02562755) (65). Preclinical studies of hepatocellular carcinoma lysing herpes simplex virus (oHSV) show that oHSV is highly selective for killing hepatocellular carcinoma (76).

However, to date, only three OVs have been approved globally for the treatment of advanced cancer (77). Despite the multi-mechanism therapeutic effect of OVs, the number of patients fully responding to OV monotherapy is small, so the effect of monotherapy is limited. It is continuously proven that the combination of OVs with other treatment modalities can unlock the therapeutic potential and improve the therapeutic efficacy (75). In addition to the combination of immunotherapy and anti-angiogenesis inhibitors we mentioned earlier, epigenetic dysregulation also plays a key role in hepatocarcinogenesis by altering gene expression through various mechanisms (78), so the combination of epigenetic modulators can also be considered (63). In addition to this, it can be used in combination with pericyte transfer (ACT), chimeric antigen receptor T cells (CAR-T) (79), bispecific T cell conjugates (BiTEs), and cancer vaccines (69).

Efficacy and safety of oncolytic viruses

OVs are a drug with great therapeutic potential, but there are still many issues that need to be addressed, such as viral transmission, dosing, antiviral immunity, etc. (80). In solid tumors, OVs must bypass a series of barriers to reach the tumor site, so overcoming the physical barriers of the tumor microenvironment such as the extracellular matrix (ECM) to viral delivery is a great challenge. ECM consists of proteoglycans that can block the anticancer drug in solid tumors distribution. Therefore, during treatment, ECM degrading enzymes including collagenase and hyaluronidase can be administered to achieve ECM reorganization and promote the spread of the virus within the tumor on the one hand, and OVs expressing ECM degrading enzymes can be designed for use on the other hand. Pre-existing immunity to the virus also reduces the effectiveness of oncolytic viruses therapy and can be circumvented by increasing the dose of systemic administration of OVs and co-administration of cyclophosphamide (64). In order to better target hepatocellular carcinoma with oncolytic viruses, it has been demonstrated that the use of a cationic galactosylated polymer (Gal32-b-Agm29) as a vector allows systemic delivery of oncolytic viruses in hepatocellular carcinoma cell lines. OVs complexed with Gal32-b-Agm29 enables easier entry of viral cells into hepatocellular carcinoma cells, enhances viral replication, and ultimately leads to hepatocellular carcinoma cell lysis and the occurrence of a higher immunogenic cell death induction program (81). More future research is needed on how to safely address other clinical studies.

Anti-anomalous proliferation of blood vessels

Hepatocellular carcinoma is a highly vascularized tumor. At the tumor site, hypoxia induces tumor cells and stromal cells to secrete a variety of pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and matrix metalloproteinase (MMP) (82), leading to vascular proliferation, and the abnormally proliferating vessels provide tumor development providing nutrients for tumor development. The theory is that controlling the rate of angiogenesis so that tumor growth lacks nutritional support will slow down the growth of the tumor. The VEGF pathway is not only a key regulator of tumor angiogenesis, but also has the ability to inhibit the infiltration and function of cytotoxic T lymphocytes by affecting immune cells in the myeloid and lymphoid lineages (83). VEGF inhibits the maturation of dendritic cells (DCs) by activating NF-κB and suppresses the activation of T cells by promoting the production of indoleamine 2,3-dioxygenase (IDO), as well as the induction of Treg cells. VEGF also regulates immunity in hepatocellular carcinoma by inducing the expression of immunosuppressive receptors, including PD-1, lymphocyte activation gene 3, T-cell immunoglobulin and mucin domain 3 (82), promoting CD8+ T-cell failure and tumor escape free escape (Figure 3). Therefore, anti-angiogenic therapy can be an idea for the treatment of liver cancer. Anti-angiogenesis can induce normalization of tumor

