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A year in review: Discussions in cancer immunity and immunotherapy

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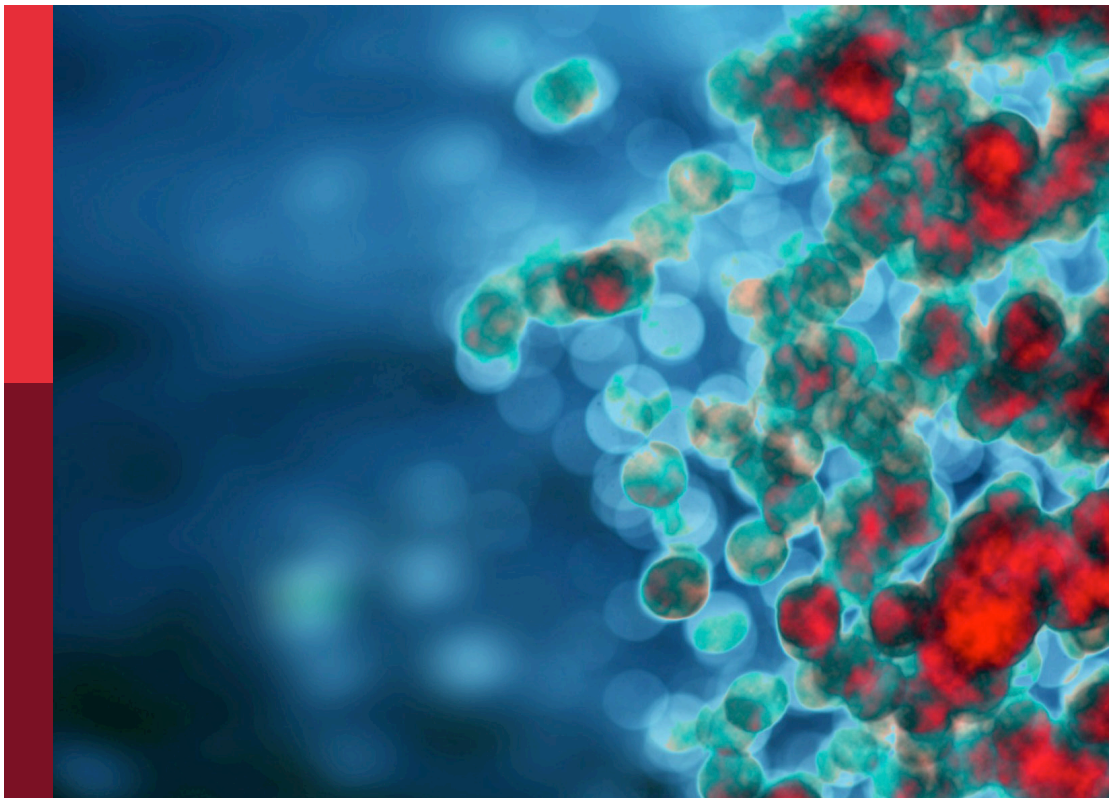

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A year in review: Discussions in cancer immunity and immunotherapy

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REVIEW
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CAR-T Cell Therapy in Hematological Malignancies: Current Opportunities and Challenges

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Chimeric antigen receptor T (CAR-T) cell therapy represents a major breakthrough in cancer treatment, and it has achieved unprecedented success in hematological malignancies, especially in relapsed/refractory (R/R) B cell malignancies. At present, CD19 and BCMA are the most common targets in CAR-T cell therapy, and numerous novel therapeutic targets are being explored. However, the adverse events related to CAR-T cell therapy might be serious or even life-threatening, such as cytokine release syndrome (CRS), CAR-T-cell-related encephalopathy syndrome (CRES), infections, cytopenia, and CRS-related coagulopathy. In addition, due to antigen escape, the limited CAR-T cell persistence, and immunosuppressive tumor microenvironment, a considerable proportion of patients relapse after CAR-T cell therapy. Thus, in this review, we focus on the progress and challenges of CAR-T cell therapy in hematological malignancies, such as attractive therapeutic targets, CAR-T related toxicities, and resistance to CAR-T cell therapy, and provide some practical recommendations.

Keywords: CAR-T cell, hematological malignancies, CAR-T related toxicities, antigen escape, immunosuppressive tumor microenvironment, combinatorial therapy

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INTRODUCTION

Traditionally, the treatment of hematological malignancies mainly includes chemotherapy, radiotherapy, and hematopoietic stem cell transplantation (HSCT). However, with advances in tumor immunology, immune targeted therapy, such as monoclonal antibodies, bispecific antibodies, antibody-drug conjugates, and chimeric antigen receptor T (CAR-T) cell therapy, has opened a new avenue for the treatment of malignancies. In particular, CAR-T cell therapy has revolutionized the treatment of hematological malignancies and achieved unprecedented responses in recent years, especially in relapsed/refractory (R/R) B-cell acute lymphocytic leukemia (B-ALL), non-Hodgkin lymphoma (NHL), and multiple myeloma (MM). At present, there are six CAR-T cell products approved by the US Food and Drug Administration (FDA) for the treatment of R/R B cell malignancies, including tisagenlecleucel (Kymriah; Novartis), axicabtagene ciloleucel (Yescarta; Gilead), brexucabtagene autoleucel (Tecartus; Gilead), lisocabtagene maraleucel (Breyanzi; Bristol Myers Squibb), idecabtagene vicleucel (Abecma; Bristol Myers

Squibb and Bluebird Bio), and ciltacabtagene autoleucel (Carvykti; Legend and Janssen), which target the most common target antigens CD19 and B cell maturation antigen (BCMA) (**Table 1** landmark clinical trials). In addition, there are a considerable number of novel targets which are being explored.

However, despite the remarkable breakthrough of CAR-T therapy in B cell malignancies, severe toxicities associated with CAR-T cell therapy may compromise its efficacy and could even progress into life-threatening conditions, such as multiple organ dysfunction, sepsis, and disseminated intravascular coagulation (DIC). Cytokine release syndrome (CRS) is the most common complication, which is a systemic inflammatory response induced by the overactivation of CAR-T cells and endogenous immune cells, such as macrophages and dendritic cells. Its manifestations are diverse and partially similar with infections. Moreover, severe CRS is correlated with the increased risk of CAR-T-cell-related encephalopathy syndrome (CRES) and coagulopathy (9). Thus, clarifying their underlying mechanisms could facilitate the prevention and management of these adverse events. In addition, the resistance after CAR T-cell therapy cannot be ignored.

Thus, this review aims to introduce the advances and challenges in CAR-T cell therapy, such as attractive therapeutic targets, toxicities related to CAR-T cell therapy, and resistance to CAR-T cell therapy, and explore their underlying mechanisms and effective treatment strategies in order to facilitate the application and management of CAR-T cell therapy.

OVERVIEW OF CAR-T CELL THERAPY

To manufacture CAR-T cells, T cells are collected from peripheral blood of patients or donors and then genetically engineered *in vitro* to express chimeric antigen receptor (CAR). Thus, CAR-T cells recognize specific surface antigens on tumor cells without antigen processing and presentation, which indicates that antigen recognition by CAR-T cells is independently of major histocompatibility complex (MHC) restriction. After genetic modifications, they undergoextensive expansion *in vitro*. Then the patients receive lymphodepleting chemotherapy to make room for these adoptive CAR-T cells, and subsequently these genetically engineered CAR-T cells are re-infused into the patients. These CAR-T cells specifically recognize target antigens and rapidly proliferate to exert anti-tumor effects *in vivo*.

The CAR structure consists of an extracellular antigen-recognition domain, a transmembrane domain, and an intracellular signaling domain. The extracellular domain, a single-chain variable fragment (scFv), is able to specifically recognize tumor surface antigens. Typically, tumor antigens are categorized into tumor-associated antigens (TAAs) and tumor specific antigens (TSAs), and most of they are TAAs. Once TAAs are identified by scFv, CAR-T cells are activated and transmit activation signals to the intracellular domain. The first-generation CAR construct contains an antigen-recognition domain scFv and an intracellular CD3ζ activation domain. Due to the absence of costimulatory signals, they exhibit the limited proliferative capacity and anti-tumor effects. The second-generation CAR construct adds a costimulatory domain, such as CD28, 4-1BB, OX40, or ICOS, which enables themselves to possess the better proliferative capacity and release more cytokines. Currently, these commercial CAR-T cell products both utilize the second-generation CAR construct. The third-generation CAR construct encompasses two distinct costimulatory molecules, such as CD28 and 4-1BB. The fourth-generation CAR construct, also named TRUCK or armored CAR, is additionally modified to secrete cytokines or express suicide genes, such as IL-7, IL-12, IL-15, IL-21, and iCaspase-9

TABLE 1 | Landmark clinical trials of FDA-approved CAR-T cell products.

CAR-T products	Target	Company	Year	Clinical trial	Indications	Response	Toxicities (Grade 3/4)	Reference
tisagenlecleucel	CD19	Novartis	2017	ELIANA	R/R B-ALL	ORR 81%, CR 60%	CRS (46%), CRES (13%) cytopenia (61%)	Maude SL et al. (1)
			2018	JULIET	R/R DLBCL	ORR 52%, CR 40%, PR 12%	CRS (22%), CRES (12%) cytopenia (32%) infections (20%)	Schuster SJ et al. (2)
			2021	ELARA	R/R FL	ORR 86%, CR 69%	CRS (49%), CRES (37%) infections (5%)	Fowler NH et al. (3)
axicabtagene ciloleucel	CD19	Gilead	2017	ZUMA-1	R/R DLBCL, transformed FL, PMBCL, and HGBCL	ORR 82%, CR 54%	CRS (13%), CRES (28%), cytopenia (78%)	Locke FL et al. (4)
brexucabtagene autoleucel	CD19	Gilead	2020	ZUMA-2	R/R MCL	ORR 85%, CR 59%,	CRS (15%), CRES (31%), cytopenia (94%), infections (32%)	Wang M et al. (5)
lisocabtagene maraleucel	CD19	Bristol Myers Squibb	2021	TRANSCEND	R/R DLBCL, HGBCL, PMBCL, and FL grade 3B	ORR 73%, CR 53%	CRS (2%), CRES (10%), cytopenia (60%)	Abramson JS et al. (6)
idecabtagene vicleucel	BCMA	Bristol Myers Squibb and Bluebird Bio	2021	KarMMa	R/R MM	ORR 73%, CR 33%	CRS (5%), CRES (3%), cytopenia (89%)	Berdeja JG et al. (7)
ciltacabtagene autoleucel	BCMA	Legend and Janssen	2022	CARTITUDE-1	R/R MM	ORR97%, CR 67%	CRS (4%), CRES (9%), cytopenia (95%)	Munshi NC et al. (8)

(10, 11) (**Figure 1**). The fourth-generation CAR-T cells may be more effective in eliminating tumor cells by activating the endogenous immune responses. However, the characteristics of fourth-generation CAR-T cells are largely unknown. In addition, the 2020 American Society of Hematology (ASH) annual meeting announced two studies about FasT CAR-T cells, including CD19-CD22 FasT dual-targeting CAR-T cells (GC022F) in B-ALL patients and BCMA-CD19 FasT dual-targeting CAR-T cells (GC012F) in R/R MM patients (12, 13). These FasT CAR-T products were manufactured in 24 to 36 hours and showed superior efficacy in preliminary studies, indicating that they might be more suitable for rapidly progressive B cell malignancies.

At present, all commercial CAR-T cell products are manufactured using autologous T lymphocytes, but high manufacturing costs, relatively longer manufacturing cycle, and decreased number and function of lymphocytes after multiline chemotherapies have restricted their further application. However, it seems that universal CAR-T (UCAR-T) cells which are derived from healthy donors are able to overcome these limitations. The large-scale production of UCAR-T cells makes them "off-the-shelf" products, which could reduce manufacturing costs and increase their accessibility. Unfortunately, these allogeneic UCAR-T cells could induce graft versus host disease (GVHD) (14). Furthermore, the host immune system is able to reject these donor-derived UCAR-T cells and impairs their persistence. In addition, due to the excellent natural killing functions of NK cells and their abundant sources, such as NK92 cell line, cord blood, peripheral blood, and induced pluripotent stem cells, as well as no induction of GVHD, CAR-NK cells are currently being explored (15).

ATTRACTIVE TARGETS FOR CAR-T CELL THERAPY IN HEMATOLOGICAL MALIGNANCIES

Currently, CD19 and BCMA are the most common targets in CAR-T cell therapy. Although anti-CD19 CAR-T cell therapy and anti-BCMA CAR-T cell therapy have achieved outstanding outcomes in B cell malignancies, relapse after CAR-T cell therapy is frequently observed. In addition, due to the antigenic heterogeneity of acute myeloid leukemia (AML) as well as the lack of CD19 expression in Hodgkin lymphoma (HL) and T cell malignancies, a number of potential targets are currently being investigated (**Figure 2**).

Targets for CAR-T Cell Therapy in B Cell Lymphoblastic Leukemia/Lymphoma

The CD19 is one of the most important target antigens in B cell malignancies, including B-ALL and NHL. In recent years, anti-CD19 CAR-T cell therapy has achieved rapid and durable responses in patients with R/R B-ALL and NHL (1–6, 16), and has dramatically altered the therapeutic landscape of B cell malignancies. Until now, four anti-CD19 CAR-T cell products have been approved by FDA for the treatment of R/R B-ALL and NHL (17). Despite the outstanding clinical results of anti-CD19 CAR-T therapy, CD19 antigen loss is frequently observed (18). Thus, the alternative targets for CAR-T cell therapy in R/R B-ALL and NHL have been explored.

CD20 is overexpressed in over 90% of B cell lymphomas and identified as an attractive target for CD20 positive B cell lymphomas, and the anti-CD20 monoclonal antibody rituximab has showed the excellent effect on NHL over the past few years. In an early clinical trial, the overall response rate (ORR) of anti-CD20

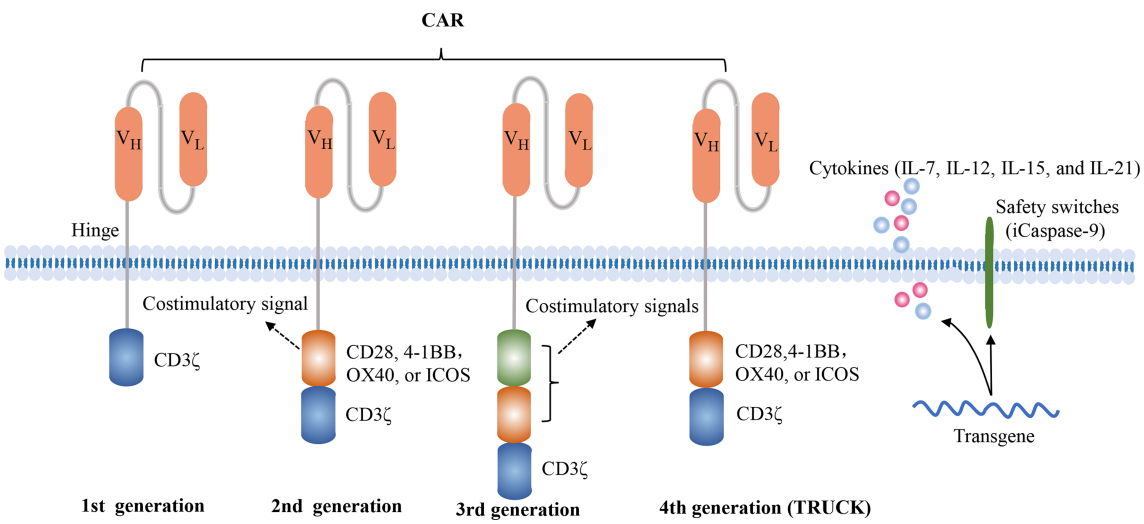

FIGURE 1 | Four generations of CAR constructs. The first-generation of CAR consists of the antigen recognition domain scFv and an intracellular T cell activation domain CD3ζ. The second-generation CAR adds a costimulatory molecule, such as CD28, 4-1BB, OX40 or ICOS, which enables the T cells to obtain a superior proliferative capacity and secrete large amounts of cytokines. The third-generation CAR contains two distinct costimulatory domains, such as CD28 and 4-1BB. The fourth-generation of CAR, also known as TRUCK or armored CAR, is additionally equipped with safety switches or engineered to secrete cytokines in order to regulate the persistence or the function of CAR-T cells, such as iCaspase-9, IL-7, IL-15, IL-21.

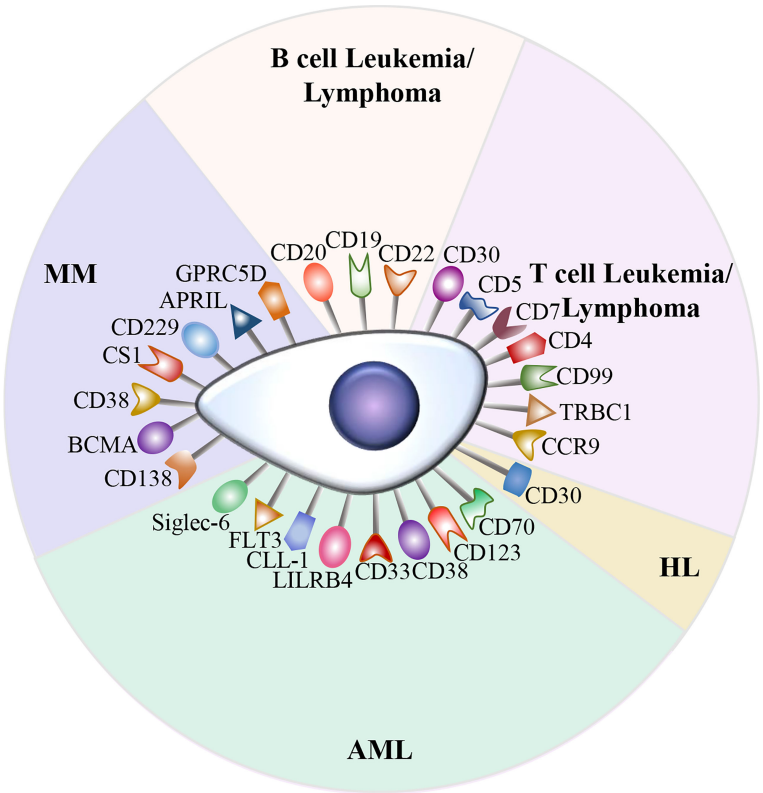

FIGURE 2 | Potential therapeutic targets in hematological malignancies. A variety of attractive targets for CAR-T cell therapy in hematological malignancies, including T and B cell leukemia/lymphoma, HL, AML, and MM.

CAR-T cell therapy in DLBCL patients was 86% (19). In subsequent phase 1 and 2 trials, the anti-CD20 CAR-T cells were administrated to 17 R/R NHL patients, and 54.5% of patients achieved complete remissions (CRs) and 12 patients remained in remission with a median follow-up of 20 months (20). To prevent antigen escape, the combination of anti-CD19 and anti-CD20 CAR-T cells for the treatment of R/R DLBCL was investigated, and this combinational therapy was demonstrated to be safe and feasible (21). CD22 is highly expressed on most B cell malignancies, including B-ALL and DLBCL (22, 23). In particular, it is restrictively expressed on normal B cells and not expressed on hematopoietic stem cells, so it is an ideal target for CAR-T cell therapy in R/R B-ALL and DLBCL. In several clinical trials, the anti-CD22 CAR-T cell therapy has shown excellent efficacy in R/R B-ALL and R/R DLBCL patients who have failed in previous anti-CD19 CAR-T cell therapy (24–26). In addition, the humanized anti-CD22 CAR- T cells exhibits potent activity against leukemia cells with low CD22 expression (27).

Targets for CAR-T Cell Therapy in T Cell Lymphoblastic Leukemia/Lymphoma

Patients with R/R T-cell acute lymphoblastic leukemia (T-ALL) and T cell lymphomas often have poor prognosis. Compared with the outstanding clinical outcomes of anti-CD19 CAR-T cell therapy in B cell malignancies, the efficacy and safety CAR-T cell therapy in T cell malignancies are largely unknown and under investigation. CD7 is highly expressed in 95% of T-ALL patients and represents a desirable target for the treatment of T-ALL (28). In an open-label and single-arm clinical trial, 2 R/R T-ALL patients were treated with allogeneic anti-CD7 CAR-T cell therapy. One patient remained in remission for over 1 year, while the other relapsed 48 days after CAR-T cell infusion (28). In another phase I clinical trial, 20 R/R T-ALL patients received donor-derived anti-CD7 CAR-T cell infusion, and 90% of participants achieved CRs (29). In addition, a case study reported that an 11-year-old T-ALL patient who didn't respond to induction failure was treated with autologous anti-CD7 CAR-T cell therapy, and he achieved remission on day 17 and subsequently underwent HSCT (30). CD5 is expressed in approximately 85% of T cell malignancies, such as T-cell lymphoblastic lymphoma (T-LBL) and peripheral T-cell lymphoma (PTCL). A recent study has demonstrated that anti-CD5 CAR-T cells effectively eliminated malignant T cells (31). In a phase I clinical trial, a refractory T-LBL patient with central nervous system (CNS) infiltration received the anti-CD5 CAR-T cell therapy and achieved CR within 4 weeks (32). Additionally, anti-CD4 CAR-T cells showed superior activity against T cell malignancies in preclinical studies (33). However, all three targets CD4, CD5 and CD7 are expressed on normal T cells, so targeting them may result in the depletion of normal T cells

and the fratricide of CAR-T cells (34). Malignant T cells express T cell receptor β-chain constant domains 1 (TRBC1), so anti-TRBC1 CAR-T cells are able to selectively eliminate TRBC1 positive malignant T cells. Importantly, it could retain a majority of normal T cells *in vivo* (35). CD99 is highly expressed in newly diagnosed T-ALL patients, and it represents a novel target for T-ALL (36). Furthermore, chemokine receptor CCR9 is expressed in over 70% of T-ALL patients, and only on less than 5% of normal T cells. In addition, it is correlated with multidrug resistance and poor prognosis. Thus, it represents an ideal target for CCR9 positive T-ALL. In preclinical studies, anti-CCR9 CAR-T cells exhibited potent anti-leukemic activity and were resistant to fratricide (37).

In addition, CD30 is highly expressed in anaplastic large-cell lymphoma (ALCL) and is variably expressed in PTCL subtypes. In particular, CD30 is restrictively expressed on normal T cells. Thus, CD30 is an ideal target for these lymphoma subtypes, and the anti-CD30 antibody-drug conjugate brentuximab vedotin (BV) has shown a high response rate in newly diagnosed PTCL patients (38, 39). Given the encouraging clinical efficacy of BV, the anti-CD30 CAR-T cells were developed and exhibited remarkable cytotoxicity against CD30 positive lymphomas in preclinical studies (40, 41).

Targets for CAR-T Cell Therapy in Hodgkin Lymphoma

Anti-CD19 CAR-T cell therapy has shown excellent results in R/R B cell NHL. However, HL lacks the expression of CD19. Interestingly, CD30 is universally expressed in classical HL. Currently, several clinical trials have been carried out to evaluate the safety and efficacy of anti-CD30 CAR-T cell therapy in R/R HL (42–45). In a clinical trial from China, 5 of 6 HL patients achieved CRs after the infusion of the third-generation anti-CD30 CAR-T cells, and the long-term remission lasted over 24 months in 3 patients (45). In another phase 1/2 clinical trial, 27 patients were treated with the anti-CD30 CAR-T cells and 67% of patients achieved CRs within 6 weeks, but 63% of patients experienced disease progression with a median follow-up of 9.5 months (46). In addition, the expression of CD30 in HL was down-regulated after anti-CD30 CAR-T cell therapy (47).

Targets for CAR-T Cell Therapy in Acute Myeloid Leukemia

AML is the most common acute adult leukemia. Unfortunately, due to antigenic heterogeneity, the CAR-T cell therapy in AML has not achieved the same success as ALL. Recently, CD123, CD33, CD38, CD70, C-type lectin-like molecule-1(CLL-1), leukocyte immunoglobulin-like receptor-B4 (LILRB4), FMS-like tyrosine kinase 3 (FLT3) and sialic acid-binding immunoglobulin-like lectin 6 (Siglec-6) have been explored. CD123 and CD33 are highly expressed on leukemic stem cells in over 80% of AML patients, but they are expressed on hematopoietic stem cells as well (48). Accordingly, targeting them could increase the risk of long-term myelosuppression (49). To decrease hematological toxicity, a rapidly switchable universal anti-CD123 CAR-T cells were prepared (50, 51). In a small study, 3 R/R AML patients were treated with this universal anti-CD123 CAR-T cells, and all of them achieved a clinical response with the rapid hematologic recovery after the withdrawal of switch-mediated co-stimulation (52). In another phase 1 trial, 3 R/R AML patients received autologous anti-CD33 CAR-T cell infusion. Unfortunately, all of them died from disease progression (53). CD38 is expressed on most AML blast cells, and anti-CD38 CAR-T cell therapy was demonstrated to be effective in relapsed AML after allogeneic HSCT (54). CLL-1, which has been identified as an myeloid cell surface marker, is overexpressed on leukemic stem cells (55). Importantly, it is absent on hematopoietic stem cells. The CLL-1-targeted CAR-T cells specifically eliminated CLL-1 positive leukemia in preclinical studies (56, 57). CD70 is expressed on AML blasts but not on normal myeloid cells, making it a promising target for the treatment of AML (58, 59). Currently, the safety and efficacy of anti-CD70 CAR-T cell therapy are under investigation. In addition, LILRB4 is highly expressed on monocytic AML cells, and it is an attractive target for monocytic AML (60). Siglec-6 is expressed in approximately 60% of AML patients and absent on normal hematopoietic stem and progenitor cells. In preclinical studies, Siglec-6 CAR T cells effectively eliminated AML blasts in an AML mouse xenotransplantation model (61). Thus, it could serve as a well-validated target for CAR-T cell therapy in AML. In addition, nucleophosmin 1 (NPM1) mutations have been observed in 30%-35% of AML patients, and they are considered to be initiating mutations in leukemic cells. In preclinical mouse models, CAR-T cells targeting a nucleophosmin neoepitope which is presented by HLA-A2 exhibited potent specific anti-leukemia activity (62).

FLT3 is a transmembrane tyrosine kinase expressed on malignant blasts in approximately 30% of AML patients. FLT3 mutations include point mutations and an internal tandem duplication (ITD), and FLT3-ITD is correlated with poor prognosis. In preclinical studies, the FLT3-targeted CAR-T cells successfully eliminated FLT3 positive AML cells (63, 64), and the FLT3 inhibitor crenolanib promoted their anti-tumor effects (64). Unfortunately, FLT3 is also expressed on normal hematopoietic stem and progenitor cells, so the FLT3-targeted CAR-T cells may affect normal hematopoiesis (64, 65).

Targets for CAR-T Cell Therapy in Multiple Myeloma

MM remains an incurable plasma cell malignancy. With the application of novel agents, such as proteasome inhibitors, immunomodulatory drugs, and anti-CD38 monoclonal antibodies, MM patients have significantly improved survival outcomes (66). However, almost all MM patients inevitably relapse. BCMA is highly selectively expressed on malignant plasma cells, so it represents one of the most promising therapeutic targets for MM. At present, anti-BCMA CAR-T cell therapy has been demonstrated to be effective in R/R MM and achieved unprecedented responses (7, 8, 67–69), and two anti-BCMA CAR-T cell products, idecabtagene vicleucel and

ciltacabtagene autoleucel, have been approved by the FDA for the treatment of R/R MM (7, 70). Furthermore, the anti-BCMA CAR-T cell therapy is effective in R/R MM patients with extramedullary disease (71–73). However, some MM patients still relapse after anti-BCMA CAR-T therapy, and BCMA expression is downregulated under therapeutic pressure. Therefore, new target antigens are required (74).

Currently, several potential target antigens have been investigated, such as CD38, CD138, CD229, SLAMF7, a proliferation-inducing ligand (APRIL), and G protein-coupled receptor, class C group 5 member D (GPRC5D). CD138 is highly expressed on MM cells and promote their survival and proliferation. In a preclinical study, anti-CD138 CAR-T cells effectively eliminated MM cells (75). In a small clinical trial, 5 patients received anti-CD138 CAR-T cell therapy, and 4 of them had a clinical response and remained stable for at least three months (76). CD38 is not only highly expressed on MM cells, but also expressed on hematopoietic cells and activated lymphocytes cells (77). Unfortunately, although the anti-CD38 CAR-T cells exhibited significant anti-tumor effects in mouse models, they impaired normal hematopoietic cells and lymphocytes (78). Clinically, CD38 is frequently combined with other targets, such as BCMA and CD138, to produce bispecific CAR-T cells, thereby reducing the risk of antigen escape (79, 80). CD229 is a surface antigen highly expressed on MM cells (81). The anti-CD229 CAR-T cells effectively eliminated MM cells in preclinical studies (82). SLAMF7, also known as CS1, is highly expressed in over 95% of MM patients. Similar to CD38, SLAMF7 is also expressed on normal lymphocytes, including activated T cells, NK cells, and B cells (83). Thus, SLAMF7 CAR-T cells could kill normal lymphocytes and increase the risk of CAR-T cell fratricide (84). Currently, the clinical trial of SLAMF7 CAR-T cells is ongoing (85). APRIL is a natural ligand which could directly bind to BCMA and transmembrane activator and CAML interactor (TACI). Thus, APRIL-targeted CAR-T cells recognize both BCMA and TACI expressed on MM cells, which may decrease the risk of antigen escape (86), and preserving its trimeric conformation could improve the anti-tumor activities (87). In addition, TGPRC5D is expressed on more than 50% of CD138 positive malignant plasma cells in bone marrow of MM patients, which also represents a potential target for the treatment of MM (88).

TOXICITIES RELATED TO CAR-T CELL THERAPY AND THEIR UNDERLYING MECHANISMS

Although CAR-T cell therapy has achieved great success in hematological malignancies, the adverse events related to CAR-T cell therapy remain to be a major challenge, such as CRS, CRES, B cell aplasia, cytopenia, and CRS-related coagulopathy. Without active and effective interventions, these complications might be life-threatening. In order to effectively manage these complications, it is important to explore their underlying mechanisms and recognize them in early stages.

Cytokine Release Syndrome

CRS is one of the most common toxicities of CAR-T cell therapy. The incidence of CRS depends on a variety of factors, including disease characteristics, CAR structure, tumor burden, and CAR-T cell doses (89). The clinical manifestations of CRS are diverse, but they are frequently characterized by fever, fatigue, myalgia, poor appetite, hypoxia, hypotension, and even organ dysfunction. If left untreated, it might rapidly progress into life-threatening conditions, such as hemodynamic instability and multiple organ dysfunction. However, the recent study revealed that the patients with ≥ grade 2 CRS had higher rates of remission and longer progression-free survival (PFS) compared with those with < grade 2 CRS, which indicates that appropriate CRS might facilitate the efficacy of CAR-T therapy (90). Because CRS are primarily mediated by IL-6, IL-6 receptor antagonist tocilizumab is mainly recommended to relieve the clinical symptoms of CRS. According to different CRS grading, different treatment regimens are adopted. The symptomatic treatment and the supportive treatment are indicated for grade 1 CRS. Tocilizumab and corticosteroids are recommended for grade 3 and 4 CRS as well as grade 2 CRS accompanied by severe symptoms. Moreover, IL-1 is another important cytokine involved in CRS and CRES, and IL-1 receptor antagonist anakinra has been demonstrated to ameliorate both CRS and CRES (91–95). Furthermore, GM-CSF deficiency or inhibition not only can alleviate CRS and CRES by inhibiting the local infiltration of myeloid cells and T cells, but also enhance the anti-tumor effects of CAR-T cells (96, 97). Besides, the severity of CRS is positively associated with the patient's tumor burden (89). To reduce tumor burden, traditional chemotherapy and radiotherapy could serve as the effective bridging strategies before CAR-T cell infusion.

The detailed mechanisms of CRS remain incompletely understood. After recognizing target antigens, CAR-T cells are rapidly activated and secrete a large amount of granzyme, perforin, IFN-γ, and TNF-α. Perforin forms pores on tumor cell membrane and allows granzyme B to enter tumor cells. Granzyme B activates GSDME which is widely expressed on CD19 positive malignant B cells, resulting in tumor cell pyroptosis and the release of danger associated molecular patterns (DAMPs), such as high-mobility group box 1 (HMGB1) (98–101). Then DAMPs recruit and activate endogenous innate immune cells, such as macrophages and dendritic cells, thereby amplifying inflammatory responses and increasing the release of cytokines, including IL-1β and IL-6. Currently, it has been demonstrated that macrophages and monocytes rather than CAR-T cells are the major sources of these cytokines and contribute to CRS (91, 92, 102, 103). In addition, the CD40 and CD40 ligand (CD40L) interactions between CAR-T cells and host antigen-presenting cells (APCs) as well as tumor cells also play an important role in immune activation and the release of cytokines (104–106). CD40 is expressed on multiple APCs, including B cells, macrophages, dendritic cells (DCs), and monocytes, and highly expressed in a variety of hematological malignancies, such as NHL, AML, MM (105–108). CD40L is highly expressed on activated T cells,

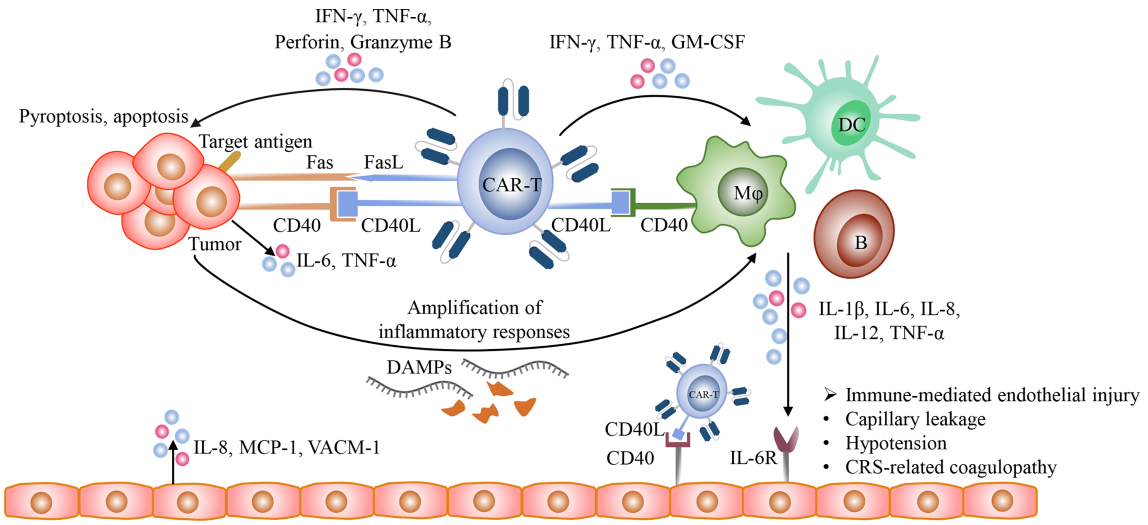

FIGURE 3 | The mechanisms of CRS. After the recognition of target antigens, CAR T-cells rapidly proliferate and release multiple cytotoxic molecules, such as granzyme, perforin, IFN-γ, and TNF-α, and upregulate the expression of CD40L and Fas ligand (FasL), and eventually induce pyroptosis and apoptosis of tumor cells. Besides, the CD40/CD40L interactions between tumor cells and CAR T-cells promote Fas-mediated apoptosis. Then the lysed tumor cells release large amounts of DAMPs, such as HMGB1, which could activate innate immune cells, including macrophages and dendritic cells, further amplifying inflammatory responses. In addition, the CD40/CD40L interactions participate in the activation of various immune cells, including T cells, B cells, macrophages, dendritic cells, and conditional innate immune cells such as endothelial cells. The activated CAR-T cells with the increased expression of CD40L could activate macrophages and endothelial cells and promote their production of pro-inflammatory cytokines in a CD40-dependent manner. The cytokines released from activated immune cells could bind to their receptors on endothelial cells and then mediate endothelial dysfunction, resulting in capillary leakage and the release of procoagulant factors.

including CAR-T cells (104, 109). The contact-dependent CD40/CD40L interactions enhance the antigen presentation of APCs and CD40 positive malignancies and promote their secretion of cytokines, such as IL-1β, IL-6, and TNF-α (110, 111) (**Figure 3**). In addition, because endothelial cells act as conditional innate immune cells and express CD40, the CD40/CD40L interactions also participate in the activation of endothelial cells.

Since lymphoma lesions are mostly localized, CRS in lymphoma exhibits some distinct manifestations, such as local CRS, which is characterized by local redness, swelling, and heat. Then, CAR-T cells persistently expand and overflow into the circulation, and experience redistribution as well as mediating tissue damage. Finally, inflammation subsides and the impaired tissues are gradually repaired (112). Thus, the early management of local CRS is helpful to reduce the occurrence of subsequent systemic CRS.

CAR-T-Cell-Related Encephalopathy Syndrome

CRES, also known as CAR-T cell-related neurotoxicity, is another common toxicity during CAR-T cell therapy. It usually occurs simultaneously with CRS or later than CRS. The manifestations of CRES include headache, dizziness, delirium, seizures and cerebral edema. Due to the lack of suitable animal models, the underlying pathological mechanisms of CRES are not fully understood. Severe CRS, high tumor burden, and excessive CAR-T cell expansion might be correlated with the increased risk of CRES. Currently, immune-mediated endothelial activation is a well-established mechanism involved in the occurrence of CRES (113–115). Upon the recognition of target antigens, CAR-T cells rapidly expand and secrete cytokines to activate endogenous immune cells, such as macrophages, which in turn release large amounts of cytokines and activate cerebral microvascular endothelial cells, eventually resulting in the disruption of tight junctions and the increased blood-brain barrier (BBB) permeability (114, 115). Then, the high concentrations of serum cytokines enter the BBB by passive diffusion, and the elevated levels of pro-inflammatory cytokines in CSF seem to be associated with CRES, including IL-1β, IL6, IL-8, IFN-γ, GM-CSF, MCP-1, and granzyme B (116–118). In addition, it has been demonstrated that T cells and macrophages, including CAR-T cells, could infiltrate into the CNS due to the disruption of the BBB (118–121). These infiltrated immune cells and cytokines could induce the activation of microglia which are brain-resident macrophages, further amplifying local inflammatory responses and eventually resulting in neurotoxicity (115, 119, 122–124). Thus, immune-mediated endothelial injury is a trigger factor for CRES (113–115, 125). As tocilizumab couldn't cross the BBB, it exhibits the limited efficacy in the management of CRES. Given the increased CNS penetration of corticosteroids, it is recommended for the treatment of CRES, and it could not affect the proliferation and the anti-tumor effects of CAR-T cells (126).

B/T Cell Aplasia and Infections

CD19 and CD20 are expressed on multiple differentiated B-lineage cells as well as malignant B cells, and represent attractive

targets for CAR-T cell therapy in B-ALL and lymphoma. BCMA, a plasma cell-selective protein, is highly expressed on MM cells as well as mature B cells and normal plasma cells. Thus, CD19-targeted, CD20-targeted and BCMA-targeted CAR T cells exhibit superior anti-tumor activity in B cell malignancies (1, 3, 67, 127), but they attack normal B cells as well, which could result in impaired humoral immunity, such as B cell aplasia and hypogammaglobulinemia (128–131). Moreover, lymphodepleting chemotherapy prior to CAR-T cell infusion could also impair host immunity. Due to impaired host immunity, these individuals are more susceptible to infections (132). It has been demonstrated that most of infection events occur during the first 30 days of CAR-T cell infusion, and the bacterial infection predominates, mainly including bloodstream infection and respiratory infection (133). In a phase 1/2 study, 31% of the patients who received the anti-CD19 CAR-T therapy experienced infections between day 31 and day 180 (134). Thus, the long term follow-up and the detection of gamma globulin levels might be helpful. To restore humoral immunity, immunoglobulin supplementation is essential for these immune-compromised individuals. In addition, the high-dose CAR-T cell infusion seems to be associated with the infections after CAR-T cell infusion (134, 135). Thus, CAR-T cells can be administrated in a dose-escalation regimen. Furthermore, CAR-T cell therapy increases the risk of HBV reactivation in patients with resolved HBV infections due to persistent B-cell aplasia, so antiviral prophylaxis and regular monitoring of the virus are recommended (128, 136, 137). Unfortunately, with the application of CAR-T cell therapy in T cell malignancies, T cell aplasia might be observed because a majority of target antigens are co-expressed on normal T cells (34).

However, it is difficult to differentiate between CRS and infections due to the similar clinical manifestations, such as pyrexia and the elevated levels of pro-inflammatory cytokines and C-reactive protein (CRP). Moreover, CRS is likely to occur simultaneously with infections. In order to avoid the life-threatening infections during CAR-T cell therapy, the early recognition and management of infections is important. Nevertheless, it is usually not timely to identify the infection by blood culture (133). Thus, the detection of special biomarkers or the establishment of a prediction model for infection is critical. IL-6 is one of the key cytokines involved in the infection-induced cytokine storm and CAR-T cell therapy-induced CRS. Typically, the elevation of serum IL-6 associated with CRS occurs within 3 weeks after CAR-T cell infusion, so the "double peaks of IL-6" is identified as one of the characteristics of life-threatening infections. Compared with blood culture, it seems that employing the "double peaks of IL-6" pattern to predict the life-threatening infection is faster (135). When infection is suspected, empiric anti-infective treatment should be initiated immediately once blood and sputum samples are collected for the detection of pathogenic microorganisms, especially in neutropenic patients. In addition, Herpesvirus infections have been occasionally observed in several clinical trials (138–140). To prevent herpesvirus infections, it's recommended that acyclovir 400 mg should be prophylactically administrated twice daily from lymphodepletion chemotherapy to at least 6 months post CAR-T cell infusion (141).

Cytopenia

Cytopenia is frequently observed during CAR-T cell therapy and lasts for several days to months, including anemia, thrombocytopenia, and leukopenia, and the incidence of cytopenia range from 30% to 100% in clinical trials (2, 29, 72, 79, 142, 143). It has been demonstrated that cytopenia is associated with severe CRS (144–146). Under inflammatory conditions, CD40 is significantly up-regulated on granulocytic progenitor/precursor cells which also express low levels of CD40L, and the CD40/CD40L interactions between granulocytic progenitor/precursor cells significantly promote their own apoptosis (147). In addition, the pro-inflammatory cytokines, such as IL-1, TNF-α, and HMGB1, could suppress erythropoietin production (148, 149), and the activated macrophages could destroy erythrocytes (150). The limited hematopoietic capacity mediated by prior chemotherapy and HSCT might be involved in cytopenia as well (151–153). Furthermore, some target antigens are co-expressed on normal hematopoietic stem or progenitor cells, so CAR-T cells could directly mediate the destruction of hematopoietic cells (48, 49). Clinically, red blood cell and platelet transfusions and hematopoietic growth factors, such as granulocyte colony-stimulating factor (G-CSF) and thrombopoietin (TPO), as well as TPO receptor agonists and sirolimus, are able to ameliorate cytopenia (154, 155).