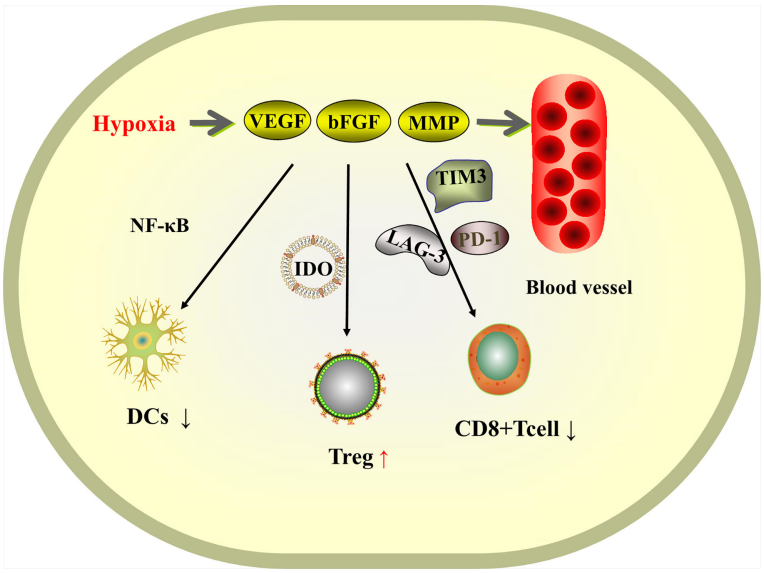

FIGURE 3
Schematic diagram of the mechanism of tissue hypoxia-induced VEGF-promoted tumor vascular proliferation and immunosuppression.

vascular structure, remove blood vessels necessary for tumor growth and metastasis, and also promote antigen presentation and activation of cytotoxic CD8+ t cells (84), reprogramming the tumor immune microenvironment (85) and transforming immunosuppression into immune stimulation, thus improving the immunosuppressive microenvironment of tumors.

However, anti-VEGF antibody monotherapy has failed to produce satisfactory antitumor efficacy in human HCC patients so far (84). Therefore, a combination of anti-angiogenic therapy and immunotherapy can be considered, where on the one hand immunotherapy enhances the efficacy of vascular endothelial factor inhibitors, on the other hand vascular endothelial factor inhibitors alleviate resistance to immunotherapy.

Atezolizumab (anti-PD-L1) and bevacizumab (vascular endothelial growth factor (VEGF) inhibitor) have been shown to be efficacious (86, 87), and their combination has demonstrated antitumor activity and safety in a phase 1b trial in patients with unresectable hepatocellular carcinoma. In patients with unresectable hepatocellular carcinoma, atezolizumab and bevacizumab had better overall survival and progression-free survival than sorafenib (28, 88), and the combination of atezolizumab + bevacizumab had longer progression-free survival than atezolizumab treatment alone (89).

Lenvatinib is a multitargeted inhibitor of multiple growth factor receptors, including vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), and the proto-oncogenes RET and KIT (90). Abnormally activated FGF signaling can directly drive cell proliferation and survival, promoting tumor angiogenesis and progression. Lenvatinib inhibits the vascular endothelial growth factor receptor and fibroblast growth factor bodies, and this dual-target inhibition effect enhances the antitumor activity of anti-Lenvatinib in HCC, while also strengthening the efficacy of PD -1 antibodies. A growing body of evidence suggests that Lenvatinib in combination with anti-PD-1 antibody significantly inhibits tumor growth *in vivo*, induces long-term immune memory, and has no significant adverse effects (91). Preliminary data from a clinical trial showed an objective remission rate (ORR) of 46% for Lenvatinib in combination with pembrolizumab (PD-1 antibody), with better response rates and duration of response (90). In July 2019, based on the results of KEYNOTE-524/Study 116 (NCT03006926), the FDA announced the approval of Lenvatinib in combination with pembrolizumab for the treatment of HCC (92). In addition, the efficacy of nivolumab and Lenvatinib has been confirmed, but more data are needed to validate (83).

It is worth noting that if anti-VEGF therapy causes excessive vascular pruning, it will aggravate tumor hypoxia, so we should reasonably apply anti-VEGF drug doses to normalize dysfunctional tumor vessels, improve tumor perfusion and alleviate tumor hypoxia (85).

Discussion

As a serious global health problem with poor prognosis and high mortality rate, there has been tremendous progress in recent years in understanding the pathogenesis, early detection

and diagnosis (93), staging and treatment of hepatocellular carcinoma (94). Research advances in the use of molecularly targeted agents (MTAs) and immune checkpoint inhibitors have significantly improved the prognosis of patients with this disease (95), demonstrating superior survival benefits, durable responses, and a manageable safety profile in advanced HCC. Oncolytic viruses, cancer vaccines (96), pericyte therapy (97), photothermal therapy (PTT) and photodynamic therapy (PDT) (98), and nanotechnology are also being explored. However, due to the specific immune tolerance of the liver (99) and the complexity of the tumor microenvironment, the treatment of hepatocellular carcinoma remains a great challenge, and continuous research, including single-cell sequencing, is needed in the future to explore new immunotherapeutic targets and personalized treatment protocols (100). In addition to this, the development of diagnostic, prognostic and biomarker prediction for hepatocellular carcinoma and other cancers using artificial intelligence is an exciting prospect (101). The role of menopausal hormones in reducing the risk of liver cancer still needs to be explored (102). With the development of science and technology and the advancement of research methods, the efficacy of treatment for liver cancer is also expected to be improved in the future.

Author contributions

HW, FS, and LZ concepted and designed the review. FS, SZ and MZ wrote the manuscript. HW, ZP, LX, and LZ revised the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Villanueva A. Hepatocellular carcinoma. *N Engl J Med* (2019) 380(15):1450–62. doi: 10.1056/NEJMra1713263

2. Anwanwan D, Singh SK, Singh S, Saikam V, Singh R. Challenges in liver cancer and possible treatment approaches. *Biochim Biophys Acta Rev Cancer* (2020) 1873(1):188314. doi: 10.1016/j.bbcan.2019.188314

3. Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers* (2021) 7(1):6. doi: 10.1038/s41572-020-00240-3

4. Bresnahan E, Ramadori P, Heikenwalder M, Zender L, Lujambio A. Novel patient-derived preclinical models of liver cancer. *J Hepatol* (2020) 72(2):239–49. doi: 10.1016/j.jhep.2019.09.028

5. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet* (2018) 391 (10127):1301–14. doi: 10.1016/S0140-6736(18)30010-2

6. Zhang L, Ding J, Li HY, Wang ZH, Wu J. Immunotherapy for advanced hepatocellular carcinoma, where are we? *Biochim Biophys Acta Rev Cancer* (2020) 1874(2):188441. doi: 10.1016/j.bbcan.2020.188441

7. Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. *Nat Rev Gastroenterol Hepatol* (2019) 16(10):589–604. doi: 10.1038/s41575-019-0186-y

8. Temraz S, Nassar F, Kreidieh F, Mukherji D, Shamseddine A, Nasr R. Hepatocellular carcinoma immunotherapy and the potential influence of gut microbiome. *Int J Mol Sci* (2021) 22(15):7800. doi: 10.3390/ijms22157800

9. Greten TF, Lai CW, Li G, Staveley-O'Carroll KF. Targeted and immune-based therapies for hepatocellular carcinoma. *Gastroenterology* (2019) 156(2):510–24. doi: 10.1053/j.gastro.2018.09.051

10. Waidmann O. Recent developments with immunotherapy for hepatocellular carcinoma. *Expert Opin Biol Ther* (2018) 18(8):905–10. doi: 10.1080/14712598.2018.1499722

11. Zeng F, Zhang Y, Han X, Zeng M, Gao Y, Weng J. Employing hypoxia characterization to predict tumour immune microenvironment, treatment sensitivity and prognosis in hepatocellular carcinoma. *Comput Struct Biotechnol J* (2021) 19:2775–89. doi: 10.1016/j.csbj.2021.03.033

12. Maggs L, Ferrone S. Improving the clinical significance of preclinical immunotherapy studies through incorporating tumor microenvironment-like conditions. *Clin Cancer Res* (2020) 26(17):4448–53. doi: 10.1158/1078-0432.CCR-20-0358

13. Bamodu OA, Chang HL, Ong JR, Lee WH, Yeh CT, Tsai JT. Elevated PDK1 expression drives PI3K/AKT/MTOR signaling promotes radiation-resistant and dedifferentiated phenotype of hepatocellular carcinoma. *Cells* (2020) 9(3):746. doi: 10.3390/cells9030746

14. Faivre S, Rimassa L, Finn RS. Molecular therapies for HCC: Looking outside the box. *J Hepatol* (2020) 72(2):342–52. doi: 10.1016/j.jhep.2019.09.010

15. Li X, Li C, Zhang L, Wu M, Cao K, Jiang F, et al. The significance of exosomes in the development and treatment of hepatocellular carcinoma. *Mol Cancer* (2020) 19(1):1. doi: 10.1186/s12943-019-1085-0

16. Wu Q, Zhou L, Lv D, Zhu X, Tang H. Exosome-mediated communication in the tumor microenvironment contributes to hepatocellular carcinoma development and progression. *J Hematol Oncol* (2019) 12(1):53. doi: 10.1186/s13045-019-0739-0

17. Barry AE, Baldeosingh R, Lamm R, Patel K, Zhang K, Dominguez DA, et al. Hepatic stellate cells and hepatocarcinogenesis. *Front Cell Dev Biol* (2020) 8:709. doi: 10.3389/fcell.2020.00709

18. Pinato DJ, Guerra N, Fessas P, Murphy R, Mineo T, Mauri FA, et al. Immune-based therapies for hepatocellular carcinoma. *Oncogene* (2020) 39 (18):3620–37. doi: 10.1038/s41388-020-1249-9