CRS-related Coagulopathy

As a newly identified toxicity, coagulopathy is frequently observed within 1 month after CAR-T cell infusion (156, 157). Its severity shows a positive correlation with CRS grade as well as the levels of IL-6 (9), so it's also known as CRS-related coagulopathy. The recent studies have reported that the incidence of coagulopathy during CAR-T therapy is approximately 50% (9, 156). There are multiple abnormal coagulation parameters in patients with CRS-related coagulopathy, mainly characterized by the elevated levels of D-dimer, the increased fibrinogen degradation products, and the decreased levels of fibrinogen as well as the prolonged prothrombin time. The progress of CRS-related coagulopathy can be divided into three stages, including hypercoagulable stage, consumptive hypo-coagulable stage, and hyperfibrinolysis stage (9). At hypercoagulable stage, the patients mainly present with excessive micro-thrombosis, which can be treated with anticoagulant drugs, such as low-molecular-weight heparin. At consumptive hypo-coagulable stage, the individuals exhibit bleeding, accompanied by the decreased fibrinogen levels and the prolonged APTT and PT, so replacement treatment is required. At hyperfibrinolysis stage, the fibrinogen level is substantially decreased and the level of D-Dimer is significantly up-regulated. Once the fibrinogen level in plasma is lower than 1.5 g/L, the fibrinogen concentrate or cryoprecipitate replacement should be administrated (**Table 2**

TABLE 2 | Toxicities related to CAR-T cell therapy and effective solutions.

Toxicities	Manifestations	Solutions	Reference
CRS	grade 1 CRS: fever, fatigue, myalgia, nausea, and/or malaise	supportive care	(117)
	grade 2 or higher CRS: fever, hypoxia, hypotension, and organ dysfunction	tocilizumab, corticosteroids, and intensive care	
CRES	headache, dizziness, delirium, seizures, cerebral edema, and coma	tocilizumab, corticosteroids, anakinra, and intensive care	(93, 95, 117)
B cell aplasia	hypogammaglobulinemia	immunoglobulin infusion	(128, 136, 137, 141)
	HBV reactivation	antiviral prophylaxis	
	herpesvirus infections	acyclovir	
Cytopenia	anemia	red blood cell transfusions	(154)
	leukopenia	granulocyte colony-stimulating factor, protective isolation	
	thrombocytopenia	platelet transfusions, thrombopoietin, romiplostim	
CRS-related coagulopathy	hypercoagulable stage: extensive micro-thrombosis, normal/ shortened APTT and PT	anticoagulant treatment: low-molecular weight heparin	(9)
	consumptive hypo-coagulable stage: hemorrhage, decreased fibrinogen levels, and prolonged APTT and PT	anticoagulant treatment combined with replacement treatment: low-molecular weight heparin, fresh-frozen plasma, cryoprecipitate, fibrinogen concentrate	
	Hyperfibrinolysis stage: hemorrhage, hypofibrinogenemia, significantly increased levels of D-Dimer and FDP, and prolonged APTT and PT	replacement treatment and antifibrinolytic treatment: cryoprecipitate, fibrinogen concentrate	

toxicities related to CAR-T cell therapy) Given that cytokine storm plays an essential role in CRS-related coagulopathy, the early and effective management of CRS might be helpful to reduce the incidence of coagulopathy. Without timely and effective intervention, a part of patients with coagulopathy may further develop disseminated intravascular coagulation (DIC), accompanied by the poor prognosis (9, 156, 158).

The mechanisms of CRS-related coagulopathy remain unclear. The activated platelets, monocytes, and endothelial cells as well as the CD40/CD40L interactions between them may collectively contribute to CRS-related coagulopathy. The CD40L expressed on activated CAR-T cells induces platelet activation in a CD40-independent manner in blood circulation (159). The activated platelets are prone to the form monocyte-platelet aggregates (MPAs) and are highly express CD40L, which could induce the expression of tissue factor (TF) in monocytes and endothelial cells through the direct interaction with CD40 (159–162). TF could activate the extrinsic coagulation cascade, and monocytes are the major sources of TF. Besides the CD40/CD40L interactions, there are also a variety of inducers could stimulate monocytes to upregulate the expression of TF, such as C-reactive protein (CRP), IL-1β and TNF-α (163–165). In addition, the CD40/CD40L interactions between them promote the excessive production of cytokines as well, such as IL-1β, TNF-α, and IL-6. High levels of cytokines further mediate endothelial injury and result in the release of TF, the procoagulant particles Weibel-Palade bodies (WPBs), and von Willebrand factor (VWF) as well as the exposure of the collagen fibers. The exposed collagen fibers trigger intrinsic coagulation pathway. In addition, cytokines IL-6, TNF-α, and IFN-γcan directly inhibit the production and activity of ADAMTS13, which contributes to the elevated levels VWF in blood and promote platelet adhesion and aggregation (166, 167). Moreover, serious liver damage induced by CAR-T cell therapy influences the production of coagulation factors, and some patients with hematological malignancies had be in a hypercoagulable state prior to CAR-T cell therapy (168) (**Figure 4**).

RESISTANCE TO CAR-T CELL THERAPY AND POTENTIAL EFFECTIVE STRATEGIES

However, with the widespread application of CAR-T cell therapy in R/R B-cell malignancies, a considerable proportion of patients relapse after CAR-T cell therapy. There are multiple factors which contribute to relapse after CAR-T cell therapy, including antigen escape, the limited CAR-T cell persistence, and immunosuppressive tumor microenvironment. There are some therapeutic strategies to overcoming the resistance to CAR-T cell therapy, including the application of bispecific or armored CAR-T cells, optimizing the CAR structure, combining CAR-T cell therapy with other approaches, such as small-molecule drugs, localized radiotherapy, and oncolytic viruses.

Overcoming Antigen Escape

Although CAR-T cell therapy has made impressive achievements in R/R B cell malignancies, a majority of patients still relapse (143). One of the primary mechanisms of relapse after CAR-T cell therapy is antigen loss (169, 170). The antigen mutations under therapeutic pressure of CAR-T cell therapy are the most common mechanisms of antigen loss, including splice variants, lineage switching, and biallelic mutations (171–173). In addition to antigen mutations, the lower antigen density on the surface of induced by the endocytosis of CAR-T cells could promote tumor immune escape as well (174). Last but not least, a study has reported that the anti-CD19 CAR was incidentally transferred into a leukemic B cell during CAR-T cell manufacturing and then bound to the CD19 epitope on leukemic blasts, so "epitope-

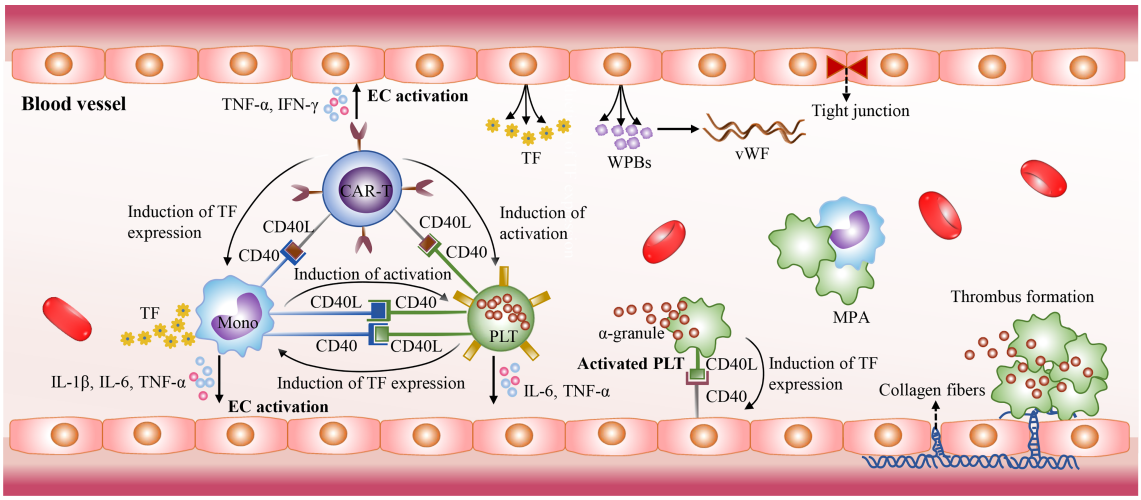

FIGURE 4 | The mechanisms of CRS-related coagulopathy. The CD40/CD40L interactions also play an essential role in CRS-related coagulopathy. The activated CAR-T cells with high CD40L expression mediate platelet activation in a CD40-independent manner. Then the activated platelets express high levels of CD40L. It could stimulate endothelial cell activation, and induce the expression of TF in monocytes and endothelial cells through direct interaction with CD40. Then TF triggers the extrinsic coagulation cascade. In addition, the CD40/CD40L interactions stimulate the excessive release of cytokines, such as IL-1β, TNF-α, and IL-6. High levels of cytokines further mediate endothelial injury and promote the release of TF and Weibel-Palade bodies (WPBs). The WPBs contains von Willebrand factor (VWF) which plays an essential role in platelet adhesion and aggregation. Due to endothelial injury, collagen fibers are exposed and activate intrinsic coagulation pathway.

masking" prevented leukemic blasts from being recognized by anti-CD19 CAR-T cells (175).

Targeting distinct antigens is one of the most effective approaches to overcome antigen-negative relapse. The dual-targeting CAR-T cells which could recognize two distinct target antigens have been demonstrated to reduce the risk of antigen-negative relapse, such as the bispecific CAR-T cells in B cell lymphoma/leukemia and the APRIL-based CAR-T cells targeting both BCMA and TACI in MM (86, 176–180). The multi-targeted CAR-T cells have also been explored. It has been demonstrated that the tri-specific CD19-CD20-CD22-targeting CAR-T cells could rapidly eliminate B cell lymphoma in a preclinical study (181), and BAFF ligand-based CAR-T cells simultaneously target three receptors, including BAFF-R, BCMA, and TACI (182). In addition to simultaneously targeting different antigens, increasing immunogenicity of target cells might be a feasible strategy. For example, small molecule γ-secretase inhibitors could reduce the shedding of BCMA and promote the recognition of MM cells by CAR-T cells (183).

The γδ T cells (γδ T) are a small population of peripheral blood cytotoxic T cells, which express both T cell receptors (TCRs) and natural killer receptors (NKRs), and involved in anti-tumor immunity. In particular, NKRs expressed on γδ T cells play a major role in tumor cell recognition in hematological malignancies (184–186). Thus, besides antigen recognition mediated by the scFvs, γδ CAR-T cells could also recognize antigen-negative leukemia cells *via* NKRs in an MHC-independent manner (187). Moreover, γδ T cells did not induce graft-versus-host disease (GVHD) in allogeneic and HLA-haploidentical hematopoietic stem cell transplantation, which indicates that γδ T cells don't trigger alloreactivity (188, 189). Thus, they are more suitable for the development of universal CAR-T cells.

Regulating the Persistence of CAR-T Cells

The short-term persistence of CAR-T cells limits their anti-tumor efficacy and may result in antigen-positive relapse. There are multiple strategies to improve the persistence of CAR-T cells, such as optimizing CAR-T cell construct, utilizing memory T cells, and rationally designing the ratio of CD4 to CD8 CAR-T cells (190). To date, CD28 and 4-1BB are the most common co-stimulatory molecules in CAR-T cell products. However, it has been demonstrated that 4-1BB co-stimulation could ameliorate CAR-T cell exhaustion compared with CD28 co-stimulation (191, 192). Remarkably, combining CD28 and 4-1BB could simultaneously augment the anti-tumor effects and increase the persistence of CAR-T cells (193–196). In addition, the CAR-T cell structure can be optimized by the fully humanized CARs. The humanized CAR-T cells could avoid the rejection by the host immune system, and they were still effective in R/R patients who have failed in prior murine CAR-T cell therapy (68, 197). $CD4^+$ T cells exhibit developmental plasticity and can directly kill tumor cells (198), but they eliminate tumor cells at the slower rate and release the lower levels of Granzyme B than $CD8^+$ T cells. Thus, $CD4^+$ CAR-T cells exhibit a superior persistence (199–202), and the ratio of CD4/CD8 CAR-T cells may influence the therapeutic efficacy. Currently, CD4/CD8 CAR-T cells at a 1:1 ratio have been demonstrated to exert excellent anti-tumor effects (200).

CAR-T cells have been considered as "living drugs", but they lack the precise regulation. Given that the excessive expansion of

CAR-T cells could lead to the life-threatening CRS, so it is necessary to regulate the expansion and persistence of CAR-T cells to mitigate unexpected or severe toxicities through the addition of the safety switches. The well-known inducible caspase 9 (iCasp9) suicide gene and the small molecule control systems have been explored (203, 204). In small molecule control systems, the FDA-approved small molecule drugs act as the key switches to specifically regulate antigen recognition or deplete CAR-T cells, such as lenalidomide, methotrexate, alemtuzumab, rituximab, and cetuximab (31, 63, 205–208), as well as orthogonal IL-2 (205, 206). The above-mentioned monoclonal antibodies mediate the depletion of CAR-T cells through antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) (63, 209). Nevertheless, the depletion of CAR-T cells is slow in this strategy, which may be not suitable for patients with severe toxicities.

Improving Anti-Tumor Efficacy of CAR-T Cell Therapy Through Combinatorial Strategies

The low activity of CAR-T cells could also limit the efficacy of CAR-T cell therapy. Multiple immune-stimulatory molecules, including certain cytokines or co-stimulatory molecules, have been demonstrated to play an important role in regulating the development and function of T cells, such as IL-7, IL-12, IL-15, IL-18, IL-21, and CD40L (109, 210–214). They could promote the robust expansion of the CAR-T cells and increase memory-phenotype CAR-T cells as well as improving their persistence (215–218). In addition to adding these exogenous cytokines, the genetic modifications to constitutively express these immune-stimulatory molecules or their receptors could also improve the function of CAR-T cells (10, 213, 219, 220). These fourth-generation CAR-T cells have showed the improved anti-tumor activities by stimulating the activation of themselves or endogenous immune cells in a autocrine or paracrine manner (11, 220–222).

To improve the efficacy of CAR-T cell therapy, combining CAR-T cell therapy with small-molecule drugs appears to be promising and may produce synergistic effects. The selective inhibitors of nuclear export selinexor, lenalidomide and carfilzomib have been approved for the treatment of MM (223, 224). Intriguingly, combining them with CAR-T cells also achieved encouraging outcomes, with the improved cytotoxic activity and cytokine production of CAR-T cells (225, 226). In particular, the recent clinical studies showed that the R/R MM patients resistant to anti-BCMA CAR-T cell therapy could also benefit from selinexor-based regimens and carfilzomib-based regimens (227, 228), and a study reported that anti-BCMA CAR-T cell therapy combined with lenalidomide was effective in the R/R MM patients who had previously relapsed after anti-BCMA CAR-T cell therapy (229). Ibrutinib, a well-known Bruton's tyrosine kinase inhibitor, has been approved for the treatment of CLL and MCL. Importantly, Ibrutinib not only improved CAR T cell-anti-tumor efficacy in both preclinical and clinical studies, but also reduced the risk of severe CRS (230–233). In addition, it has been demonstrated that demethylating agents azacitidine and decitabine could enhance cytotoxic effect of CAR-T cells as well (234–236). Besides, CAR-T cell therapy in combination with inhibitors of antiapoptotic proteins could overcome the resistance induced by antiapoptotic proteins (237). However, CAR-T cell therapy combined with small-molecule drugs is still in its infancy, and numerous combinational strategies are being explored.

Additionally, the localized radiotherapy could serve as a well-tolerated and effective bridging strategy between the leukapheresis and CAR-T cell infusion for lymphoma or MM patients with bulky disease (238–240). On the one hand, this combinational therapy could prevent disease progression and reduce tumor burden; On the other hand, it may sensitize the CAR-T cells through the abscopal effect, which may be associated with the upregulation of intratumoral chemokines and cytokines, the release of neo-antigens, and the activation of endogenous immune cells (241–245). Nonetheless, the optimal irradiation dose and fractionation remain to be identified.

Overcoming Immunosuppressive Microenvironment

While directly targeting tumor cells is important, it is also critical to overcome the immunosuppressive tumor microenvironment. Although tumor microenvironment is believed to play a relatively minor role in drug resistance in hematological malignancies, MM, leukemia, and lymphoma microenvironment also contains tumor supportive components, such as stromal cells, myeloid-derived suppressor cells, regulatory T-cells, tumor-associated macrophages, and tumor-associated neutrophils (246–250), which interact closely with malignant cells and promote their survival as well as immune escape (250–253). In addition, these immunosuppressive components impair the cytotoxic effects of CAR-T cells and result in CAR-T cell exhaustion (248, 249). Therefore, it is also necessary to overcome the immunosuppressive microenvironment in hematological malignancies. In addition to armored CAR-T cells, CAR-T cell therapy in combination with checkpoint blockades or oncolytic viruses also appears to be an appealing strategy.

The PD-1/PD-L1 pathway plays a major role in T cell exhaustion and represents a major mechanism of tumor immune escape. Thus, blockage of PD-1/PD-L1 interaction could promote the immune system to fight against cancer cells, and PD-1 blockade has achieved tremendous success in diverse tumor types in recent years, especially in lymphoma (254, 255). PD-1 blockade is usually administrated in combination with conventional chemotherapy or other immunotherapies. Currently, multiple studies have explored the combination therapy with CAR-T cells and PD-1 blockade (**Table 3** combinatorial strategies with CAR-T cell therapy). In a clinical trial enrolled 11 NHL patients, 45.5% of patients achieved CRs after this combinatorial therapy, and the toxicities were well-tolerable (256, 257). The mechanisms may be mainly attributed to the decreased CAR-T cell exhaustion (258–260). In addition, the CAR-T cells which could secrete the PD-1 blocking scFv have been developed. The preclinical study demonstrated that the efficacy of such CAR-T cells was equally effective or superior to the combinational therapy of CAR-T cells

TABLE 3 | Combinatorial strategies with CAR-T cell therapy reported in clinical studies.

Combinatorial approach	Disease	Target	Reference
PD-1 blockade	R/R NHL	CD19	(256, 257)
Ibrutinib	R/R CLL, MCL, FL	CD19	(230, 231, 233)
Radiotherapy	R/R NHL	CD19	(238–241)
Decitabine	R/R NHL	CD19	(236)
Lenalidomide	R/R MM	BCMA	(229)
Selinexor-based regimens	R/R MM	BCMA	(228)
Carfilzomib-based regimens	R/R MM	BCMA	(227)

and PD-1 inhibitor (261). TIM-3 is another inhibitory immune checkpoint, and the combination of TIM-3 blockade with CAR-T cells exerts synergistic anti-tumor activity as well (262).

The combination of CAR-T cell therapy and oncolytic viruses is an innovative strategy to overcome immunosuppressive microenvironment. The virus-infected tumor cells which carry pathogen-associated molecular patterns (PAMPs) could recruit host immune cells and thereby promote the recognition of TAAs by the host immune system and the oncolytic viruses also can be genetically modified with immune-stimulatory molecules to enhance the anti-tumor activity of CAR-T cells (263, 264). Besides, oncolytic viruses directly lyse tumor cells and result in the release of TAAs and damage-associated molecular patterns (DAMPs), which could increase tumor immunogenicity and activate APCs through pattern recognition receptors (PRRs), eventually activating tumor-specific T cells (265–267).

In addition, the armored CAR-T cells which express the immune-regulatory molecules, such as IL-15, IL-18, CD40L as well as TGF-β dominant-negative receptor II, are able to remodel the tumor microenvironment (211, 219, 220, 268). Oncometabolites in the tumor microenvironment could inhibit the metabolism and function of CAR-T cells, so suppressing the accumulation oncometabolites is a potential therapeutic option. Kynurenine (Kyn) is an oncometabolite which exists in various hematopoietic malignancies, such as lymphoma and leukemia, and the enzyme kynurenine catalyzes the degradation of Kyn. Thus, the anti-CD19 CAR-T cells were genetically modified with the enzyme kynurenine gene, and they exhibited the improved anti-tumor activity against Nalm6-GL cells in the immunosuppressive tumor microenvironment with high Kyn (269). Interestingly, some target antigens are co-expressed on immunosuppressive cells in tumor microenvironment, so targeting these antigens can simultaneously eliminate malignant cells and immunosuppressive cells. For example, anti-CD123 CAR-T cells target both malignant cells and TAMs in HL (270).

CONCLUSIONS

There are many challenges and opportunities presented by CAR-T cell therapy. With the identification of novel therapeutic targets and the optimization of CAR constructs, CAR-T cell therapy will have broader clinical applications, beyond hematological malignancies. However, with the rapid commercialization of CAR-T cell therapy, it poses a significant challenge for the management of CAR-T cell therapy, such as the toxicities associated with CAR-T therapy and relapse after CAR T-cell therapy. Therefore, exploring their underlying mechanisms and overcoming these limitations will help R/R patients gain more benefits from this promising. Currently, multiple combinatorial approaches with CAR-T cell therapy are being explored and seem to be promising immunotherapy. In addition, UCAR-T cells and CAR-NK cells also show great potential in cancer treatment due to their low manufacturing costs and off-the-shelf availability.

AUTHOR CONTRIBUTIONS

YX, XZ, and LZ designed the manuscript. XZ, HZ, and SC drafted the manuscript and created the figures. XZ and YX revised the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. 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(5):439–48. doi: 10.1056/NEJMoa1709866
2. Schuster SJ, Bishop MR, Tam CS, Waller EK, Borchmann P, McGuirk JP, et al. Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma. *N Engl J Med* (2019) 380:45–56. doi: 10.1056/NEJMoa1804980
3. Fowler NH, Dickinson M, Dreyling M, Martinez-Lopez J, Kolstad A, Butler J, et al. Tisagenlecleucel in Adult Relapsed or Refractory Follicular Lymphoma: The Phase 2 ELARA Trial. *Nat Med* (2021) 28(2):325–32. doi: 10.1038/s41591-021-01622-0
4. Locke FL, Ghobadi A, Jacobson CA, Miklos DB, Lekakis LJ, Oluwole OO, et al. Long-Term Safety and Activity of Axicabtagene Ciloleucel in Refractory Large B-Cell Lymphoma (ZUMA-1): A Single-Arm, Multicentre, Phase 1-2 Trial. *Lancet Oncol* (2019) 20(1):31–42. doi: 10.1016/S1470-2045(18)30864-7

5. Wang M, Munoz J, Goy A, Locke FL, Jacobson CA, Hill BT, et al. KTE-X19 CAR T-Cell Therapy in Relapsed or Refractory Mantle-Cell Lymphoma. *N Engl J Med* (2020) 382(14):1331–42. doi: 10.1056/NEJMoa1914347
6. Abramson JS, Palomba ML, Gordon LI, Lunning MA, Wang M, Arnason J, et al. Lisocabtagene Maraleucel for Patients With Relapsed or Refractory Large B-Cell Lymphomas (TRANSCEND NHL 001): A Multicentre Seamless Design Study. *Lancet* (2020) 396(10254):839–52. doi: 10.1016/S0140-6736(20)31366-0
7. Berdeja JG, Madduri D, Usmani SZ, Jakubowiak A, Agha M, Cohen AD, et al. Ciltacabtagene Autoleucel, a B-Cell Maturation Antigen-Directed Chimeric Antigen Receptor T-Cell Therapy in Patients With Relapsed or Refractory Multiple Myeloma (CARTITUDE-1): A Phase 1b/2 Open-Label Study. *Lancet* (2021) 398(10297):314–24. doi: 10.1016/S0140-6736(21)00933-8
8. Munshi NC, Anderson LDJr, Shah N, Madduri D, Berdeja J, Lonial S, et al. Idecabtagene Vicleucel in Relapsed and Refractory Multiple Myeloma. *N Engl J Med* (2021) 384(8):705–16. doi: 10.1056/NEJMoa2024850
9. Jiang H, Liu L, Guo T, Wu Y, Ai L, Deng J, et al. Improving the Safety of CAR-T Cell Therapy by Controlling CRS-Related Coagulopathy. *Ann Hematol* (2019) 98(7):1721–32. doi: 10.1007/s00277-019-03685-z
10. Lanitis E, Rota G, Kosti P, Ronet C, Spill A, Seijo B, et al. Optimized Gene Engineering of Murine CAR-T Cells Reveals the Beneficial Effects of IL-15 Coexpression. *J Exp Med* (2021) 218(2):e20192203. doi: 10.1084/jem.20192203
11. Duan D, Wang K, Wei C, Feng D, Liu Y, He Q, et al. The BCMA-Targeted Fourth-Generation CAR-T Cells Secreting IL-7 and CCL19 for Therapy of Refractory/Recurrent Multiple Myeloma. *Front Immunol* (2021) 12:609421. doi: 10.3389/fimmu.2021.609421
12. Yang J, Jiang P, Zhang X, Li J, Wu Y, Xu L, et al. Successful 24-Hours Manufacture of Anti-CD19/CD22 Dual Chimeric Antigen Receptor (CAR) T Cell Therapy for B-Cell Acute Lymphoblastic Leukemia (B-ALL). *Blood* (2020) 136 (Supplement 1):2–3. doi: 10.1182/blood-2020-136866
13. Jiang H, Dong B, Gao L, Liu L, Ge J, He A, et al. Clinical Results of a Multicenter Study of the First-in-Human Dual BCMA and CD19 Targeted Novel Platform Fast CAR-T Cell Therapy for Patients with Relapsed/Refractory Multiple Myeloma. *Blood* (2020) 136 (Supplement 1):25–6. doi: 10.1182/blood-2020-136866
14. Hu Y, Wang J, Wei G, Yu J, Luo Y, Shi J, et al. A Retrospective Comparison of Allogenic and Autologous Chimeric Antigen Receptor T Cell Therapy Targeting CD19 in Patients With Relapsed/Refractory Acute Lymphoblastic Leukemia. *Bone Marrow Transplant* (2019) 54(8):1208–17. doi: 10.1038/s41409-018-0403-2
15. 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(6):545–53. doi: 10.1056/NEJMoa1910607
16. Jacobson CA, Chavez JC, Sehgal AR, William BM, Munoz J, Salles G, et al. Axicabtagene Ciloleucel in Relapsed or Refractory Indolent non-Hodgkin Lymphoma (ZUMA-5): A Single-Arm, Multicentre, Phase 2 Trial. *Lancet Oncol* (2022) 23(1):91–103. doi: 10.1016/S1470-2045(21)00591-X
17. Zelenetz AD, Gordon LI, Chang JE, Christian B, Abramson JS, Advani RH, et al. NCCN Guidelines® Insights: B-Cell Lymphomas, Version 5.2021. *J Natl Compr Canc Netw* (2021) 19(11):1218–30. doi: 10.6004/jnccn.2021.0054
18. Pan J, Yang JF, Deng BP, Zhao XJ, Zhang X, Lin YH, et al. High Efficacy and Safety of Low Dose CD19 Directed CAR-T Cell Therapy in 51 Refractory or Relapsed B Acute Lymphoblastic Leukemia Patients. *Leukemia* (2017) 31:2587–93. doi: 10.1038/leu.2017.145
19. Wang Y, Zhang WY, Han QW, Liu Y, Dai HR, Guo YL, et al. Effective Response and Delayed Toxicities of Refractory Advanced Diffuse Large B-Cell Lymphoma Treated by CD20-Directed Chimeric Antigen Receptor-Modified T Cells. *Clin Immunol* (2014) 155(2):160–75. doi: 10.1016/j.clim.2014.10.002
20. Zhang WY, Liu Y, Wang Y, Wang CM, Yang QM, Zhu HL, et al. Long-Term Safety and Efficacy of CART-20 Cells in Patients With Refractory or Relapsed B-Cell non-Hodgkin Lymphoma: 5-Years Follow-Up Results of the Phase I and IIa Trials. *Signal Transduct Target Ther* (2017) 2:17054. doi: 10.1038/sigtrans.2017.54
21. Sang W, Shi M, Yang J, Cao J, Xu L, Yan D, et al. Phase II Trial of Co-Administration of CD19- and CD20-Targeted Chimeric Antigen Receptor T Cells for Relapsed and Refractory Diffuse Large B Cell Lymphoma. *Cancer Med* (2020) 9(16):5827–38. doi: 10.1002/cam4.3259
22. Olejniczak SH, Stewart CC, Donohue K, Czuczman MS. A Quantitative Exploration of Surface Antigen Expression in Common B-Cell Malignancies Using Flow Cytometry. *Immunol Invest* (2006) 35(1):93–114. doi: 10.1080/08820130500496878
23. Haso W, Lee DW, Shah NN, Stetler-Stevenson M, Yuan CM, Pastan IH, et al. Anti-CD22-Chimeric Antigen Receptors Targeting B-Cell Precursor Acute Lymphoblastic Leukemia. *Blood* (2013) 121(7):1165–74. doi: 10.1182/blood-2012-06-438002
24. Pan J, Niu Q, Deng B, Liu S, Wu T, Gao Z, et al. CD22 CAR T-Cell Therapy in Refractory or Relapsed B Acute Lymphoblastic Leukemia. *Leukemia* (2019) 33(12):2854–66. doi: 10.1038/s41375-019-0488-7
25. Fry TJ, Shah NN, Orentas RJ, Stetler-Stevenson M, Yuan CM, Ramakrishna S, et al. CD22-Targeted CAR T Cells Induce Remission in B-ALL That is Naive or Resistant to CD19-Targeted CAR Immunotherapy. *Nat Med* (2018) 24(1):20–8. doi: 10.1038/nm.4441
26. Zhu H, Deng H, Mu J, Lyu C, Jiang Y, Deng Q. Anti-CD22 CAR-T Cell Therapy as a Salvage Treatment in B Cell Malignancies Refractory or Relapsed After Anti-CD19 CAR-T Therapy. *Onco Targets Ther* (2021) 14:4023–37. doi: 10.2147/OTT.S312904
27. Tan Y, Cai H, Li C, Deng B, Song W, Ling Z, et al. A Novel Full-Human CD22-CAR T Cell Therapy With Potent Activity Against CD22 Low B-ALL. *Blood Cancer J* (2021) 11(4):71. doi: 10.1038/s41408-021-00465-9
28. Li S, Wang X, Yuan Z, Liu L, Luo L, Li Y, et al. Eradication of T-ALL Cells by CD7-Targeted Universal CAR-T Cells and Initial Test of Ruxolitinib-Based CRS Management. *Clin Cancer Res* (2021) 27(5):1242–6. doi: 10.1158/1078-0432.CCR-20-1271
29. Pan J, Tan Y, Wang G, Deng B, Ling Z, Song W, et al. Donor-Derived CD7 Chimeric Antigen Receptor T Cells for T-Cell Acute Lymphoblastic Leukemia: First-In-Human, Phase I Trial. *J Clin Oncol* (2021) 39 (30):3340–51. doi: 10.1200/JCO.21.00389
30. Xie L, Ma L, Liu S, Chang L, Wen F. Chimeric Antigen Receptor T Cells Targeting CD7 in a Child With High-Risk T-Cell Acute Lymphoblastic Leukemia. *Int Immunopharmacol* (2021) 96:107731. doi: 10.1016/j.intimp.2021.107731
31. Wada M, Zhang H, Fang L, Feng J, Tse CO, Zhang W, et al. Characterization of an Anti-CD5 Directed CAR T-Cell Against T-Cell Malignancies. *Stem Cell Rev Rep* (2020) 16(2):369–84. doi: 10.1007/s12015-019-09937-9
32. Feng J, Xu H, Cinquina A, Wu Z, Chen Q, Zhang P, et al. Treatment of Aggressive T Cell Lymphoblastic Lymphoma/leukemia Using Anti-CD5 CAR T Cells. *Stem Cell Rev Rep* (2021) 17(2):652–61. doi: 10.1007/s12015-020-10092-9
33. Pinz K, Liu H, Golightly M, Jares A, Lan F, Zieve GW, et al. Preclinical Targeting of Human T-Cell Malignancies Using CD4-Specific Chimeric Antigen Receptor (CAR)-Engineered T Cells. *Leukemia* (2016) 30(3):701–7. doi: 10.1038/leu.2015.311
34. Alcantara M, Tesio M, June CH, Houot R. CAR T-Cells for T-Cell Malignancies: Challenges in Distinguishing Between Therapeutic, Normal, and Neoplastic T-Cells. *Leukemia* (2018) 32(11):2307–15. doi: 10.1038/s41375-018-0285-8
35. Maciocia PM, Wawrzyniecka PA, Philip B, Ricciardelli I, Akarca AU, Onuoha SC, et al. Targeting the T cell Receptor β-Chain Constant Region for Immunotherapy of T Cell Malignancies. *Nat Med* (2017) 23(12):1416–23. doi: 10.1038/nm.4444
36. Shi J, Zhang Z, Cen H, Wu H, Zhang S, Liu J, et al. CAR T Cells Targeting CD99 as an Approach to Eradicate T-Cell Acute Lymphoblastic Leukemia Without Normal Blood Cells Toxicity. *J Hematol Oncol* (2021) 14(1):162. doi: 10.1186/s13045-021-01178-z
37. Maciocia PM, Wawrzyniecka PA, Maciocia NC, Burley A, Karpanasamy T, Devereaux S, et al. Anti-CCR9 Chimeric Antigen Receptor T Cells for T Cell Acute Lymphoblastic Leukemia. *Blood* (2022), blood.2021013648. doi: 10.1182/blood.2021013648
38. Horwitz S, O'Connor OA, Pro B, Illidge T, Fanale M, Advani R, et al. Brentuximab Vedotin With Chemotherapy for CD30-Positive Peripheral T-Cell Lymphoma (ECHELON-2): A Global, Double-Blind, Randomised,

Phase 3 Trial. *Lancet* (2019) 393:229–40. doi: 10.1016/S0140-6736(18)32984-2
39. Fanale MA, Horwitz SM, Forero-Torres A, Bartlett NL, Advani RH, Pro B, et al. Five-Year Outcomes for Frontline Brentuximab Vedotin With CHP for CD30-Expressing Peripheral T-Cell Lymphomas. *Blood* (2018) 131(19):2120–4. doi: 10.1182/blood-2017-12-821009
40. Guercio M, Orlando D, Di Cecca S, Sinibaldi M, Boffa I, Caruso S, et al. CD28.OX40 Co-Stimulatory Combination is Associated With Long *In Vivo* Persistence and High Activity of CAR.CD30 T-Cells. *Haematologica* (2021) 106(4):987–99. doi: 10.3324/haematol.2019.231183
41. Wu Y, Chen D, Lu Y, Dong SC, Ma R, Tang WY, et al. A New Immunotherapy Strategy Targeted CD30 in Peripheral T-Cell Lymphomas: CAR-Modified T-Cell Therapy Based on CD30 mAb. *Cancer Gene Ther* (2022) 29(2):167–77. doi: 10.1038/s41417-021-00295-8
42. Wang CM, Wu ZQ, Wang Y, Guo YL, Dai HR, Wang XH, et al. Autologous T Cells Expressing CD30 Chimeric Antigen Receptors for Relapsed or Refractory Hodgkin Lymphoma: An Open-Label Phase I Trial. *Clin Cancer Res* (2017) 23(5):1156–66. doi: 10.1158/1078-0432.CCR-16-1365
43. Ramos CA, Grover NS, Beaven AW, Lulla PD, Wu MF, Ivanova A, et al. Anti-CD30 CAR-T Cell Therapy in Relapsed and Refractory Hodgkin Lymphoma. *J Clin Oncol* (2020) 38(32):3794–804. doi: 10.1200/JCO.20.01342
44. Ramos CA, Ballard B, Zhang H, Dakhova O, Gee AP, Mei Z, et al. Clinical and Immunological Responses After CD30-Specific Chimeric Antigen Receptor-Redirected Lymphocytes. *J Clin Invest* (2017) 127(9):3462–71. doi: 10.1172/JCI94306
45. Wang D, Zeng C, Xu B, Xu JH, Wang J, Jiang LJ, et al. Anti-CD30 Chimeric Antigen Receptor T Cell Therapy for Relapsed/Refractory CD30+ Lymphoma Patients. *Blood Cancer J* (2020) 10(1):8. doi: 10.1038/s41408-020-0274-9
46. Voorhees TJ, Zhao B, Oldan J, Hucks G, Khandani A, Dittus C, et al. Pretherapy Metabolic Tumor Volume is Associated With Response to CD30 CAR T Cells in Hodgkin Lymphoma. *Blood Adv* (2022) 6(4):1255–63. doi: 10.1182/bloodadvances.2021005385
47. Kim DH, Vega F. Relapsed Classic Hodgkin Lymphoma With Decreased CD30 Expression After Brentuximab and Anti-CD30 CAR-T Therapies. *Blood* (2022) 139(6):951. doi: 10.1182/blood.2021013881
48. Nguyen DH, Ball ED, Varki A. Myeloid Precursors and Acute Myeloid Leukemia Cells Express Multiple CD33-Related Siglecs. *Exp Hematol* (2006) 34:728–35. doi: 10.1016/j.exphem.2006.03.003
49. Baroni ML, Sanchez Martinez D, Gutierrez Aguera F, Roca Ho H, Castella M, Zanetti SR, et al. 41BB-Based and CD28-Based CD123-Redirected T-Cells Ablate Human Normal Hematopoiesis *In Vivo*. *J Immunother Cancer* (2020) 8(1):e000845. doi: 10.1136/jitc-2020-000845
50. Loff S, Dietrich J, Meyer JE, Riewaldt J, Spehr J, von Bonin M, et al. Rapidly Switchable Universal CAR-T Cells for Treatment of CD123-Positive Leukemia. *Mol Ther Oncolytics* (2020) 17:408–20. doi: 10.1016/j.omto.2020.04.009
51. Meyer JE, Loff S, Dietrich J, Spehr J, Jurado Jiménez G, von Bonin M, et al. Evaluation of Switch-Mediated Costimulation in Trans on Universal CAR-T Cells (UniCAR) Targeting CD123-Positive AML. *Oncoimmunology* (2021) 10(1):1945804. doi: 10.1080/2162402X.2021.1945804
52. Wermke M, Kraus S, Ehninger A, Bargou RC, Goebeler ME, Middeke JM, et al. Proof of Concept for a Rapidly Switchable Universal CAR-T Platform With UniCAR-T-CD123 in Relapsed/Refractory AML. *Blood* (2021) 137(22):3145–8. doi: 10.1182/blood.2020009759
53. Tambaro FP, Singh H, Jones E, Rytting M, Mahadeo KM, Thompson P, et al. Autologous CD33-CAR-T Cells for Treatment of Relapsed/Refractory Acute Myelogenous Leukemia. *Leukemia* (2021) 35(11):3282–6. doi: 10.1038/s41375-021-01232-2
54. Cui Q, Qian C, Xu N, Kang L, Dai H, Cui W, et al. CD38-Directed CAR-T Cell Therapy: A Novel Immunotherapy Strategy for Relapsed Acute Myeloid Leukemia After Allogeneic Hematopoietic Stem Cell Transplantation. *J Hematol Oncol* (2021) 14(1):82. doi: 10.1186/s13045-021-01092-4
55. Bakker AB, van den Oudenrijn S, Bakker AQ, Feller N, van Meijer M, Bia JA, et al. C-Type Lectin-Like Molecule-1: A Novel Myeloid Cell Surface Marker Associated With Acute Myeloid Leukemia. *Cancer Res* (2004) 64:8443–50. doi: 10.1158/0008-5472.CAN-04-1659
56. Wang J, Chen S, Xiao W, Li W, Wang L, Yang S, et al. CAR-T Cells Targeting CLL-1 as an Approach to Treat Acute Myeloid Leukemia. *J Hematol Oncol* (2018) 11:7. doi: 10.1186/s13045-017-0553-5
57. Lin G, Zhang Y, Yu L, Wu D. Cytotoxic Effect of CLL-1 CAR-T Cell Immunotherapy With PD-1 Silencing on Relapsed/Refractory Acute Myeloid Leukemia. *Mol Med Rep* (2021) 23(3):208. doi: 10.3892/mmr.2021.11847
58. Sauer T, Parikh K, Sharma S, Omer B, Sedloev D, Chen Q, et al. CD70-Specific CAR T Cells Have Potent Activity Against Acute Myeloid Leukemia Without HSC Toxicity. *Blood* (2021) 138(4):318–30. doi: 10.1182/blood.2020008221
59. Riether C, Pabst T, Höpner S, Bacher U, Hinterbrandner M, Banz Y, et al. Targeting CD70 With Cusatuzumab Eliminates Acute Myeloid Leukemia Stem Cells in Patients Treated With Hypomethylating Agents. *Nat Med* (2020) 26(9):1459–67. doi: 10.1038/s41591-020-0910-8
60. John S, Chen H, Deng M, Gui X, Wu G, Chen W, et al. A Novel Anti-LILRB4 CAR-T Cell for the Treatment of Monocytic AML. *Mol Ther* (2018) 26(10):2487–95. doi: 10.1016/j.ymthe.2018.08.001
61. Jetani H, Navarro-Bailón A, Maucher M, Frenz S, Verbruggen C, Yeguas A, et al. Siglec-6 is a Novel Target for CAR T-Cell Therapy in Acute Myeloid Leukemia. *Blood* (2021) 138(19):1830–42. doi: 10.1182/blood.2020009192
62. Xie G, Ivica NA, Jia B, Li Y, Dong H, Liang Y, et al. CAR-T Cells Targeting a Nucleophosmin Neoepitope Exhibit Potent Specific Activity in Mouse Models of Acute Myeloid Leukaemia. *Nat BioMed Eng* (2021) 5(5):399–413. doi: 10.1038/s41551-020-00625-5
63. Sommer C, Cheng HY, Nguyen D, Dettling D, Yeung YA, Sutton J, et al. Allogeneic FLT3 CAR T Cells With an Off-Switch Exhibit Potent Activity Against AML and Can Be Depleted to Expedite Bone Marrow Recovery. *Mol Ther* (2020) 28(10):2237–51. doi: 10.1016/j.ymthe.2020.06.022
64. Jetani H, Garcia-Cadenas I, Nerreter T, Thomas S, Rydzek J, Meijide JB, et al. CAR T-Cells Targeting FLT3 Have Potent Activity Against FLT3-ITD+ AML and Act Synergistically With the FLT3-Inhibitor Crenolanib. *Leukemia* (2018) 32(5):1168–79. doi: 10.1038/s41375-018-0009-0
65. Kikushige Y, Yoshimoto G, Miyamoto T, Iino T, Mori Y, Iwasaki H, et al. Human Flt3 is Expressed at the Hematopoietic Stem Cell and the Granulocyte/Macrophage Progenitor Stages to Maintain Cell Survival. *J Immunol* (2008) 180:7358–67. doi: 10.4049/jimmunol.180.11.7358
66. Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, et al. International Myeloma Working Group Updated Criteria for the Diagnosis of Multiple Myeloma. *Lancet Oncol* (2014) 15(12):e538–48. doi: 10.1016/S1470-2045(14)70442-5
67. Shah N, Chari A, Scott E, Mezzi K, Usmani SZ. B-Cell Maturation Antigen (BCMA) in Multiple Myeloma: Rationale for Targeting and Current Therapeutic Approaches. *Leukemia* (2020) 34(4):985–1005. doi: 10.1038/s41375-020-0734-z
68. Wang D, Wang J, Hu G, Wang W, Xiao Y, Cai H, et al. A Phase 1 Study of a Novel Fully Human BCMA-Targeting CAR (CT103A) in Patients With Relapsed/Refractory Multiple Myeloma. *Blood* (2021) 137(21):2890–901. doi: 10.1182/blood.2020008936
69. Li C, Cao W, Que Y, Wang Q, Xiao Y, Gu C, et al. A Phase I Study of Anti-BCMA CAR T Cell Therapy in Relapsed/Refractory Multiple Myeloma and Plasma Cell Leukemia. *Clin Transl Med* (2021) 11(3):e346. doi: 10.1002/ctm2.346
70. Sharma P, Kanapuru B, George B, Lin X, Xu Z, Bryan WW, et al. FDA Approval Summary: Idecabtagene Vicleucel for Relapsed or Refractory Multiple Myeloma. *Clin Cancer Res* (2022) 28(9): 1759–64. doi: 10.1158/1078-0432.CCR-21-3803
71. Deng H, Liu M, Yuan T, Zhang H, Cui R, Li J, et al. Efficacy of Humanized Anti-BCMA CAR T Cell Therapy in Relapsed/Refractory Multiple Myeloma Patients With and Without Extramedullary Disease. *Front Immunol* (2021) 12:720571. doi: 10.3389/fimmu.2021.720571
72. Que Y, Xu M, Xu Y, Almeida VDF, Zhu L, Wang Z, et al. Anti-BCMA CAR-T Cell Therapy in Relapsed/Refractory Multiple Myeloma Patients With Extramedullary Disease: A Single Center Analysis of Two Clinical Trials. *Front Immunol* (2021) 12:755866. doi: 10.3389/fimmu.2021.755866
73. Oriol A, Abril L, Torrent A, Ibarra G, Ribera JM. The Role of Idecabtagene Vicleucel in Patients With Heavily Pretreated Refractory Multiple Myeloma.

Ther Adv Hematol (2021) 12:20406207211019622. doi: 10.1177/20406207211019622
74. van de Donk NWCJ, Usmani SZ, Yong K. CAR T-Cell Therapy for Multiple Myeloma: State of the Art and Prospects. *Lancet Haematol* (2021) 8(6):e446–61. doi: 10.1016/S2352-3026(21)00057-0
75. Sun C, Mahendravada A, Ballard B, Kale B, Ramos C, West J, et al. Safety and Efficacy of Targeting CD138 With a Chimeric Antigen Receptor for the Treatment of Multiple Myeloma. *Oncotarget* (2019) 10:2369–83. doi: 10.18632/oncotarget.26792
76. Guo B, Chen M, Han Q, Hui F, Dai H, Zhang W, et al. CD138-Directed Adoptive Immunotherapy of Chimeric Antigen Receptor (CAR)-Modified T Cells for Multiple Myeloma. *J Cell Immunother* (2016) 2:28–35. doi: 10.1016/j.jocit.2014.11.001
77. van de Donk NW, Janmaat ML, Mutis T, Lammerts van Bueren JJ, Ahmadi T, Sasser AK, et al. Monoclonal Antibodies Targeting CD38 in Hematological Malignancies and Beyond. *Immunol Rev* (2016) 270(1):95–112. doi: 10.1111/imr.12389
78. Drent E, Groen RW, Noort WA, Themeli M, Lammerts van Bueren JJ, Parren PW, et al. Pre-Clinical Evaluation of CD38 Chimeric Antigen Receptor Engineered T Cells for the Treatment of Multiple Myeloma. *Haematologica* (2016) 101:616–25. doi: 10.3324/haematol.2015.137620
79. Tang Y, Yin H, Zhao X, Jin D, Liang Y, Xiong T, et al. High Efficacy and Safety of CD38 and BCMA Bispecific CAR-T in Relapsed or Refractory Multiple Myeloma. *J Exp Clin Cancer Res* (2022) 41(1):2. doi: 10.1186/s13046-021-02214-z
80. Mei H, Li C, Jiang H, Zhao X, Huang Z, Jin D, et al. A Bispecific CAR-T Cell Therapy Targeting BCMA and CD38 in Relapsed or Refractory Multiple Myeloma. *J Hematol Oncol* (2021) 14(1):161. doi: 10.1186/s13045-021-01170-7
81. Atanackovic D, Panse J, Hildebrandt Y, Jadczak A, Kobold S, Cao Y, et al. Surface Molecule CD229 as a Novel Target for the Diagnosis and Treatment of Multiple Myeloma. *Haematologica* (2011) 96(10):1512–20. doi: 10.3324/haematol.2010.036814
82. Radhakrishnan SV, Luetkens T, Scherer SD, Davis P, Vander Mause ER, Olson ML, et al. CD229 CAR T Cells Eliminate Multiple Myeloma and Tumor Propagating Cells Without Fratricide. *Nat Commun* (2020) 11(1):798. doi: 10.1038/s41467-020-14619-z
83. Hsi ED, Steinle R, Balasa B, Szmania S, Draksharapu A, Shum BP, et al. CS1, a Potential New Therapeutic Antibody Target for the Treatment of Multiple Myeloma. *Clin Cancer Res* (2008) 14:2775–84. doi: 10.1158/1078-0432.CCR-07-4246
84. Gogishvili T, Danhof S, Prommersberger S, Rydzek J, Schreder M, Brede C, et al. SLAMF7-CAR T Cells Eliminate Myeloma and Confer Selective Fratricide of SLAMF7(+) Normal Lymphocytes. *Blood* (2017) 130:2838–47. doi: 10.1182/blood-2017-04-778423
85. Prommersberger S, Reiser M, Beckmann J, Danhof S, Amberger M, Quade-Lyssy P, et al. CARAMBA: A First-in-Human Clinical Trial With SLAMF7 CAR-T Cells Prepared by Virus-Free Sleeping Beauty Gene Transfer to Treat Multiple Myeloma. *Gene Ther* (2021) 28(9):560–71. doi: 10.1038/s41434-021-00254-w
86. Lee L, Draper B, Chaplin N, Philip B, Chin M, Galas-Filipowicz D, et al. An APRIL-Based Chimeric Antigen Receptor for Dual Targeting of BCMA and TACI in Multiple Myeloma. *Blood* (2018) 131(7):746–58. doi: 10.1182/blood-2017-05-781351
87. Schmidts A, Ormhøj M, Choi BD, Taylor AO, Bouffard AA, Scarfò I, et al. Rational Design of a Trimeric APRIL-Based CAR-Binding Domain Enables Efficient Targeting of Multiple Myeloma. *Blood Adv* (2019) 3(21):3248–60. doi: 10.1182/bloodadvances.2019000703
88. Smith EL, Harrington K, Staehr M, Masakayan R, Jones J, Long TJ, et al. GPRC5D is a Target for the Immunotherapy of Multiple Myeloma With Rationally Designed CAR T Cells. *Sci Transl Med* (2019) 11(485):eaau7746. doi: 10.1126/scitranslmed.aau7746
89. Obstfeld AE, Frey NV, Mansfield K, Lacey SF, June CH, Porter DL, et al. Cytokine Release Syndrome Associated With Chimeric-Antigen Receptor T-Cell Therapy: Clinicopathological Insights. *Blood* (2017) 130(23):2569–72. doi: 10.1182/blood-2017-08-802413
90. Dong R, Jiang S, Chen Y, Ma Y, Sun L, Xing C, et al. Prognostic Significance of Cytokine Release Syndrome in B Cell Hematological Malignancies Patients After Chimeric Antigen Receptor T Cell Therapy. *J Interferon Cytokine Res* (2021) 41(12):469–76. doi: 10.1089/jir.2021.0057
91. Norelli M, Camisa B, Barbiera G, Falcone L, Purevdorj A, Genua M, et al. Monocyte-Derived IL-1 and IL-6 are Differentially Required for Cytokine-Release Syndrome and Neurotoxicity Due to CAR T Cells. *Nat Med* (2018) 24(6):739–48. doi: 10.1038/s41591-018-0036-4
92. Giavridis T, van der Stegen SJC, Eyquem J, Hamieh M, Piersigilli A, Sadelain M. CAR T Cell-Induced Cytokine Release Syndrome is Mediated by Macrophages and Abated by IL-1 Blockade. *Nat Med* (2018) 24(6):731–8. doi: 10.1038/s41591-018-0041-7
93. Wehrli M, Gallagher K, Chen YB, Leick MB, McAfee SL, El-Jawahri AR, et al. Single-Center Experience Using Anakinra for Steroid-Refractory Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS). *J Immunother Cancer* (2022) 10(1):e003847. doi: 10.1136/jitc-2021-003847
94. Mehta P, Cron RQ, Hartwell J, Manson JJ, Tattersall RS. Silencing the Cytokine Storm: The Use of Intravenous Anakinra in Haemophagocytic Lymphohistiocytosis or Macrophage Activation Syndrome. *Lancet Rheumatol* (2020) 2(6):e358–67. doi: 10.1016/S2665-9913(20)30096-5
95. Strati P, Ahmed S, Kebriaei P, Nastoupil LJ, Claussen CM, Watson G, et al. Clinical Efficacy of Anakinra to Mitigate CAR T-Cell Therapy-Associated Toxicity in Large B-Cell Lymphoma. *Blood Adv* (2020) 4(13):3123–7. doi: 10.1182/bloodadvances.2020002328
96. Sterner RM, Sakemura R, Cox MJ, Yang N, Khadka RH, Forsman CL, et al. GM-CSF Inhibition Reduces Cytokine Release Syndrome and Neuroinflammation But Enhances CAR-T Cell Function in Xenografts. *Blood* (2019) 133(7):697–709. doi: 10.1182/blood-2018-10-881722
97. Sachdeva M, Duchateau P, Depil S, Poirot L, Valton J. Granulocyte-Macrophage Colony-Stimulating Factor Inactivation in CAR T-Cells Prevents Monocyte-Dependent Release of Key Cytokine Release Syndrome Mediators. *J Biol Chem* (2019) 294(14):5430–7. doi: 10.1074/jbc.AC119.007558
98. 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
99. Gong T, Liu L, Jiang W, Zhou R. DAMP-Sensing Receptors in Sterile Inflammation and Inflammatory Diseases. *Nat Rev Immunol* (2020) 20(2):95–112. doi: 10.1038/s41577-019-0215-7
100. Broz P, Pelegrín P, Shao F. The Gasdermins, a Protein Family Executing Cell Death and Inflammation. *Nat Rev Immunol* (2020) 20(3):143–57. doi: 10.1038/s41577-019-0228-2
101. Deng T, Tang C, Zhang G, Wan X. DAMPs Released by Pyroptotic Cells as Major Contributors and Therapeutic Targets for CAR-T-Related Toxicities. *Cell Death Dis* (2021) 12(1):129. doi: 10.1038/s41419-021-03428-x
102. Singh N, Hofmann TJ, Gershenson Z, Levine BL, Grupp SA, Teachey DT. Monocyte Lineage-Derived IL-6 Does Not Affect Chimeric Antigen Receptor T-Cell Function. *Cytotherapy* (2017) 19(7):867–80. doi: 10.1016/j.jcyt.2017.04.001
103. Hao Z, Li R, Meng L, Han Z, Hong Z. Macrophage, the Potential Key Mediator in CAR-T Related CRS. *Exp Hematol Oncol* (2020) 9:15. doi: 10.1186/s40164-020-00171-5
104. Elgueta R, Benson MJ, de Vries VC, Wasiuk A, Guo Y, Noelle RJ. Molecular Mechanism and Function of CD40/CD40L Engagement in the Immune System. *Immunol Rev* (2009) 229(1):152–72. doi: 10.1111/j.1600-065X.2009.00782.x
105. Aldinucci D, Poletto D, Nanni P, Degan M, Rupolo M, Pinto A, et al. CD40L Induces Proliferation, Self-Renewal, Rescue From Apoptosis, and Production of Cytokines by CD40-Expressing AML Blasts. *Exp Hematol* (2002) 30(11):1283–92. doi: 10.1016/s0301-472x(02)00921-9
106. Qi C, Tian S, Wang J, Ma H, Qian K, Zhang X. Co-Expression of CD40/CD40L on XG1 Multiple Myeloma Cells Promotes IL-6 Autocrine Function. *Cancer Invest* (2015) 33(1):6–15. doi: 10.3109/07357907.2014.988340
107. Pellat-Deceunynck C, Amiot M, Robillard N, Wijdenes J, Bataille R. CD11a-CD18 and CD102 Interactions Mediate Human Myeloma Cell Growth Arrest Induced by CD40 Stimulation. *Cancer Res* (1996) 56:1909–16.
108. Teoh G, Tai YT, Urashima M, Shirahama S, Matsuzaki M, Chauhan D, et al. CD40 Activation Mediates P53-Dependent Cell Cycle Regulation in Human Multiple Myeloma Cell Lines. *Blood* (2000) 95(3):1039–46. doi: 10.1182/blood.V95.3.1039.003k02_1039_1046

109. Tang T, Cheng X, Truong B, Sun L, Yang X, Wang H. Molecular Basis and Therapeutic Implications of CD40/CD40L Immune Checkpoint. *Pharmacol Ther* (2021) 219:107709. doi: 10.1016/j.pharmthera.2020.107709
110. Bullock TNJ. CD40 Stimulation as a Molecular Adjuvant for Cancer Vaccines and Other Immunotherapies. *Cell Mol Immunol* (2022) 19 (1):14–22. doi: 10.1038/s41423-021-00734-4
111. van Mierlo GJ, den Boer AT, Medema JP, van der Voort EI, Fransen MF, Offringa R, et al. CD40 Stimulation Leads to Effective Therapy of CD40(-) Tumors Through Induction of Strong Systemic Cytotoxic T Lymphocyte Immunity. *Proc Natl Acad Sci USA* (2002) 99(8):5561–6. doi: 10.1073/pnas.082107699
112. Wei J, Liu Y, Wang C, Zhang Y, Tong C, Dai G, et al. The Model of Cytokine Release Syndrome in CAR T-Cell Treatment for B-Cell non-Hodgkin Lymphoma. *Signal Transduct Target Ther* (2020) 5(1):134. doi: 10.1038/s41392-020-00256-x
113. Gust J, Taraseviciute A, Turtle CJ. Neurotoxicity Associated With CD19-Targeted CAR-T Cell Therapies. *CNS Drugs* (2018) 32(12):1091–101. doi: 10.1007/s40263-018-0582-9
114. Mackall CL, Miklos DB. CNS Endothelial Cell Activation Emerges as a Driver of CAR T Cell-Associated Neurotoxicity. *Cancer Discov* (2017) 7 (12):1371–3. doi: 10.1158/2159-8290.CD-17-1084
115. Gust J, Hay KA, Hanafi LA, Li D, Myerson D, Gonzalez-Cuyar LF, et al. Endothelial Activation and Blood-Brain Barrier Disruption in Neurotoxicity After Adoptive Immunotherapy With CD19 CAR-T Cells. *Cancer Discov* (2017) 7(12):1404–19. doi: 10.1158/2159-8290.CD-17-0698
116. Gust J, Ponce R, Liles WC, Garden GA, Turtle CJ. Cytokines in CAR T Cell-Associated Neurotoxicity. *Front Immunol* (2020) 11:577027. doi: 10.3389/fimmu.2020.577027
117. Morris EC, Neelapu SS, Giavridis T, Sadelain M. Cytokine Release Syndrome and Associated Neurotoxicity in Cancer Immunotherapy. *Nat Rev Immunol* (2022) 22(2):85–96. doi: 10.1038/s41577-021-00547-6
118. Deng Q, Han G, Puebla-Osorio N, Ma MCJ, Strati P, Chasen B, et al. Characteristics of anti-CD19 CAR T cell infusion products associated with efficacy and toxicity in patients with large B cell lymphomas. *Nat Med* (2020) 26(12):1878–87. doi: 10.1038/s41591-020-1061-7
119. Santomasso BD, Park JH, Salloum D, Riviere I, Flynn J, Mead E, et al. Clinical and Biological Correlates of Neurotoxicity Associated With CAR T-Cell Therapy in Patients With B-Cell Acute Lymphoblastic Leukemia. *Cancer Discov* (2018) 8(8):958–71. doi: 10.1158/2159-8290.CD-17-1319
120. Taraseviciute A, Tkachev V, Ponce R, Turtle CJ, Snyder JM, Liggitt HD, et al. Chimeric Antigen Receptor T Cell-Mediated Neurotoxicity in Nonhuman Primates. *Cancer Discov* (2018) 8(6):750–63. doi: 10.1158/2159-8290.CD-17-1368
121. Hu Y, Sun J, Wu Z, Yu J, Cui Q, Pu C, et al. Predominant Cerebral Cytokine Release Syndrome in CD19-Directed Chimeric Antigen Receptor-Modified T Cell Therapy. *J Hematol Oncol* (2016) 9(1):70. doi: 10.1186/s13045-016-0299-5
122. Gomez-Nicola D, Valle-Argos B, Nieto-Sampedro M. Blockade of IL-15 Activity Inhibits Microglial Activation Through the NFkappaB, P38, and ERK1/2 Pathways, Reducing Cytokine and Chemokine Release. *Glia* (2010) 58(3):264–76. doi: 10.1002/glia.20920
123. Shiomi A, Usui T. Pivotal Roles of GM-CSF in Autoimmunity and Inflammation. *Mediat Inflammation* (2015) 2015:568543. doi: 10.1155/2015/568543
124. Ponomarev ED, Shriver LP, Maresz K, Pedras-Vasconcelos J, Verthelyi D, Dittel BN. GM-CSF Production by Autoreactive T Cells is Required for the Activation of Microglial Cells and the Onset of Experimental Autoimmune Encephalomyelitis. *J Immunol* (2007) 178(1):39–48. doi: 10.4049/jimmunol.178.1.39
125. Galea I. The Blood-Brain Barrier in Systemic Infection and Inflammation. *Cell Mol Immunol* (2021) 18(11):2489–501. doi: 10.1038/s41423-021-00757-x
126. Liu S, Deng B, Yin Z, Pan J, Lin Y, Ling Z, et al. Corticosteroids do Not Influence the Efficacy and Kinetics of CAR-T Cells for B-Cell Acute Lymphoblastic Leukemia. *Blood Cancer J* (2020) 10(2):15. doi: 10.1038/s41408-020-0280-y
127. Sanchez L, Dardac A, Madduri D, Richard S, Richter J. B-Cell Maturation Antigen (BCMA) in Multiple Myeloma: The New Frontier of Targeted Therapies. *Ther Adv Hematol* (2021) 12:2040620721989585. doi: 10.1177/2040620721989585
128. Li P, Zhou L, Ye S, Zhang W, Wang J, Tang X, et al. Risk of HBV Reactivation in Patients With Resolved HBV Infection Receiving Anti-CD19 Chimeric Antigen Receptor T Cell Therapy Without Antiviral Prophylaxis. *Front Immunol* (2021) 12:638678. doi: 10.3389/fimmu.2021.638678
129. Spiegel JY, Craig J, Claire GK, Frank MJ, Muffly L, Shiraz P, et al. Immune Reconstitution and Infectious Complications Following Axicabtagene Ciloleucel Therapy for Large B-Cell Lymphoma. *Blood Adv* (2021) 5 (1):143–55. doi: 10.1182/bloodadvances.2020002732
130. Hartmann J, Schüßler-Lenz M, Bondanza A, Buchholz CJ. Clinical Development of CAR T Cells-Challenges and Opportunities in Translating Innovative Treatment Concepts. *EMBO Mol Med* (2017) 9(9):1183–97. doi: 10.15252/emmm.201607485
131. Pfeiffer A, Thalheimer FB, Hartmann S, Frank AM, Bender RR, Danisch S, et al. *In Vivo* Generation of Human CD19-CAR T Cells Results in B-Cell Depletion and Signs of Cytokine Release Syndrome. *EMBO Mol Med* (2018) 10(11):e9158. doi: 10.15252/emmm.201809158
132. Wei J, Zhao J, Han M, Meng F, Zhou J. SARS-CoV-2 Infection in Immunocompromised Patients: Humoral Versus Cell-Mediated Immunity. *J Immunother Cancer* (2020) 8(2):e000862. doi: 10.1136/jitc-2020-000862
133. Park JH, Romero FA, Taur Y, Sadelain M, Brentjens RJ, Hohl TM, et al. Cytokine Release Syndrome Grade as a Predictive Marker for Infections in Patients With Relapsed or Refractory B-Cell Acute Lymphoblastic Leukemia Treated With Chimeric Antigen Receptor T Cells. *Clin Infect Dis* (2018) 67 (4):533–40. doi: 10.1093/cid/ciy152
134. Hill JA, Li D, Hay KA, Green ML, Cherian S, Chen X, et al. Infectious Complications of CD19-Targeted Chimeric Antigen Receptor-Modified T-Cell Immunotherapy. *Blood* (2018) 131(1):121–30. doi: 10.1182/blood-2017-07-793760
135. Luo H, Wang N, Huang L, Zhou X, Jin J, Li C, et al. Inflammatory Signatures for Quick Diagnosis of Life-Threatening Infection During the CAR T-Cell Therapy. *J Immunother Cancer* (2019) 7(1):271. doi: 10.1186/s40425-019-0767-x
136. Han L, Zhou J, Zhou K, Zhu X, Zhao L, Fang B, et al. Safety and Efficacy of CAR-T Cell Targeting BCMA in Patients With Multiple Myeloma Coinfected With Chronic Hepatitis B Virus. *J Immunother Cancer* (2020) 8(2):e000927. doi: 10.1136/jitc-2020-000927
137. Wang D, Mao X, Que Y, Xu M, Cheng Y, Huang L, et al. Viral Infection/Reactivation During Long-Term Follow-Up in Multiple Myeloma Patients With Anti-BCMA CAR Therapy. *Blood Cancer J* (2021) 11(10):168. doi: 10.1038/s41408-021-00563-8
138. Strati P, Varma A, Adkins S, Nastoupil LJ, Westin J, Hagemeister FB, et al. Hematopoietic Recovery and Immune Reconstitution After Axicabtagene Ciloleucel in Patients With Large B-Cell Lymphoma. *Haematologica* (2021) 106(10):2667–72. doi: 10.3324/haematol.2020.254045
139. Yang C, Lei W, Xie H, Wu G, Wei J, Liang A, et al. Sustained Remission of Relapsed or Refractory Mantle Cell Lymphoma After 4-1bb-Based CD19-Directed CAR-T Therapy. *Onco Targets Ther* (2020) 13:12163–8. doi: 10.2147/OTT.S280535
140. Xu J, Chen LJ, Yang SS, Sun Y, Wu W, Liu YF, et al. Exploratory Trial of a Biepitopic CAR T-Targeting B Cell Maturation Antigen in Relapsed/Refractory Multiple Myeloma. *Proc Natl Acad Sci USA* (2019) 116 (19):9543–51. doi: 10.1073/pnas.1819745116
141. Hill JA, Seo SK. How I Prevent Infections in Patients Receiving CD19-Targeted Chimeric Antigen Receptor T Cells for B-Cell Malignancies. *Blood* (2020) 136(8):925–35. doi: 10.1182/blood.2019004000
142. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, et al. Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. *N Engl J Med* (2017) 377(26):2531–44. doi: 10.1056/NEJMoa1707447
143. 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(18):1726–37. doi: 10.1056/NEJMoa1817226
144. Rejeski K, Perez A, Sesques P, Hoster E, Berger C, Jentzsch L, et al. CAR-HEMATOTOX: A Model for CAR T-Cell-Related Hematologic Toxicity in Relapsed/Refractory Large B-Cell Lymphoma. *Blood* (2021) 138(24):2499–513. doi: 10.1182/blood.2020010543

145. Juluri KR, Wu V, Voutsinas JM, Hou J, Hirayama AV, Mullane E, et al. Severe Cytokine Release Syndrome is Associated With Hematologic Toxicity Following CD19 CAR T-Cell Therapy. *Blood Adv* (2022) 6(7):2055–68. doi: 10.1182/bloodadvances.2020004142
146. Jain T, Knezevic A, Pennisi M, Chen Y, Ruiz JD, Purdon TJ, et al. Hematopoietic Recovery in Patients Receiving Chimeric Antigen Receptor T-Cell Therapy for Hematologic Malignancies. *Blood Adv* (2020) 4 (15):3776–87. doi: 10.1182/bloodadvances.2020002509
147. Mavroudi I, Papadaki V, Pyrovolaki K, Katonis P, Eliopoulos AG, Papadaki HA. The CD40/CD40 Ligand Interactions Exert Pleiotropic Effects on Bone Marrow Granulopoiesis. *J Leukoc Biol* (2011) 89(5):771–83. doi: 10.1189/jlb.0610330
148. Dulmovits BM, Tang Y, Papoin J, He M, Li J, Yang H, et al. HMGB1-Mediated Restriction of EPO Signaling Contributes to Anemia of Inflammation. *Blood* (2022) 139(21): 3181–93. doi: 10.1182/blood.2021012048
149. Jelkmann W. Proinflammatory Cytokines Lowering Erythropoietin Production. *J Interferon Cytokine Res* (1998) 18(8):555–119. doi: 10.1089/jir.1998.18.555
150. Ganz T. Anemia of Inflammation. *N Engl J Med* (2019) 381(12):1148–57. doi: 10.1056/NEJMra1804281
151. Schubert ML, Dietrich S, Stilgenbauer S, Schmitt A, Pavel P, Kunz A, et al. Feasibility and Safety of CD19 Chimeric Antigen Receptor T Cell Treatment for B Cell Lymphoma Relapse After Allogeneic Hematopoietic Stem Cell Transplantation. *Biol Blood Marrow Transplant* (2020) 26(9):1575–80. doi: 10.1016/j.bbmt.2020.04.025
152. Fried S, Avigdor A, Bielorai B, Meir A, Besser MJ, Schachter J, et al. Early and Late Hematologic Toxicity Following CD19 CAR-T Cells. *Bone Marrow Transplant* (2019) 54(10):1643–50. doi: 10.1038/s41409-019-0487-3
153. Jain MD, Davila ML. Concise Review: Emerging Principles From the Clinical Application of Chimeric Antigen Receptor T Cell Therapies for B Cell Malignancies. *Stem Cells* (2018) 36(1):36–44. doi: 10.1002/stem.2715
154. Baur R, Jitschin R, Kharboutli S, Stoll A, Völkl S, Büttner-Herold M, et al. Thrombopoietin Receptor Agonists for Acquired Thrombocytopenia Following Anti-CD19 CAR-T-Cell Therapy: A Case Report. *J Immunother Cancer* (2021) 9(7):e002721. doi: 10.1136/jitc-2021-002721
155. Xing L, Wang Y, Liu H, Gao S, Shao Q, Yue L, et al. Case Report: Sirolimus Alleviates Persistent Cytopenia After CD19 CAR-T-Cell Therapy. *Front Oncol* (2021) 11:798352. doi: 10.3389/fonc.2021.798352
156. Wang Y, Qi K, Cheng H, Cao J, Shi M, Qiao J, et al. Coagulation Disorders After Chimeric Antigen Receptor T Cell Therapy: Analysis of 100 Patients With Relapsed and Refractory Hematologic Malignancies. *Biol Blood Marrow Transplant* (2020) 26(5):865–75. doi: 10.1016/j.bbmt.2019.11.027
157. Johnsrud A, Craig J, Baird J, Spiegel J, Muffly L, Zehnder J, et al. Incidence and Risk Factors Associated With Bleeding and Thrombosis Following Chimeric Antigen Receptor T-Cell Therapy. *Blood Adv* (2021) 5(21):4465–75. doi: 10.1182/bloodadvances.2021004716
158. Shao M, Yu Q, Teng X, Guo X, Wei G, Xu H, et al. CRS-Related Coagulopathy in BCMA Targeted CAR-T Therapy: A Retrospective Analysis in a Phase I/II Clinical Trial. *Bone Marrow Transplant* (2021) 56 (7):1642–50. doi: 10.1038/s41409-021-01226-9
159. Danese S, de la Motte C, Reyes BM, Sans M, Levine AD, Fiocchi C. Cutting Edge: T Cells Trigger CD40-Dependent Platelet Activation and Granular RANTES Release: A Novel Pathway for Immune Response Amplification. *J Immunol* (2004) 172:2011–5. doi: 10.4049/jimmunol.172.4.2011
160. Lindmark E, Tenno T, Siegbahn A. Role of Platelet P-Selectin and CD40 Ligand in the Induction of Monocytic Tissue Factor Expression. *Arterioscler Thromb Vasc Biol* (2000) 20(10):2322–8. doi: 10.1161/01.atv.20.10.2322
161. Hottz ED, Azevedo-Quintanilha IG, Palhinha L, Teixeira L, Barreto EA, Pão CRR, et al. Platelet Activation and Platelet-Monocyte Aggregate Formation Trigger Tissue Factor Expression in Patients With Severe COVID-19. *Blood* (2020) 136(11):1330–41. doi: 10.1182/blood.2020007252
162. Henn V, Slupsky JR, Gräfe M, Anagnostopoulos I, Förster R, Müller-Berghaus G, et al. CD40 Ligand on Activated Platelets Triggers an Inflammatory Reaction of Endothelial Cells. *Nature* (1998) 391(6667):591–4. doi: 10.1038/35393
163. Cermak J, Key NS, Bach RR, Balla J, Jacob HS, Vercellotti GM. C-Reactive Protein Induces Human Peripheral Blood Monocytes to Synthesize Tissue Factor. *Blood* (1993) 82(2):513–20. doi: 10.1182/blood.V82.2.513.513
164. Khan MM, Liu Y, Khan ME, Gilman ML, Khan ST, Bromberg M, et al. Upregulation of Tissue Factor in Monocytes by Cleaved High Molecular Weight Kininogen is Dependent on TNF-Alpha and IL-1beta. *Am J Physiol Heart Circ Physiol* (2010) 298(2):H652–8. doi: 10.1152/ajpheart.00825.2009
165. Mach F, Schönbeck U, Bonnefoy JY, Pober JS, Libby P. Activation of Monocyte/Macrophage Functions Related to Acute Atheroma Complication by Ligation of CD40: Induction of Collagenase, Stromelysin, and Tissue Factor. *Circulation* (1997) 96(2):396–9. doi: 10.1161/01.cir.96.2.396
166. Bernardo A, Ball C, Nolasco L, Moake JF, Dong JF. Effects of Inflammatory Cytokines on the Release and Cleavage of the Endothelial Cell-Derived Ultralarge Von Willebrand Factor Multimers Under Flow. *Blood* (2004) 104 (1):100–6. doi: 10.1182/blood-2004-01-0107
167. Cao WJ, Niiya M, Zheng XW, Shang DZ, Zheng XL. Inflammatory Cytokines Inhibit ADAMTS13 Synthesis in Hepatic Stellate Cells and Endothelial Cells. *J Thromb Haemost* (2008) 6(7):1233–5. doi: 10.1111/j.1538-7836.2008.02989.x
168. Ikezoe T. Advances in the Diagnosis and Treatment of Disseminated Intravascular Coagulation in Haematological Malignancies. *Int J Hematol* (2021) 113(1):34–44. doi: 10.1007/s12185-020-02992-w
169. Guo Z, Tu S, Yu S, Wu L, Pan W, Chang N, et al. Preclinical and Clinical Advances in Dual-Target Chimeric Antigen Receptor Therapy for Hematological Malignancies. *Cancer Sci* (2021) 112(4):1357–68. doi: 10.1111/cas.14799
170. Neelapu SS, Ghobadi A, Jacobson CA, Miklos DB, Lekakis LJ, Oluwole O, et al. 2-Year Follow-Up and High-Risk Subset Analysis of Zuma-1, the Pivotal Study of Axicabtagene Ciloleucel (Axi-Cel) in Patients With Refractory Large B Cell Lymphoma. *Biol Blood Marrow Tr* (2019) 25(3): S65. doi: 10.1016/j.bbmt.2018.12.148
171. Orlando EJ, Han X, Tribouley C, Wood PA, Leary RJ, Riester M, et al. Genetic Mechanisms of Target Antigen Loss in CAR19 Therapy of Acute Lymphoblastic Leukemia. *Nat Med* (2018) 24(10):1504–6. doi: 10.1038/s41591-018-0146-z
172. Jacoby E, Nguyen SM, Fountaine TJ, Welp K, Gryder B, Qin H, et al. CD19 CAR Immune Pressure Induces B-Precursor Acute Lymphoblastic Leukaemia Lineage Switch Exposing Inherent Leukaemic Plasticity. *Nat Commun* (2016) 7:12320. doi: 10.1038/ncomms12320
173. Samur MK, Fulciniti M, Aktas Samur A, Bazarbachi AH, Tai YT, Prabhala R, et al. Biallelic Loss of BCMA as a Resistance Mechanism to CAR T Cell Therapy in a Patient With Multiple Myeloma. *Nat Commun* (2021) 12 (1):868. doi: 10.1038/s41467-021-21177-5
174. Hamieh M, Dobrin A, Cabriolu A, van der Stegen SJC, Giavridis T, Mansilla-Soto J, et al. CAR T Cell Trogocytosis and Cooperative Killing Regulate Tumour Antigen Escape. *Nature* (2019) 568(7750):112–6. doi: 10.1038/s41586-019-1054-1
175. Ruella M, Xu J, Barrett DM, Fraietta JA, Reich TJ, Ambrose DE, et al. Induction of Resistance to Chimeric Antigen Receptor T Cell Therapy by Transduction of a Single Leukemic B Cell. *Nat Med* (2018) 24(10):1499–503. doi: 10.1038/s41591-018-0201-9
176. Zanetti SR, Velasco-Hernandez T, Gutierrez-Agüera F, Díaz VM, Romecín PA, Roca-Ho H, et al. A Novel and Efficient Tandem CD19- and CD22-Directed CAR for B Cell ALL. *Mol Ther* (2022) 30(2):550–63. doi: 10.1016/j.ymthe.2021.08.033
177. Tong C, Zhang Y, Liu Y, Ji X, Zhang W, Guo Y, et al. Optimized Tandem CD19/CD20 CAR-Engineered T Cells in Refractory/Relapsed B-Cell Lymphoma. *Blood* (2020) 136(14):1632–44. doi: 10.1182/blood.2020005278
178. Shah NN, Johnson BD, Schneider D, Zhu F, Szabo A, Keever-Taylor CA, et al. Bispecific Anti-CD20, Anti-CD19 CAR T Cells for Relapsed B Cell Malignancies: A Phase 1 Dose Escalation and Expansion Trial. *Nat Med* (2020) 26(10):1569–75. doi: 10.1038/s41591-020-1081-3
179. Wei G, Zhang Y, Zhao H, Wang Y, Liu Y, Liang B, et al. CD19/CD22 Dual-Targeted CAR T-Cell Therapy for Relapsed/Refractory Aggressive B-Cell Lymphoma: A Safety and Efficacy Study. *Cancer Immunol Res* (2021) 9 (9):1061–70. doi: 10.1158/2326-6066.CIR-20-0675

180. Dai H, Wu Z, Jia H, Tong C, Guo Y, Ti D, et al. Bispecific CAR-T Cells Targeting Both CD19 and CD22 for Therapy of Adults With Relapsed or Refractory B Cell Acute Lymphoblastic Leukemia. *J Hematol Oncol* (2020) 13 (1):30. doi: 10.1186/s13045-020-00856-8
181. Schneider D, Xiong Y, Wu D, Hu P, Alabanza L, Steimle B, et al. Trispecific CD19-CD20-CD22-Targeting duoCAR-T Cells Eliminate Antigen-Heterogeneous B Cell Tumors in Preclinical Models. *Sci Transl Med* (2021) 13(586):eabc6401. doi: 10.1126/scitranslmed.abc6401
182. Wong DP, Roy NK, Zhang K, Anukanth A, Asthana A, Shirkey-Son NJ, et al. A BAFF Ligand-Based CAR-T Cell Targeting Three Receptors and Multiple B Cell Cancers. *Nat Commun* (2022) 13(1):217. doi: 10.1038/s41467-021-27853-w
183. Pont MJ, Hill T, Cole GO, Abbott JJ, Kelliher J, Salter AI, et al. Gamma-Secretase Inhibition Increases Efficacy of BCMA-Specific Chimeric Antigen Receptor T Cells in Multiple Myeloma. *Blood* (2019) 134(19):1585–97. doi: 10.1182/blood.2019000050
184. Correia DV, Lopes A, Silva-Santos B. Tumor Cell Recognition by γδ T Lymphocytes: T-Cell Receptor vs. NK-Cell Receptors. *Oncoimmunology* (2013) 2(1):e22892. doi: 10.4161/onci.22892
185. Lança T, Correia DV, Moita CF, Raquel H, Neves-Costa A, Ferreira C, et al. The MHC Class Ib Protein ULBP1 is a Nonredundant Determinant of Leukemia/Lymphoma Susceptibility to Gammadelta T-Cell Cytotoxicity. *Blood* (2010) 115(12):2407–11. doi: 10.1182/blood-2009-08-237123
186. Pistoia V, Tumino N, Vacca P, Veneziani I, Moretta A, Locatelli F, et al. Human γδ T-Cells: From Surface Receptors to the Therapy of High-Risk Leukemias. *Front Immunol* (2018) 9:984. doi: 10.3389/fimmu.2018.00984
187. Rozenbaum M, Meir A, Aharony Y, Itzhaki O, Schachter J, Bank I, et al. Gamma-Delta CAR-T Cells Show CAR-Directed and Independent Activity Against Leukemia. *Front Immunol* (2020) 11:1347. doi: 10.3389/fimmu.2020.01347
188. Airoldi I, Bertaina A, Prigione I, Zorzoli A, Pagliara D, Cocco C, et al. γδ T-Cell Reconstitution After HLA-Haploidentical Hematopoietic Transplantation Depleted of TCR-αβ+/CD19+ Lymphocytes. *Blood* (2015) 125(15):2349–58. doi: 10.1182/blood-2014-09-599423
189. Handgretinger R, Schilbach K. The Potential Role of γδ T Cells After Allogeneic HCT for Leukemia. *Blood* (2018) 131(10):1063–72. doi: 10.1182/blood-2017-08-752162
190. McLellan AD, Ali Hosseini Rad SM. Chimeric Antigen Receptor T Cell Persistence and Memory Cell Formation. *Immunol Cell Biol* (2019) 97 (7):664–74. doi: 10.1111/imcb.12254
191. Long AH, Haso WM, Shern JF, Wanhainen KM, Murgai M, Ingaramo M, et al. 4-1BB Costimulation Ameliorates T Cell Exhaustion Induced by Tonic Signaling of Chimeric Antigen Receptors. *Nat Med* (2015) 21(6):581–90. doi: 10.1038/nm.3838
192. Li G, Boucher JC, Kotani H, Park K, Zhang Y, Shrestha B, et al. 4-1BB Enhancement of CAR T Function Requires NF-κb and TRAFs. *JCI Insight* (2018) 3(18):e121322. doi: 10.1172/jci.insight.121322
193. Zhao Z, Condomines M, van der Stegen SJC, Perna F, Kloss CC, Gunset G, et al. Structural Design of Engineered Costimulation Determines Tumor Rejection Kinetics and Persistence of CAR T Cells. *Cancer Cell* (2015) 28 (4):415–28. doi: 10.1016/j.ccell.2015.09.004
194. Roselli E, Boucher JC, Li G, Kotani H, Spitler K, Reid K, et al. 4-1BB and Optimized CD28 Co-Stimulation Enhances Function of Human Mono-Specific and Bi-Specific Third-Generation CAR T Cells. *J Immunother Cancer* (2021) 9(10):e003354. doi: 10.1136/jitc-2021-003354
195. Weinkove R, George P, Dasyam N, McLellan AD. Selecting Costimulatory Domains for Chimeric Antigen Receptors: Functional and Clinical Considerations. *Clin Transl Immunol* (2019) 8(5):e1049. doi: 10.1002/cti2.1049
196. Ramos CA, Rouce R, Robertson CS, Reyna A, Narala N, Vyas G, et al. In Vivo Fate and Activity of Second- Versus Third-Generation CD19-Specific CAR-T Cells in B Cell Non-Hodgkin's Lymphomas. *Mol Ther* (2018) 26(12):2727–37. doi: 10.1016/j.ymthe.2018.09.009
197. Myers RM, Li Y, Barz Leahy A, Barrett DM, Teachey DT, Callahan C, et al. Humanized CD19-Targeted Chimeric Antigen Receptor (CAR) T Cells in CAR-Naive and CAR-Exposed Children and Young Adults With Relapsed or Refractory Acute Lymphoblastic Leukemia. *J Clin Oncol* (2021) 39 (27):3044–55. doi: 10.1200/JCO.20.03458
198. Taniuchi I. CD4 Helper and CD8 Cytotoxic T Cell Differentiation. *Annu Rev Immunol* (2018) 36:579–601. doi: 10.1146/annurev-immunol-042617-053411
199. Agarwal S, Hanauer JDS, Frank AM, Riechert V, Thalheimer FB, Buchholz CJ. *In Vivo* Generation of CAR T Cells Selectively in Human CD4+ Lymphocytes. *Mol Ther* (2020) 28(8):1783–94. doi: 10.1016/j.ymthe.2020.05.005
200. Turtle CJ, Hanafi LA, Berger C, Gooley TA, Cherian S, Hudecek M, et al. CD19 CAR-T Cells of Defined CD4+:CD8+ Composition in Adult B Cell ALL Patients. *J Clin Invest* (2016) 126(6):2123–38. doi: 10.1172/JCI85309
201. Liadi I, Singh H, Romain G, Rey-Villamizar N, Merouane A, Adolacion JR, et al. Individual Motile CD4(+) T Cells Can Participate in Efficient Multikilling Through Conjugation to Multiple Tumor Cells. *Cancer Immunol Res* (2015) 3(5):473–82. doi: 10.1158/2326-6066.CIR-14-0195
202. Melenhorst JJ, Chen GM, Wang M, Porter DL, Chen C, Collins MA, et al. Decade-Long Leukaemia Remissions With Persistence of CD4+ CAR T Cells. *Nature* (2022) 602(7897):503–9. doi: 10.1038/s41586-021-04390-6
203. Diaconu I, Ballard B, Zhang M, Chen Y, West J, Dotti G, et al. Inducible Caspase-9 Selectively Modulates the Toxicities of CD19-Specific Chimeric Antigen Receptor-Modified T Cells. *Mol Ther* (2017) 25(3):580–92. doi: 10.1016/j.ymthe.2017.01.011
204. Wu CY, Roybal KT, Puchner EM, Onuffer J, Lim WA. Remote Control of Therapeutic T Cells Through a Small Molecule-Gated Chimeric Receptor. *Science* (2015) 350(6258):aab4077. doi: 10.1126/science.aab4077
205. Aspuria PJ, Vivona S, Bauer M, Semana M, Ratti N, McCauley S, et al. An Orthogonal IL-2 and IL-2rβ System Drives Persistence and Activation of CAR T Cells and Clearance of Bulky Lymphoma. *Sci Transl Med* (2021) 13 (625):eabg7565. doi: 10.1126/scitranslmed.abg7565
206. Zhang Q, Hresko ME, Picton LK, Su L, Hollander MJ, Nunez-Cruz S, et al. A Human Orthogonal IL-2 and IL-2rβ System Enhances CAR T Cell Expansion and Antitumor Activity in a Murine Model of Leukemia. *Sci Transl Med* (2021) 13(625):eabg6986. doi: 10.1126/scitranslmed.abg6986
207. Jan M, Scarfò I, Larson RC, Walker A, Schmidts A, Guirguis AA, et al. Reversible ON- and OFF-Switch Chimeric Antigen Receptors Controlled by Lenalidomide. *Sci Transl Med* (2021) 13(575):eabb6295. doi: 10.1126/scitranslmed.abb6295
208. Park S, Pascua E, Lindquist KC, Kimberlin C, Deng X, Mak YSL, et al. Direct Control of CAR T Cells Through Small Molecule-Regulated Antibodies. *Nat Commun* (2021) 12(1):710. doi: 10.1038/s41467-020-20671-6
209. Paszkiewicz PJ, Fräßle SP, Srivastava S, Sommermeyer D, Hudecek M, Drexler I, et al. Targeted Antibody-Mediated Depletion of Murine CD19 CAR T Cells Permanently Reverses B Cell Aplasia. *J Clin Invest* (2016) 126 (11):4262–72. doi: 10.1172/JCI84813
210. Cieri N, Camisa B, Cocchiarella F, Forcato M, Oliveira G, Provasi E, et al. IL-7 and IL-15 Instruct the Generation of Human Memory Stem T Cells From Naive Precursors. *Blood* (2013) 121(4):573–84. doi: 10.1182/blood-2012-05-431718
211. 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(7):2130–41. doi: 10.1016/j.celrep.2018.04.051
212. Ma A, Koka R, Burkett P. Diverse Functions of IL-2, IL-15, and IL-7 in Lymphoid Homeostasis. *Annu Rev Immunol* (2006) 24:657–79. doi: 10.1146/annurev.immunol.24.021605.090727
213. Shum T, Omer B, Tashiro H, Kruse RL, Wagner DL, Parikh K, et al. Constitutive Signaling From an Engineered IL7 Receptor Promotes Durable Tumor Elimination by Tumor-Redirected T Cells. *Cancer Discov* (2017) 7 (11):1238–47. doi: 10.1158/2159-8290.CD-17-0538
214. Cui W, Liu Y, Weinstein JS, Craft J, Kaech SM. An Interleukin-21-Interleukin-10-STAT3 Pathway is Critical for Functional Maturation of Memory CD8+ T Cells. *Immunity* (2011) 35(5):792–805. doi: 10.1016/j.immuni.2011.09.017
215. Battram AM, Bachiller M, Lopez V, Fernández de Larrea C, Urbano-Ispizua A, Martín-Antonio B. IL-15 Enhances the Persistence and Function of BCMA-Targeting CAR-T Cells Compared to IL-2 or IL-15/IL-7 by Limiting CAR-T Cell Dysfunction and Differentiation. *Cancers (Basel)* (2021) 13(14):3534. doi: 10.3390/cancers13143534