19. Hao X, Sun G, Zhang Y, Kong X, Rong D, Song J, et al. Targeting immune cells in the tumor microenvironment of HCC: New opportunities and challenges. *Front Cell Dev Biol* (2021) 9:775462. doi: 10.3389/fcell.2021.775462

20. da Costa VR, Araldi RP, Vigerelli H, D'Ámelio F, Mendes TB, Gonzaga V, et al. Exosomes in the tumor microenvironment: From biology to clinical applications. *Cells* (2021) 10(10):2617. doi: 10.3390/cells10102617

21. Sun W, Fu S, Wu S, Tu R. Growing evidence of exosomal MicroRNA-related metastasis of hepatocellular carcinoma. *BioMed Res Int* (2020) 2020:4501454. doi: 10.1155/2020/4501454

22. Fu X, Liu M, Qu S, Ma J, Zhang Y, Shi T, et al. Exosomal microRNA-32-5p induces multidrug resistance in hepatocellular carcinoma *via* the PI3K/Akt pathway. *J Exp Clin Cancer Res* (2018) 37(1):52. doi: 10.1186/s13046-018-0677-7

23. Yugawa K, Yoshizumi T, Mano Y, Itoh S, Harada N, Ikegami T, et al. Cancer-associated fibroblasts promote hepatocellular carcinoma progression

through downregulation of exosomal miR-150-3p. *Eur J Surg Oncol* (2021) 47 (2):384–93. doi: 10.1016/j.ejso.2020.08.002

24. Federico P, Petrillo A, Giordano P, Bosso D, Fabbrocini A, Ottaviano M, et al. Immune checkpoint inhibitors in hepatocellular carcinoma: Current status and novel perspectives. *Cancers (Basel)* (2020) 12(10):3025. doi: 10.3390/cancers12103025

25. Brown ZJ, Greten TF, Heinrich B. Adjuvant treatment of hepatocellular carcinoma: Prospect of immunotherapy. *Hepatology* (2019) 70(4):1437–42. doi: 10.1002/hep.30633

26. Nakano S, Eso Y, Okada H, Takai A, Takahashi K, Seno H. Recent advances in immunotherapy for hepatocellular carcinoma. *Cancers (Basel)* (2020) 12(4):775. doi: 10.3390/cancers12040775

27. Hayashi H, Nakagawa K. Combination therapy with PD-1 or PD-L1 inhibitors for cancer. *Int J Clin Oncol* (2020) 25(5):818–30. doi: 10.1007/s10147-019-01548-1

28. Ahn E, Araki K, Hashimoto M, Li W, Riley JL, Cheung J, et al. Role of PD-1 during effector CD8 T cell differentiation. *Proc Natl Acad Sci* (2018) 115(18):4749–54. doi: 10.1073/pnas.1718217115

29. Chan LL, Chan SL. Emerging immune checkpoint inhibitors for the treatment of hepatocellular carcinoma. *Expert Opin Emerg Drugs* (2021) 26 (1):39–52. doi: 10.1080/14728214.2021.1902503

30. Xu W, Liu K, Chen M, Sun JY, McCaughan GW, Lu XJ, et al. Immunotherapy for hepatocellular carcinoma: recent advances and future perspectives. *Ther Adv Med Oncol* (2019) 11:1758835919862692. doi: 10.1177/1758835919862692

31. Khan AA, Liu ZK, Xu X. Recent advances in immunotherapy for hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int* (2021) 20(6):511–20. doi: 10.1016/j.hbpd.2021.06.010

32. Sangro B, Gomez-Martin C, de la Mata M, Inarrairaegui M, Garralda E, Barrera P, et al. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis c. *J Hepatol* (2013) 59(1):81–8. doi: 10.1016/j.jhep.2013.02.022

33. Qin S, Xu L, Yi M, Yu S, Wu K, Luo S. Novel immune checkpoint targets: moving beyond PD-1 and CTLA-4. *Mol Cancer* (2019) 18(1):155. doi: 10.1186/s12943-019-1091-2

34. Chiu DK, Yuen VW, Cheu JW, Wei LL, Ting V, Fehlings M, et al. Hepatocellular carcinoma cells up-regulate PVRL1, stabilizing PVR and inhibiting the cytotoxic T-cell response *via* TIGIT to mediate tumor resistance to PD1 inhibitors in mice. *Gastroenterology* (2020) 159(2):609–23. doi: 10.1053/j.gastro.2020.03.074