216. Zhou J, Jin L, Wang F, Zhang Y, Liu B, Zhao T. Chimeric Antigen Receptor T (CAR-T) Cells Expanded With IL-7/IL-15 Mediate Superior Antitumor Effects. *Protein Cell* (2019) 10(10):764–9. doi: 10.1007/s13238-019-0643-y
217. Alizadeh D, Wong RA, Yang X, Wang D, Pecoraro JR, Kuo CF, et al. IL15 Enhances CAR-T Cell Antitumor Activity by Reducing Mtorc1 Activity and Preserving Their Stem Cell Memory Phenotype. *Cancer Immunol Res* (2019) 7(5):759–72. doi: 10.1158/2326-6066.CIR-18-0466
218. Loschinski R, Böttcher M, Stoll A, Bruns H, Mackensen A, Mougiakakos D. IL-21 Modulates Memory and Exhaustion Phenotype of T-Cells in a Fatty Acid Oxidation-Dependent Manner. *Oncotarget* (2018) 9(17):13125–38. doi: 10.18632/oncotarget.24442
219. Curran KJ, Seinstra BA, Nikhamin Y, Yeh R, Usachenko Y, van Leeuwen DG, et al. Enhancing Antitumor Efficacy of Chimeric Antigen Receptor T Cells Through Constitutive CD40L Expression. *Mol Ther* (2015) 23(4):769–78. doi: 10.1038/mt.2015.4
220. Kuhn NF, Purdon TJ, van Leeuwen DG, Lopez AV, Curran KJ, Daniyan AF, et al. CD40 Ligand-Modified Chimeric Antigen Receptor T Cells Enhance Antitumor Function by Eliciting an Endogenous Antitumor Response. *Cancer Cell* (2019) 35(3):473–488.e6. doi: 10.1016/j.ccell.2019.02.006
221. 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(6):1160–70. doi: 10.1038/leu.2010.75
222. Štach M, Ptáčková P, Mucha M, Musil J, Klener P, Otáhal P. Inducible Secretion of IL-21 Augments Anti-Tumor Activity of Piggybac-Manufactured Chimeric Antigen Receptor T Cells. *Cytotherapy* (2020) 22 (12):744–54. doi: 10.1016/j.jcyt.2020.08.005
223. Azmi AS, Uddin MH, Mohammad RM. The Nuclear Export Protein XPO1-From Biology to Targeted Therapy. *Nat Rev Clin Oncol* (2021) 18(3):152–69. doi: 10.1038/s41571-020-00442-4
224. Syed YY. Selinexor: First Global Approval. *Drugs* (2019) 79(13):1485–94. doi: 10.1007/s40265-019-01188-9
225. Wang S, Sellner L, Wang L, Sauer T, Neuber B, Gong W, et al. Combining Selective Inhibitors of Nuclear Export (SINEs) With Chimeric Antigen Receptor (CAR) T Cells for CD19-positive Malignancies. *Oncol Rep* (2021) 46(2):170. doi: 10.3892/or.2021.8121
226. Works M, Soni N, Hauskins C, Sierra C, Baturevych A, Jones JC, et al. Anti-B-Cell Maturation Antigen Chimeric Antigen Receptor T Cell Function Against Multiple Myeloma Is Enhanced in the Presence of Lenalidomide. *Mol Cancer Ther* (2019) 1818(12):2246–57. doi: 10.1158/1535-7163.MCT-18-1146
227. Parrondo RD, Sam K, Rasheed A, Alegria V, Sher T, Roy V, et al. Subsequent Anti-Myeloma Therapy After Idecabtagene Vicleucel Treatment in Patients With Relapsed/Refractory Multiple Myeloma: A Single Center Analysis. *Blood Cancer J* (2022) 12(4):66. doi: 10.1038/s41408-022-00662-0
228. Chari A, Vogl DT, Jagannath S, Jasielec J, Unger TJ, DeCastro A, et al. Selinexor-Based Regimens for the Treatment of Myeloma Refractory to Chimeric Antigen Receptor T Cell Therapy. *Br J Haematol* (2020) 189(4): e126–30. doi: 10.1111/bjh.16550
229. Zhao G, Wei R, Feng L, Wu Y, He F, Xiao M, et al. Lenalidomide Enhances the Efficacy of Anti-BCMA CAR-T Treatment in Relapsed/Refractory Multiple Myeloma: A Case Report and Revies of the Literature. *Cancer Immunol Immunother* (2022) 71(1):39–44. doi: 10.1007/s00262-021-02959-8
230. Gauthier J, Hirayama AV, Purushe J, Hay KA, Lymp J, Li DH, et al. Feasibility and Efficacy of CD19-Targeted CAR T Cells With Concurrent Ibrutinib for CLL After Ibrutinib Failure. *Blood* (2020) 135(19):1650–60. doi: 10.1182/blood.2019002936
231. Liu M, Deng H, Mu J, Li Q, Pu Y, Jiang Y, et al. Ibrutinib Improves the Efficacy of Anti-CD19-CAR T-Cell Therapy in Patients With Refractory non-Hodgkin Lymphoma. *Cancer Sci* (2021) 112(7):2642–51. doi: 10.1111/cas.14915
232. Liu M, Wang X, Li Z, Zhang R, Mu J, Jiang Y, et al. Synergistic Effect of Ibrutinib and CD19 CAR-T Cells on Raji Cells *In Vivo* and *In Vitro*. *Cancer Sci* (2020) 111(11):4051–60. doi: 10.1111/cas.14638
233. Gill SI, Frey NV, Hexner E, Metzger S, O'Brien M, Hwang WT, et al. Anti-CD19 CAR T Cells in Combination With Ibrutinib for the Treatment of Chronic Lymphocytic Leukemia. *Blood Adv* (2022). doi: bloodadvances.2022007317 doi: 10.1182/bloodadvances.2022007317
234. You L, Han Q, Zhu L, Zhu Y, Bao C, Yang C, et al. Decitabine-Mediated Epigenetic Reprograming Enhances Anti-Leukemia Efficacy of CD123-Targeted Chimeric Antigen Receptor T-Cells. *Front Immunol* (2020) 11:1787. doi: 10.3389/fimmu.2020.01787
235. El Khawanky N, Hughes A, Yu W, Myburgh R, Matschulla T, Taromi S, et al. Demethylating Therapy Increases Anti-CD123 CAR T Cell Cytotoxicity Against Acute Myeloid Leukemia. *Nat Commun* (2021) 12(1):6436. doi: 10.1038/s41467-021-26683-0
236. Li S, Xue L, Wang M, Qiang P, Xu H, Zhang X, et al. Decitabine Enhances Cytotoxic Effect of T Cells With an Anti-CD19 Chimeric Antigen Receptor in Treatment of Lymphoma. *Onco Targets Ther* (2019) 12:5627–38. doi: 10.2147/OTT.S198567
237. Holthof LC, van der Schans JJ, Katsarou A, Poels R, Gelderloos AT, Drent E, et al. Bone Marrow Mesenchymal Stromal Cells Can Render Multiple Myeloma Cells Resistant to Cytotoxic Machinery of CAR T Cells Through Inhibition of Apoptosis. *Clin Cancer Res* (2021) 27(13):3793–803. doi: 10.1158/1078-0432.CCR-20-2188
238. Pinnix CC, Gunther JR, Dabaja BS, Strati P, Fang P, Hawkins MC, et al. Bridging Therapy Prior to Axicabtagene Ciloleucel for Relapsed/Refractory Large B-Cell Lymphoma. *Blood Adv* (2020) 4(13):2871–83. doi: 10.1182/bloodadvances.2020001837
239. Wright CM, LaRiviere MJ, Baron JA, Uche C, Xiao Y, Arscott WT, et al. Bridging Radiation Therapy Before Commercial Chimeric Antigen Receptor T-Cell Therapy for Relapsed or Refractory Aggressive B-Cell Lymphoma. *Int J Radiat Oncol Biol Phys* (2020) 108(1):178–88. doi: 10.1016/j.ijrobp.2020.05.014
240. Sim AJ, Jain MD, Figura NB, Chavez JC, Shah BD, Khimani F, et al. Radiation Therapy as a Bridging Strategy for CAR T Cell Therapy With Axicabtagene Ciloleucel in Diffuse Large B-Cell Lymphoma. *Int J Radiat Oncol Biol Phys* (2019) 105(5):1012–21. doi: 10.1016/j.ijrobp.2019.05.065
241. Smith EL, Mailankody S, Staehr M, Wang X, Senechal B, Purdon TJ, et al. BCMA-Targeted CAR T-Cell Therapy Plus Radiotherapy for the Treatment of Refractory Myeloma Reveals Potential Synergy. *Cancer Immunol Res* (2019) 7(7):1047–53. doi: 10.1158/2326-6066.CIR-18-0551
242. DeSelm C, Palomba ML, Yahalom J, Hamieh M, Eyquem J, Rajasekhar VK, et al. Low-Dose Radiation Conditioning Enables CAR T Cells to Mitigate Antigen Escape. *Mol Ther* (2018) 26(11):2542–52. doi: 10.1016/j.ymthe.2018.09.008
243. Pocaterra A, Catucci M, Mondino A. Adoptive T Cell Therapy of Solid Tumors: Time to Team Up With Immunogenic Chemo/Radiotherapy. *Curr Opin Immunol* (2021) 74:53–9. doi: 10.1016/j.coi.2021.10.004
244. Spiotto M, Fu YX, Weichselbaum RR. The Intersection of Radiotherapy and Immunotherapy: Mechanisms and Clinical Implications. *Sci Immunol* (2016) 1(3):EAAG1266. doi: 10.1126/sciimmunol.aag1266
245. Sridharan V, Margalit DN, Lynch SA, Severgnini M, Zhou J, Chau NG, et al. Definitive Chemoradiation Alters the Immunologic Landscape and Immune Checkpoints in Head and Neck Cancer. *Br J Cancer* (2016) 115(2):252–60. doi: 10.1038/bjc.2016.166
246. Asimakopoulos F, Hope C, Johnson MG, Pagenkopf A, Gromek K, Nagel B. Extracellular Matrix and the Myeloid-in-Myeloma Compartment: Balancing Tolerogenic and Immunogenic Inflammation in the Myeloma Niche. *J Leukoc Biol* (2017) 102(2):265–75. doi: 10.1189/jlb.3MR1116-468R
247. Pimenta DB, Varela VA, Datoguia TS, Caraciolo VB, Lopes GH, Pereira WO. The Bone Marrow Microenvironment Mechanisms in Acute Myeloid Leukemia. *Front Cell Dev Biol* (2021) 9:764698. doi: 10.3389/fcell.2021.764698
248. Epperly R, Gottschalk S, Velasquez MP. A Bump in the Road: How the Hostile AML Microenvironment Affects CAR T Cell Therapy. *Front Oncol* (2020) 10:262. doi: 10.3389/fonc.2020.00262
249. Holthof LC, Mutis T. Challenges for Immunotherapy in Multiple Myeloma: Bone Marrow Microenvironment-Mediated Immune Suppression and Immune Resistance. *Cancers (Basel)* (2020) 12(4):988. doi: 10.3390/cancers12040988
250. Autio M, Leivonen SK, Brück O, Karjalainen-Lindsberg ML, Pellinen T, Leppä S. Clinical Impact of Immune Cells and Their Spatial Interactions in Diffuse Large B-Cell Lymphoma Microenvironment. *Clin Cancer Res* (2022) 28(4):781–92. doi: 10.1158/1078-0432.CCR-21-3140

251. Kline J, Godfrey J, Ansell SM. The Immune Landscape and Response to Immune Checkpoint Blockade Therapy in Lymphoma. *Blood* (2020) 135 (8):523–33. doi: 10.1182/blood.2019000847
252. Crespo J, Sun H, Welling TH, Tian Z, Zhou W. T Cell Anergy, Exhaustion, Senescence, and Stemness in the Tumor Microenvironment. *Curr Opin Immunol* (2013) 25:214–21. doi: 10.1016/j.coi.2012.12.003
253. Dhakal B, Hari PN, Usmani SZ, Hamadani M. Chimeric Antigen Receptor T Cell Therapy in Multiple Myeloma: Promise and Challenges. *Bone Marrow Transplant* (2021) 56(1):9–19. doi: 10.1038/s41409-020-01023-w
254. Hatic H, Sampat D, Goyal G. Immune Checkpoint Inhibitors in Lymphoma: Challenges and Opportunities. *Ann Transl Med* (2021) 9(12):1037. doi: 10.1038/s41409-020-01023-w
255. Song MK, Park BB, Uhm J. Understanding Immune Evasion and Therapeutic Targeting Associated With PD-1/PD-L1 Pathway in Diffuse Large B-Cell Lymphoma. *Int J Mol Sci* (2019) 20(6):1326. doi: 10.3390/ijms20061326
256. Cao Y, Lu W, Sun R, Jin X, Cheng L, He X, et al. Anti-CD19 Chimeric Antigen Receptor T Cells in Combination With Nivolumab are Safe and Effective Against Relapsed/Refractory B-Cell non-Hodgkin Lymphoma. *Front Oncol* (2019) 9:767. doi: 10.3389/fonc.2019.00767
257. Wang J, Deng Q, Jiang YY, Zhang R, Zhu HB, Meng JX, et al. CAR-T 19 Combined With Reduced-Dose PD-1 Blockade Therapy for Treatment of Refractory Follicular Lymphoma: A Case Report. *Oncol Lett* (2019) 18 (5):4415–20. doi: 10.3892/ol.2019.10783
258. Song W, Zhang M. Use of CAR-T Cell Therapy, PD-1 Blockade, and Their Combination for the Treatment of Hematological Malignancies. *Clin Immunol* (2020) 214:108382. doi: 10.1016/j.clim.2020.108382
259. John LB, Kershaw MH, Darcy PK. Blockade of PD-1 Immunosuppression Boosts CAR T-Cell Therapy. *Oncoimmunology* (2013) 2(10):e26286. doi: 10.4161/onci.26286
260. Zhang R, Deng Q, Jiang YY, Zhu HB, Wang J, Zhao MF. Effect and Changes in PD-1 Expression of CD19 CAR-T Cells From T Cells Highly Expressing PD-1 Combined With Reduced-Dose PD-1 Inhibitor. *Oncol Rep* (2019) 41 (6):3455–63. doi: 10.3892/or.2019.7096
261. Rafiq S, Yeku OO, Jackson HJ, Purdon TJ, van Leeuwen DG, Drakes DJ, et al. Targeted Delivery of a PD-1-Blocking scFv by CAR-T Cells Enhances Anti-Tumor Efficacy *In Vivo*. *Nat Biotechnol* (2018) 36(9):847–56. doi: 10.1038/nbt.4195
262. Kenderian SS, Ruella M, Shestova O, Klichinsky M, Kim M, Porter DL, et al. Identification of PD1 and TIM3 As Checkpoints That Limit Chimeric Antigen Receptor T Cell Efficacy in Leukemia. *Biol Blood Marrow Transplant* (2016) 22(3):S19–21. doi: 10.1016/j.bbmt.2015.11.291
263. Wenthe J, Naseri S, Labani-Motlagh A, Enblad G, Wikström KI, Eriksson E, et al. Boosting CAR T-Cell Responses in Lymphoma by Simultaneous Targeting of CD40/4-1BB Using Oncolytic Viral Gene Therapy. *Cancer Immunol Immunother* (2021) 70(10):2851–65. doi: 10.1007/s00262-021-02895-7
264. Eriksson E, Milenova I, Wenthe J, Ståhle M, Leja-Jarblad J, Ullenhag G, et al. Shaping the Tumor Stroma and Sparking Immune Activation by CD40 and 4-1BB Signaling Induced by an Armed Oncolytic Virus. *Clin Cancer Res* (2017) 23(19):5846–57. doi: 10.1158/1078-0432.CCR-17-0285
265. Jiang H, Fueyo J. Healing After Death: Antitumor Immunity Induced by Oncolytic Adenoviral Therapy. *Oncoimmunology* (2014) 3(7):e947872. doi: 10.4161/21624011
266. Workenhe ST, Mossman KL. Rewiring Cancer Cell Death to Enhance Oncolytic Viro-Immunotherapy. *Oncoimmunology* (2013) 2(12):e27138. doi: 10.4161/onci.27138
267. Wenthe J, Naseri S, Hellström AC, Wiklund HJ, Eriksson E, Loskog A. Immunostimulatory Oncolytic Virotherapy for Multiple Myeloma Targeting 4-1BB and/or CD40. *Cancer Gene Ther* (2020) 27(12):948–59. doi: 10.1038/s41417-020-0176-9
268. Alabanza LM, Xiong Y, Vu B, Webster B, Wu D, Hu P, et al. Armored BCMA CAR T Cells Eliminate Multiple Myeloma and Are Resistant to the Suppressive Effects of TGF-β. *Front Immunol* (2022) 13:832645. doi: 10.3389/fimmu.2022.832645
269. Yang Q, Hao J, Chi M, Wang Y, Xin B, Huang J, et al. Superior Antitumor Immunotherapy Efficacy of Kynureninase Modified CAR-T Cells Through Targeting Kynurenine Metabolism. *Oncoimmunology* (2022) 11(1):2055703. doi: 10.1080/2162402X.2022.2055703
270. Ruella M, Klichinsky M, Kenderian SS, Shestova O, Ziober A, Kraft DO, et al. Overcoming the Immunosuppressive Tumor Microenvironment of Hodgkin Lymphoma Using Chimeric Antigen Receptor T Cells. *Cancer Discov* (2017) 7:1154–67. doi: 10.1158/2159-8290.CD-16-0850

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The expression pattern of Immune checkpoints after chemo/radiotherapy in the tumor microenvironment

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As a disease with the highest disease-associated burden worldwide, cancer has been the main subject of a considerable proportion of medical research in recent years, intending to find more effective therapeutic approaches with fewer side effects. Combining conventional methods with newer biologically based treatments such as immunotherapy can be a promising approach to treating different tumors. The concept of "cancer immunoediting" that occurs in the field of the tumor microenvironment (TME) is the aspect of cancer therapy that has not been at the center of attention. One group of the role players of the so-called immunoediting process are the immune checkpoint molecules that exert either co-stimulatory or co-inhibitory effects in the anti-tumor immunity of the host. It involves alterations in a wide variety of immunologic pathways. Recent studies have proven that conventional cancer therapies, such as chemotherapy, radiotherapy, or a combination of them, i.e., chemoradiotherapy, alter the "immune compartment" of the TME. The mentioned changes encompass a wide range of variations, including the changes in the density and immunologic type of the tumor-infiltrating lymphocytes (TILs) and the alterations in the expression patterns of the different immune checkpoints. These rearrangements can have either anti-tumor immunity empowering or immune attenuating sequels. Thus, recognizing the consequences of various chemo(radio)therapeutic regimens in the TME seems to be of great significance in the evolution of therapeutic approaches. Therefore, the present review intends to summarize how chemo (radio)therapy affects the TME and specifically some of the most important,

well-known immune checkpoints' expressions according to the recent studies in this field.

KEYWORDS

cancer therapy, tumor microenvironment, chemo(radio)therapy, immune checkpoints, combination therapy

Introduction

Cancer is the second-most common etiology of death worldwide after cardiac disease (1). Cancers cause the most disease-associated burden among different diseases all around the world, which is about 244.6 million Disability-Adjusted Life Years (DALYs), even more than ischemic heart disease (IHD) (2). Despite significant improvements in cancer therapy, it is still one of the leading health issues. So the explorations to find different solutions for this problem are ongoing. Our immune system combats cancer through various mechanisms involving different types of immune cells and molecules, such as cytokines and immune checkpoints. Malignant tumor cells use a wide variety of mechanisms to avoid and attenuate the immune system, which leads to uncontrolled proliferation of the cells, invasion and metastasis of the tumor, and at last, morbidity and mortality of cancer (3). The field of this battle between the host's immune system and the tumor is known as the tumor microenvironment (TME), which is composed of different compartments such as the tumor and immune parts (4, 5). The tumor cells form and modulate the TME and dominate other components such as infiltrated immune cells and molecules (6). Immunotherapy is a relatively novel method of cancer therapy compared to conventional therapies such as chemo(radio)therapy. It acts by blocking the function of inhibitory immune checkpoints present on the various types of malignant and immune cells in the TME (7). Several studies have proven the efficacy of immunotherapy in treating different cancers. We can point to studies on various types of malignancies, including melanoma (8), non-small cell lung carcinoma (NSCLC) (9), head and neck malignancies (10), urinary tract cancers (11), colorectal carcinoma (CRC) (12), hepatocellular carcinoma (HCC) (13), Merkel cell carcinoma (14), and Hodgkin lymphoma (15). However, significant responses to immunotherapy are currently just seen in a limited number of cancers and patients. It indicates a need for searching for and designing more novel therapeutic strategies (16). One of these recently described novel approaches is the concept of "combination therapy."

In this approach, we benefit from two or more mechanistically different methods such as immunotherapy and chemo(radio) therapy or surgery to induce synergistic, additive, and more robust attacks combating cancer (17–20). Combining conventional chemo(radio)therapeutic methods with immunotherapy seems to be one of the promising approaches. The TME characteristics differ widely across different types of cancers. Several studies have shown that various chemo(radio) therapy regimens alter the TME. The quality and pattern of these changes are associated with the type of tumor and the agents used during treatment (21). To design more effective combination therapies, we need to become more familiar with the exact properties of the TME across different tumors and with the changes induced by the chemo(radio)therapy. Many studies have demonstrated the alterations in the expression patterns of the immune checkpoints, as the crucial immunomodulatory molecules in the TME, in response to different chemo(radio) therapeutic regimens (22). Increasing our knowledge about the exclusive characteristics of the immune checkpoints, their mechanism(s) of function, and the related molecular pathways can help us design more efficient blocking agents. These immune-checkpoint inhibitors (ICIs) can be utilized as complementary therapy based on the changes caused by the conventional approaches, specifically chemo(radio)therapy. In the current study, we have reviewed the detailed properties of the TME and mentioned the bilateral role of the immune checkpoints in immune system-tumor interactions. Also, we evaluated the studies that assessed the changes caused by adjuvant and neoadjuvant chemo (radio)therapeutic therapies in the expression patterns of clinically valuable immune checkpoints.

Tumor microenvironment - a key player in the immunoediting process and anti-tumor immunity

The concept of immune surveillance is the process of removing cancerous cells by the immune system based on recognizing specifically expressed neoantigens and stress-induced molecules in tumor cells. Lewis Thomas described this concept clearly and experimentally in the late 1950s for the first time (23, 24). Cancer immunoediting is a relatively new and more comprehensive concept, comprised of three phases:

elimination phase (involving immune surveillance), equilibrium phase, and escape phase (3). Immune cells and factors put as much pressure as possible on tumor cells that survived the previous stage in the equilibrium phase. A significant population of cancerous cells is destroyed in this course, while a proportion develops new mutations making them resistant to the immune system's attack. In the final escape phase, tumor variants that have become unsusceptible to the immune attacks extend in an unrestrained pattern (25). As a result, immunologically carved tumors expand steadily and become clinically evident (26). A wide variety of mechanisms altogether lead to the formation of tumor escape. These include decreased immune recognition by losing strong neoantigens, MHC class I, and co-stimulatory molecules. The other mechanism is increased resistance to cellular death by overexpression of anti-apoptotic molecules like Bcl-2. Tumors form an immunosuppressive tumor microenvironment (TME) by secreting cytokines like TGF-β and overexpressing co-inhibitory immune checkpoints such as Programmed Cell Death Protein 1(PD-1, CD279)/Programmed Death-ligand 1 (PD-L1), T cell Immunoglobulin domain, and Mucin domain 3 (TIM-3, CD366)/Galectin9, and Lymphocyte Activation Gene 3 (LAG-3) (27, 28).

The tumor microenvironment is a unique environment that arises in the context of tumor progression due to tumor-host interactions. It is composed of different elements such as proliferating tumor cells, tumor stroma, infiltrating immune cells, blood vessels, and related tissue cells (Figure 1). TME is constructed, reformed, and controlled by the tumor at all times and has dominance over molecular and cellular events happening in neighboring tissues (25). Types of immune cells from both innate and adaptive parts are present in the TME (29). Natural killer (NK) cells are the innate immune system's main effectors, constituting the first line of defense against tumors (30). Despite their ability to kill circulating cancerous cells, NK cell's significance for battling and destroying established solid tumors seems to be unsure in the result of several mechanisms compromising their capacity to eliminate solid tumor cells, such as their inability to penetrate the core of the tumor and various immunoediting events leading to tumor escape (31). Research assessing immunophenotypes of several types of solid tumors in

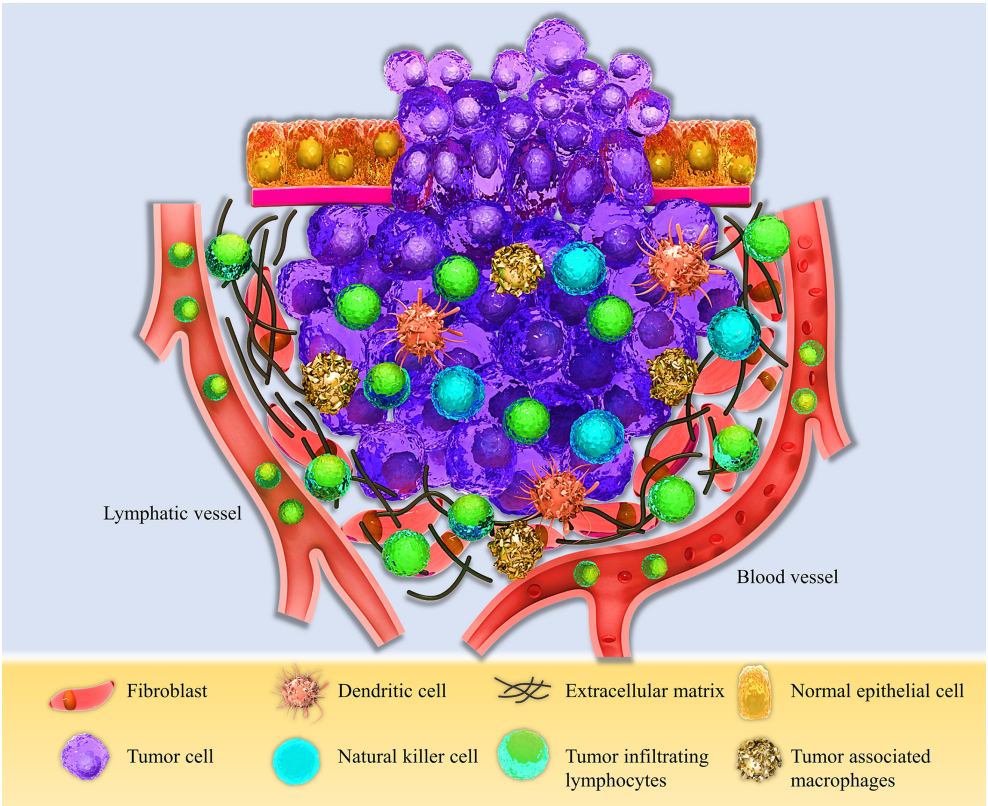

FIGURE 1
Schematic view of the Tumor microenvironment (TME). The TME consists of different compartments, including the proliferating tumor cells, tumor site, and tumor-infiltrating immune cells, such as Dendritic cells (DCs), Natural killer cells (NK cells), Tumor-associated macrophages (TAMs), and Tumor-infiltrating lymphocytes (TILs), and the stromal part containing fibroblasts, extracellular matrix, and lymphatic and blood vessels.

a wide population of patients with different types of cancers has shown some evidence of a T-cell infiltrated phenotype (32–34). Tumor-infiltrating lymphocytes (TILs) with various $CD4^+$ to $CD8^+$ T cells proportions build up a major part of TME. $CD8^+$ cytotoxic T lymphocytes (CTLs) have been historically considered the pivotal cells in the immune system's battle against tumors because of their ability to detect MHC class I mediated presentation of the intracellular antigens, expressed by all tumor cell types (35). $CD4^+$ T helper cells (Th) also have a crucial role in immune defense against malignancies by various mechanisms such as activating antigen-specific effector cells and alarming innate immune cells such as macrophages, mast cells, and eosinophils (36, 37). These cells are activated in two main ways, directly by MHC class II expressing tumor cells and indirectly by antigen-presenting cells (APCs) present at the TME, such as dendritic cells (DCs) (38). Antigen-primed Th cells can directly activate tumor-antigen-specific CTLs through different routes such as direct interaction, improving CTL activity by co-stimulatory molecules on the surface of CTL, like CD127, CD34, and MHC class II, and enhancing CTL growth by secreting cytokines such as IL-12 (39).

In conditions associated with chronic inflammation like cancer and chronic infection, persistent antigen presentation and stimulation of T cell receptor (TCR) leading to activation of CTLs results in a gradual decrease in the effector activity of CTLs that finally disturbs response to tumors and infections. This phenomenon is called exhaustion (40–42). In this process, inhibitory molecules such as PD-1, Cytotoxic T lymphocyte Antigen-4 (CTLA-4, CD152), LAG-3, TIM-3, CD160, and T cell Immunoreceptor with Ig and ITIM domain (TIGIT) are significantly overexpressed in exhausted T cells, so they do not respond properly to the stimulation of TCR by presented antigens (43, 44). Exhausted CD8+ T cells do not proliferate well because they have impaired killing activity and secrete Low levels of effector cytokines such as INF-γ and TNF-α (45). The other subsets of T cells in TME are regulatory T cells (Treg) expressing the Foxp3 (Forkhead Box P3) molecule as their primary marker. These cells play a central role in stabilizing immune homeostasis and preventing autoimmunity (46). Considering their ability to avoid self-antigen responses, they may restrict anti-tumor immune response by different mechanisms such as activating inhibitory molecules mentioned before, like CTLA-4 (47–49). Plenty of studies have shown considerable infiltration of Treg cells into different types of tumors, such as in the head and neck, breast, lung, gastrointestinal tract, liver, pancreas, and ovary. On this basis, depleting TME from Tregs or manipulating their function in a specific manner can experimentally induce efficacious tumor immunity (50). Basic science findings clarifying the molecular and cellular mechanisms involved in T cell biology, as the facts mentioned above, have given rise to new therapeutic approaches toward malignancies, including immune checkpoint blocking by immunotherapy (51).

Immune-checkpoints: A wide variety of molecules with a bilateral role in tumor-immune system battle

An appropriately working immune system protects the body from foreign pathogens and developing malignant tumors (52). Activation of immune effectors such as T cells is tightly controlled to prevent malfunctions such as dysregulations leading to autoimmunity. T lymphocytes need at least two stimulatory signals to be activated. The first signal is provided when the T cell receptor (TCR) recognizes the specific antigen the MHC molecule presents. A co-stimulatory signal is also needed to activate the T cell fully. For instance, CD80 or CD86 molecules on the surface of APCs interact with the CD28 molecule on the T cell and give rise to the co-stimulatory signal (53, 54). Also, in addition to immune checkpoints, different kinds of cytokines play crucial roles in this process (Figure 2).

Immune checkpoint receptors are in the membrane of various immune cells, mainly T cells and NK cells. When these cells face the specific antigens and previously mentioned ligands on the APCs, such as macrophages and DCs or the cancerous cells, they induce some signals which can be positive and stimulatory or negative and inhibitory. These signals originate from the interaction between these immune checkpoint receptors on the target cells and their ligands, i.e., checkpoint molecules on the effector cells. These negative and positive regulations exerted by the immune checkpoints and their receptors play a crucial role in stabilizing immune balance and homeostasis in the normal physiologic condition (55, 56).

Molecules such as CTLA-4, PD-1, TIM-3, TIGITT, and LAG-3 are checkpoint receptors with an immunosuppressive role. They generate inhibitory signals that avoid the full activation of effector cells, such as the CTLs. So, in the tumor-immune system battle, these molecules lead to immune exhaustion and provide a mechanism for the immune evasion of the tumors, suppressing the immune system's anti-tumor potentiality (57, 58). Among checkpoint receptors with a co-stimulatory function are glucocorticoid-induced TNFR-related protein (GITR), CD 27, CD40, and OX40 from the superfamily of tumor necrosis factor receptors (TNFR). CD28 and inducible T cell co-stimulator (ICOS) are also stimulatory checkpoint receptors belonging to the B7-CD28 superfamily (59). The incompetent function of these molecules in the effector T cells recognizing the neoantigens expressed by the malignant cells in the TME weakens the anti-tumor immune response providing another opportunity for the immune evasion of the tumors. The concept of immunotherapy is based on the knowledge gained through the years about the two categories of checkpoint receptors and their ligands mentioned above. Based on these two groups of immune checkpoint functions, i.e., their either co-inhibitory or co-stimulatory function, there exist two approaches

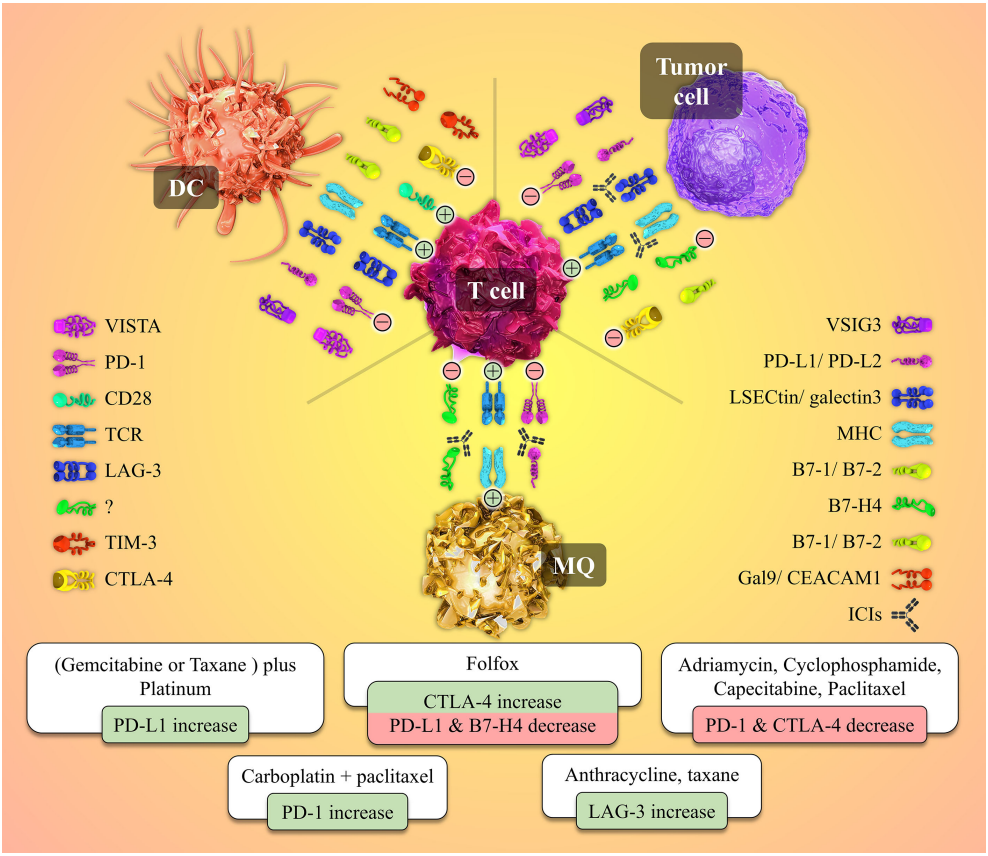

FIGURE 2
Cell to cell interactions and the role of the immune-checkpoint molecules and their receptors in the tumor microenvironment besides the patterns of immune-checkpoints expression patterns' changes post-NAC with different chemotherapeutic agents. Tumor site T-cells need two activating signals to defend against and kill the tumor cells (shown by plus mark in a circle). The first signal is provided by the interaction between the T cell receptor (TCR) and its specific antigen presented by the MHC molecule on the Antigen-presenting cells (APCs) or the tumor cell. The second signal is a co-stimulatory one originating from CD28 and B7-1/B7-2 molecules interaction. Tumor cells overexpress inhibitory immune checkpoints to produce inhibitory signals and neutralize the positive ones (shown by the negative mark in a circle). Immune checkpoint inhibitors (ICIs), as a wide variety of drugs used in the immunotherapy of cancers, block the mentioned co-inhibitory function of the checkpoint molecules. Different chemotherapeutic agents alter the expression patterns of immune-checkpoint molecules by whether down-regulating or up-regulating the expression of these immune markers.

toward modulating and boosting the immune system to defend against malignancies more efficiently. One approach is to inhibit and antagonize the inhibitory checkpoints to prevent T cell exhaustion. It neutralizes the immunosuppressive effects. Immune checkpoint inhibitors (ICIs) are monoclonal antibodies (mAbs) developed against various classical and recently discovered inhibitory immune checkpoints (Figure 2) (60–62). Some of these ICIs are FDA-approved and widely used in treating different kinds of tumors such as melanoma, small and non-small-cell lung cancers, renal cell carcinoma, and gastric cancers (63).

The second approach in immunotherapy is based on the concept that augmenting the stimulatory functions of co-stimulatory immune checkpoints can empower the effector cells such as CTLs in the TME. It leads to more effective killing of the tumor cells. It is achievable by designing and utilizing agonistic antibodies that improve the positive signaling of these checkpoint molecules. It leads to more effective immune responses against malignancies (55).

The second approach has more extensive effects on the T cells than the first approach. It originates from different types of tumors expressing inhibitory immune checkpoints in different patterns. So, inhibition of a particular checkpoint molecule by the specific ICI is only beneficial when the targeted tumor significantly expresses that molecule at high levels (55). Despite broader effects, the second approach is accompanied by more risk of dangerous adverse effects such as multiple organ failure due to cytokine storm caused by CD28 activating antibody, theralizumab (64). This phenomenon has restricted the clinical use of this approach.

There are some cardinal problems with using ICIs. The first issue is that the quantity of the T cells present in the TME is a restricting factor. Low numbers of the TILs in the tumor compartment of the TME weakens the response of the tumor to the ICIs. The other problem is the adaptation of cancerous cells to a specific ICI by upregulating other co-inhibitory immune checkpoints that preserves the negative signals and avoids reversing TILs exhaustion. The latter problem can be solved by designing and utilizing bispecific antibodies (bsAb) that target two checkpoints simultaneously. Some of these bsAbs are in the market now (65, 66).

To solve the first problem, i.e., low numbers of TILs in the tumor site, the immunogenicity of the cancerous cells should be improved. Prompting immunologically mediated tumor cell death by taking advantage of cytotoxic methods or procedures targeting specific immune molecules can increase immunogenicity. The approach to combining other biological and non-biological therapies with immune checkpoint inhibition by ICIs to improve its efficacy is indeed the so-called "combination therapies." Among non-biological therapeutic procedures are surgery, chemotherapy, and radiotherapy (67–69). Anti-cancer vaccines, antibodies against cytokines, oncolytic virotherapy, natural or synthetic cytokines, and chimeric antigen receptor (CAR) T cells are biological methods used in combination with ICIs (70–74). According to all we mentioned above, it is clear that cancer treatment is now multidimensional. Combining conventional approaches such as chemotherapy, radiotherapy, and surgery with relatively new therapies like immunotherapy and other biological methods may help us achieve better results by positively modifying the prognosis of different types of cancers (38). We need to improve our knowledge about the induced alterations in the TME of various tumors in response to adjuvant therapies. It helps us choose the most effective adjuvant therapies as different regimens used in the chemo(radio)therapy of cancers have diverse effects. Also, it can lead us to design more effective and specific combination therapies consisting of immunotherapy and conventional therapies. It is the first goal of this study. In the ongoing parts, we have reviewed the history, expression distribution, function, and changes in expression of some of the most important and clinically targeted immune checkpoints.

PD-1

Programmed death-1 (PD-1) molecule, as a member of the immunoglobulin gene superfamily, was first discovered in 1992 (75). PD-1 is expressed on the surface of particular subsets of T cells and also non-T cell subsets like B cells and NK cells (Table 1) (16). This co-inhibitory immune checkpoint has a

TABLE 1 A summary of the immune checkpoints and their expression changes pattern in response to chemo/radio therapy.