35. Lee JB, Ha SJ, Kim HR. Clinical insights into novel immune checkpoint inhibitors. *Front Pharmacol* (2021) 12:681320. doi: 10.3389/fphar.2021.681320

36. Wolf Y, Anderson AC, Kuchroo VK. TIM3 comes of age as an inhibitory receptor. *Nat Rev Immunol* (2020) 20(3):173–85. doi: 10.1038/s41577-019-0224-6

37. Cersosimo RJ. Systemic targeted and immunotherapy for advanced hepatocellular carcinoma. *Am J Health Syst Pharm* (2021) 78(3):187–202. doi: 10.1093/ajhp/zxaa365

38. Huang X, Zhang X, Bai X, Liang T. Blocking PD-L1 for anti-liver cancer immunity: USP22 represents a critical cotarget. *Cell Mol Immunol* (2020) 17 (7):677–9. doi: 10.1038/s41423-019-0348-4

39. Ling S, Li J, Shan Q, Dai H, Lu D, Wen X, et al. USP22 mediates the multidrug resistance of hepatocellular carcinoma *via* the SIRT1/AKT/MRP1 signaling pathway. *Mol Oncol* (2017) 11(6):682–95. doi: 10.1002/1878-0261.12067

40. Xiang J, Zhang N, Sun H, Su L, Zhang C, Xu H, et al. Disruption of SIRT7 increases the efficacy of checkpoint inhibitor *via* MEF2D regulation of programmed cell death 1 ligand 1 in hepatocellular carcinoma cells. *Gastroenterology* (2020) 158(3):664–78.e24. doi: 10.1053/j.gastro.2019.10.025

41. El Dika I, Khalil DN, Abou-Alfa GK. Immune checkpoint inhibitors for hepatocellular carcinoma. *Cancer* (2019) 125(19):3312–9. doi: 10.1002/cncr.32076

42. Cheng AL, Hsu C, Chan SL, Choo SP, Kudo M. Challenges of combination therapy with immune checkpoint inhibitors for hepatocellular carcinoma. *J Hepatol* (2020) 72(2):307–19. doi: 10.1016/j.jhep.2019.09.025

43. Zongyi Y, Xiaowu L. Immunotherapy for hepatocellular carcinoma. *Cancer Lett* (2020) 470:8–17. doi: 10.1016/j.canlet.2019.12.002

44. Li H, Li CW, Li X, Ding Q, Guo L, Liu S, et al. MET inhibitors promote liver tumor evasion of the immune response by stabilizing PDL1. *Gastroenterology* (2019) 156(6):1849–61.e13. doi: 10.1053/j.gastro.2019.01.252

45. Gnoni A, Licchetta A, Memeo R, Argentiero A, Solimando AG, Longo V, et al. Role of BRAF in hepatocellular carcinoma: A rationale for future targeted cancer therapies. *Medicina (Kaunas)* (2019) 55(12):754. doi: 10.3390/medicina55120754

46. Han P, Li H, Jiang X, Zhai B, Tan G, Zhao D, et al. Dual inhibition of akt and c-met as a second-line therapy following acquired resistance to sorafenib in hepatocellular carcinoma cells. *Mol Oncol* (2017) 11(3):320–34. doi: 10.1002/1878-0261.12039

47. Oliveres H, Pineda E, Maurel J. MET inhibitors in cancer: pitfalls and challenges. *Expert Opin Investig Drugs* (2020) 29(1):73–85. doi: 10.1080/13543784.2020.1699532

48. Rezasoltani S, Yadegar A, Asadzadeh Aghdaei H, Reza Zali M. Modulatory effects of gut microbiome in cancer immunotherapy: A novel paradigm for blockade of immune checkpoint inhibitors. *Cancer Med* (2021) 10(3):1141–54. doi: 10.1002/cam4.3694

49. Jiang JW, Chen XH, Ren Z, Zheng SS. Gut microbial dysbiosis associates hepatocellular carcinoma *via* the gut-liver axis. *Hepatobiliary Pancreat Dis Int* (2019) 18(1):19–27. doi: 10.1016/j.hbpd.2018.11.002

50. Yu LX, Schwabe RF. The gut microbiome and liver cancer: mechanisms and clinical translation. *Nat Rev Gastroenterol Hepatol* (2017) 14(9):527–39. doi: 10.1038/nrgastro.2017.72

51. Schwabe RF, Greten TF. Gut microbiome in HCC - mechanisms, diagnosis and therapy. *J Hepatol* (2020) 72(2):230–8. doi: 10.1016/j.jhep.2019.08.016

52. Thomas CE, Luu HN, Wang R, Xie G, Adams-Haduch J, Jin A, et al. Association between pre-diagnostic serum bile acids and hepatocellular carcinoma: The Singapore Chinese health study. *Cancers (Basel)* (2021) 13(11):2648. doi: 10.3390/cancers13112648

53. Petrick JL, Florio AA, Koshiol J, Pfeiffer RM, Yang B, Yu K, et al. Prediagnostic concentrations of circulating bile acids and hepatocellular carcinoma risk: REVEAL-HBV and HCV studies. *Int J Cancer* (2020) 147 (10):2743–53. doi: 10.1002/ijc.33051

54. Chen YH, Wu WK, Wu MS. Microbiota-associated therapy for non-alcoholic steatohepatitis-induced liver cancer: A review. *Int J Mol Sci* (2020) 21 (17):5999. doi: 10.3390/ijms21175999

55. Delaune V, Orci LA, Lacotte S, Peloso A, Schrenzel J, Lazarevic V, et al. Fecal microbiota transplantation: a promising strategy in preventing the progression of non-alcoholic steatohepatitis and improving the anti-cancer immune response. *Expert Opin Biol Ther* (2018) 18(10):1061–71. doi: 10.1080/14712598.2018.1518424

56. Zheng Y, Wang T, Tu X, Huang Y, Zhang H, Tan D, et al. Gut microbiome affects the response to anti-PD-1 immunotherapy in patients with hepatocellular carcinoma. *J Immunother Cancer* (2019) 7(1):193. doi: 10.1186/s40425-019-0650-9

57. Wang Y, Ma R, Liu F, Lee SA, Zhang L. Modulation of gut microbiota: A novel paradigm of enhancing the efficacy of programmed death-1 and programmed death ligand-1 blockade therapy. *Front Immunol* (2018) 9:374. doi: 10.3389/fimmu.2018.00374

58. Li L, Ye J. Characterization of gut microbiota in patients with primary hepatocellular carcinoma received immune checkpoint inhibitors: A Chinese population-based study. *Med (Baltimore)* (2020) 99(37):e21788. doi: 10.1097/MD.0000000000021788

59. Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J* (2021) 11(4):69. doi: 10.1038/s41408-021-00459-7

60. Zeng P, Shen D, Zeng CH, Chang XF, Teng GJ. Emerging opportunities for combining locoregional therapy with immune checkpoint inhibitors in hepatocellular carcinoma. *Curr Oncol Rep* (2020) 22(8):76. doi: 10.1007/s11912-020-00943-6

61. Kim GH, Kim JH, Kim PH, Chu HH, Gwon DI, Ko HK. Emerging trends in the treatment of advanced hepatocellular carcinoma: A radiological perspective. *Korean J Radiol* (2021) 22(11):1822–33. doi: 10.3348/kjr.2021.0229

62. Oura K, Morishita A, Tani J, Masaki T. Tumor immune microenvironment and immunosuppressive therapy in hepatocellular carcinoma: A review. *Int J Mol Sci* (2021) 22(11):5801. doi: 10.3390/ijms22115801

63. Chianese A, Santella B, Ambrosino A, Stelitano D, Rinaldi L, Galdiero M, et al. Oncolytic viruses in combination therapeutic approaches with epigenetic modulators: Past, present, and future perspectives. *Cancers (Basel)* (2021) 13 (11):2761. doi: 10.3390/cancers13112761

64. Abd-Aziz N, Poh CL. Development of oncolytic viruses for cancer therapy. *Transl Res* (2021) 237:98–123. doi: 10.1016/j.trsl.2021.04.008

65. Zhang Y, Li Y, Chen K, Qian L, Wang P. Oncolytic virotherapy reverses the immunosuppressive tumor microenvironment and its potential in combination with immunotherapy. *Cancer Cell Int* (2021) 21(1):262. doi: 10.1186/s12935-021-01972-2

66. Yang L, Gu X, Yu J, Ge S, Fan X. Oncolytic virotherapy: From bench to bedside. *Front Cell Dev Biol* (2021) 9:790150. doi: 10.3389/fcell.2021.790150

67. Chaurasiya S, Chen NG, Fong Y. Oncolytic viruses and immunity. *Curr Opin Immunol* (2018) 51:83–90. doi: 10.1016/j.coi.2018.03.008

68. Twumasi-Boateng K, Pettigrew JL, Kwok YYE, Bell JC, Nelson BH. Oncolytic viruses as engineering platforms for combination immunotherapy. *Nat Rev Cancer* (2018) 18(7):419–32. doi: 10.1038/s41568-018-0009-4