Immune checkpoint molecules	Ligands	Distribution of the receptors	Function	Immune checkpoint inhibitor (ICI) drugs and some of the related clinical trials on ICIs.	The dominant pattern of expression changes post-NAC (R)
PD-1	PD-L1 (CD272) PD-L2 (CD273)	T cell subsets (TILs, Tregs, Effector T cells) Non-T cell subsets (NK cells, B cells, subsets of DCs)	Co-inhibitory effect by the PD-1/ PD-L1 signaling pathway	Nivolumab (NCT01721759), Pembrolizumab (NCT02256436), Cemiplimab (NCT03002376)	A significant increase in expression levels was seen in most of the studies
PD-L1	PD-1 B7-1 (CD80)	T cells, B cells, NK cells, Monocytes, DCs	Co-inhibitory effect by the PD-1/ PD-L1 signaling pathway	Durvalumab (NCT02639065), Avelumab (NCT03704467), Atezolizumab (NCT02425891)	A significant increase in expression levels was seen in most of the studies
CTLA-4	B7-1 (CD80) B7-2 (CD86)	Tregs Activated T cells	Co-inhibitory function by reducing IL-2 production, inhibiting T-cell proliferation, and eliminating B7-1,2 on APCs	Ipilimumab (NCT02279732), Tremelimumab (NCT01853618)	Opposing results in expression level alterations were seen, indicating a need for more studies
LAG-3 (CD233)	MHC-II, LSEctin, Galectin-3, FGLP-1	TILs, NK cells, B cells, DCs	Inhibitory regulatory effect on T-cell proliferation and DC activation	Eftilagimod alpha (NCT00349934), Relatlimab (NCT04611126), LAG525 (NCT03499899), MK4280 (NCT03598608), Sym022 (NCT03489369), REGN3767 (NCT03005782), TSR-033 (NCT02817633)	An increase in the expression levels was detected in the studies, but the number of studies was limited
B7-H4	B7-H4 receptor (Not well-known)	Cancerous cells (as in ovarian, uterus, and lung tumors), TAMs	Inhibitory function on activated effector T cells by decreasing IL-2 production and inducing cell-cycle arrest	FPA-150 (alsevalimab) (currently in phase Ia/Ib of the clinical trial in solid tumors,NCT03514121)	A single study demonstrated a decrease in expression levels that was associated with a better prognosis

The table represents a summary of the characteristics of the immune checkpoints, some of the ICI drugs, and related clinical trials evaluating their efficacy. Also, the dominant pattern of immune checkpoints' expression changes in response to chemo/radio therapy due to the studies mentioned in previous sections on each immune checkpoint is presented.

crucial role in stabilizing peripheral immune tolerance. For example, its knockout in C57BL/6 mice leads to an autoimmune pathology resembling what occurs in lupus erythematosus pathogenesis (76). PD-1, despite its name, has no role in the cell death induction process/apoptosis (47). This immune checkpoint has two ligands, PD-ligand-1 (PD-L1), expressed by a wide variety of somatic cells in response to pro-inflammatory cytokines, and PD-L2 (CD273 or B7-DC), with more restricted antigen-presenting expression (77). Activation of the PD-1 signaling pathway gives rise to transcriptional and epigenetics alterations in T cells, which finally leads to a decrease in the production of proteins such as inflammatory cytokines and finally T cell "exhaustion" in the TME (78).

Considering the PD-1/PD-L1 axis and its role in T cell anergy, several monoclonal antibodies have been designed to target these immune checkpoints. Some are FDA-approved, such as durvalumab, nivolumab, and pembrolizumab, which are currently used in immunotherapy of several types of cancers (Table 1) (60, 79–82). For example, CheckMate063, a phase2, single-arm trial, proved the activity and safety of nivolumab for patients with advanced, refractory NSCLC (83). Another clinical trial study demonstrated a reduced rate of death in advanced urothelial carcinoma patients with disease progression, during or following chemotherapy, as a result of treatment with pembrolizumab (Hazard Ratio (HR) = 0.73) (84). Realizing changes in PD-1 expression in the TME in response to chemo (radio)therapy across different types of tumors may help us design better combination therapies for managing the cancers. In recent years a limited number of studies have done this (Table 1).

According to a systematic review by Van den Ende et al., eight studies had assessed alterations in PD-1 expression patterns in response to chemo(radio)therapy until January 2019. Seven of these studies compared the level of PD-1 expression in the TME of pre-treatment to a post-treatment tissue. Also, one of them compared treated vs. untreated groups of a cohort. A total of five of these studies had statistically significant results. A significant increase in PD-1 expression was seen in four single studies in patients with ovarian cancer, breast cancer, non-small cell lung carcinoma (NSCLC), and glioblastoma. Also, a significant decrease after treatment was observed in a study on patients with breast cancer (85). In the latter study, breast tumor specimens of 33 women were evaluated immunohistochemically before and after neoadjuvant chemotherapy (NAC) with a regimen consisting of Adriamycin, cyclophosphamide, capecitabine, and paclitaxel. The results showed a significant decrease in the PD-1$^+$ T-cells population, but this reduction did not have a remarkable association with prognosis and complete pathological response (pCR) (86). A study on patients with stage II-III NSCLC compared two treated and non-treated groups based on receiving or not receiving NAC regimens including carboplatin plus paclitaxel or pemetrexed and cisplatin plus gemcitabine. Immunohistochemistry (IHC) analysis revealed a higher density of PD-1 expressing antigen-experienced and memory antigen-experienced cells (87). In a cohort study by Lo and colleagues on post-NAC tumor samples of 90 patients with high-grade serous carcinoma (HGSC) of the ovary, despite the rise seen in density of the favorable tumor-infiltrating T cells and B cells, no remarkable changes were seen in patients' survivals after NAC with paclitaxel plus carboplatin. They hypothesized that this poor association could be attributable to the probable immunosuppressive effects of chemotherapy on the TME.

Assessment of the changes in the expression level of the inhibitory immune markers clarified that levels of IDO-1, FOXP3, and PD-L1 did not differ notably pre- and post-NAC. In contrast, PD-1 levels showed a considerable and significant increase in post-NAC samples compared with pre-NAC ones. This finding meant that an increase in the number of tumor-infiltrating lymphocytes (TILs) expressing PD-1 (as a co-inhibitory immune checkpoint) has occurred and neutralized the positive immune-stimulatory effects of the chemotherapy (88). Miyazaki et al. assessed the alterations in the expression of immune markers containing PD-1 and PD-L1 in initially and secondary resected samples of glioblastoma (GBM) from 16 patients who received chemotherapeutic agent temozolomide (TMZ) combined with fractioned radiotherapy (FRT) after the first surgery, before recurrent tumor surgery. IHC assays revealed significantly increased staining scores for CD3, CD8, and PD-1 in secondary resected specimens. Based on the PD-1 staining score, patients were categorized into low or high PD-1 score groups. Assessments to determine the prognostic value of PD-1 expression score in these two groups showed that a high PD-1 expression score was accompanied by longer progression-free survival (PFS), shorter survival after recurrence, and briefly poor prognosis (89). Considering what was mentioned above about the inhibitory role of the PD-1 pathway, which leads to T cell exhaustion and formation of an immunosuppressive context in the TME, and also paying attention to the predominance of the increasing pattern in PD-1 expression in the TME after chemo(radio) therapy in some solid tumors, it seems that combining these conditional treatments with immunotherapeutic agents inhibiting PD-1 specifically, may promote the efficacy of these anti-tumor approaches and improve the prognosis of many cancers.

PD-L1

Programmed death-ligand 1 (PD-L1), also known as CD274 and B7 homolog 1 (B7-H1), is a member of the B7 family of type 1 transmembrane protein receptors. B7-H1 gene was discovered and cloned by Dong et al. 1999 (90), and its name changed to PD-L1 after recognizing its interaction with previously known PD-1 molecule (91). This protein is expressed in the many immune cells subtypes, including T cells, B cells, NK cells,

Monocytes, and APCs, such as dendritic cells (DCs) and macrophages (Table 1) (53, 63). Expression of PD-L1 is increased following stimulation of some cell types by pro-inflammatory cytokines such as IFN-γ and IL-4 (68). As mentioned before, evidence indicates that activation of the PD-1/PD-L1 signaling pathway suppresses T cell-mediated immunologic responses in peripheral tissues and avoids effector T cells giving rise to tissue damage, the process described as immune "tolerance" (92, 93). Considering this crucial role of the PD-1/PD-L1 pathway, it is expected that cancerous cells use this property as an evasion mechanism halting the immune system's anti-tumor function (92, 94). It has been proven that a wide variety of tumors upregulate the expression of PD-L1 on the surface of their cells as a mechanism to evade the immune system (95). Thus, inhibiting PD-L1 through designing and utilizing specified monoclonal antibodies (mAbs) has been widely brought into play in cancer immunotherapy in recent years. Some of these immune checkpoint inhibitors (ICI) like Durvalumab, Avelumab, and Atezolizumab are FDA-approved (Table 1) (51, 96, 97). A clinical trial study (NCT02639065) of Durvalumab on thirty-seven patients with esophageal cancer showed a relapse-free survival (RFS) rate of 73% (98). Similar to other immune markers and elements and as a consequence of alterations in the TME, several studies have demonstrated variations in PD-L1 expression patterns after traditional cancer treatments, including chemotherapy, radiotherapy, or a combination of them (Table 1). According to a systematic review by Van den Ende and colleagues, until January 2019, 48 studies had evaluated PD-L1 expression changes in response to common chemotherapeutic regimens, radiotherapy, or a combination of these approaches. Statistical analysis revealed that 30 articles reported higher expression of PD-L1 comparing pre-treatment and post-treatment specimens or comparing treated vs. untreated patients' samples in cohorts. Just half of these increases were statistically significant. Among these studies are fluoropyrimidine-based neoadjuvant chemoradiotherapy of rectal cancer in 3 individual studies, two studies on ovarian cancer treated with carboplatin/paclitaxel regimen, two studies on head and neck squamous cell carcinoma with two different NCT regimens based on cisplatin or docetaxel, platinum, and fluorouracil and single studies on mesothelioma of the pleura, NSCLC, and basal cell carcinoma (BCC). Conversely, only eight studies reported decreases in the level of PD-L1 in post-treatment samples. Six studies demonstrated significant reductions, including single studies on FOLFOX-based treated rectal cancer, vinorelbine-based treated NSCLC, and nasopharyngeal cancer treated with chemoradiotherapy or radiotherapy alone (85). In a study by Lim et al. on 123 patients with rectal cancer, they compared pre- and post-NCT specimens immunohistochemically to assess the effects of NCT on the expression of PD-L1 and CD8+ TILs in the TME. Results demonstrated a rise in the expression levels of PD-L1 and the density of $CD8^+$ TILs in post-NCT biopsies. Patients with high expression of PD-L1 pre- and post-NCT showed a lesser rise in $CD8^+$ cells, and their overall survival and disease-free periods were significantly poorer. These findings may indicate the potentiality of applying combined methods such as simultaneous therapy with NCT and immune-checkpoint inhibitors (99). Ogura and colleagues did a similar study on 287 patients with rectal cancer evaluating PD-L1 expression and $CD8^+$ cells density in the stromal and tumor compartments of the TME before and after chemoradiotherapy (CRT). This study showed an increase in PD-L1 expression on the stromal immune cells but not on the tumor cells. This finding was correlated with a high count of the tumor area's $CD8^+$ cells pre-CRT and high stromal density of $CD8^+$ cells post-CRT (100). Song et al. carried out a study on 76 patients with squamous cell carcinoma (SCC) of the lung, comparing PD-L1 expression levels pre- and post-NCT with gemcitabine or Taxane plus platinum agent. Results demonstrated a significant up-regulation in PD-L1 expression post-NAC. PD-L1 positive patients had a poorer prognosis with shorter overall survivals (101). As mentioned before, some studies have paradoxically reported a reduction pattern in the expression of PD-L1 post-CRT. For instance, in a study by Zhang et al. on 109 patients with rectal cancer, the proportion of $PD\text{-}L1^+$ TILs were significantly lower in post-NCT (FOLFOX with or without radiotherapy) specimens associated with poorer prognosis. The precise mechanism for this alteration was not found, and a probable unknown stimulatory role for the PD-1/PD-L1 signaling pathway was suggested as an underlying mechanism (102). The noticeable point is that the chemotherapeutic regimens used in these studies with paradoxical findings differed from each other. So, the kind of applied chemotherapeutic agents can be an impressive factor altering TME positively or negatively in the case of every single immune cell and immune marker such as PD-L1. However, further investigations are needed to assess and confirm this hypothesis.

CTLA-4

Activation of T-cell is a relatively sophisticated process that needs more than one stimulatory signal. One of the components is the co-stimulatory signal induced by the interaction of B7-1 (CD80) or B7-2 (CD86) molecules on the APCs with the CD28 molecules on the T-cells, which gives rise to signaling within the T cells. The consequences of this signaling include the proliferation of the T-cells, improved survival and differentiation *via* synthesizing and secreting growth cytokines such as IL-2, overexpressing genes involved in cell survival, and improving energy metabolism (103).

CTLA-4 is a CD28 homolog with more affinity to the B7 molecule. Binding CTLA-4 to B7, opposite to what CD28 does, not only does not lead to a stimulatory signal but also produces a

co-inhibitory signal that results in limited Il-2 production, restricted T-cell proliferation, and lower survival (Table 1) (104, 105). CTLA-4 eliminates B7-1 and B7-2 molecules from the membrane of APCs *via* trans-endocytosis and produces its function inhibitory effect through signaling independent mechanism (106). $CD4^+$ Regulatory T cells (Treg) need CTLA-4's appropriate function to establish and preserve immune tolerance. Blocking CTLA-4 leads to Treg dysfunction and leads to multi-organ autoimmunity (107, 108). Ipilimumab is an FDA-approved anti-CTLA-4 monoclonal antibody (mAb) from the IgG-1 subclass, and its effect on melanoma metastasis has been evaluated. However, some studies, including a clinical trial (NCT02279732) evaluating the effect of combination therapy of ipilimumab with chemotherapy in patients with squamous lung cancer, have demonstrated that adding this mAb to the chemotherapeutic regimen does not alter the median OS significantly (HR=0.91) (109). Tremelimumab is another mAb developed against CTLA-4 with the same binding affinity (Table 1) (110, 111). A clinical trial study (NCT01853618) in patients with HCC introduced tremelimumab as a potential novel treatment for advanced HCC (112). Two mechanisms have been suggested on how these mAbs work, one of them emphasizing the inhibitory effect of mAbs on CTLA-4, which leads to enhancement of the CD28/B7 binding. The other suggestion proposes that these mAbs exhaust the Tregs in the TME (113, 114).

Due to a systematic review, four studies involving two studies on rectal cancer, a study on breast cancer, and another single study on esophageal cancer have evaluated the changes in the expression level of CTLA-4 in the TME after CRT or NCT alone until January 2019. Only two of these mentioned studies, including one on rectal cancer patients and the other on breast cancer, had significant results, however, opposing alterations in CTLA-4 expression (85). Kaewkangsadan and colleagues designed and conducted a study on sixteen patients with large and locally advanced breast cancers (LLABCs). They used a chemotherapy regimen of adriamycin, cyclophosphamide, capecitabine, and paclitaxel as the NCT. Then they evaluated the alterations that occurred in the TME of specimens and the association of these changes with the prognosis of the disease by comparing pre- and post-therapy biopsies. Different types of TILs and immune markers were studied. The results demonstrated that NCT agents employed in the study maintained the CD8+ TILs population. A significant decrease was seen in the number of circulating and tumor-infiltrating FOXP3+ and stromal CTLA-4+ T cells. These changes reduce the secretion of inhibitory cytokines such as IL-10 and TGF-β. No changes happened in the population of intratumoral $CD8^+$and $CTLA4^+$ T cells. Also, the analysis showed that high levels of CTLA-4+ T cells in the stromal compartment were significantly associated with pCR. However, there was no similar relation between intratumoral CTLA4+ T cells and the pCR (86).

In another study, Zhang et al. assessed the effect of two different methods of neoadjuvant therapy on the TME cells and immune markers such as CTLA-4 on 109 patients with rectal cancer. A group of patients received the FOLFOX regimen as the NCT. The other group received neoadjuvant chemoradiotherapy (NACR) consisting of FOLFOX plus radiotherapy. Overall, the results clarified that the expression level of CTLA-4 in both groups was significantly higher post-neoadjuvant therapy. The NACR group showed higher levels of CTLA-4 expression compared with the other group in a meaningful manner. They attributed this finding to the immune system's response to radiation exposure to avoid the autoimmunity caused by radiation. This study also showed a strong correlation between CTLA4+ and FOXP3+ TILs. It can be related to the increase in the number of Tregs in response to radiotherapy. Despite these changes, there was no significant relationship between CTLA-4 levels and the quality of response to the therapies (102). Overall, considering what was said above, only a few studies have evaluated the CTLA-4 expression levels alterations in response to chemo(radio)therapy till now. So, it seems that there is a need for more and more studies about this key immune checkpoint to help us make firm statements on how and by which mechanisms different types of neoadjuvant regimens change the expression of CTLA-4, what is the clinical significance of these patterns and their effect on the prognosis of various types of cancers and the overall survival (Table 1).

LAG-3

Lymphocyte activating gene 3 (LAG-3), also known as CD233, is another immune checkpoint from the immunoglobulin superfamily. It was identified by Triebel and colleagues in 1990 (115). This molecule is expressed on TILs, NK cells, B cells, and DCs (116–119). LAG-3, with structural similarity and a close gene placement to the CD4 gene, has more affinity to binding MHC class II molecule (120). During the last years, several ligands have been introduced for LAG-3 as MHC-II, LSECtin, Galectin-3, and fibrinogen-like protein 1 (FGL1) (Table 1) (121–123). The detailed mechanisms of the LAG-3 function have not been known yet. However, this immune checkpoint exerts an inhibitory regulatory effect in activating T-cells that restrains autoimmunity and saves tissues (124). Co-expression of LAG-3 and PD-1 on TILs in the TME gives rise to T cell exhaustion and the consequent unlimited tumor growth (57). Some studies have demonstrated improvement in anti-tumor immunity by inhibiting the PD-L1 and LAG-3 simultaneously using bispecific antibodies (125). So, inhibiting LAG-3 enhances the immune system's anti-tumor function by improving the effectiveness of other types of immunotherapy (126). Some mAbs have been developed against LAG-3 (Table 1) (127). These mAbs block the

interaction between LAG-3 and MHC-II in the TME and improve the induction of apoptosis in the tumor cells. For example, LAG-3-Ig fusion proteins like IMP321 or eftilagimod alpha increase the expression of co-stimulatory molecules and IL-12 secretion that, finally enhances tumor immunity (78, 128). A clinical trial study (NCT00349934) in metastatic breast cancer patients demonstrated that a combination of eftilagimod alpha and paclitaxel empowered immune responses and doubled the tumor response rate (129). Relatimab, another LAG-3 blocking mAb, is currently being used widely in clinical trials, such as the study on metastatic ovarian cancer to improve the progression-free survival (PFS) of the patients (NCT04611126). Another Anti-LAG-3 mAb, Sym022, has been evaluated in some clinical trials, including a study on patients with locally advanced/unresectable or metastatic solid tumors or lymphomas (NCT03489369). Only a few studies have investigated the alterations in LAG-3 expression patterns in the TME post-NCT and its relationship with the disease prognosis (Table 1). Wang et al. studied the effect of NCT with an Anthracycline/Taxane-based regimen on the expression levels of LAG-3 and some other checkpoint molecules and their prognostic value in 148 patients with Triple-negative breast cancer (TNBC). Results of the study demonstrated an increase in expression levels of four molecules: CD8, PD-1, PD-L1, and LAG-3. A significant increase occurred in the LAG-3 levels post-NCT. There was also a significant correlation between high LAG-3, PD-1, and PD-L1 levels in pre-NCT biopsies. Nevertheless, high levels of LAG-3 on TILs in post-NCT samples demonstrated remarkable differences in nodal status and PD-l expression levels. At last high numbers of CD8+ TILs and nodal status were introduced as the definite factors altering the prognosis of tumor post-NCT. Also, high expression levels of LAG-3, particularly combined with high levels of PD-1, were other poor prognostic predictors (130). In another study, Bottai et al. assessed the TILs by evaluating the density of CD4+, CD8+, and FOXP3+ T cells. They also determined levels of expression of some immune checkpoints, including LAG-3 and PD-1, in the specimens of TNBCs from patients who underwent operation post-NCT. The results revealed that high quantities of stromal TILs were an independent good prognostic predictor correlated with high expression levels of the LAG-3 and PD-1. However, there was no significant association between these molecules' expression and patients' outcomes (131). The controversy between the results of the two mentioned studies may be attributable to the surgical intervention involved in the latter study or may be due to the tumor heterogeneity. These are just hypotheses and need more evaluation to be confirmed. Considering what we discussed above, we need more studies to evaluate the changes in LAG-3 expression patterns in response to NCT, determine the exact mechanisms of its action, and determine its effect on the prognosis across different cancers.

B7-H4

B7-H4, as a member of the B7 family, is a transmembrane protein discovered by three different teams of researchers in 2003 (132–134). Also, Salceda and colleagues isolated the molecule again later in 2005. They researched to identify the overexpressed genes in cancers, focusing on gynecologic ones (135). There exists much inconsistency in the expression and distribution of B7-H4 across various types of tumor cells and normal cells (Table 1). Some studies have identified this molecule's mRNA in different normal tissues in the human as the ovary, testis, pancreas, lung, spleen, and liver (136). However, the IHC studies for this molecule on normal tissues were negative, which indicates the firm translational control on this immune checkpoint. The same study demonstrated the expression of B7-H4 on a significant percentage of ovarian and lung tumor biopsies (133). Other studies also identified the molecule on uterus, colon, and breast tumors specimens. The intensity of the staining and expression was correlated with the cancer stage. Other cancers, such as gastric, kidney, and liver tumors, did not show similar results (137, 138). Expression levels of B7-H4 alter in a dynamic pattern along with the changes in the TME. High levels of Treg-induced production of some cytokines such as IL-6 and IL-10 by tumor-associated macrophages (TAMs) give rise to the overexpression of B7-H4 on the surface of TAMs. Some studies have shown a negative correlation between Treg count and the level of expression of B7-H4 on TAMs with the tumor prognosis (139–141). Many studies have suggested the inhibitory effect of B7-H4 on activated effector T cells *via* different mechanisms such as reduced IL-2 secretion that leads to diminished cell proliferation. Also, inducing cell cycle arrest is another mechanism (142). Several studies assessed the effects of B7-H4 expression on cancer cells *in vitro* and *in vivo*. The results clarified that B7-H4 empowers the tumors in many aspects, as preventing the apoptosis of the cancerous cells, augmenting proliferation and cell adhesion, and finally increasing the ability of migration, invasion, and metastasis (135, 143–146). B7-H4 overexpression in lung adenocarcinoma gives rise to an immunosuppressive TME (147). Until now, researchers have developed and used different antibodies against B7-H4 in several studies, including a clinical trial assessing the effect of an anti-B7-H4 drug, FPA150, in patients with advanced solid tumors (NCT03514121). (Table 1) (148, 149). Immune system augmenting effects such as increased IL-2 production reversed inhibitory effects of B7-H4 on effector T cells. It increased T cell proliferation, indicating the promising results of bringing these blocking antibodies into play (150). Only a few studies have evaluated the changes caused by conventional cancer therapies such as chemotherapy and radiotherapy in the expression level of B7-H4 in the TME (Table 1). Maskey et al. performed a study to evaluate the effect of NCT on TILs and B7-H4 expression in patients with gastric cancer. The other goal was to determine the

cells and markers associated with the overall survival. They also evaluated their impact on the prognosis of the disease. To do this, they assessed and compared the expression of different subsets of TILs and the levels of B7-H4 molecule in two groups of patients with gastric cancer. One of these groups went under the NCT (NCT group) before the surgery, while the other did not (nNCT group). The regimen used for NCT was the FOLFOX regimen. The number of participants was 102. The results, achieved by the IHC analysis on the post-surgery biopsies, indicated that the NCT group had significantly lower levels of expression of B7-H4 molecule but higher levels of $CD4^+$ and $CD8^+$ TILs. More analysis demonstrated that NCT alone had no significant effect on the overall survival (OS). However, patients with lower expression of B7-H4 in the NCT group had significantly higher OS. So, the level of expression of B7-H4 is associated with the prognosis of the disease in patients with gastric cancer. However, TILs levels do not correlate with the disease prognosis (151). It seems that we need more research across different types of tumors to assess the exact effects of adjuvant therapies on the expression of B7-H4 in the TME and to determine its effect on the response to therapies. Also, determining the relationship between the expression of B7-H4 and disease prognosis is very crucial.

The role of personalized medicine in combination therapy

Despite the wide use of the various kinds of targeted cancer therapies, such as immunotherapy, and also their combination with traditional ones, including chemotherapy, radiotherapy, and surgery, a remarkable proportion of the patients getting these therapies do not achieve the optimal cure, i.e., they show full resistance at the first steps or face the tumor relapse after the primary success (152). Many studies have demonstrated that the underlying etiology of this failure is the intra-population diversities, including their specific genetic composition that gives rise to heterogeneities in their "omics" data, besides the environmental factors. Omics, including the terms such as transcriptomic and proteomic, are indeed the connectors of the genotype of each individual to his phenotype. For example, a transcriptome is the whole mRNA of a subject or specimen. Methods such as microarray analysis and RNA sequencing techniques help us provide the transcriptomic information we need about the level of expression of different biomarkers and proteins, such as immune checkpoints. Also, the proteome is the entire protein expressed by a cell or a tissue, such as a tumor sample. Tools like mass spectrometry provide proteomic data about the characteristics of the proteins, such as expression amounts, post-translational alterations, cellular sites, and types of interactions between different proteins (153). Metabolomics is about recognizing and analyzing the metabolites, i.e., the mid-level molecules produced by the metabolic reactions. Metabolites are affected by genetic and environmental factors simultaneously, so a complete analysis of them can enlighten the specific response of an individual to a drug (154). The different omics techniques mentioned above are, in fact, the primary data collecting tools for a relatively new approach to cancer treatment, i.e., "precision medicine" or "personalized medicine." The growing field of personalized medicine can be considered a revolution concerning cancer therapy, emphasizing designing and developing specific treatments for an individual or a group of patients based on data demonstrating their unique genetic, physiologic, and environmental features (155). The mentioned data can help us predict the response of different patients with diverse characteristics to a specific treatment shifting the trend of using generic medicine for all the patients of a particular disease to a specified and precise approach. The TME heterogeneity is one of the cardinal factors that bring about dissimilar responses in different individuals getting the same treatment, whether a single or a combination therapy (156). Cancer vaccines, mAbs (including ICIs), and CAR T-cells are among the personalized medicine-based therapies currently being used. As we mentioned in the previous sections, immune checkpoints blocking agents or ICIs are currently among the widely used drugs in both immunotherapeutic strategies and combination therapies. From the perspective of personalized medicine, to get more benefit from using ICIs and other target therapies, we need data and diagnostics to assess the possibility of a suitable response from a particular individual's tumor. So, we need more studies to clarify the details of the immunologic pathways in which the immune checkpoints are involved, intending to recognize and introduce more biomarkers and other diagnostic elements that can help us anticipate a patient's response to a particular drug or treatment (16). Only a few studies exist about personalizing traditional cancer therapies while personalizing these methods seems necessary due to their role and importance in different therapeutic approaches, such as in developing combination therapies. Wang et al. suggested that performing an appropriate diagnostic process before therapy can help us execute personalized cancer chemotherapy (157). Identifying biomarkers using "omics" technologies, especially proteomics, can be useful in evaluating the possibility of good responses to chemotherapy. Culturing a patient's cancer cells to determine drug sensitivity is another method for assessing the probability of favorable responses to chemotherapeutic agents (158).

The main concept of this paper, i.e., evaluation of the alterations in immune checkpoint molecules' expression patterns in response to chemo/radiotherapy, is somehow related to personalized medicine. Considering what we mentioned about the TME features and its role in anti-tumor immunity, also its changes in response to traditional adjuvant therapies, including the alterations in immune checkpoints expressions, and paying attention to what we mentioned about

personalized medicine in this part, it seems logical to involve the patient(s) individual features, such as omics and physiologic characteristics in designing more specialized and effective combination therapies for various types of cancers.

microRNA's targeting of IC

We employed a miRNA target prediction approach to consider the involvement of miRNAs during IC targeting and modulation of predicted miRNAs upon chemotherapy or radiotherapy. miRWalk v.3 was used to predict miRNAs with the ability to target IC (159). Also, the miRTarBase database of experimentally validated miRNA-gene targeting was employed to confirm the predicted interactions (160) (Figure 3). Then, the alteration of resultant miRNAs was considered by pieces of literature review. It is found that miR-194-3p is significantly down-regulated in docetaxel-resistant colon cancer cells. In addition, over-expressed miR-194-3p could promote SW620/docetaxel and SW480/docetaxel apoptosis and improve their docetaxel sensitivities. In addition, over-expressed miR-194-3p promoted docetaxel sensitivity of colon cancer cells by negatively regulating KLK10 (161). Overall, it was predicted that miR-193-3p could target PD-L1 and be involved in the activity of docetaxel. The results of a recent study highlighted the tumor suppressor roles of miRNA-486-5p mimic in bladder cancer carcinogenesis, identifying miRNA-486-5p mimic as an important therapeutic target in bladder cancer. Also, the results revealed that miRNA-486-5p mimic could increase cisplatin sensitivity in different bladder cancer cell lines and provide a better outcome for chemotherapy with cisplatin (162). A study conducted by Jin et al. showed the downregulation of miR-486-5p in nonsmall-cell lung cancer tissues compared with normal lung tissues and lower levels of miR-486-5p indicated a poorer prognosis for patients with nonsmall-cell lung cancer in terms of overall survival. Furthermore, this study demonstrated that miR-486-5p increased the sensitivity of A549 cells to cisplatin and inhibited EMT by directly targeting TWF1 (163). Also, it was predicted that miR-486-5p could target CD40 and involved in the activity of cisplatin. miR-761 expression is negatively associated with the expression of FOXM1 in colorectal cancer tissues. Elevated expression of FOXM1

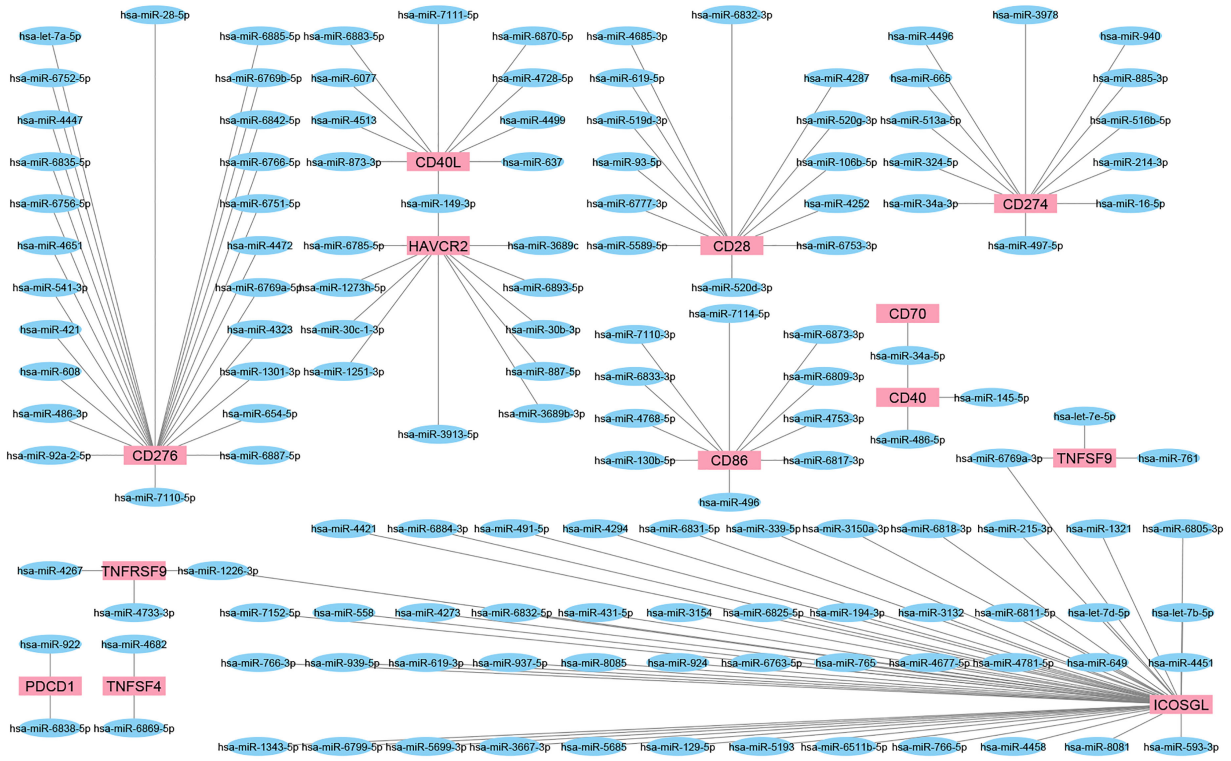

FIGURE 3
The interaction network of miRNAs and IC. These network shows the predicted interaction between some ICs and miRNAs. Targets or so-called IC are shown as red rectangles and predicted miRNAs as blue ellipses. Most miRNAs could target a single IC; however, miR-149-3p, miR-34a-5p, miR-6769a-3p, and miR-1226-3p could interact with more than one IC.

suppressed the sensitivity of miR-761-overexpressing HT29 cells to 5-FU. It is also indicated that FOXM1 overexpression promoted cell proliferation, cycle, and invasion of miR-761-overexpressing HT29 cells. These data suggested that miR-761 played a tumor suppressor miRNA in colorectal cancer progression, and reduced miR-761 expression might be a major mechanism for 5-FU resistance in the colorectal cancer cell (164). Besides, it was predicted that miR-761 could target CD137L and be involved in the activity of 5-FU. A recent study indicated that miR-93-5p reduces the proliferation and migratory capacity of breast cancer cells and increases the ratio of apoptotic cells. Increasing apoptosis by overexpression of miR-93-5p may increase radiosensitivity in breast cancer cells (165). In addition, it was predicted that miR-93-5p could target CD28 and be involved in the activity of radiotherapy. It is demonstrated that CARM1 is highly expressed in cervical cancer tissues and radio-resistant cervical cancer cells, while miR-16-5p expression is low.

Under irradiation, up-regulation of CARM1 can induce radiotherapy resistance of cervical cancer cells, while overexpression of miR-16-5p or CARM1 knockdown could inhibit the survival of CC cell and induced apoptosis. Therefore, CARM1 was verified as a target for miR-16-5p. Besides, up-regulation of CARM1 reversed the increase in radiosensitivity induced by miR-16-5p (166). In addition, it was predicted that miR-16-5p could target PD-L1 and involved in the activity of radiotherapy. Additionally, it is reported that ionizing radiation (IR) exposure impaired lung cancer cell viability and found that miR-339-5p is a novel IR-inducible miRNA. Overexpression of miR-339-5p enhanced radiosensitivity of A549 and H460 cells by inhibiting cell viability, increasing apoptosis, inducing cell cycle arrest, and suppressing cell proliferation. Further exploration validated that miR-339-5p can target phosphatases of regenerating liver-1 (PRL-1) in lung cancer cells (167). Besides, it was predicted that miR-339-5p could target ICOSL and involved in the activity of radiotherapy.

Conclusion and future perspective

Over past decades, significant advances have been made in cancer treatment, such as immunotherapeutic approaches using ICIs. Despite this, only limited types of cancers and a limited number of patients take advantage of immunotherapy. To design more effective therapies, we need to recognize the changes occurring in the TME across different types of tumors in response to various treatments. In this study, we have reviewed the alterations in the expression patterns of well-known and relatively newly found immune checkpoints during various RCTs. Different studies demonstrate that many factors such as the type of tumor and the type of chemo(radio)therapeutic regimen can influence the immune checkpoint expression patterns. Evaluating the results of different studies showed that the changes in immune markers in the TME are dependent on the number of TILs present in the tumor to a great degree. Tumors with higher numbers of TILs become more active, expressing higher levels of immune molecules and neoantigens post-NCT. These tumors are usually referred to as hot tumors, such as ovarian, rectal, and pancreatic tumors, and seem to be more promising targets for immunotherapy post-NCT and surgery. There have been many studies on some of the immune checkpoints, such as PD-L1. Most of them have shown the upregulation of this molecule following NCT. However, there are only a few studies with inconsistent results about other immune checkpoints, such as CTLA-4, LAG-3, and B7-H4. It indicates a need for designing more comprehensive studies. A remarkable number of studies showed that some changes in the immune checkpoints' expression patterns were associated with the prognosis of the disease. It shows the necessity of becoming more knowledgeable about the alterations happening in the TME and its different elements, such as the checkpoint molecules in response to different chemo (radio)therapeutic approaches. Also, the number of studies about the effect of chemo(radio)therapeutic neoadjuvant therapies on the expression patterns of co-stimulatory immune checkpoints in the TME seems insufficient. So, there is a need for more studies to prepare the field for designing better combination therapies consistent with the concept of personalized medicine. The other point not noted in the mentioned studies is the link and association between the expression patterns of different immune checkpoints. Some studies have demonstrated a correlation and association between immune checkpoints' genes originating from the "co-expression gene networks" (168, 169). This fact indeed gives us some clues which guide us toward considering immune checkpoints as a connected network rather than single independent genes. However, almost all the studies reviewed in this paper had not noticed this determining point. So, we must pay attention to the co-expression gene networks, i.e., the linkage between immune checkpoints' genes, that brings about their probable simultaneous and correlated up-regulation or down-regulation in the TME before and after interventions such as chemo/radiotherapy to develop more effective and promising combination therapies.

Author contributions

HH, the first author of the manuscript, searched, collected papers, and wrote the initial version of the manuscript. ZA, AD, and ADu left comments, and revised the manuscript. NR, SN, and OB contributed to manuscript preparation. AB provided figures. NS and BB the corresponding author of the manuscript supervised the project and also contributed to the revising of the

main text of the manuscript. All authors have read and agreed to the published version of the manuscript.

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. Avgerinos KI, Spyrou N, Mantzoros CS, Dalamaga M. Obesity and cancer risk: Emerging biological mechanisms and perspectives. *Metabolism* (2019) 92:121–35. doi: 10.1016/j.metabol.2018.11.001

2. Mattiuzzi C, Lippi G. Current cancer epidemiology. *J Epidemiol Global Health* (2019) 9:217. doi: 10.2991/jegh.k.191008.001

3. Kim R. Cancer immunoediting: from immune surveillance to immune escape. *Cancer Immunother* (2007), 9–27. doi: 10.1016/B978-012372551-6/50066-3

4. Hernández-Camarero P, López-Ruiz E, Marchal JA, Perán M. Cancer: a mirrored room between tumor bulk and tumor microenvironment. *J Exp Clin Cancer Res* (2021) 40:217. doi: 10.1186/s13046-021-02022-5

5. Xiao Y, Yu D. Tumor microenvironment as a therapeutic target in cancer. *Pharmacol Ther* (2021) 221:107753. doi: 10.1016/j.pharmthera.2020.107753

6. Whiteside T. The tumor microenvironment and its role in promoting tumor growth. *Oncogene* (2008) 27:5904–12. doi: 10.1038/onc.2008.271

7. Wieder T, Eigentler T, Brenner E, Röcken M. Immune checkpoint blockade therapy. *J Allergy Clin Immunol* (2018) 142:1403–14. doi: 10.1016/j.jaci.2018.02.042

8. Hodi FS, O'Day SJ, Mcdermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* (2010) 363:711–23. doi: 10.1056/NEJMoa1003466

9. Peters S, Kerr KM, Stahel R. PD-1 blockade in advanced NSCLC: A focus on pembrolizumab. *Cancer Treat Rev* (2018) 62:39–49. doi: 10.1016/j.ctrv.2017.10.002

10. Fuereder T. Immunotherapy for head and neck squamous cell carcinoma. *Memo* (2016) 9:66–9. doi: 10.1007/s12254-016-0270-8

11. Kitano S. [Development of immune checkpoint inhibitors]. *Rinsho Ketsueki* (2017) 58:966–76. doi: 10.11406/rinketsu.58.966

12. Payandeh Z, Khalili S, Somi MH, Mard-Soltani M, Baghbanzadeh A, Hajiasgharzadeh K, et al. PD-1/PD-L1-dependent immune response in colorectal cancer. *J Cell Physiol* (2020) 235:5461–75. doi: 10.1002/jcp.29494

13. Longo V, Gnoni A, Casadei Gardini A, Pisconti S, Licchetta A, Scartozzi M, et al. Immunotherapeutic approaches for hepatocellular carcinoma. *Oncotarget* (2017) 8:33897–910. doi: 10.18632/oncotarget.15406

14. Nghiem P, Bhatia S, Lipson EJ, Sharfman WH, Kudchadkar RR, Brohl AS, et al. Durable tumor regression and overall survival in patients with advanced merkel cell carcinoma receiving pembrolizumab as first-line therapy. *J Clin Oncol* (2019) 37:693–702. doi: 10.1200/JCO.18.01896

15. Liu D, Jenkins RW, Sullivan RJ. Mechanisms of resistance to immune checkpoint blockade. *Am J Clin Dermatol* (2019) 20:41–54. doi: 10.1007/s40257-018-0389-y

16. Wei SC, Duffy CR, Allison JP. Fundamental mechanisms of immune checkpoint blockade therapy. *Cancer Discovery* (2018) 8:1069–86. doi: 10.1158/2159-8290.CD-18-0367

17. Mokhtari RB, Homayouni TS, Baluch N, Morgatskaya E, Kumar S, Das B, et al. Combination therapy in combating cancer. *Oncotarget* (2017) 8:38022. doi: 10.18632/oncotarget.16723

18. Zhu S, Zhang T, Zheng L, Liu H, Song W, Liu D, et al. Combination strategies to maximize the benefits of cancer immunotherapy. *J Hematol Oncol* (2021) 14:156. doi: 10.1186/s13045-021-01164-5

19. Najafi M, Majidpoor J, Toolee H, Mortezaee K. The current knowledge concerning solid cancer and therapy. *J Biochem Mol Toxicol* (2021) 35:e22900. doi: 10.1002/jbt.22900

20. Liang JL, Luo GF, Chen WH, Zhang XZ. Recent advances in engineered materials for immunotherapy-involved combination cancer therapy. *Adv Mater* (2021) 33:e2007630. doi: 10.1002/adma.202007630

21. Klemm F, Joyce JA. Microenvironmental regulation of therapeutic response in cancer. *Trends Cell Biol* (2015) 25:198–213. doi: 10.1016/j.tcb.2014.11.006

22. Hirata E, Sahai E. Tumor microenvironment and differential responses to therapy. *Cold Spring Harbor Perspect Med* (2017) 7:a026781. doi: 10.1101/cshperspect.a026781

23. Thomas L. Reaction to homologous tissue antigens in relation to hypersensitivity, cellular and humoral aspects to the hypersensitivity state. *Cell Humoral Aspects Hypersensitivity State* (1959) 529.

24. Swann JB, Smyth MJ. Immune surveillance of tumors. *J Clin Invest* (2007) 117:1137–46. doi: 10.1172/JCI31405

25. 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

26. Mittal D, Gubin MM, Schreiber RD, Smyth MJ. New insights into cancer immunoediting and its three component phases–elimination, equilibrium and escape. *Curr Opin Immunol* (2014) 27:16–25. doi: 10.1016/j.coi.2014.01.004

27. 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

28. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity* (2004) 21:137–48. doi: 10.1016/j.immuni.2004.07.017

29. Ren C, Li J, Zhou Y, Zhang S, Wang Q. Typical tumor immune microenvironment status determine prognosis in lung adenocarcinoma. *Transl Oncol* (2022) 18:101367. doi: 10.1016/j.tranon.2022.101367

30. Russo E, Laffranchi M, Tomaipitinca L, Del Prete A, Santoni A, Sozzani S, et al. NK cell anti-tumor surveillance in a myeloid cell-shaped environment. *Front Immunol* (2021) 12:787116. doi: 10.3389/fimmu.2021.787116

31. Guillerey C. NK cells in the tumor microenvironment. (2020). Tumor Microenvironment. Springer. doi: 10.1007/978-3-030-49270-0_4

32. Gajewski TF, Schreiber H, Fu Y-X. Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol* (2013) 14:1014–22. doi: 10.1038/ni.2703

33. Xie Q, Ding J, Chen Y. Role of CD8(+) T lymphocyte cells: Interplay with stromal cells in tumor microenvironment. *Acta Pharm Sin B* (2021) 11:1365–78. doi: 10.1016/j.apsb.2021.03.027

34. Xie Y, Xie F, Zhang L, Zhou X, Huang J, Wang F, et al. Targeted anti-tumor immunotherapy using tumor infiltrating cells. *Adv Sci (Weinh)* (2021) 8:e2101672. doi: 10.1002/advs.202101672

35. Martínez-Lostao L, Anel A, Pardo J. How do cytotoxic lymphocytes kill cancer cells? *Clin Cancer Res* (2015) 21:5047–56. doi: 10.1158/1078-0432.CCR-15-0685

36. Surman DR, Dudley ME, Overwijk WW, Restifo NP. Cutting edge: CD4+ T cell control of CD8+ T cell reactivity to a model tumor antigen. *J Immunol* (2000) 164:562–5. doi: 10.4049/jimmunol.164.2.562

37. Nishimura T, Iwakabe K, Sekimoto M, Ohmi Y, Yahata T, Nakui M, et al. Distinct role of antigen-specific T helper type 1 (Th1) and Th2 cells in tumor eradication *in vivo*. *J Exp Med* (1999) 190:617–28. doi: 10.1084/jem.190.5.617

38. Miao Q, Zhang L, Zheng X, Jiang K, Wu B, Lin G. Transformation of a cold to hot tumor and a durable response to immunotherapy in a patient with non-small cell lung cancer after chemoradiotherapy: a case report. *Ann Palliat Med* (2021) 10:4982–6. doi: 10.21037/apm-21-761

39. Knutson KL, Disis M. Tumor antigen-specific T helper cells in cancer immunity and immunotherapy. *Cancer Immunol Immunother* (2005) 54:721–8. doi: 10.1007/s00262-004-0653-2

40. Dolina JS, van Braeckel-Budimir N, Thomas GD, Salek-Ardakani S. CD8(+) T cell exhaustion in cancer. *Front Immunol* (2021) 12:715234. doi: 10.3389/fimmu.2021.715234

41. Zheng L, Qin S, Si W, Wang A, Xing B, Gao R, et al. Pan-cancer single-cell landscape of tumor-infiltrating T cells. *Science* (2021) 374:abe6474. doi: 10.1126/science.abe6474

42. Gonzalez NM, Zou D, Gu A, Chen W. Schrödinger's T cells: Molecular insights into stemness and exhaustion. *Front Immunol* (2021) 12:725618. doi: 10.3389/fimmu.2021.725618

43. Ando M, Ito M, Srirat T, Kondo T, Yoshimura A. Memory T cell, exhaustion, and tumor immunity. *Immunol Med* (2020) 43:1–9. doi: 10.1080/25785826.2019.1698261

44. Sadreddini S, Baradaran B, Aghebati-Maleki A, Sadreddini S, Shanehbandi D, Fotouhi A, et al. Immune checkpoint blockade opens a new way to cancer immunotherapy. *J Cell Physiol* (2019) 234:8541–9. doi: 10.1002/jcp.27816

45. Martinez GJ, Pereira RM, Äijö T, Kim EY, Marangoni F, Pipkin ME, et al. The transcription factor NFAT promotes exhaustion of activated CD8+ T cells. *Immunity* (2015) 42:265–78. doi: 10.1016/j.immuni.2015.01.006

46. Hatzioannou A, Boumpas A, Papadopoulou M, Papafragkos I, Varveri A, Alissafi T, et al. Regulatory T cells in autoimmunity and cancer: A duplicitous lifestyle. *Front Immunol* (2021) 12:731947. doi: 10.3389/fimmu.2021.731947

47. Wing JB, Tanaka A, Sakaguchi S. Human FOXP3+ regulatory T cell heterogeneity and function in autoimmunity and cancer. *Immunity* (2019) 50:302–16. doi: 10.1016/j.immuni.2019.01.020

48. Scott EN, Gocher AM, Workman CJ, Vignali DAA. Regulatory T cells: Barriers of immune infiltration into the tumor microenvironment. *Front Immunol* (2021) 12:702726. doi: 10.3389/fimmu.2021.702726

49. Zhulai G, Oleinik E. Targeting regulatory T cells in anti-PD-1/PD-L1 cancer immunotherapy. *Scand J Immunol* (2022) 95:e13129. doi: 10.1111/sji.13129

50. Tanaka A, Sakaguchi S. Regulatory T cells in cancer immunotherapy. *Cell Res* (2017) 27:109–18. doi: 10.1038/cr.2016.151

51. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nat Rev Immunol* (2020) 20:651–68. doi: 10.1038/s41577-020-0306-5

52. Natoli G, Ostuni R. Adaptation and memory in immune responses. *Nat Immunol* (2019) 20:783–92. doi: 10.1038/s41590-019-0399-9

53. Van Gool S, Barcy S, Devos S, Vandenberghe P, Ceuppens J, Thielemans K, et al. CD80 (B7-1) and CD86 (B7-2): potential targets for immunotherapy? *Res Immunol* (1996) 146:183–96. doi: 10.1016/0923-2494(96)80256-2

54. Reeves E, James E. Antigen processing and immune regulation in the response to tumours. *Immunology* (2017) 150:16–24. doi: 10.1111/imm.12675

55. Marhelava K, Pilch Z, Bajor M, Graczyk-Jarzynka A, Zagozdzon R. Targeting negative and positive immune checkpoints with monoclonal antibodies in therapy of cancer. *Cancers* (2019) 11:1756. doi: 10.3390/cancers11111756

56. Jacquelot N, Ghaedi M, Warner K, Chung DC, Crome SQ, Ohashi PS. Immune checkpoints and innate lymphoid cells-new avenues for cancer immunotherapy. *Cancers (Basel)* (2021) 13:5967. doi: 10.3390/cancers13235967

57. Anderson AC, Joller N, Kuchroo VK. Lag-3, Tim-3, and TIGIT: co-inhibitory receptors with specialized functions in immune regulation. *Immunity* (2016) 44:989–1004. doi: 10.1016/j.immuni.2016.05.001

58. Shin DS, Ribas A. The evolution of checkpoint blockade as a cancer therapy: what's here, what's next? *Curr Opin Immunol* (2015) 33:23–35. doi: 10.1016/j.coi.2015.01.006

59. Donini C, D'Ambrosio L, Grignani G, Aglietta M, Sangiolo D. Next generation immune-checkpoints for cancer therapy. *J Thorac Dis* (2018) 10:S1581. doi: 10.21037/jtd.2018.02.79

60. Wang Y, Wang M, Wu HX, Xu RH. Advancing to the era of cancer immunotherapy. *Cancer Commun (Lond)* (2021) 41:803–29. doi: 10.1002/cac2.12178

61. Dhar R, Seethy A, Singh S, Pethusamy K, Srivastava T, Talukdar J, et al. Cancer immunotherapy: Recent advances and challenges. *J Cancer Res Ther* (2021) 17:834–44. doi: 10.4103/jcrt.JCRT_1241_20

62. Yap TA, Parkes EE, Peng W, Moyers JT, Curran MA, Tawbi HA. Development of immunotherapy combination strategies in cancer. *Cancer Discovery* (2021) 11:1368–97. doi: 10.1158/2159-8290.CD-20-1209

63. Zarour HM. Reversing T-cell dysfunction and exhaustion in cancer. *Clin Cancer Res* (2016) 22:1856–64. doi: 10.1158/1078-0432.CCR-15-1849

64. Suntharalingam G, Perry MR, Ward S, Brett SJ, Castello-Cortes A, Brunner MD, et al. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *New Engl J Med* (2006) 355:1018–28. doi: 10.1056/NEJMoa063842

65. Labrijn AF, Janmaat ML, Reichert JM, Parren PW. Bispecific antibodies: a mechanistic review of the pipeline. *Nat Rev Drug Discovery* (2019) 18:585–608. doi: 10.1038/s41573-019-0028-1

66. Chen L, Douglass J, Kleinberg L, Ye X, Marciscano AE, Forde PM, et al. Concurrent immune checkpoint inhibitors and stereotactic radiosurgery for brain metastases in non-small cell lung cancer, melanoma, and renal cell carcinoma. *Int J Radiat Oncol Biol Phys* (2018) 100:916–25. doi: 10.1016/j.ijrobp.2017.11.041

67. Hu ZI, Ho AY, Mcarthur HL. Combined radiation therapy and immune checkpoint blockade therapy for breast cancer. *Int J Radiat Oncol Biol Phys* (2017) 99:153–64. doi: 10.1016/j.ijrobp.2017.05.029

68. Dafni U, Tsourti Z, Vervita K, Peters S. Immune checkpoint inhibitors, alone or in combination with chemotherapy, as first-line treatment for advanced non-small cell lung cancer. a systematic review and network meta-analysis. *Lung Cancer* (2019) 134:127–40. doi: 10.1016/j.lungcan.2019.05.029

69. Geynisman DM, Chien C-R, Smieliauskas F, Shen C, Shih Y-CT. Economic evaluation of therapeutic cancer vaccines and immunotherapy: A systematic review. *Hum Vaccines Immunother* (2014) 10:3415–24. doi: 10.4161/hv.29407

70. Marchini A, Scott EM, Rommelaere J. Overcoming barriers in oncolytic virotherapy with HDAC inhibitors and immune checkpoint blockade. *Viruses* (2016) 8:9. doi: 10.3390/v8010009

71. Rahimi Kalateh Shah Mohammad G, Ghahremanloo A, Soltani A, Fathi E, Hashemy SI. Cytokines as potential combination agents with PD-1/PD-L1 blockade for cancer treatment. *J Cell Physiol* (2020) 235:5449–60. doi: 10.1002/jcp.29491

72. Yoon DH, Osborn MJ, Tolar J, Kim CJ. Incorporation of immune checkpoint blockade into chimeric antigen receptor T cells (CAR-ts): combination or built-in CAR-T. *Int J Mol Sci* (2018) 19:340. doi: 10.3390/ijms19020340

73. Ishida Y. PD-1: its discovery, involvement in cancer immunotherapy, and beyond. *Cells* (2020) 9:1376. doi: 10.3390/cells9061376

74. Taefehshokr N, Baradaran B, Baghbanzadeh A, Taefehshokr S. Promising approaches in cancer immunotherapy. *Immunobiology* (2020) 225:151875. doi: 10.1016/j.imbio.2019.11.010

75. Okazaki T, Iwai Y, Honjo T. New regulatory co-receptors: inducible co-stimulator and PD-1. *Curr Opin Immunol* (2002) 14:779–82. doi: 10.1016/S0952-7915(02)00398-9

76. Nishimura H, Okazaki T, Tanaka Y, Nakatani K, Hara M, Matsumori A, et al. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science* (2001) 291:319–22. doi: 10.1126/science.291.5502.319

77. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science* (2018) 359:1350–5. doi: 10.1126/science.aar4060

78. Hosseinkhani N, Derakhshani A, Kooshkaki O, Abdoli SHADBAD M, Hajiasgharzadeh K, Baghbanzadeh A, et al. Immune checkpoints and CAR-T cells: the pioneers in future cancer therapies? *Int J Mol Sci* (2020) 21:8305. doi: 10.3390/ijms21218305

79. Kim N, Kim HS. Targeting checkpoint receptors and molecules for therapeutic modulation of natural killer cells. *Front Immunol* (2018) 9:2041. doi: 10.3389/fimmu.2018.02041

80. Wang J, Li J, Tang G, Tian Y, Su S, Li Y. Clinical outcomes and influencing factors of PD-1/PD-L1 in hepatocellular carcinoma. *Oncol Lett* (2021) 21:279. doi: 10.3892/ol.2021.12540

81. Chen J, Wang J, Xu H. Comparison of atezolizumab, durvalumab, pembrolizumab, and nivolumab as first-line treatment in patients with extensive-stage small cell lung cancer: A systematic review and network meta-analysis. *Med (Baltimore)* (2021) 100:e25180. doi: 10.1097/MD.0000000000025180

82. Peyrottes A, Ouzaid I, Califano G, Hermieu JF, Xylinas E. Neoadjuvant immunotherapy for muscle-invasive bladder cancer. *Medicina (Kaunas)* (2021) 57:769. doi: 10.3390/medicina57080769

83. Rizvi NA, Mazières J, Planchard D, Stinchcombe TE, Dy GK, Antonia SJ, et al. Activity and safety of nivolumab, an anti-PD-1 immune checkpoint inhibitor, for patients with advanced, refractory squamous non-small-cell lung cancer (CheckMate 063): a phase 2, single-arm trial. *Lancet Oncol* (2015) 16:257–65. doi: 10.1016/S1470-2045(15)70054-9

84. Narayan V, Kahlmeyer A, Dahm P, Skoetz N, Risk MC, Bongiorno C, et al. Pembrolizumab monotherapy versus chemotherapy for treatment of advanced urothelial carcinoma with disease progression during or following platinum-containing chemotherapy. a cochrane rapid review. *Cochrane Database Syst Rev* (2018) 7:Cd012838. doi: 10.1002/14651858.CD012838.pub2

85. Van Den Ende T, Van Den Boorn HG, Hoonhout NM, Van Etten-Jamaludin FS, Meijer SL, Derks S, et al. Priming the tumor immune microenvironment with chemo (radio) therapy: a systematic review across tumor types. *Biochim Biophys Acta (BBA) Reviews Cancer* (2020) 1874:188386. doi: 10.1016/j.bbcan.2020.188386

86. Kaewkangsadan V, Verma C, Eremin JM, Cowley G, Ilyas M, Eremin O. Crucial contributions by T lymphocytes (effector, regulatory, and checkpoint inhibitor) and cytokines (TH1, TH2, and TH17) to a pathological complete

response induced by neoadjuvant chemotherapy in women with breast cancer. *J Immunol Res* (2016) 2016:25. doi: 10.1155/2016/4757405

87. Parra ER, Villalobos P, Behrens C, Jiang M, Pataer A, Swisher SG, et al. Effect of neoadjuvant chemotherapy on the immune microenvironment in non–small cell lung carcinomas as determined by multiplex immunofluorescence and image analysis approaches. *J Immunotherapy Cancer* (2018) 6:1–11. doi: 10.1186/s40425-018-0368-0

88. Lo CS, Sanii S, Kroeger DR, Milne K, Talhouk A, Chiu DS, et al. Neoadjuvant chemotherapy of ovarian cancer results in three patterns of tumor-infiltrating lymphocyte response with distinct implications for immunotherapy. *Clin Cancer Res* (2017) 23:925–34. doi: 10.1158/1078-0432.CCR-16-1433

89. Miyazaki T, Ishikawa E, Matsuda M, Akutsu H, Osuka S, Sakamoto N, et al. Assessment of PD-1 positive cells on initial and secondary resected tumor specimens of newly diagnosed glioblastoma and its implications on patient outcome. *J Neuro Oncol* (2017) 133:277–85. doi: 10.1007/s11060-017-2451-7

90. Dong H., Zhu G., Tamada K., Chen L. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nature Med* (1999) 5(12):1365–69.

91. Akinleye A, Rasool Z. Immune checkpoint inhibitors of PD-L1 as cancer therapeutics. *J Hematol Oncol* (2019) 12:1–13. doi: 10.1186/s13045-019-0779-5

92. Keir ME, Francisco LM, Sharpe AH. PD-1 and its ligands in T-cell immunity. *Curr Opin Immunol* (2007) 19:309–14. doi: 10.1016/j.coi.2007.04.012

93. Lotfinejad P, Kazemi T, Mokhtarzadeh A, Shanehbandi D, Jadidi Niaragh F, Safaei S, et al. PD-1/PD-L1 axis importance and tumor microenvironment immune cells. *Life Sci* (2020) 259:118297. doi: 10.1016/j.lfs.2020.118297

94. Keir ME, Liang SC, Guleria I, Latchman YE, Qipo A, Albacker LA, et al. Tissue expression of PD-L1 mediates peripheral T cell tolerance. *J Exp Med* (2006) 203:883–95. doi: 10.1084/jem.20051776

95. Wang Y, Wang H, Yao H, Li C, Fang J-Y, Xu J. Regulation of PD-L1: emerging routes for targeting tumor immune evasion. *Front Pharmacol* (2018) 9:536. doi: 10.3389/fphar.2018.00536

96. Maiorano BA, de Giorgi U, Ciardiello D, Schinzari G, Cisternino A, Tortora G, et al. Immune-checkpoint inhibitors in advanced bladder cancer: Seize the day. *Biomedicines* (2022) 10:411. doi: 10.3390/biomedicines10020411

97. Jin H, D'Urso V, Neuteboom B, Mckenna SD, Schweickhardt R, Gross AW, et al. Avelumab internalization by human circulating immune cells is mediated by both fc gamma receptor and PD-L1 binding. *Oncoimmunology* (2021) 10:1958590. doi: 10.1080/2162402X.2021.1958590

98. Mamdani H, Schneider B, Perkins SM, Burney HN, Kasi PM, Abushahin LI, et al. A phase II trial of adjuvant durvalumab following trimodality therapy for locally advanced esophageal and gastroesophageal junction adenocarcinoma: A big ten cancer research consortium study. *Front Oncol* (2021) 11:736620. doi: 10.3389/fonc.2021.736620

99. Lim YJ, Koh J, Kim S, Jeon S-R, Chie EK, Kim K, et al. Chemoradiation-induced alteration of programmed death-ligand 1 and CD8+ tumor-infiltrating lymphocytes identified patients with poor prognosis in rectal cancer: A matched comparison analysis. *Int J Radiat Oncol Biol Phys* (2017) 99:1216–24. doi: 10.1016/j.ijrobp.2017.07.004

100. Ogura A, Akiyoshi T, Yamamoto N, Kawachi H, Ishikawa Y, Mori S, et al. Pattern of programmed cell death-ligand 1 expression and CD8-positive T-cell infiltration before and after chemoradiotherapy in rectal cancer. *Eur J Cancer* (2018) 91:11–20. doi: 10.1016/j.ejca.2017.12.005

101. Song Z, Yu X, Zhang Y. Altered expression of programmed death-ligand 1 after neo-adjuvant chemotherapy in patients with lung squamous cell carcinoma. *Lung Cancer* (2016) 99:166–71. doi: 10.1016/j.lungcan.2016.07.013

102. Zhang S, Bai W, Tong X, Bu P, Xu J, Xi Y. Correlation between tumor microenvironment–associated factors and the efficacy and prognosis of neoadjuvant therapy for rectal cancer. *Oncol Lett* (2019) 17:1062–70. doi: 10.3892/ol.2018.9682

103. Buchbinder EI, Desai A. CTLA-4 and PD-1 pathways: similarities, differences, and implications of their inhibition. *Am J Clin Oncol* (2016) 39:98. doi: 10.1097/COC.0000000000000239

104. Chambers CA, Kuhns MS, Egen JG, Allison JP. CTLA-4-mediated inhibition in regulation of T cell responses: mechanisms and manipulation in tumor immunotherapy. *Annu Rev Immunol* (2001) 19:565–94. doi: 10.1146/annurev.immunol.19.1.565

105. Collins AV, Brodie DW, Gilbert RJ, Iaboni A, Manso-Sancho R, Walse B, et al. The interaction properties of costimulatory molecules revisited. *Immunity* (2002) 17:201–10. doi: 10.1016/S1074-7613(02)00362-X

106. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science* (2015) 348:69–74. doi: 10.1126/science.aaa4971

107. Friedline RH, Brown DS, Nguyen H, Kornfeld H, Lee J, Zhang Y, et al. CD4 + regulatory T cells require CTLA-4 for the maintenance of systemic tolerance. *J Exp Med* (2009) 206:421–34. doi: 10.1084/jem.20081811

108. Read S, Greenwald R, Izcue A, Robinson N, Mandelbrot D, Francisco L, et al. Blockade of CTLA-4 on CD4+ CD25+ regulatory T cells abrogates their function *in vivo*. *J Immunol* (2006) 177:4376–83. doi: 10.4049/jimmunol.177.7.4376

109. Govindan R, Szczesna A, Ahn MJ, Schneider CP, Gonzalez Mella PF, Barlesi F, et al. Phase III trial of ipilimumab combined with paclitaxel and carboplatin in advanced squamous non-Small-Cell lung cancer. *J Clin Oncol* (2017) 35:3449–57. doi: 10.1200/JCO.2016.71.7629

110. He M, Chai Y, Qi J, Zhang CW, Tong Z, Shi Y, et al. Remarkably similar CTLA-4 binding properties of therapeutic ipilimumab and tremelimumab antibodies. *Oncotarget* (2017) 8:67129. doi: 10.18632/oncotarget.18004

111. Kooshkaki O, Derakhshani A, Hosseinkhani N, Torabi M, Safaei S, Brunetti O, et al. Combination of ipilimumab and nivolumab in cancers: From clinical practice to ongoing clinical trials. *Int J Mol Sci* (2020) 21:4427. doi: 10.3390/ijms21124427

112. Duffy AG, Ulahannan SV, Makorova-Rusher O, Rahma O, Wedemeyer H, Pratt D, et al. Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma. *J Hepatol* (2017) 66:545–51. doi: 10.1016/j.jhep.2016.10.029

113. Vargas FA, Furness AJ, Litchfield K, Joshi K, Rosenthal R, Ghorani E, et al. Fc effector function contributes to the activity of human anti-CTLA-4 antibodies. *Cancer Cell* (2018) 33:649–663.e4. doi: 10.1016/j.ccell.2018.02.010

114. Hargadon KM, Johnson CE, Williams CJ. Immune checkpoint blockade therapy for cancer: an overview of FDA-approved immune checkpoint inhibitors. *Int Immunopharmacol* (2018) 62:29–39. doi: 10.1016/j.intimp.2018.06.001

115. Triebel F, Jitsukawa S, Baixeras E, Roman-Roman S, Genevee C, Viegas-Pequignot E, et al. LAG-3, a novel lymphocyte activation gene closely related to CD4. *J Exp Med* (1990) 171:1393–405. doi: 10.1084/jem.171.5.1393

116. Kisielow M, Kisielow J, Capoferri-Sollami G, Karjalainen K. Expression of lymphocyte activation gene 3 (LAG-3) on b cells is induced by T cells. *Eur J Immunol* (2005) 35:2081–8. doi: 10.1002/eji.200526090

117. Grosso JF, Kelleher CC, Harris TJ, Maris CH, Hipkiss EL, de Marzo A, et al. LAG-3 regulates CD8+ T cell accumulation and effector function in murine self-and tumor-tolerance systems. *J Clin Invest* (2007) 117:3383–92. doi: 10.1172/JCI31184

118. Andreae S, Buisson S, Triebel FDR. MHC class II signal transduction in human dendritic cells induced by a natural ligand, the LAG-3 protein (CD223). *Blood* (2003) 102:2130–7. doi: 10.1182/blood-2003-01-0273

119. Lythgoe MP, Liu DSK, Annels NE, Krell J, Frampton AE. Gene of the month: lymphocyte-activation gene 3 (LAG-3). *J Clin Pathol* (2021) 74:543–7. doi: 10.1136/jclinpath-2021-207517

120. Ruffo E, Wu RC, Bruno TC, Workman CJ, Vignali DA. Lymphocyte-activation gene 3 (LAG3): the next immune checkpoint receptor. *Semin Immunol* (2019) 42:101305. doi: 10.1016/j.smim.2019.101305

121. Kouo T, Huang L, Pucsek AB, Cao M, Solt S, Armstrong T, et al. Galectin-3 shapes antitumor immune responses by suppressing CD8+ T cells *via* LAG-3 and inhibiting expansion of plasmacytoid dendritic cells. *Cancer Immunol Res* (2015) 3:412–23. doi: 10.1158/2326-6066.CIR-14-0150

122. 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

123. Xu F, Liu J, Liu D, Liu B, Wang M, Hu Z, et al. LSECtin expressed on melanoma cells promotes tumor progression by inhibiting antitumor T-cell responses. *Cancer Res* (2014) 74:3418–28. doi: 10.1158/0008-5472.CAN-13-2690

124. Goldberg MV, Drake CG. LAG-3 in cancer immunotherapy. *Cancer Immunol Immunother* (2010) 344:269–78. doi: 10.1007/82_2010_114

125. Jiang H, Ni H, Zhang P, Guo X, Wu M, Shen H, et al. PD-L1/LAG-3 bispecific antibody enhances tumor-specific immunity. *Oncoimmunology* (2021) 10:1943180. doi: 10.1080/2162402X.2021.1943180

126. D'Arrigo P, Tufano M, Rea A, Vigorito V, Novizio N, Russo S, et al. Manipulation of the immune system for cancer defeat: a focus on the T cell inhibitory checkpoint molecules. *Curr medicinal Chem* (2020) 27:2402–48. doi: 10.2174/0929867325666181106114421

127. Sordo-Bahamonde C, Lorenzo-Herrero S, González-Rodríguez AP, Payer ÁR, González-García E, López-Soto A, et al. LAG-3 blockade with relatlimab (BMS-986016) restores anti-leukemic responses in chronic lymphocytic leukemia. *Cancers (Basel)* (2021) 13:2112. doi: 10.3390/cancers13092112

128. Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell* (2015) 161:205–14. doi: 10.1016/j.cell.2015.03.030

129. Brignone C, Gutierrez M, Mefti F, Brain E, Jarcau R, Cvitkovic F, et al. First-line chemoimmunotherapy in metastatic breast carcinoma: combination of paclitaxel and IMP321 (LAG-3Ig) enhances immune responses and antitumor activity. *J Transl Med* (2010) 8:71. doi: 10.1186/1479-5876-8-71

130. Wang Y, Dong T, Xuan Q, Zhao H, Qin L, Zhang Q. Lymphocyte-activation gene-3 expression and prognostic value in neoadjuvant-treated triple-

negative breast cancer. *J Breast Cancer* (2018) 21:124–33. doi: 10.4048/jbc.2018.21.2.124

131. Bottai G, Raschioni C, Losurdo A, Di Tommaso L, Tinterri C, Torrisi R, et al. An immune stratification reveals a subset of PD-1/LAG-3 double-positive triple-negative breast cancers. *Breast Cancer Res* (2016) 18:1–10. doi: 10.1186/s13058-016-0783-4

132. Prasad DV, Richards S, Mai XM, Dong C. B7S1, a novel B7 family member that negatively regulates T cell activation. *Immunity* (2003) 18:863–73. doi: 10.1016/S1074-7613(03)00147-X

133. Sica GL, Choi I-H, Zhu G, Tamada K, Wang S-D, Tamura H, et al. B7-H4, a molecule of the B7 family, negatively regulates T cell immunity. *Immunity* (2003) 18:849–61. doi: 10.1016/S1074-7613(03)00152-3

134. Zang X, Kim J, Murphy K, Waitz R, Allison JP. B7x: a widely expressed B7 family member that inhibits T cell activation. *Proc Natl Acad Sci* (2003) 100:10388–92. doi: 10.1073/pnas.1434299100

135. Salceda S, Tang T, Kmet M, Munteanu A, Ghosh M, Macina R, et al. The immunomodulatory protein B7-H4 is overexpressed in breast and ovarian cancers and promotes epithelial cell transformation. *Exp Cell Res* (2005) 306:128–41. doi: 10.1016/j.yexcr.2005.01.018

136. Choi I-H, Zhu G, Sica GL, Strome SE, Cheville JC, Lau JS, et al. Genomic organization and expression analysis of B7-H4, an immune inhibitory molecule of the B7 family. *J Immunol* (2003) 171:4650–4. doi: 10.4049/jimmunol.171.9.4650

137. Qian Y, Shen L, Cheng L, Wu Z, Yao H. B7-H4 expression in various tumors determined using a novel developed monoclonal antibody. *Clin Exp Med* (2011) 11:163–70. doi: 10.1007/s10238-010-0125-2

138. Simon I, Zhuo S, Corral L, Diamandis EP, Sarno MJ, Wolfert RL, et al. B7-h4 is a novel membrane-bound protein and a candidate serum and tissue biomarker for ovarian cancer. *Cancer Res* (2006) 66:1570–5. doi: 10.1158/0008-5472.CAN-04-3550

139. Kryczek I, Zou L, Rodriguez P, Zhu G, Wei S, Mottram P, et al. B7-H4 expression identifies a novel suppressive macrophage population in human ovarian carcinoma. *J Exp Med* (2006) 203:871–81. doi: 10.1084/jem.20050930

140. Kryczek I, Wei S, Zou L, Zhu G, Mottram P, Xu H, et al. Cutting edge: induction of B7-H4 on APCs through IL-10: novel suppressive mode for regulatory T cells. *J Immunol* (2006) 177:40–4. doi: 10.4049/jimmunol.177.1.40

141. Kryczek I, Wei S, Zhu G, Myers L, Mottram P, Cheng P, et al. Relationship between B7-H4, regulatory T cells, and patient outcome in human ovarian carcinoma. *Cancer Res* (2007) 67:8900–5. doi: 10.1158/0008-5472.CAN-07-1866

142. Smith JB, Stashwick C, Powell JR DJ. B7-H4 as a potential target for immunotherapy for gynecologic cancers: a closer look. *Gynecol Oncol* (2014) 134:181–9. doi: 10.1016/j.ygyno.2014.03.553

143. Abadi YM, Jeon H, Ohaegbulam KC, Scandiuzzi L, Ghosh K, Hofmeyer KA, et al. Host b7x promotes pulmonary metastasis of breast cancer. *J Immunol* (2013) 190:3806–14. doi: 10.4049/jimmunol.1202439

144. Yin Y, Shi L, Yang J, Wang H, Yang H, Wang Q. B7 family member H4 induces epithelial-mesenchymal transition and promotes the proliferation, migration and invasion of colorectal cancer cells. *Bioengineered* (2022) 13:107–18. doi: 10.1080/21655979.2021.2009411

145. Wu H, Wang X, Mo N, Zhang L, Yuan X, Lü Z. B7-homolog 4 promotes epithelial-mesenchymal transition and invasion of bladder cancer cells *via* activation of nuclear factor-κB. *Oncol Res* (2018) 26:1267–74. doi: 10.3727/096504018X15172227703244

146. Li C, Zhan Y, Ma X, Fang H, Gai X. B7-H4 facilitates proliferation and metastasis of colorectal carcinoma cell through PI3K/Akt/mTOR signaling pathway. *Clin Exp Med* (2020) 20:79–86. doi: 10.1007/s10238-019-00590-7

147. Lu Y, Wu F, Cao Q, Sun Y, Huang M, Xiao J, et al. B7-H4 is increased in lung adenocarcinoma harboring EGFR-activating mutations and contributes to immunosuppression. *Oncogene* (2022) 41:704–17. doi: 10.1038/s41388-021-02124-6

148. Miao G, Sun X. Development of a novel anti-B7-H4 antibody enhances anti-tumor immune response of human T cells. *BioMed Pharmacother* (2021) 141:111913. doi: 10.1016/j.biopha.2021.111913

149. Dangaj D, Lanitis E, Zhao A, Joshi S, Cheng Y, Sandaltzopoulos R, et al. Novel recombinant human b7-h4 antibodies overcome tumoral immune escape to potentiate T-cell antitumor responses. *Cancer Res* (2013) 73:4820–9. doi: 10.1158/0008-5472.CAN-12-3457

150. Podojil JR, Miller SD. Potential targeting of B7-H4 for the treatment of cancer. *Immunol Rev* (2017) 276:40–51. doi: 10.1111/imr.12530

151. Maskey N, Li K, Hu M, Xu Z, Peng C, Yu F, et al. Impact of neoadjuvant chemotherapy on lymphocytes and co-inhibitory B7-H4 molecule in gastric cancer: low B7-H4 expression associates with favorable prognosis. *Tumor Biol* (2014) 35:11837–43. doi: 10.1007/s13277-014-2410-2

152. Erfanian N, Derakhshani A, Nasseri S, Fereidouni M, Baradaran B, Jalili Tabrizi N, et al. Immunotherapy of cancer in single-cell RNA sequencing era: A precision medicine perspective. *BioMed Pharmacother* (2022) 146:112558. doi: 10.1016/j.biopha.2021.112558

153. Larance M, Lamond AI. Multidimensional proteomics for cell biology. *Nat Rev Mol Cell Biol* (2015) 16:269–80. doi: 10.1038/nrm3970

154. Roessner U, Bowne J. What is metabolomics all about? *Biotechniques* (2009) 46:363–5. doi: 10.2144/000113133

155. Krzyszczyk P, Acevedo A, Davidoff EJ, Timmins LM, Marrero-Berrios I, Patel M, et al. The growing role of precision and personalized medicine for cancer treatment. *Technol (Singap World Sci)* (2018) 6:79–100. doi: 10.1142/S2339547818300020

156. Runa F, Hamalian S, Meade K, Shisgal P, Gray PC, Kelber JA. Tumor microenvironment heterogeneity: challenges and opportunities. *Curr Mol Biol Rep* (2017) 3:218–29. doi: 10.1007/s40610-017-0073-7

157. Wang Z, Liu X, Ho RL, Lam CW, Chow MS. Precision or personalized medicine for cancer chemotherapy: Is there a role for herbal medicine. *Molecules* (2016) 21:889. doi: 10.3390/molecules21070889

158. Schrag D, Garewal HS, Burstein HJ, Samson DJ, Von Hoff DD, Somerfield MR. American Society of clinical oncology technology assessment: chemotherapy sensitivity and resistance assays. *J Clin Oncol* (2004) 22:3631–8. doi: 10.1200/JCO.2004.05.065

159. Sticht C, de la Torre C, Parveen A, Gretz N. miRWalk: an online resource for prediction of microRNA binding sites. *PLoS One* (2018) 13:e0206239. doi: 10.1371/journal.pone.0206239

160. Huang H-Y, Lin Y.-C.-D., Li J, Huang K-Y, Shrestha S, Hong H-C, et al. miRTarBase 2020: updates to the experimentally validated microRNA–target interaction database. *Nucleic Acids Res* (2020) 48:D148–54. doi: 10.1093/nar/gkz896

161. Zhao J, Wang Y, Wang Y, Gao J, Wu X, Li H. miR-194-3p represses the docetaxel resistance in colon cancer by targeting KLK10. *Pathol Res Pract* (2022) 236:153962. doi: 10.1016/j.prp.2022.153962

162. Salimian J, Baradaran B, Azimzadeh Jamalkandi S, Moridikia A, Ahmadi A. MiR-486-5p enhances cisplatin sensitivity of human muscle-invasive bladder cancer cells by induction of apoptosis and down-regulation of metastatic genes. *Urol Oncol* (2020) 38:738.e9–738.e21. doi: 10.1016/j.urolonc.2020.05.008

163. Jin X, Pang W, Zhang Q, Huang H. MicroRNA-486-5p improves nonsmall-cell lung cancer chemotherapy sensitivity and inhibits epithelial-mesenchymal transition by targeting twinfilin actin binding protein 1. *J Int Med Res* (2019) 47:3745–56. doi: 10.1177/0300060519850739

164. Cao S, Lin L, Xia X, Wu H. MicroRNA-761 promotes the sensitivity of colorectal cancer cells to 5-fluorouracil through targeting FOXM1. *Oncotarget* (2018) 9:321–31. doi: 10.18632/oncotarget.20109

165. Pan C, Sun G, Sha M, Wang P, Gu Y, Ni Q. Investigation of miR-93-5p and its effect on the radiosensitivity of breast cancer. *Cell Cycle* (2021) 20:1173–80. doi: 10.1080/15384101.2021.1930356

166. Zhang S, Wang W, Wu X, Liu W, Ding F. miR-16-5p modulates the radiosensitivity of cervical cancer cells *via* regulating coactivator-associated arginine methyltransferase 1. *Pathol Int* (2020) 70:12–20. doi: 10.1111/pin.12867

167. Wang J, Jiang M, Xia S. miR-339-5p increases radiosensitivity of lung cancer cells by targeting phosphatases of regenerating liver-1 (PRL-1). *Med Sci Monit* (2018) 24:8408–16. doi: 10.12659/MSM.910808

168. Tu L, Guan R, Yang H, Zhou Y, Hong W, Ma L, et al. Assessment of the expression of the immune checkpoint molecules PD-1, CTLA4, TIM-3 and LAG-3 across different cancers in relation to treatment response, tumor-infiltrating immune cells and survival. *Int J Cancer* (2020) 147:423–39. doi: 10.1002/ijc.32785

169. Nirschl CJ, Drake CG. Molecular pathways: coexpression of immune checkpoint molecules: signaling pathways and implications for cancer immunotherapy. *Clin Cancer Res* (2013) 19:4917–24. doi: 10.1158/1078-0432.CCR-12-1972

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The therapeutic potential of TREM2 in cancer

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Cancer continues to be a substantial health concern and a leading cause of death in the United States and around the world. Therefore, it is important to continue to explore the potential of novel therapeutic targets and combinatorial therapies. Triggering receptor expressed on myeloid cells 2 (TREM2) is a transmembrane receptor of the immunoglobulin superfamily that associates with DNAX activation protein (DAP) 12 and DAP10 to propagate signals within the cell. TREM2 has primarily been recognized for its expression on cells in the monocyte-macrophage lineage, with the majority of work focusing on microglial function in Alzheimer's Disease. However, expansion of TREM2 research into the field of cancer has revealed that epithelial tumor cells as well as intratumoral macrophages and myeloid regulatory cells also express TREM2. In this review, we discuss evidence that TREM2 contributes to tumor suppressing or oncogenic activity when expressed by epithelial tumor cells. In addition, we discuss the immunosuppressive role of TREM2-expressing intratumoral macrophages, and the therapeutic potential of targeting TREM2 in combination with immune checkpoint therapy. Overall, the literature reveals TREM2 could be considered a novel therapeutic target for certain types of cancer.

KEYWORDS

TREM2 (triggering receptor expressed on myeloid cells 2), immunotherapy, tumor associated macrophage (TAM), immunosuppression, tumor infiltrating lymphocyte (TIL)

Introduction

Triggering receptor expressed on myeloid cells 2 (TREM2) is a transmembrane receptor of the immunoglobulin superfamily that binds an array of ligands including bacteria and polyanionic molecules (1), DNA (2), lipoproteins (3), phospholipids (4) and sulfoglicolipids such as Sulfavant A (5). TREM2 itself does not contain intrinsic signaling capabilities; therefore, it associates with the adaptor proteins DNAX activation protein (DAP) 12 and DAP10; which, upon TREM2-ligand interaction are phosphorylated and propagate signals within the cell (6). TREM2-ligand interaction and subsequent ITAM phosphorylation of DAP12, which is the primary adaptor protein for TREM2, results in

activation of Syk, which leads to the phosphorylation of ERK1/2, PLCγ1, and Cbl (7, 8). In contrast, activation of DAP10 results in recruitment of PI3K and activation of Grb2, leading to Akt and ERK signaling respectively (6). While the strength and direction of TREM2 downstream signaling is differentially modulated upon interaction with various ligands, many aspects of TREM2 interaction and the downstream signals propagated remain to be fully understood (7). In addition to signaling through TREM2-ligand interaction and propagation of signals through DAP12 and DAP10, cleavage of TREM2 by a disintegrin and metalloproteinase (ADAM) proteases results in soluble TREM2 (sTREM2), which can act as a signaling molecule. ADAM 10 and 17 cleave human TREM2 at the H157-S158 peptide bond to release the ectodomain of TREM2 (9). Treatment with sTREM2 in *in vitro* studies has resulted in ERK and MAPK14 activation in bone marrow derived macrophages (10) and NF-κB activation in microglia; however, the receptors for sTREM2 remain unidentified (11).

Generally, TREM2 is appreciated for its expression on the surface of cells in the monocyte-macrophage lineage, such as microglia and osteoclasts, with implications for neurodegenerative diseases (12–15) and bone disorders (16, 17). However, more recently, TREM2 has been identified on certain epithelial-derived cancer cells and its expression influences their behavior. This review will focus on the role of TREM2 in cancer, including patient survival data and TREM2 expression in human tumor samples, as well as a discussion of the potentially oncogenic or tumor suppressive roles of TREM2 when expressed by the epithelial tumor cells. In addition, it will provide information on the immunosuppressive environment created by tumor infiltrating immune cells expressing TREM2.

TREM2 discovery and early implications to human health

TREM2 was first discovered in human monocyte-derived dendritic cells (DCs), where its expression promoted DC survival and upregulation of CCR7, major histocompatibility complex class II, CD86, and CD40 (8). However, TREM2 was first implicated in human health and disease when variants of TREM2 and its adaptor protein DAP12 were identified in families with Nasu-Hakola Disease, which is also known as polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy. Patients with Nasu-Hakola Disease are homozygous for loss-of-function mutations in either DAP12 or TREM2, and the disease is characterized by presenile dementia and bone cysts (18–20). With the realization of the importance of TREM2 in neuronal health, investigators have also shown that TREM2 plays a protective function against development of Alzheimer's Disease. Microglia in the brain express TREM2 and mutations of TREM2 impact its ability to bind ligands, diminish microglial activation, and accelerate progression of Alzheimer's Disease (21). Up to this point, the majority of work on TREM2 has been conducted in the context of Nasu-Hakola Disease and Alzheimer's Disease and the role of TREM2 in these settings has recently been reviewed elsewhere (22, 23). However, the importance of TREM2 in cancer has recently come to light, although it is not yet widely studied or understood, hence the focus on cancer for this review.

TREM2 expression in human tumors and correlations with human survival data

TREM2 in human tumor samples

Multiple studies have analyzed TREM2 mRNA expression and protein expression in human tumor tissue compared to normal tissue as summarized in Table 1. However, the data from different groups are sometimes contradictory even within the same cancers, such as hepatocellular carcinoma (HCC) and gastric cancer. In both of these cases, studies have analyzed TREM2 expression and reported opposite findings despite using similar methods. This highlights the heterogeneity of human tumors as well as the need for further investigation and understanding of the role of TREM2 in cancer.

An important first step to understanding the role of TREM2 in cancer was to determine which cell type expresses TREM2 in the tumor microenvironment (TME). A study on TREM2 in HCC observed increased TREM2 expression in HCC samples compared to surrounding normal tissue, and IHC staining revealed the TREM2-expressing cells morphologically resembled macrophages (25). Analysis of single cell sequencing from human HCC livers confirmed this morphological observation as the data demonstrated prominent *TREM2* expression in the macrophages (25). These findings have been corroborated by more recent analysis of single cell RNA sequencing that confirmed specific expression of *TREM2* in macrophages in HCC (27). Similarly, Molgora et al. observed increased TREM2 staining in macrophages, as determined by cell morphology, in 75% of carcinomas from various primary sites compared to normal tissue (34). IHC of primary carcinomas and melanomas demonstrated co-expression of TREM2 with macrophage markers CD163, CD68, MAF-B, CSF1R, and MITF; however, the study did not specify the types of cancer analyzed (34).