69. Shi T, Song X, Wang Y, Liu F, Wei J. Combining oncolytic viruses with cancer immunotherapy: Establishing a new generation of cancer treatment. *Front Immunol* (2020) 11:683. doi: 10.3389/fimmu.2020.00683

70. Lawler SE, Speranza MC, Cho CF, Chiocca EA. Oncolytic viruses in cancer treatment: A review. *JAMA Oncol* (2017) 3(6):841–9. doi: 10.1001/jamaoncol.2016.2064

71. Breitbach CJ, Arulanandam R, De Silva N, Thorne SH, Patt R, Daneshmand M, et al. Oncolytic vaccinia virus disrupts tumor-associated vasculature in humans. *Cancer Res* (2013) 73(4):1265–75. doi: 10.1158/0008-5472.CAN-12-2687

72. Berkey SE, Thorne SH, Bartlett DL. Oncolytic virotherapy and the tumor microenvironment. *Adv Exp Med Biol* (2017) 1036:157–72. doi: 10.1007/978-3-319-67577-0_11

73. Muller L, Berkeley R, Barr T, Ilett E, Errington-Mais F. Past, present and future of oncolytic reovirus. *Cancers (Basel)* (2020) 12(11):3219. doi: 10.3390/cancers12113219

74. Samson A, Bentham MJ, Scott K, Nuovo G, Bloy A, Appleton E, et al. Oncolytic reovirus as a combined antiviral and anti-tumour agent for the treatment of liver cancer. *Gut* (2018) 67(3):562–73. doi: 10.1136/gutjnl-2016-312009

75. Malfitano AM, Di Somma S, Iannuzzi CA, Pentimalli F, Portella G. Virotherapy: From single agents to combinatorial treatments. *Biochem Pharmacol* (2020) 177:113986. doi: 10.1016/j.bcp.2020.113986

76. Yamada T, Hamano Y, Hasegawa N, Seo E, Fukuda K, Yokoyama KK, et al. Oncolytic virotherapy and gene therapy strategies for hepatobiliary cancers. *Curr Cancer Drug Targets* (2018) 18(2):188–201. doi: 10.2174/1568009617666170330123841

77. Macedo N, Miller DM, Haq R, Kaufman HL. Clinical landscape of oncolytic virus research in 2020. *J Immunother Cancer* (2020) 8(2):e001486. doi: 10.1136/jitc-2020-001486

78. Rebouissou S, Nault JC. Advances in molecular classification and precision oncology in hepatocellular carcinoma. *J Hepatol* (2020) 72(2):215–29. doi: 10.1016/j.jhep.2019.08.017

79. Watanabe N, McKenna MK, Rosewell Shaw A, Suzuki M. Clinical CAR-T cell and oncolytic virotherapy for cancer treatment. *Mol Ther* (2021) 29(2):505–20. doi: 10.1016/j.ymthe.2020.10.023

80. Zheng M, Huang J, Tong A, Yang H. Oncolytic viruses for cancer therapy: Barriers and recent advances. *Mol Ther Oncolytics* (2019) 15:234–47. doi: 10.1016/j.omto.2019.10.007

81. Garofalo M, Bellato F, Magliocca S, Malfanti A, Kuryk L, Rinner B, et al. Polymer coated oncolytic adenovirus to selectively target hepatocellular carcinoma cells. *Pharmaceutics* (2021) 13(7):949. doi: 10.3390/pharmaceutics13070949

82. Yi M, Jiao D, Qin S, Chu Q, Wu K, Li A. Synergistic effect of immune checkpoint blockade and anti-angiogenesis in cancer treatment. *Mol Cancer* (2019) 18(1):60. doi: 10.1186/s12943-019-0974-6

83. Kudo M. Scientific rationale for combined immunotherapy with PD-1/PD-L1 antibodies and VEGF inhibitors in advanced hepatocellular carcinoma. *Cancers (Basel)* (2020) 12(5). doi: 10.3390/cancers12051089

84. Song Y, Fu Y, Xie Q, Zhu B, Wang J, Zhang B. Anti-angiogenic agents in combination with immune checkpoint inhibitors: A promising strategy for cancer treatment. *Front Immunol* (2020) 11:1956. doi: 10.3389/fimmu.2020.01956

85. Nishida N. Clinical implications of the dual blockade of the PD-1/PD-L1 and vascular endothelial growth factor axes in the treatment of hepatocellular carcinoma. *Hepatobiliary Surg Nutr* (2020) 9(5):640–3. doi: 10.21037/hbsn.2019.10.18