Interestingly, analysis of liver, lung, and lymph node metastases originating from ovarian serous and breast carcinoma and colorectal and lung adenocarcinoma by IHC demonstrate specific $TREM2^{+}$ staining within the metastatic nodules and not in the surrounding normal tissue (34). Spatial analysis of TREM2 expression by IHC within tumors shows

TREM2$^+$ macrophages are primarily localized in the tumor nest in hepatocellular, lung, and pancreatic carcinomas. In other cancers, TREM2$^+$ macrophages were found within both the tumor nest and tumor stroma (32). Although these studies indicate TREM2 is expressed on tumor associated macrophages (TAMs), the conclusions of these studies do not exclude that other cell types in the tumor might also express TREM2.

Human survival data

In a systematic pan-cancer analysis of TREM2 across 33 cancer types, Cheng et al. identified positive and negative associations of TREM2 expression with prognosis in different cancers using data extracted from the TCGA. Kaplan Meier analysis indicated high TREM2 expression was associated with

TABLE 1 TREM2 expression in human tumors.

Cancer type	mRNA expression	Protein expression	Reference
Hepatocellular Carcinoma	Decreased (qRT-PCR)	Decreased (WB, IHC)	Tang et al. (24)
	Increased (TCGA)	Increased (IHC)	Esparza-Baquer et al. (25)
	Increased (TCGA)	Increased (IHC)	Cheng et al. (26)
	Increased (scRNAseq)	Increased (IHC)	Zhou et al. (27)
Gastric cancer	Increased (qRT-PCR)	Increased (IHC)	Zhang et al. (28)
	not assessed	Decreased (IHC)	Tang et al. (24)
	Increased (TCGA)	NA	Cheng et al. (26)
Pancreatic cancer	not assessed	Increased (IHC)	Tang et al. (24)
Glioma	Increased (qRT-PCR, TCGA)	Increased (IHC)	Wang et al. (29)
Glioblastoma multiforme	Increased (TCGA)	NA	Cheng et al. (26)
Renal cell carcinoma	Increased (qRT-PCR)	Increased (WB)	Zhang et al. (30)
Kidney renal clear cell carcinoma	Increased (TCGA)	NA	Cheng et al. (26)
Kidney renal papillary cell carcinoma	Increased (TCGA)	NA	Cheng et al. (26)
Kidney chromophobe	Increased (TCGA)	NA	Cheng et al. (26)
Colon cancer	NA	Decreased (IHC)	Kim et al. (31)
	Increased (TCGA)	Increased (IHC)	Cheng et al. (26)
Head and neck squamous cell carcinoma	Increased (TCGA)	Increased (IHC)	Cheng et al. (26)
Uterine corpus endometrial carcinoma	Increased (TCGA)	NA	Cheng et al. (26)
Cholangiocarcinoma	Increased (TCGA)	NA	Cheng et al. (26)
Prostate adenocarcinoma	Increased (TCGA)	NA	Cheng et al. (26)
Bladder urothelial carcinoma	Increased (TCGA)	NA	Cheng et al. (26)
Breast cancer	Increased (TCGA)	Increased (IHC)	Cheng et al. (26)
	Increased (TCGA)	NA	Nalio Ramos et al. (32)
Cervical squamous cell carcinoma and endocervical adenocarcinoma	Increased (TCGA)	Increased (IHC)	Cheng et al. (26)
Thyroid carcinoma	Increased (TCGA)	NA	Cheng et al. (26)
Esophageal carcinoma	Increased (TCGA)	NA	Cheng et al. (26)
Lung squamous cell carcinoma	Decreased (TCGA)	Decreased (IHC)	Cheng et al. (26)
Non-small cell lung cancer	Decreased (TCGA)	Decreased (IHC)	Cheng et al. (26)
	NA	Increased (FC)	Zhang et al. (33)

qRT-PCR, quantitative real time polymerase chain reaction; TCGA, The Cancer Genome Atlas (transcript level); WB, western blot; IHC, immunohistochemistry; scRNAseq, single cell RNA sequencing; FC, flow cytometry; NA, not assessed.
Increased or decreased expression of TREM2 in comparison to normal tissue is indicated for each cancer type at the mRNA and protein expression levels. The method of detection is also noted.

better overall survival in cervical squamous cell carcinoma, endocervical adenocarcinoma, lymphoid neoplasm diffuse large B-cell lymphoma, lung adenocarcinoma, thyroid carcinoma, and skin cutaneous melanoma (26). In contrast, the study found that high TREM2 expression was associated with worse overall survival in lower grade glioma, liver hepatocellular carcinoma, and kidney renal clear cell carcinoma (26). The seeming discrepancy between TREM2 benefitting or worsening patient prognosis in different types of cancer may at first seem puzzling; however, the investigators dove deeper to better understand TREM2 in each cancer type. Gene expression data and protein-level data from IHC demonstrate differences in TREM2 expression between cancer types with the highest expression in glioblastoma multiforme and the lowest in acute myeloid leukemia. IHC implementation to compare TREM2 expression between normal and tumor tissue showed increased TREM2 expression in the tumor tissue compared to normal tissue in many cancers. However, in other cancers, such as lung squamous cell carcinoma, TREM2 staining was moderate in the normal tissue and weak in the tumor tissue. These deviations in expression of TREM2 indicate that TREM2 may serve distinct roles and may exhibit differing levels of influence in distinct types of cancer. By probing associations between TREM2 and tumor mutation burden, immune scores, tumor stage, DNA methylation, and infiltration of immune cells the investigators highlight the heterogeneity among tumor types. TREM2 may interact differently with each of these factors, thus summing to differing prognoses in patients.

While this pan-cancer analysis is a great resource, there are also previous smaller studies that individually confirm or contradict the findings from the pan-cancer analysis. In agreement with this study, Wang et al. previously demonstrated an association between high TREM2 and worse overall survival in glioma (29). This same association has also been shown in gastric cancer (28), colorectal cancer (CRC) (34), triple negative breast cancer (34) and luminal breast cancer (32), suggesting TREM2 contributes to oncogenic activity in these cancer types. However, in disagreement with the systematic pan-cancer analysis, a previously published study by Tang et al. demonstrated increased TREM2 expression correlates with better overall survival in HCC (24), indicating TREM2 may contribute to tumor suppressing activity in HCC. The discrepancy between the two studies may be explained by use of data from two distinct cohorts of patients. The pan-cancer analysis utilized the TCGA while Tang et al. evaluated a cohort of 250 patients with HCC whose surgically resected samples and survival data were obtained and analyzed by the investigators. Furthermore, Tang et al. collected primary tumor, matched non-tumor liver tissue and venous metastasis from the subjects, which indicates all tumor specimens had metastasized, thus skewing the data set. Another more recent study demonstrated high levels of $TREM2^{+}$ TAMs predicted worse overall survival in both lung adenocarcinoma and lung squamous cell carcinoma, which is also in disagreement with the pan-cancer analysis. This may be accounted for by the specific attention of some groups to macrophage expression of TREM2 rather than overall expression of TREM2.

As discussed in the sections below, the *in vitro* and *in vivo* data from mouse studies do not always align with the human survival data. In the data covered in this review, this is the case specifically in the models of CRC (31), which may be due to the inability of the mouse model to fully recapitulate the human disease. However, this discrepancy could also be related to the method of *TREM2* expression analysis in these Kaplan Meier plots. The data used to generate these plots originate from bulk RNA sequencing. Tumors are comprised of a heterogeneous milieu of cells and *TREM2* can be expressed by the cancer cells or by other immune cell populations such as TAMs. Therefore, the Kaplan Meier plots may be useful as a starting point to evaluate overarching trends, but not as useful for the purpose of delineating the specific role of *TREM2* within the epithelial tumor cells versus the immune cells in the TME. As we learn more about the roles of TREM2 expressed by differing cell types, the utilization of scRNAseq may be critical to advancing our knowledge and understanding of TREM2 in cancer.

TREM2 in cancer progression

Due to the heterogeneous cellular composition of tumors, the contribution of different proteins on the various cell types complicates therapeutic strategies. When considering TREM2 as a potential therapeutic target, it is critical to understand its functions and properties in different contexts and to consider the cell-type expressing TREM2. The emerging body of literature on the subject of TREM2 expression by the epithelial tumor cells is seemingly contradictory, with some studies suggesting TREM2 contributes to tumor suppressive activity and other studies suggesting it supports oncogenic activity. However, the literature consistently reports that TREM2 expression by immune cells creates an immunosuppressive environment that allows the cancer cells to thrive. In subsequent sections, we summarize what is known about the immunosuppressive and oncogenic roles of TREM2 and suggest areas for future investigation.

TREM2 contributes to either tumor suppressing or oncogenic activity in different types of cancer

The case for TREM2 contributing to oncogenic activity

Data on TREM2 expression in epithelial tumor cells is still limited, but two studies, one in renal cell carcinoma (RCC) and

one in glioma have been conducted that point to an oncogenic role of TREM2. The RCC study utilized the ACHN and Caki-2 RCC cell lines (30) and the glioma study used the U87 and U373 glioma cell lines (29). In both studies, silencing of *TREM2* resulted in decreased cell proliferation and increased apoptosis *in vitro* and decreased tumor volume *in vivo* with subcutaneous cell injection models (29, 30). Additionally, in the RCC model, silencing of *TREM2 in vitro* resulted in a decrease of *Bcl2*, a regulator of apoptosis, an increase in the apoptosis genes *Bax* and *Casp3*, and a decrease in the proliferation marker PCNA, measured at both the gene and protein expression levels (30). In the glioma study, silencing of *TREM2* led to a decrease in cell adhesion as well as decreases in the migratory and invasive capacities of both cell lines (29). This work suggests epithelial TREM2 contributes to oncogenic activity in the context of RCC and glioma.

The case for TREM2 contributing to tumor suppressing activity

In vitro and *in vivo* studies suggest that TREM2 may contribute to tumor suppressing activity in CRC and HCC. Using HT29 CRC cells, Kim and colleagues demonstrated that antibody-mediated *TREM2* neutralization resulted in increased cell proliferation, induction of the S phase of the cell cycle, increased cell migration, and increased invasive capacity. Conversely, *TREM2*-overexpressing MC38 CRC cells resulted in decreased tumor volume following subcutaneous injection in mice (31). Together these data suggest that epithelial TREM2 may support tumor suppressing activity in CRC. Similarly, in a mouse model of HCC, knockdown of *TREM2* resulted in increased cell viability, increased migratory and invasive capacities, as well as decreased epithelial markers with an increase in mesenchymal markers (24). Subcutaneous injection of cells with *TREM2* knockdown resulted in increased tumor volume suggesting TREM2 may also contribute to tumor suppressing activity in HCC. To further support this conclusion, the same study found that overexpression of TREM2 resulted in opposing results from the knockdown conditions (24). Another study of HCC utilized carcinogen-induced models of HCC in TREM2 deficient mice to probe the function of TREM2 in HCC. The investigators found that mice globally-deficient for TREM2, thus not expressing TREM2 on the epithelial tumor cells or any other cell type within the TME, developed an increased number of tumors of all sizes following carcinogen (DEN: diethylnitrosamine) administration and also developed an increased number of as well as larger tumors in fibrosis associated HCC models (25). While these data overall support TREM2 contributing to tumor suppressing activity in HCC and CRC, mechanistic conclusions are clouded by the global deletion of TREM2. All studies are summarized in Table 2.

Cancer-associated fibroblast TREM2 expression may modulate paracrine signaling and tumorigenicity

Perugorria et al. previously showed that TREM2 can be expressed in activated hepatic stellate cells (HSCs) in the context of liver injury, which modulates toll like receptor-mediated inflammation (35). Therefore, following the observation that TREM2 deficient mice developed an increased number of tumors in carcinogen-induced models of HCC, the investigators interrogated how TREM2 expressed by HSCs would impact tumorigenicity. The researchers evaluated tumorigenicity utilizing a hanging droplet liver cancer spheroid growth assay. Consistent with findings from the *TREM2* deficient carcinogen-induced models of HCC, conditioned media from *TREM2*-overexpressing HSCs suppressed spheroid growth (25). Further analysis revealed that *TREM2* overexpression in HSCs attenuated

TABLE 2 Summary of mouse studies with supporting evidence for TREM2 contributing to tumor suppressing or oncogenic activity in different types of cancer.

	Cancer type	Cancer model	Phenotype	Reference
Tumor Suppressive Activity	HCC	s.c. TREM2 KD	Increase tumor size	Tang et al. (24)
		s.c. TREM2 OE	Decrease tumor size	
		i.v. (tail vein) TREM2 OE	Suppress lung metastasis	
	HCC	DEN-induced carcinogenesis, *Trem2*$^{-/-}$ mice	Increase tumor number	Esparza-Baquer et al. (25)
		DEN/CCl_4-induced carcinogenesis, *Trem2*$^{-/-}$ mice	Increase tumor number	
		TAA-Induced carcinogenesis, *Trem2*$^{-/-}$ mice	Increase tumor volume	
	CRC	s.c. TREM2 OE	Decrease tumor volume	Kim et al. (31)
Oncogenic activity	Glioma	s.c. TREM2 KD	Decrease tumor volume	Wang et al. (29)
	RCC	s.c. TREM2 KD	Decrease tumor volume	Zhang et al. (30)

HCC, hepatocellular carcinoma; CRC, colorectal cancer; RCC, renal cell carcinoma; s.c., subcutaneous injection of cancer cells; i.v., intravenous; KD, knockdown; OE, overexpression; DEN, diethylnitrosamine; CCl_4, carbon tetrachloride; TAA, thioacetamide.

expression of multiple canonical Wnt ligands, which may have contributed to spheroid growth suppression. Although these data are limited to HCC, they open up the prospect that activated cancer-associated fibroblasts in other cancer types may express TREM2, and in doing so may modulate paracrine signaling to the surrounding cells. However, it is of note that these studies were only conducted *in vitro* and thus, future studies should include further analysis of cancer associated fibroblasts in both HCC and other types of cancer both *in vitro* and *in vivo*.

TREM2 expression and functions within the immune cell populations of the TME

Expression of TREM2 by myeloid cells in the TME creates an immunosuppressive environment

$CD8^+$ cytotoxic T lymphocytes are key immune cells for controlling tumor growth by killing cancer cells that express major histocompatibility complex class I molecules. However, immunosuppressive crosstalk between cancer cells and other cell types within the TME, such as cancer-associated fibroblasts, regulatory T cells, and M2-polarized macrophages, can suppress the effector functions of $CD8^+$ T cells (36). Given that analysis of human tumor samples from various primary carcinomas including those of skin, liver, lung, breast, bladder, colon, stomach, pancreas, and kidney contain $TREM2^+$ macrophages in 75% of samples (34), there is reason to consider that TREM2 expression contributes to the immunosuppressive phenotype. In settings of infection, TREM2 enhances phagocytosis and reduces pro-inflammatory cytokine secretion by macrophages, thus serving an immunoregulatory role (7). Current knowledge suggests that TREM2 expression on cells of the monocyte-macrophage lineage may also serve an immunoregulatory role in cancer, creating an immunosuppressive environment. Recent work by Drake and colleagues uncovered a tumor-specific $C1Q^+TREM2^+APOE^+$ macrophage population in clear cell renal carcinoma associated with post-surgical disease recurrence for patients (37). This suggests that TREM2 expression in tumor-specific macrophages is associated with a pro-tumorigenic environment.

TREM2 has primarily been shown to be expressed on the surface of cells in the monocyte-macrophage lineage, including microglia (38), osteoclasts (39), and other macrophages such Kupffer cells (40) and lipid associated macrophages (LAMs) in adipose tissue (41). Studies have used scRNAseq to understand how *TREM2* deficiency impacts the myeloid compartment in the TME as well as tumor progression. One study identified two populations of tumor-infiltrating myeloid suppressive cells that express TREM2 in a subcutaneous MCA-205 fibrosarcoma model: a TAM population and a myeloid regulatory cell population (42). A second study employed a subcutaneous MCA/1956 model in $Trem2^{+/+}$ and $Trem2^{-/-}$ mice to understand how TREM2 deficiency impacts the myeloid compartment. The initial analysis of all $CD45^+$ cells demonstrated that *Trem2* was expressed on all macrophage clusters albeit at varying levels, but no TREM2 was detected in DCs or lymphoid cells. Further re-clustering of macrophages identified specific macrophage clusters with high expression of *Trem2* in the $Trem2^{+/+}$ mice. The presence of these specific macrophage clusters was significantly diminished in the $Trem2^{-/-}$ mice suggesting that TREM2 may be responsible for sustaining these populations of macrophages (34). This demonstrates that TREM2 deficiency impacts the restructuring of the myeloid cell compartment within a tumor.

Following these observations, tumor growth was evaluated in $Trem2^{-/-}$ and $Trem2^{+/+}$ mice utilizing a subcutaneous injection model with either MCA-205 (42) or MCA/1956 (34) sarcoma cell lines. Tumor growth attenuation was observed in the $Trem2^{-/-}$ mice compared to the $Trem2^{+/+}$ mice with both cell lines (34, 42). Likewise, Molgora et al. observed tumor growth attenuation in a MC38 CRC subcutaneous model and an orthotopic mammary model in $Trem2^{-/-}$ mice (34). The TREM2 expression profile on the cancer cells injected in these studies was not reported. Both studies attribute the reduction in tumor growth to the lack of TREM2 expression on the immune cells and thus a reduced ability of the immune cells to create an immunosuppressive environment. It is important to note, given the differential effects by tumor type above, that both of these papers utilized sarcoma models in their scRNAseq studies, which are mesenchymal rather than epithelial derived tumors. scRNAseq was not performed for the MC38 CRC subcutaneous model or the orthotopic mammary model.

More recent studies utilizing scRNAseq have begun to elucidate and characterize TREM2-expressing macrophages in epithelial tumors. $TREM2^+$ macrophages identified in the lungs of a mouse mammary tumor model demonstrated a gene expression profile akin to LAMs with positive enrichment for pathways associated with cholesterol and lipid metabolism (43). Interestingly, these LAMs are increased in the lungs of mammary tumor-bearing mice compared to non-tumor bearing mice and are enriched for protumorigenic pathways related to negative regulation of T-cell responses, epithelial-mesenchymal transition, and endothelial cell proliferation (43). An increased presence of these LAMs in the lungs at a premetastatic time point suggests an immunosuppressive preparation of the metastatic niche. In complement to this study, a TREM2-expressing macrophage subpopulation in HCC patient tissues were reported to resemble hepatic LAMs with upregulation of immunosuppressive pathways such as Treg recruitment and angiogenesis stimulation (27). $TREM2^+$ TAMs in non-small cell lung cancer (NSCLC) patient tissues were also enriched for fatty acid metabolism and protumorigenic pathways (33).

While there is much to learn about the role of TREM2 in cancer, some key studies indicate that TREM2 is involved in suppressing the function of $CD8^+$ T cells as well as inhibiting their proliferation, which would argue for myeloid-targeted inhibition of TREM2 at least in some cancers.

Impairment of $CD8^+$ T cells by $TREM2^+$ myeloid cells and recruitment of T regulatory cells may provide a mechanistic link for immunosuppression

Although T cells don't express TREM2, it is possible they can be impacted by TREM2 expression on other cell types within the TME. Subcutaneous MCA/1956 tumors in mice deficient for TREM2 displayed an increase in $CD8^+$ T cells as a percent of all tumor infiltrating T cells compared to wild-type mice. These $CD8^+$ T cells were deemed activated based on PD-1 expression and tumor growth was restrained in the TREM2 deficient mice. Administration of an anti-CD8 monoclonal antibody in both *Trem2*$^{+/+}$ and *Trem2*$^{-/-}$ mice accelerated tumor growth compared to the controls (34). In another study utilizing subcutaneous injection of MCA-205 fibrosarcoma cells in *Trem2*$^{-/-}$ mice, the investigators not only observed a reduction in tumor growth, but also an expansion of the natural killer and cytotoxic T cell population accompanied by a decrease in dysfunctional $CD8^+$ T cells (42). Therefore, these data suggest that tumor growth attenuation in the TREM2 deficient conditions is mechanistically linked to the activation of $CD8^+$ T cells. Additionally, the data suggest that the presence of $TREM2^+$ cells in the tumor stroma contributes to the suppression of these cytotoxic T lymphocytes and their ability to control tumor growth.

In addition to the impaired effector functions of cytotoxic T lymphocytes, the proliferation of these cells may be diminished by the expression of TREM2 on cells in the TME. Bone marrow derived DCs were induced to express TREM2 by culturing with conditioned media from 3LL lung cancer cells. Yao et al. found that T cells co-cultured with *Trem2*$^+$ DCs exhibited lower levels of proliferation compared to T cells co-cultured with *Trem2* deficient DCs or a combination of $TREM2^+$ DCs with anti-TREM2 mAb (44). In another recent study, N9 macrophages either positive or deficient for TREM2 were co-cultured with $CD8^+$ T cells. The results demonstrate that co-culture of the $CD8^+$ T cells with *Trem2*$^{+/+}$ N9 cells suppresses T cell proliferation in a manner at least comparable to treatment with transforming growth factor β (42).

These observations have also been corroborated in human tumor tissue. Assessment of fresh NSCLC patient samples by flow cytometry revealed that tumors with high $TREM2^+$ TAM infiltration exhibited a decrease in $CD8^+$ T cells expressing CD107a, perforin 1, and tumor necrosis factor-α suggesting a decrease in effector function (33). This is corroborated in *ex vivo* studies where co-culture of $CD8^+$ T cells with $TREM2^+$ TAMs also resulted in decreased T cell proliferation and reduction of CD107a, perforin 1, and tumor necrosis factor-α production (33).

In addition to reduction of $CD8^+$ T cell proliferation and effector function, two independent studies in NSCLC and HCC suggest immunosuppressive $TREM2^+$ TAMs are also involved in recruiting T regulatory cells (Tregs). Implementation of CellPhoneDB, which infers cell-cell communication networks from scRNAseq data, to HCC patient samples revealed interaction of $TREM2^+$ LAM-like cells with Tregs *via* the CCL20/CXCL9/CXCL10/CXCL12-CXCR3 axis, suggesting recruitment of Tregs by the $TREM2^+$ LAM-like cells by means of migration-related chemokines (27). Assessment of cell-cell interactions in NSCLC patient sample scRNAseq data identified an interaction between IL1β and IL1R from $TREM2^+$ TAMs and $FOXP3^+$ Tregs, respectively (33). Further, NSCLC samples with high $TREM2^+$ TAMs exhibited an increase in transforming growth factor-β-expressing $FOXP3^+$ Tregs by flow cytometry (33), and $TREM2^+CD163^+$ macrophages were found to colocalize with $FOXP3^+$ Tregs within HCC tumors (27). These data suggest decreased effector functions of $CD8^+$ T cells may be due in part to the infiltration and function of Tregs. The interplay between stromal cells in the TME is complex, and while it is unlikely that TREM2 impacts the tumor *via* a single mechanism, these data indicate that key mechanisms are through suppression of cytotoxic T lymphocytes and recruitment of regulatory T cells.

TREM2 expression may confer resistance to immune checkpoint therapy

As noted, TREM2 expression by myeloid cells can impact T cell activation and proliferation; thus, it is not surprising that TREM2 may be able to serve as a biomarker for tumor burden and high TREM2 expression may confer resistance to immune checkpoint therapy (ICT). Yao et al. found an increase of TREM2 positive monocytes in the peripheral blood of lung cancer patients and in both the peripheral blood and lungs of tumor-bearing mice (44). Furthermore, TREM2 expression on macrophages in lung cancer patient samples increased with both pathological staging of disease as well as degree of lymph node metastasis (44). Lung cancer patients that responded to chemotherapy with a reduction of tumor burden displayed a decrease in TREM2 positive monocytes in the peripheral blood. Additionally, lung cancer patients that underwent surgical tumor resection, and thus had a reduced tumor burden, also displayed a decrease in TREM2 positive monocytes in the peripheral blood (44).

NSCLC patients with high $TREM2^+$ TAM infiltration had a lower objective response rate to ICT compared to patients with low numbers of $TREM2^+$ TAMs and were more likely to experience tumor progression following PD-1 blockade (33). Analysis of scRNAseq data from melanoma patients divided into

subgroups of responders and non-responders to ICT revealed a significant enrichment of macrophages with high expression of TREM2 in non-responders, implying that macrophage cell populations with high expression of TREM2 may precipitate ICT resistance (45). Overall, these studies demonstrate an upregulation of TREM2 on TAMs with increasing disease severity and in patients non-responsive to ICT.

TREM2 modulation enhances anti-PD-1 therapy

Molgora et al. probed whether neutralization of TREM2 in combination with ICT improves tumor response to treatment (34). The investigators first established that treatment with αPD-1 in TREM2 deficient mice led to further tumor control and regression in sarcoma and CRC models. Furthermore, subsequent treatment of wild-type mice with combined αTREM2 and αPD-1 mAb led to complete reduction of tumor burden in all mice tested. This demonstrates that deficiency of TREM2 or treatment with anti-TREM2 mAb augments the efficacy of αPD-1 ICT.

Currently, a Phase I clinical trial (ClinicalTrials.gov identifier: NCT04691375) for a TREM2 mAb administered either as a single agent or in combination with pembrolizumab (αPD-1) is underway. The subjects in this clinical trial have locally advanced and/or metastatic solid tumors that are refractory or relapsed to standard of care treatment (46). This specific TREM2 mAb, PY314, is a depleting antibody designed to deplete tumor associated macrophages expressing TREM2. This study was initiated in October of 2020 and the estimated completion date is October 2023 (46)

Conclusion and future directions

When considering novel therapeutic targets, it is important to consider how different cell types may respond to therapy and thus impact the patient's overall response and outcome. As covered in this review and summarized in Figure 1, TREM2 is expressed by multiple cell types within the TME. TREM2 may have tumor cell intrinsic functions in addition to its role in stromal cells and fibroblasts that could be tumor suppressive or oncogenic depending on the type of cancer. Therefore, moving forward it is imperative that we better understand the mechanisms by which TREM2 contributes to tumor suppressive or oncogenic activity in the cancer types discussed in this review. Within this review, discrepancies are unsurprisingly found between *in vitro* studies, mouse models, and human data. As future studies are conducted, use of *in vitro*

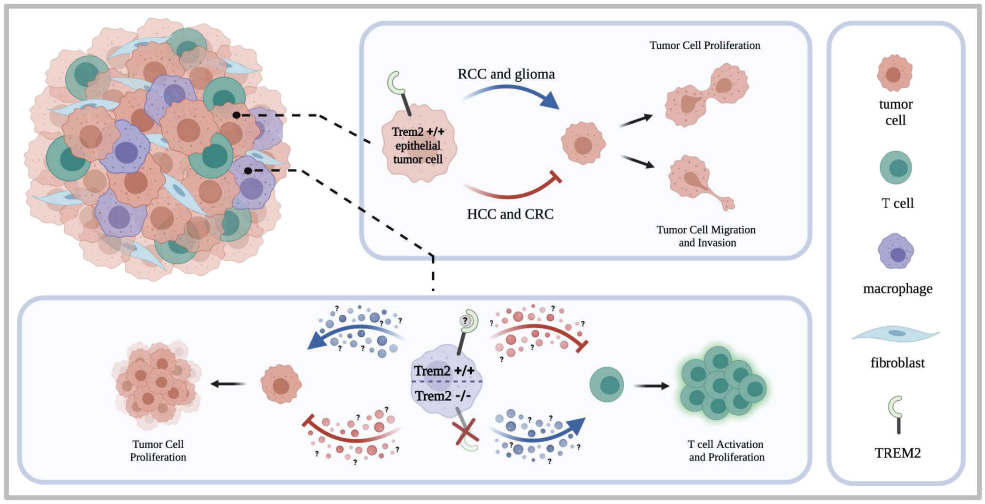

FIGURE 1
TREM2 in heterogeneous tumors. Tumors are composed of a heterogeneous makeup of cells including the tumor cells, tumor associated macrophages, T cells, and tumor associated fibro blasts. Studies have shown that TREM2 can be expressed by both epithelial tumor cells and infiltrating immune cells such as macrophages. When expressed by the epithelial tumor cells, studies indicate that TREM2 supports oncogenic activity in renal cell carcinoma and glioma but contributes to tumor suppressing activity in renal cell carcinoma. Thus, contributing to oncogenic or tumor suppressing activity, TREM2 can enhance or inhibit tumor cell proliferation and tumor cell migration and invasion. When expressed by infiltrating immune cells such as macrophages, the literature indicates that TREM2 expressing immune cells contribute to creating an immunosuppressive environment by inhibiting T cell activation and proliferation. Consequently, tumor cell proliferation is then enhanced. When TREM2 is knocked out on the tumor infiltrating immune cells, increased T cell activation and proliferation is observed accompanied by a decrease in tumor growth.

methods and cell lines should be accompanied with complementary *in vivo* and human data to ensure the rigor of the data. Additionally, we need to understand the tumor cell intrinsic role of TREM2 in more types of solid cancers such as breast cancer. This understanding is key since anti-TREM2 mAb treatment is already undergoing clinical testing for a variety of solid tumor types. Overall, the data indicate that high expression of TREM2 on cells of the monocyte-macrophage lineage creates an immunosuppressive environment in which T cells are less activated and their proliferation is suppressed. Therefore, this pro-tumoral role of TREM2 is the current prevailing opinion in the literature. Thus, inhibition or blockade of TREM2 may be an effective therapeutic strategy. However, depending on the role in tumor cells, blockade of TREM2 may still be clinically unfavorable. With these varying results, it is of the utmost importance to continue to uncover the role of TREM2 in cancer.

Author contributions

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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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References

1. Daws MR, Sullam PM, Niemi EC, Chen TT, Tchao NK, Seaman WE. Pattern recognition by TREM-2: Binding of anionic ligands. *J Immunol* (2003) 171:594–9. doi: 10.4049/jimmunol.171.2.594

2. Kawabori M, Kacimi R, Kauppinen T, Calosing C, Kim JY, Hsieh CL, et al. Triggering receptor expressed on myeloid cells 2 (TREM2) deficiency attenuates phagocytic activities of microglia and exacerbates ischemic damage in experimental stroke. *J Neurosci* (2015) 35:3384–96. doi: 10.1523/JNEUROSCI.2620-14.2015

3. Bailey CC, DeVaux LB, Farzan M. The triggering receptor expressed on myeloid cells 2 binds apolipoprotein e. *J Biol Chem* (2015) 290:26033–42. doi: 10.1074/jbc.M115.677286

4. Cannon JP, O'Driscoll M, Litman GW. Specific lipid recognition is a general feature of CD300 and TREM molecules. *Immunogenetics* (2012) 64:39–47. doi: 10.1007/s00251-011-0562-4

5. Gallo C, Manzo E, Barra G, Fioretto L, Ziaco M, Nuzzo G, et al. Sulfavant a as the first synthetic TREM2 ligand discloses a homeostatic response of dendritic cells after receptor engagement. *Cell Mol Life Sci* (2022) 79:369. doi: 10.1007/s00018-022-04297-z

6. Peng Q, Malhotra S, Torchia JA, Kerr WG. Coggeshall KM and Humphrey MB. TREM2- and DAP12-dependent activation of PI3K requires DAP10 and is inhibited by SHIP1. *Sci Signal* (2010) 3:ra38. doi: 10.1126/scisignal.2000500

7. Kober DL, Brett TJ. TREM2-ligand interactions in health and disease. *J Mol Biol* (2017) 429:1607–29. doi: 10.1016/j.jmb.2017.04.004

8. Bouchon A, Hernandez-Munain C, Cella M, Colonna M. A DAP12-mediated pathway regulates expression of CC chemokine receptor 7 and maturation of human dendritic cells. *J Exp Med* (2001) 194:1111–22. doi: 10.1084/jem.194.8.1111

9. Feuerbach D, Schindler P, Barske C, Joller S, Beng-Louka E, Worringer KA, et al. ADAM17 is the main sheddase for the generation of human triggering receptor expressed in myeloid cells (hTREM2) ectodomain and cleaves TREM2 after histidine 157. *Neurosci Lett* (2017) 660:109–14. doi: 10.1016/j.neulet.2017.09.034

10. Wu K, Byers DE, Jin X, Agapov E, Alexander-Brett J, Patel AC, et al. TREM-2 promotes macrophage survival and lung disease after respiratory viral infection. *J Exp Med* (2015) 212:681–97. doi: 10.1084/jem.20141732

11. Zhong L, Chen XF, Wang T, Wang Z, Liao C, Wang Z, et al. Soluble TREM2 induces inflammatory responses and enhances microglial survival. *J Exp Med* (2017) 214:597–607. doi: 10.1084/jem.20160844

12. Ulland TK, Song WM, Huang SC, Ulrich JD, Sergushichev A, Beatty WL, et al. TREM2 maintains microglial metabolic fitness in alzheimer's disease. *Cell* (2017) 170:649–663.e13. doi: 10.1016/j.cell.2017.07.023

13. Ulrich JD, Finn MB, Wang Y, Shen A, Mahan TE, Jiang H, et al. Altered microglial response to abeta plaques in APPPS1-21 mice heterozygous for TREM2. *Mol Neurodegener* (2014) 9:20. doi: 10.1186/1750-1326-9-20

14. Gratuze M, Leyns CEG, Holtzman DM. New insights into the role of TREM2 in alzheimer's disease. *Mol Neurodegener* (2018) 13:66. doi: 10.1186/s13024-018-0298-9

15. Jay TR, von Saucken VE, Landreth GE. TREM2 in neurodegenerative diseases. *Mol Neurodegener* (2017) 12:56. doi: 10.1186/s13024-017-0197-5

16. Otero K, Shinohara M, Zhao H, Cella M, Gilfillan S, Colucci A, et al. TREM2 and beta-catenin regulate bone homeostasis by controlling the rate of osteoclastogenesis. *J Immunol* (2012) 188:2612–21. doi: 10.4049/jimmunol.1102836

17. Jiang H, Si Y, Li Z, Huang X, Chen S, Zheng Y, et al. TREM-2 promotes acquired cholesteatoma-induced bone destruction by modulating TLR4 signaling pathway and osteoclasts activation. *Sci Rep* (2016) 6:38761. doi: 10.1038/srep38761

18. Paloneva J, Kestila M, Wu J, Salminen A, Bohling T, Ruotsalainen V, et al. Loss-of-function mutations in TYROBP (DAP12) result in a presenile dementia with bone cysts. *Nat Genet* (2000) 25:357–61. doi: 10.1038/77153

19. Paloneva J, Manninen T, Christman G, Hovanes K, Mandelin J, Adolfsson R, et al. Mutations in two genes encoding different subunits of a receptor signaling complex result in an identical disease phenotype. *Am J Hum Genet* (2002) 71:656–62. doi: 10.1086/342259

20. Klunemann HH, Ridha BH, Magy L, Wherrett JR, Hemelsoet DM, Keen RW, et al. The genetic causes of basal ganglia calcification, dementia, and bone cysts: DAP12 and TREM2. *Neurology* (2005) 64:1502–7. doi: 10.1212/01.WNL.0000160304.00003.CA

21. Yeh FL, Hansen DV, Sheng M. TREM2, microglia, and neurodegenerative diseases. *Trends Mol Med* (2017) 23:512–33. doi: 10.1016/j.molmed.2017.03.008

22. Li RY, Qin Q, Yang HC, Wang YY, Mi YX, Yin YS, et al. TREM2 in the pathogenesis of AD: A lipid metabolism regulator and potential metabolic therapeutic target. *Mol Neurodegener* (2022) 17:40. doi: 10.1186/s13024-022-00542-y

23. Filipello F, Goldsbury C, You SF, Locca A, Karch CM, Piccio L. Soluble TREM2: Innocent bystander or active player in neurological diseases? *Neurobiol Dis* (2022) 165:105630. doi: 10.1016/j.nbd.2022.105630

24. Tang W, Lv B, Yang B, Chen Y, Yuan F, Ma L, et al. TREM2 acts as a tumor suppressor in hepatocellular carcinoma by targeting the PI3K/Akt/beta-catenin pathway. *Oncogenesis* (2019) 8:9. doi: 10.1038/s41389-018-0115-x

25. Esparza-Baquer A, Labiano I, Sharif O, Agirre-Lizaso A, Oakley F, Rodrigues PM, et al. TREM-2 defends the liver against hepatocellular carcinoma through multifactorial protective mechanisms. *Gut* (2020) 70:1345-61. doi: 10.1136/gutjnl-2019-319227

26. Cheng X, Wang X, Nie K, Cheng L, Zhang Z, Hu Y, et al. Systematic pan-cancer analysis identifies TREM2 as an immunological and prognostic biomarker. *Front Immunol* (2021) 12:646523. doi: 10.3389/fimmu.2021.646523

27. Zhou L, Wang M, Guo H, Hou J, Zhang Y, Li M, et al. Integrated analysis highlights the immunosuppressive role of TREM2(+) macrophages in hepatocellular carcinoma. *Front Immunol* (2022) 13:848367. doi: 10.3389/fimmu.2022.848367

28. Zhang X, Wang W, Li P, Wang X, Ni K. High TREM2 expression correlates with poor prognosis in gastric cancer. *Hum Pathol* (2018) 72:91–9. doi: 10.1016/j.humpath.2017.10.026

29. Wang XQ, Tao BB, Li B, Wang XH, Zhang WC, Wan L, et al. Overexpression of TREM2 enhances glioma cell proliferation and invasion: A therapeutic target in human glioma. *Oncotarget* (2016) 7:2354–66. doi: 10.18632/oncotarget.6221

30. Zhang H, Sheng L, Tao J, Chen R, Li Y, Sun Z, et al. Depletion of the triggering receptor expressed on myeloid cells 2 inhibits progression of renal cell carcinoma *via* regulating related protein expression and PTEN-PI3K/Akt pathway. *Int J Oncol* (2016) 49:2498–506. doi: 10.3892/ijo.2016.3740

31. Kim SM, Kim EM, Ji KY, Lee HY, Yee SM, Woo SM, et al. TREM2 acts as a tumor suppressor in colorectal carcinoma through Wnt1/beta-catenin and erk signaling. *Cancers (Basel)* (2019) 11:1315. doi: 10.3390/cancers11091315

32. Nalio Ramos R, Missolo-Koussou Y, Gerber-Ferder Y, Bromley CP, Bugatti M, Nunez NG, et al. Tissue-resident FOLR2(+) macrophages associate with CD8 (+) T cell infiltration in human breast cancer. *Cell* (2022) 185:1189–1207.e25. doi: 10.1016/j.cell.2022.02.021

33. Zhang H, Liu Z, Wen H, Guo Y, Xu F, Zhu Q, et al. Immunosuppressive TREM2(+) macrophages are associated with undesirable prognosis and responses to anti-PD-1 immunotherapy in non-small cell lung cancer. *Cancer Immunol Immunother* (2022) 1-12. doi: 10.1007/s00262-022-03173-w

34. Molgora M, Esaulova E, Vermi W, Hou J, Chen Y, Luo J, et al. TREM2 modulation remodels the tumor myeloid landscape enhancing anti-PD-1 immunotherapy. *Cell* (2020) 182:886–900.e17. doi: 10.1016/j.cell.2020.07.013

35. Perugorria MJ, Esparza-Baquer A, Oakley F, Labiano I, Korosec A, Jais A, et al. Non-parenchymal TREM-2 protects the liver from immune-mediated hepatocellular damage. *Gut* (2019) 68:533–46. doi: 10.1136/gutjnl-2017-314107

36. Farhood B, Najafi M, Mortezaee K. CD8(+) cytotoxic T lymphocytes in cancer immunotherapy: A review. *J Cell Physiol* (2019) 234:8509–21. doi: 10.1002/jcp.27782

37. Obradovic A, Chowdhury N, Haake SM, Ager C, Wang V, Vlahos L, et al. Single-cell protein activity analysis identifies recurrence-associated renal tumor macrophages. *Cell* (2021) 184:2988–3005.e16. doi: 10.1016/j.cell.2021.04.038

38. Takahashi K, Rochford CD, Neumann H. Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. *J Exp Med* (2005) 201:647–57. doi: 10.1084/jem.20041611

39. Humphrey MB, Nakamura MC. A comprehensive review of immunoreceptor regulation of osteoclasts. *Clin Rev Allergy Immunol* (2016) 51:48–58. doi: 10.1007/s12016-015-8521-8

40. Goncalves LA, Rodrigues-Duarte L, Rodo J, Vieira de Moraes L, Marques I, Penha-Goncalves C. TREM2 governs kupffer cell activation and explains belr1 genetic resistance to malaria liver stage infection. *Proc Natl Acad Sci U S A* (2013) 110:19531–6. doi: 10.1073/pnas.1306873110

41. Jaitin DA, Adlung L, Thaiss CA, Weiner A, Li B, Descamps H, et al. Lipid-associated macrophages control metabolic homeostasis in a Trem2-dependent manner. *Cell* (2019) 178:686–698.e14. doi: 10.1016/j.cell.2019.05.054

42. Katzenelenbogen Y, Sheban F, Yalin A, Yofe I, Svetlichnyy D, Jaitin DA, et al. Coupled scRNA-seq and intracellular protein activity reveal an immunosuppressive role of TREM2 in cancer. *Cell* (2020) 182:872–885.e19. doi: 10.1016/j.cell.2020.06.032

43. Huggins DN, LaRue RS, Wang Y, Knutson TP, Xu Y, Williams JW, et al. Characterizing macrophage diversity in metastasis-bearing lungs reveals a lipid-associated macrophage subset. *Cancer Res* (2021) 81:5284–95. doi: 10.1158/0008-5472.CAN-21-0101

44. Yao Y, Li H, Chen J, Xu W, Yang G, Bao Z, et al. TREM-2 serves as a negative immune regulator through syk pathway in an IL-10 dependent manner in lung cancer. *Oncotarget* (2016) 7:29620–34. doi: 10.18632/oncotarget.8813

45. Xiong D, Wang Y, You M. A gene expression signature of TREM2(hi) macrophages and gammadelta T cells predicts immunotherapy response. *Nat Commun* (2020) 11:5084. doi: 10.1038/s41467-020-18546-x

46. ClinicalTrials.gov. A study of PY314 in subjects with advanced solid tumors. (2020).

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SNAI1-dependent upregulation of CD73 increases extracellular adenosine release to mediate immune suppression in TNBC

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Triple-negative subtype of breast cancer (TNBC) is hallmarked by frequent disease relapse and shows highest mortality rate. Although PD-1/PD-L1 immune checkpoint blockades have recently shown promising clinical benefits, the overall response rate remains largely insufficient. Hence, alternative therapeutic approaches are warranted. Given the immunosuppressive properties of CD73-mediated adenosine release, CD73 blocking approaches are emerging as attractive strategies in cancer immunotherapy. Understanding the precise mechanism regulating the expression of CD73 is required to develop effective anti-CD73-based therapy. Our previous observations demonstrate that the transcription factors driving epithelial-to-mesenchymal transition (EMT-TF) can regulate the expression of several inhibitory immune checkpoints. Here we analyzed the role of the EMT-TF SNAI1 in the regulation of CD73 in TNBC cells. We found that doxycycline-driven SNAI1 expression in the epithelial -like TNBC cell line MDA-MB-468 results in CD73 upregulation by direct binding to the CD73 proximal promoter. SNAI1-dependent upregulation of CD73 leads to increased production and release of extracellular adenosine by TNBC cells and contributes to the enhancement of TNBC immunosuppressive properties. Our data are validated in TNBC samples by showing a positive correlation between the mRNA expression of CD73 and SNAI1. Overall, our results reveal a new CD73 regulation mechanism in TNBC that participates in TNBC-mediated

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immunosuppression and paves the way for developing new treatment opportunities for CD73-positive TNBC.