86. Pinter M, Jain RK, Duda DG. The current landscape of immune checkpoint blockade in hepatocellular carcinoma: A review. *JAMA Oncol* (2021) 7(1):113–23. doi: 10.1001/jamaoncol.2020.3381

87. Plaz Torres MC, Lai Q, Piscaglia F, Caturelli E, Cabibbo G, Biasini E, et al. Treatment of hepatocellular carcinoma with immune checkpoint inhibitors and applicability of first-line Atezolizumab/Bevacizumab in a real-life setting. *J Clin Med* (2021) 10(15):3201. doi: 10.3390/jcm10153201

88. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, et al. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. *N Engl J Med* (2020) 382(20):1894–905. doi: 10.1056/NEJMoa1915745

89. Lee MS, Ryoo B-Y, Hsu C-H, Numata K, Stein S, Verret W, et al. Atezolizumab with or without bevacizumab in unresectable hepatocellular carcinoma (GO30140): an open-label, multicentre, phase 1b study. *Lancet Oncol* (2020) 21(6):808–20. doi: 10.1016/S1470-2045(20)30156-X

90. Zhao Y, Zhang YN, Wang KT, Chen L. Lenvatinib for hepatocellular carcinoma: From preclinical mechanisms to anti-cancer therapy. *Biochim Biophys Acta Rev Cancer* (2020) 1874(1):188391. doi: 10.1016/j.bbcan.2020.188391

91. Deng H, Kan A, Lyu N, Mu L, Han Y, Liu L, et al. Dual vascular endothelial growth factor receptor and fibroblast growth factor receptor inhibition elicits antitumor immunity and enhances programmed cell death-1 checkpoint blockade in hepatocellular carcinoma. *Liver Cancer* (2020) 9(3):338–57. doi: 10.1159/000505695

92. Huang A, Yang XR, Chung WY, Dennison AR, Zhou J. Targeted therapy for hepatocellular carcinoma. *Signal Transduct Target Ther* (2020) 5(1):146. doi: 10.1038/s41392-020-00264-x

93. Wang W, Wei C. Advances in the early diagnosis of hepatocellular carcinoma. *Genes Dis* (2020) 7(3):308–19. doi: 10.1016/j.gendis.2020.01.014

94. Nault JC, Cheng AL, Sangro B, Llovet JM. Milestones in the pathogenesis and management of primary liver cancer. *J Hepatol* (2020) 72(2):209–14. doi: 10.1016/j.jhep.2019.11.006

95. Nishida N. Role of oncogenic pathways on the cancer immunosuppressive microenvironment and its clinical implications in hepatocellular carcinoma. *Cancers (Basel)* (2021) 13(15):3666. doi: 10.3390/cancers13153666

96. Lurje I, Werner W, Mohr R, Roderburg C, Tacke F, Hammerich L. *In situ* vaccination as a strategy to modulate the immune microenvironment of hepatocellular carcinoma. *Front Immunol* (2021) 12:650486. doi: 10.3389/fimmu.2021.650486

97. Tagliamonte M, Mauriello A, Cavalluzzo B, Ragone C, Manolio C, Petrizzo A, et al. Tackling hepatocellular carcinoma with individual or combinatorial immunotherapy approaches. *Cancer Lett* (2020) 473:25–32. doi: 10.1016/j.canlet.2019.12.029

98. Fan Z, Zhuang C, Wang S, Zhang Y. Photodynamic and photothermal therapy of hepatocellular carcinoma. *Front Oncol* (2021) 11:787780. doi: 10.3389/fonc.2021.787780

99. Feng GS, Hanley KL, Liang Y, Lin X. Improving the efficacy of liver cancer immunotherapy: The power of combined preclinical and clinical studies. *Hepatology* (2021) 73 Suppl 1:104–14. doi: 10.1002/hep.31479

100. Woller N, Engelskircher SA, Wirth T, Wedemeyer H. Prospects and challenges for T cell-based therapies of HCC. *Cells* (2021) 10(7):1651. doi: 10.3390/cells10071651

101. Kather JN, Calderaro J. Development of AI-based pathology biomarkers in gastrointestinal and liver cancer. *Nat Rev Gastroenterol Hepatol* (2020) 17(10):591–2. doi: 10.1038/s41575-020-0343-3

102. Bagcchi S. Menopausal hormone therapy reduces liver cancer risk. *Lancet Oncol* (2016) 17(2):e50. doi: 10.1016/S1470-2045(16)00003-6

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