KEYWORDS

CD73, SNAI1, epithelial-to-mesenchymal transition, adenosine, anti-tumor immune response, immunotherapy, breast cancer, immune checkpoints

Introduction

Breast cancer is the most frequently diagnosed malignancy in women. Triple-negative is a subtype of breast cancer (TNBCs) that does not express estrogen receptors, progesterone receptors, and human epidermal growth factor receptor-2/neu (HER-2). TNBC accounts for 15% of all breast cancers exhibiting high probability of disease relapse and the highest mortality rate among breast cancer subtypes (1). TNBC patients do not benefit from hormonal therapy or HER-2 blockade, making conventional chemotherapy the only established therapeutic option which does not prevent high recurrence rates, acquired resistance, and metastasis [2].

Compared to other breast cancer subtypes, TNBCs have enhanced intra-tumoral T cell infiltration and a higher mutational burden (1, 2). Therefore, TNBCs have an increased potential to generate immunogenic mutations and are considered eligible tumors for immune checkpoint inhibition-based therapy. Recent clinical trials in TNBC patients based on PD-1/PD-L1 blockade revealed an overall response rate of 20% (3). Despite this promising clinical response, most enrolled patients showed little or no therapeutic benefit, fostering the need for alternative immunotherapeutic approaches.

The ectonucleotidase CD73 is an attractive target in cancer immunotherapy (4). CD73 is involved in generating extracellular adenosine (ADO), a potent immunosuppressive molecule for both innate and adaptive immunity (4, 5). Indeed, ADO inhibits the anti-tumor function of T and Natural Killer (NK) cells and enhances the immunosuppressive function of T regulatory cells and tumor-associated macrophages (TAM).

CD73 is upregulated in many cancer types, including breast cancer. CD73 expression is negatively regulated by estrogen receptor signaling (6). Therefore, the absence of estrogen receptors in TNBCs could contribute to CD73 expression. In addition, analysis of CD73 expression in TNBC patients shows that high CD73 is associated with decreased overall and disease-free survival and increased resistance to conventional chemotherapy (7, 8).

The molecular mechanisms involved in regulating CD73 expression are not yet fully understood. It is reported that the transcription factor Hypoxia-inducible factor (HIF)-1 is involved in directly activating CD73/NT5E expression (9, 10). However, no data are available on whether and how CD73 is regulated during tumor progression and metastatic spread.

Epithelial-to-mesenchymal transition (EMT) is a process whereby epithelial cells acquire motile and invasive mesenchymal features. EMT is driven by a series of EMT-inducing transcription factors (EMT-TFs). EMT in tumor cells is associated with increased aggressiveness, drug resistance, and immune escape. We have previously demonstrated that both PD-L1 and CD47 inhibitory immune checkpoints are upregulated in human mesenchyma -like breast cancer cell lines by mechanisms involving the EMT-TFs ZEB1 or SNAI1 (11, 12). In line with our previous work and considering the key functions of the EMT-TF SNAI1 in TNBC aggressiveness (13), we investigated the role of SNAI1 in the modulation of CD73 expression in TNBC cells and the functional impact of such modulation on the immunosuppressive properties of TNBC cells.

Materials and methods

Cell culture, treatment and transfection

Human TNBC cell lines MDA-MB-231 and MDA-MB-468 were purchased from DSMZ (Braunschweig; Germany). MDA-MB-468-iSNAI1 and MDA-MB-468-iGFP cells stably expressing doxycycline-inducible SNAI1 and GFP, respectively, were provided by Dr. Brett G. Hollier (Brisbane, Queensland, Australia).

MDA-MB-231 cells were cultured in RPMI 1640-GlutaMAX™, 10% FBS, and 1% Penicillin-Streptomycin. MDA-MB-468 cells were cultured in DMEM-HighGlucose-Glutamax, 10% FBS, and 1% Penicillin-Streptomycin. The NK92-MI cell line was cultured in RPMI 1640-GlutaMAX™, 10% FBS, 10% Horse Serum (ATCC), and 1% Penicillin-Streptomycin. The mouse TNBC cell line Py8119 was purchased from ATCC and was cultured in F-12K Medium, 5% FBS.

NK cells from healthy donors (NKD) were obtained from fresh apheresis products after Ficoll-Paque Plus centrifugation (GE Healthcare) and purification using a human NK Cell Isolation Kit (Miltenyi Biotec). Purified NKD were cultured in RPMI 1640-GlutaMAX™, 10% pooled human serum (Jacques Boy), 5% FBS, 1% Penicillin-Streptomycin, and IL-2 (150 UI/ml (Immunotools). All cells were grown at 37°C under humidified

conditions and 5% CO_2 and routinely tested for Mycoplasma free (MycoAlert Detection Kit; Lonza).

For SNAI1 induction, MDA-MB-468-iSNAI1 and MDA-MB-468-iGFP cells were seeded 24h before Doxycycline (Dox) (D9891, Sigma-Aldrich-Merck) treatment. The indicated doses of Dox were added every 48 h in fresh medium for 5 days, and cells were harvested on day 6. For rhEGF (#E9644; Sigma) treatment, MDA-MB-468 cells were seeded 24 h before rhEGF treatment. rhEGF (50 ng/ml) were added every 48 h in a fresh medium containing 0.5% FBS for 5 days, and cells were harvested on day 6.

Control CRISPR/Cas9 and SNAI1 CRISPR/Cas9 plasmids were obtained from Santa Cruz Biotechnology and transfected into Py8119 cells according to manufacturer's protocol.

Antibodies

The following antibodies for Western blot, confocal, and ChIP were from Cell Signaling: anti-SNAIL (#3879S), anti-ZEB1 (#D80D3), anti-E-cadherin (#24E10), anti-Vimentin (#D21H3) XP®. For others: Anti-β-Actin–Peroxidase (A3854; Sigma-Aldrich-Merck), Alexa 488-conjugated secondary antibody (1/400, Molecular Probes), Actin-Stain 488 Phalloidin (1/400; Cytoskeleton, Inc.). FACS and ImageStream antibodies were as follows: CD73-PE antibody (344004; 1:100, Biolegend), Alexa 633-conjugated secondary antibody (1/500; Molecular Probes), Ki67-PE (151210; 1:100; Biolegend).

Quantitative real-time PCR

Total RNA was extracted from cell lines using the Nucleospin RNA Plus Kit (Macherey-Nagel). Total RNA from 12 TNBC patients was purchased from Origene (CR561562, CR561706, CR561397, CR562540, CR562125, CR560441, CR560325, CR561546, CR561196, CR561161, CR561083, CR560707). RNA was reverse-transcribed using the Maxima First-Strand cDNA Synthesis Kit (Thermo Fischer Scientific) and amplified by qPCR using the Power SYBR Green PCR Master Mix (Eurogentec). mRNA levels of genes of interest were normalized to housekeeping 18S mRNA levels.

Western blotting

Adherent cells were lysed on ice in 62.5 mM Tris-HCl [pH 6.8], 2% w/v SDS, 10% glycerol, and 1× protease inhibitor cocktail (Thermo Fischer Scientific). Protein extracts were separated by SDS-PAGE and transferred onto nitrocellulose membranes (VWR). Primary antibodies were incubated overnight at 4°C and visualized using peroxidase-conjugated secondary antibodies (DAKO) and Western Lightning Ultra (Perkin Elmer). Blots were scanned and processed using ImageJ software.

Flow and imaging cytometry

Cells were harvested in 10 mM EDTA (Invitrogen). Surface staining was done at 4°C for 30 min. Intracellular staining was done with Cyto-Fast™ Fix/Perm Buffer Set (426803; Biolegend). Ki67 staining in NK cells was performed following ice-cold ethanol (70%) fixation. Dead cells were excluded using Live/Dead staining Kits (L34976; Thermo Fischer Scientific) or BD Via-Probe™ Cell Viability Solution (555815; Becton Dickinson). Samples were processed on a CytoFLEX flow cytometer and analyzed using CytExpert software. For imaging cytometry, cells were fixed on ice for 20min in 1% PFA after staining, resuspended in 2%FBS at a concentration of 2×10^7 cells/ml, and processed on ImageStreamX MKII (EMD Millipore) and analyzed using IDEA software.

ChIP assay

ChIP was performed on MDA-MB-468-iSNAI1 lysates using the SimpleChIP Enzymatic Chromatin IP kit (#9005; Cell Signaling). EMT-TF binding to E-box in the proximal region of human E-cadherin promoter was used as a positive control (14). SYBR Green RT-qPCR was performed using primers described in Table S1.

Extracellular adenosine measurement

Cell-conditioned media were collected and centrifuged at 2000 rpm, 4°C, for 15 min to remove cellular debris. Extracellular ADO concentration was determined based on a standard curve using Adenosine Assay Kit (K237-100; Biovision). Adenosine levels were determined according to the number of cells counted at the end of the experiment, as previously reported (9). The CD73 inhibitor APCP (M3763; Sigma) was added at 100 μM simultaneously with Dox.

Adenosine analog (CADO) treatment and immune-cytotoxicity assays

CADO (C5134; Sigma-Aldrich-Merck) was used at 5 μM or 10 μM. NK cells were treated with CADO every 2 days and harvested on days 4 or 6. Cytotoxicity assays were performed as previously described (15).

Confocal analysis

Cells were fixed for 20 min in 4% PFA at room temperature (RT), permeabilized with 0.1% Triton (10 min, RT), and blocked for 1 h with 10% FBS at RT. Antibodies were incubated for 1 h at RT, and nuclei were stained with DAPI for 5 min at RT. Images were acquired on confocal LSM880 Airy (Carl Zeiss). Scale bars were determined using ZEN 3.0 (blue edition) software.

In silico TNBC data mining

TNBC patients (n = 258) from the METABRIC dataset were downloaded from cbioportal v3.4.12. TNBC patients were defined based on their negative expression of ER, PR, and HER2. mRNA levels of *NT5E* (CD73), *SNAI1* (SNAI1), *VIM* (Vimentin), and *CDH1* (E-Cadherin) were extracted, and the co-expression was defined on cBioPortal (https://www.cbioportal.org/). The associations between *NT5E*/CD73, *SNAI1*, *VIM*, and *CDH1* expressions were analyzed by using the Pearson correlation test.

Statistical analyses

All statistical analyses were done in GraphPadPrism v8.0. Unpaired Student's t-tests or Mann-Whitney tests were used depending on whether the data presented Gaussian distribution with $P < 0.05$ considered significant. The Pearson correlation coefficient (two-tailed confidence interval of 95%) was used to assess the correlation between EMT-TF and CD73.

Results and discussion

Upregulation of CD73 in TNBC cell lines is associated with mesenchymal features

We analyzed the expression of CD73 in epithelial -like MDA-MB-468 and mesenchymal -like MDA-MB-231 TNBC cell lines. MDA-MB-468 cells express high levels of the epithelial marker E-cadherin, whereas the mesenchymal -like MDA-MB-231 cells express high levels of the mesenchymal markers ZEB1 and Vimentin (Figure S1). Using RT-qPCR, flow cytometry, and imaging cytometry, we measured *NT5E* (encoding CD73) mRNA and CD73 surface expression in both cell lines. We found that *NT5E* mRNA and CD73 cell surface protein are upregulated in mesenchymal MDA-MB-231 cells as compared to epithelial MDA-MB-468 (Figures 1A–C).

To assess the impact of EMT on CD73 expression, we used recombinant human epidermal growth factor (EGF), a potent inducer of EMT in epithelial MDA-MB-468 cells (16). Treatment of MDA-MB-468 cells with EGF for 6 days induced morphological changes consisting of a loss of cell-cell contacts and the acquisition of an elongated mesenchymal phenotype (Figure 1D, left panels). EGF also increased the expression of the mesenchymal markers SNAI1, ZEB1, and Vimentin and decreased the epithelial marker E-cadherin (Figure 1D, middle and right panels). This transition was associated with a significant increase in *NT5E* mRNA (Figure 1D, middle panel) and in the percentage (%) of CD73 positive MDA-MB-468 cells, as well as CD73 mean fluorescence intensity (MFI) (Figure 1E). Together, these results suggest that acquiring mesenchymal characteristics in TNBC cells is associated with increased CD73 expression at transcriptional and protein levels.

The EMT-TF SNAI1 is involved in the upregulation of CD73 in MDA-MB-468

SNAI1 is relatively more expressed than other EMT-TFs in TNBC (13). To assess the potential regulation of CD73 by SNAI1 in TNBC cells, we used MDA-MB-468 cells expressing Dox-inducible SNAI1 (MDA-MB-468-iSNAI1 cells). We first determined the appropriate Dox concentration to induce SNAI1 in MDA-MB-468-iSNAI1 cells. Using increasing Dox concentrations (0.25, 0.5, and 1 μg/ml), we showed a consistent and dose-dependent induction of *SNAI1* and *NT5E* mRNA (Figure S2A), indicating the potential role of SNAI1 in the transcriptional activation of *NT5E* expression.

As the maximum increase in *NT5E* expression was observed at 1 μg/ml Dox, we considered this concentration for subsequent experiments. It should be highlighted that MDA-MB-468 expressing Dox-inducible GFP (MDA-MB-468-iGFP), used as control, did not show *SNAI1* or *NT5E* induction following Dox treatment, thus ruling out any off-target effect of Dox on SNAI1 and/or NT5E expression (data not shown).

To elucidate the link between SNAI1 and *NT5E*/CD73 expression, we treated cells with Dox for 5 days to induce EMT in epithelial cells (designated as EPI). We next removed Dox from the culture medium of resulting mesenchymal-like cells (designated as EMT) to revert EMT and re-acquire an epithelial-like phenotype, designated as mesenchymal to epithelial transition (MET). Our data (Figures 2A, B) show that driving EMT in MDA-MB-468-iSNAI1 cells was associated with an increase in *SNAI1*, *ZEB1*, *VIM*, and *NT5E* and a decrease in *CDH1* expression. Immunofluorescence staining showed the acquisition of mesenchymal features under these experimental conditions, as evidenced by the nuclear accumulation of SNAI1 protein, actin microfilament remodeling, Vimentin upregulation, and E-cadherin downregulation (Figures 2C, D). All these events, observed by inducing EMT, were abrogated on day 17 following Dox removal leading to a MET switch (Figures 2A–D).

To evaluate whether the regulation of CD73 and SNAI1 following EMT and MET occurred in the same cell populations, by flow cytometry, we quantified MDA-MB-468-iSNAI1 positive cells for both CD73 and SNAI1 under EPI, EMT and MET conditions. We showed that under EPI conditions, only 2% of cells were positive for both CD73 and SNAI1 ($CD73^+$ $SNAI1^+$). The percent of $CD73^+$ $SNAI1^+$ cells significantly increased to 80% under EMT and subsequently decreased to almost 1% under MET conditions (Figure 2E and Figure S2B). Our results reported in Figure 2E, showing the regulation of CD73 under EPI, EMT, and MET conditions, were reproduced by imaging cytometry (Figure 2F). Together, our results demonstrate a positive regulation of *NT5E*/CD73 following SNAI1 induction at the transcriptional and protein level in MDA-MB-468-iSNAI1 cells. The regulation of *NT5E*/CD73 by SNAI1 is further confirmed using the additional mouse TNBC cell line Py8119 displaying several mesenchymal features

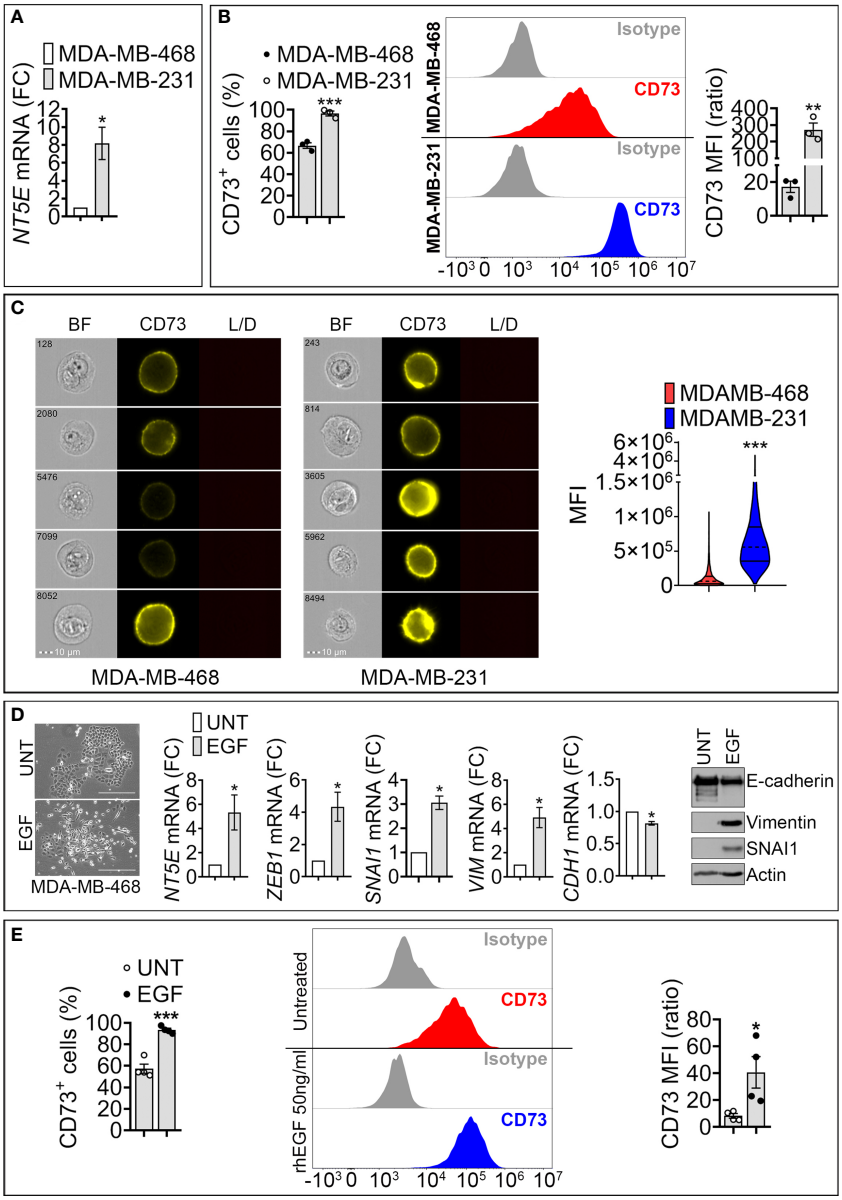

FIGURE 1
Expression of *NT5E*/CD73 mRNA and protein in mesenchymal-like MDA-MB-231 and epithelial like MDA-MB-468 TNBC cells. **(A)** RT-qPCR measurement of *NT5E* mRNA in mesenchymal-like MDA-MB-231 and epithelial-like MDA-MB-468 TNBC cell lines. NT5E expression was calculated relative to MDA-MB-468 cells. Bars represent means from four independent experiments ± SEM; *$P < 0.05$ calculated by Mann Whitney. **(B)** Flow cytometry analysis of cell surface CD73 in TNBC cells. *Left panel:* percentage of CD73-positive ($CD73^+$) cells; *Middle panel*: representative FACS histograms of indicated cells stained with control isotype or anti-CD73 antibody. *Right panel:* mean fluorescence intensity (MFI) of cell surface CD73 in MDA-MB-468 and MDA-MB-231 cells. Bars represent means from three independent experiments ± SEM, **$P < 0.01$, ***$P < 0.001$ calculated by unpaired t-test. **(C)** CD73 cell surface expression in MDA-MB-468 and MDA-MB-231 cells acquired by imaging cytometry. *Left panels:* representative images for each cell line acquired on brightfield (BF), CD73-PE, and live/dead (L/D) channels. The scale bar and event number are shown. *Right panel:* Violin plot quantification of CD73 mean fluorescence intensity (MFI) acquired by imaging cytometry in MDA-MB-468 and MDA-MB-231 cells. Results are the average of 10^4 acquisitions, ***P < 0.001 by unpaired t-test. **(D)** *Left panels:* Morphology of untreated (UNT)- and EGF (EGF)-treated MDA-MB-468 cells. Bar: 300µm. *Middle panels:* mRNA expression of CD73 and EMT markers (SNAI1, ZEB1, VIM and CDH1) in MDA-MB-468 cells treated with rhEGF (50ng/ml for 6 days). The expression level of each gene in treated (EGF) cells was calculated relative to untreated (UNT) cells. Bars represent means from four independent experiments ± SEM; *$P < 0.05$ calculated by Mann Whitney. *Right panel*: Representative Western-blot showing the protein expression of E-cadherin, SNAI1, Vimentin in (UNT)- and EGF (EGF)-treated MDA-MB-468 cells. Actin was used as a loading control. **(E)**. Flow cytometry quantification of surface CD73. *Left panel:* Percentage of CD73 positive MDA-MB-468 cells treated as described in **(D)**. *Middle panel*: representative FACS histograms of untreated or rhEGF-treated MDA-MB-468 cells stained with isotype or anti –CD73 antibody. *Right panel:* Mean fluorescence intensity (MFI) of cell surface CD73 in MDA-MB-468 treated as described in **(D)**. Bars represent means from four independent experiments ± SEM; *$P < 0.05$, and ***$P < 0.001$ are calculated by unpaired t-test.

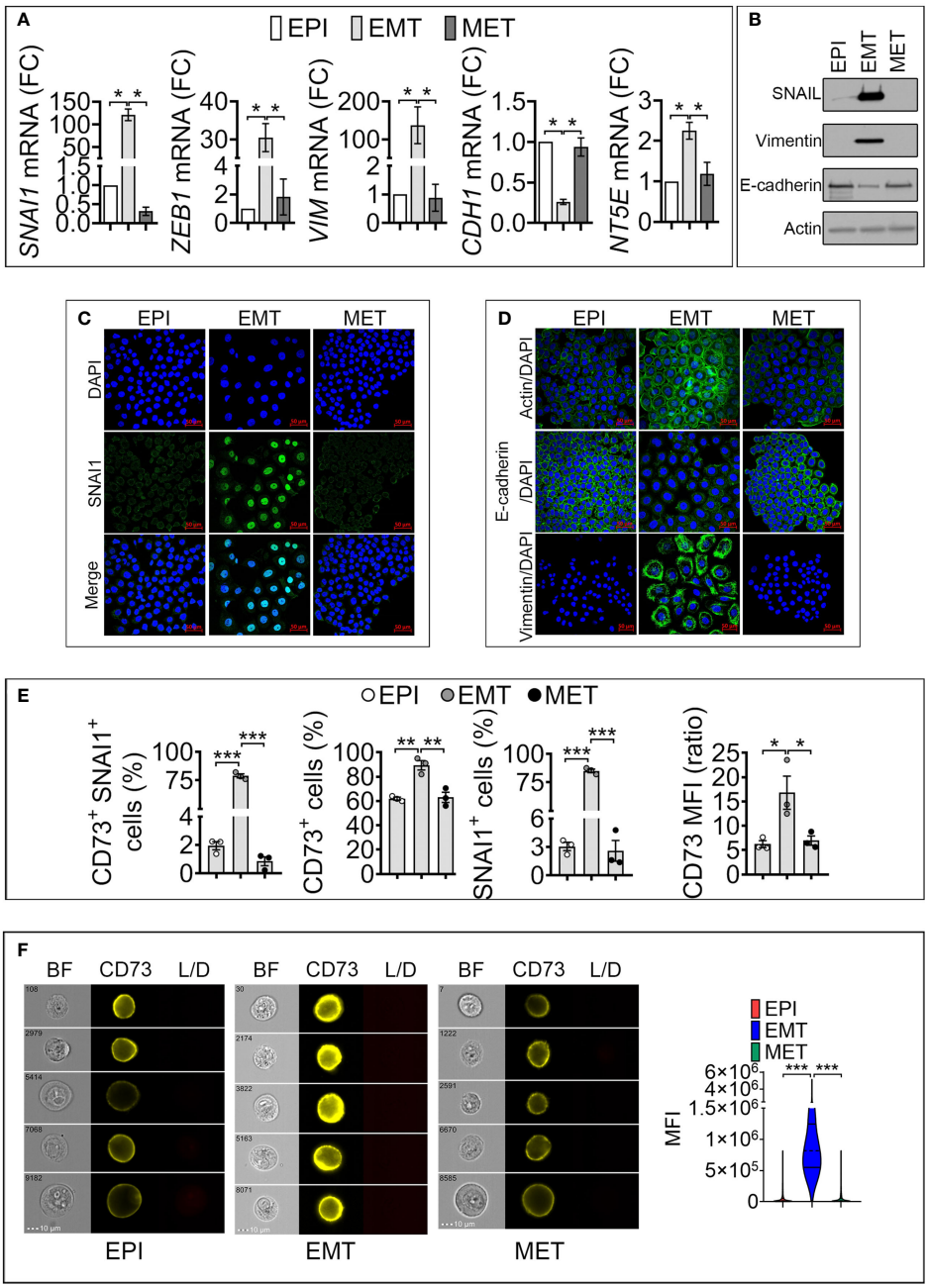

FIGURE 2
Induction of SNAI1 in TNBC MDA-MB-468-iSNAI1 cells upregulates NT5E/CD73 mRNA and protein expression. **(A)** mRNA expression of *SNAI1*, *ZEB1*, *VIM*, *CDH1 NT5E* and *NT5E* in EPI, EMT, and MET conditions. The expression of each gene in EMT and MET conditions was calculated relative to the EPI condition. Bars represent means from four independent experiments ± SEM (*$P < 0.05$ calculated by Mann Whitney). **(B)** Representative Western-blot showing the protein expression of SNAI1, Vimentin, and E-cadherin in MDA-MB-468 cells cultured under EPI, EMT, and MET conditions. Actin was used as a loading control. **(C, D)** Immunofluorescence images of SNAI1 **(C)**, Actin, E-cadherin, and Vimentin **(D)** staining (in green) in MDA-MB-468 cells cultured under EPI, EMT, or MET conditions. Nuclei are stained with DAPI (blue). The images shown are representative of two independent experiments. Scale bar: 50 µm. **(E)** Flow cytometry analysis of the percentage (%) of MDA-MB-468 cells cultured under EPI, EMT, and MET conditions that are positive for both CD73/SNAI1 (CD73+ SNAI1+) and either CD73 (CD73+) or SNAI1 (SNAI1+). CD73 MFI is reported. Bars represent means from three independent experiments ± SEM (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ by unpaired t-test). **(F)** CD73 surface expression in MDA-MB-468 cultured under EPI, EMT, and MET conditions by flow imaging cytometry. *Left panels:* representative images of 10^4 acquisitions for each condition on brightfield (BF), CD73 and live/dead (L/D) channels. The scale bar and event number are shown. *Right panel:* Violin plot quantification of CD73 mean fluorescence intensity (MFI) in MDA-MB-468. Results are the average of 10^4 events for each condition. (***$P < 0.001$ calculated by unpaired t-test).

(absence of E-cadherin expression, expression of the EMT-transcription factors SNAIL1 and ZEB1, and higher expression of N-Cadherin and Vimentin) compared to the epithelial-like Py230 cell line. Both cell lines are derived from MMTV-PyMT transgene-induced mammary tumors (17) (Figure S2C). Using CRISPR/Cas9 technology, we generated Py8119 cells expressing a truncated non-functional SNAIL1 (Py8119 Del SNAIL) (Figure S2D). Compared to control cells, we showed that Py8119 Del SNAIL cells expressed significantly lower levels of *NT5E* mRNA (Figure S2E) and protein (Figure S2F).

CD73/*NT5E* is a direct target of SNAI1 in MDA-MB-468 cells, and its expression positively correlates with SNAI1 in TNBC patients

To assess whether CD73 is a direct target of SNAI1, we analyzed *in silico* the presence of the putative SNAI1 binding motifs CAGGTG and CACCTG, called E-boxes, in the proximal promoter of the *NT5E* gene. By using the Eukaryotic Promoter Database (Swiss Institute of Bioinformatics) and fuzznuc (EMBOSS explorer) software, we identified three CAGGTG (E-box 1, 3, 5) and two CACCTG (E-box 2, 4) E-boxes in the human *NT5E* proximal promoter (Figure 3A).

We next performed ChIP on EPI and EMT cells using the SNAI1 antibody to validate our *in silico* data. Our results show a consistently increased binding of SNAI1 (three-fold) to E-box 5, similar to E-cadherin used as a positive control (Figure 3B). Our results indicate that the CD73 gene is directly targeted by SNAI1 in MDA-MB-468 cells. This result agrees with a recently published report showing the direct binding of SNAI1 on CD73 promoter in mouse breast carcinoma cells (18).

Our data support that the regulation of CD73 in cells undergoing EMT occurs by direct binding of EMT-TFs to E-box motives in the *NT5E*/CD73 proximal promoter region. Among EMT-TFs, we identified SNAI1 as a major regulator of CD73 in TNBC. However, we cannot rule out that other EMT-TFs could also be involved in CD73 regulation in TNBC and other cancer types and settings. Consistent with this, it has been reported that EMT genomic signature is associated with *NT5E* expression in human HER2-positive breast tumors, and the EMT-TF TWIST was described to upregulate CD73 in immortalized mammary epithelial cells by a mechanism that is not fully understood (19). Another possible non-mutually exclusive mechanism by which EMT regulates the expression of CD73 is through EMT-dependent induction of cytokines such as TGF-β or TNF-α, as previously described (20, 21). Nevertheless, our data, together with previous reports, highlight the prominent role of EMT in CD73 immune checkpoint upregulation.

We next investigated whether a correlation between SNAI1 and CD73 expression is observed in TNBC patients. We first analyzed by qRT-PCR *NT5E*/CD73 and *SNAI1* gene expression in tumor mRNA from 12 TNBC patients. We observed a significant positive correlation between *NT5E*/CD73 and *SNAI1* expression (Figure 3C). We next validated our data using the large TNBC cohort described in the METABRIC dataset. We selected 258 TNBC patients based on their negative ER/PR/HER2 status (Figure 3D). Our results revealed a significant and positive correlation between *NT5E* and *SNAI1* and the mesenchymal marker *VIM* in the selected TNBC patients (Figure 3E). In contrast, *NT5E* was negatively correlated with the epithelial marker *CDH1* (Figure 3E). We also found that the expression of *SNAI1* and *VIM* is consistently high in TNBC samples displaying high *NT5E* levels (Figure 3E). These results support our data and strengthen the link between EMT and CD73 expression in TNBC. Data related to TNBC patients are provided in Table S2.

SNAI1-dependent upregulation of CD73 increases the release of extracellular adenosine and mediates immunosuppression

We next assessed the functional impact of SNAI1-dependent upregulation of cell surface CD73. We first analyzed the secreted ADO level in the conditioned medium of MDA-MB-468-iSNAI1 undergoing EMT. Extracellular ADO concentration was significantly increased from 2.82 ± 0.41 to 6.49 ± 0.74 μM following SNAI1-dependent upregulation of CD73 (EMT condition) and subsequently decreased to 2.17 ± 0.54 μM after Dox removal and SNAI1 and CD73 downregulation (MET condition) (Figure 4A). The increased release of extracellular ADO by MDA-MB-468-iSNAI1 cells cultured under EMT conditions was related to increased CD73 expression because such an increase was no longer observed following treatment of cells with the CD73 inhibitor Adenosine 5'-(α,β-methylene) diphosphate (APCP) (Figure 4B).

CD73 is considered a major source of intra-tumoral ADO production (22). Although its exact concentration in the tumor microenvironment is not yet well defined, it has been proposed that ADO concentration is in the micromolar range (23). In keeping with this, we next evaluated whether the level of ADO released following SNAI1-dependent EMT was sufficient to impair the cytotoxic properties of NK cells. The rationale for using NK cells relies on establishing a positive correlation between NK cell signature genes and TNBC patient survival (24). Intra-tumoral ADO elicits an immunosuppressive effect by interacting with Adenosine receptors. Indeed, four adenosine receptors have been identified including A_1, A_{2A}, A_{2B} and A_3 (25, 26). The Adenosine A_{2A} receptor subtype is the predominant subtype found on T cells (27, 28) and NK cells (29).

We, therefore, analyzed the time- and concentration-dependent effects of the adenosine analog CADO on cytotoxic

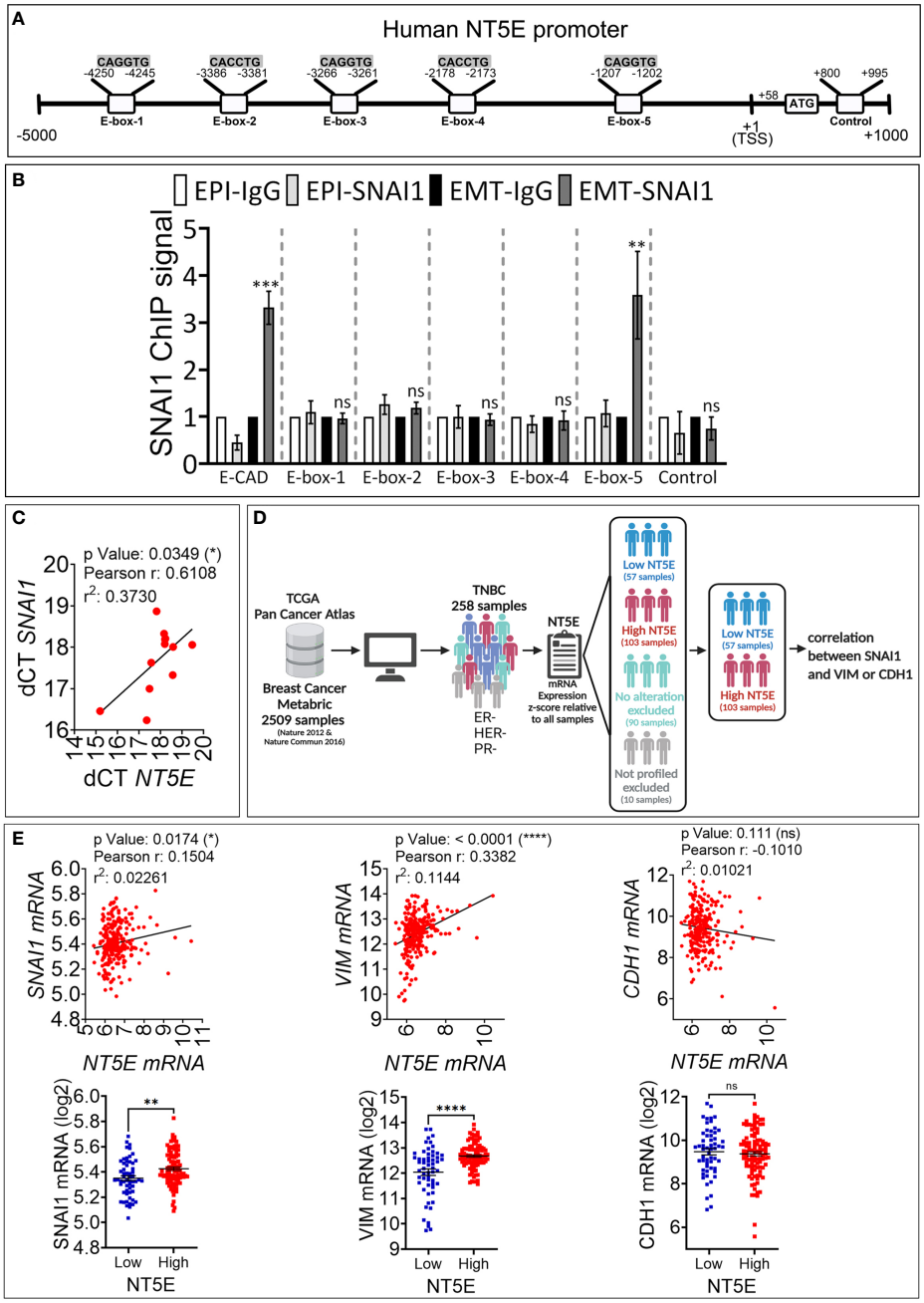

FIGURE 3
SNAI1 directly regulates CD73 expression in human TNBC MDA-MB-468-iSNAI1 cells. **(A)** Schematic representation of the different E-boxes identified *in silico* in the human CD73 promoter (CD73 mRNA, NCBI Reference Sequence: NM_002526). The transcription Start Site (TSS) and the ATG start codon are reported at positions +1 and +58, respectively. The control motif (+800 to +995) corresponds to a region containing no E-box and is used as a negative control. **(B)** ChIP was performed on MDA-MB-468-iSNAI1 cells cultured under EPI or EMT conditions using anti-SNAI1 antibodies followed by five pairs of primers flanking the identified E-boxes (E-box-1-5) or primers flanking control region. E-cadherin (E-CAD) primers were used as a positive control. For each gene, the RT-qPCR signals were normalized to control IgG. SNAI1 ChIP signal was reported as fold enrichment over IgG control. Two individual experiments (done in triplicate) were performed (*P < 0.05, **P <0.01, ***P < 0.001 calculated by unpaired t-test). **(C)** Correlation between *NT5E*/CD73 and *SNAI1 in* tumor mRNA from 12 TNBC patients. Pearson correlation coefficient (r) and P-value are shown. **(D)** Analysis process of METABRIC dataset. **(E)** Correlation between *NT5E*, *SNAI1*, *VIM*, and *CDH1* gene expression in TNBC patients from METABRIC dataset (n = 258). For each association: Upper panel: correlation between *NT5E*/CD73 and the indicated gene. Pearson coefficients and P values are shown. Lower panel: TNBC samples were separated according to *NT5E*/CD73 expression level to form high (Z score ≥ +0.5) and low (Z score ≤-0.5) *NT5E*/CD73 groups. In each group. *SNAI1*, *VIM*, and *CDH1* gene expression were evaluated. Dots represent mRNA level ± SEM (**P <0.01, ****P < 0.0001 calculated by unpaired t-test). ns, not significant.

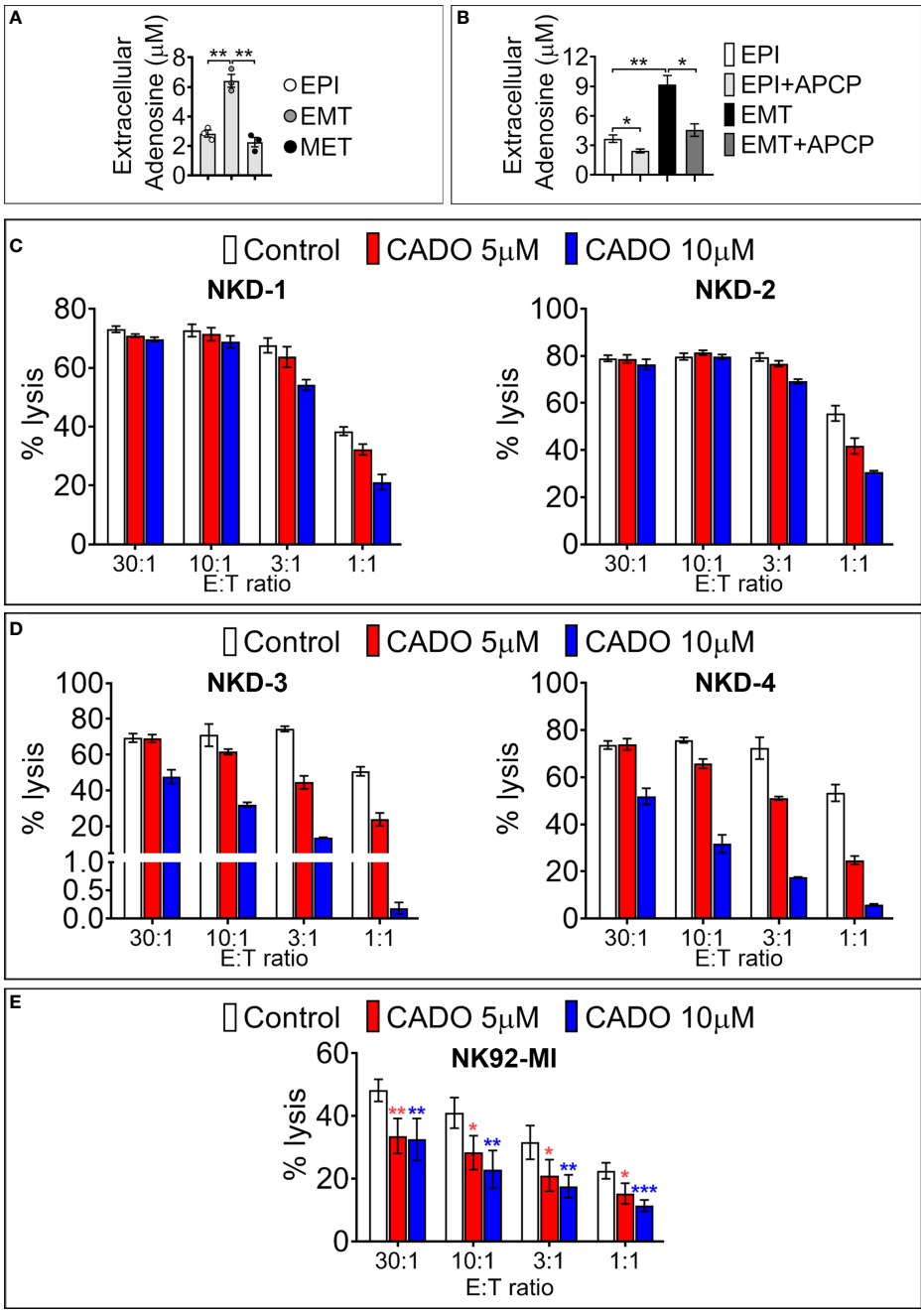

FIGURE 4
SNAI1-mediated CD73 upregulation results in increased extracellular ADO production. **(A)** Concentration of extracellular ADO released in the culture medium of MDA-MB-468-iSNAI1 cells cultured under control conditions (EPI), after 5 days of Dox (EMT), and after Dox removal (MET). Bars represent means from three independent experiments ± SEM (*P < 0.05, **P < 0.01 calculated by unpaired t-test). **(B)** Concentration of extracellular ADO released in the culture medium of MDA-MB-468-iSNAI1 cells cultured under control conditions (EPI) and after 5 days of Dox (EMT) in the absence or presence (+APCP) of APCP (100 μM). Bars represent means from three independent experiments ± SEM (*P < 0.05, **P < 0.01 calculated by unpaired t-test). **(C)** NK cells from four healthy donors (NKD-1 to 4) were pre-treated with CADO at 5 μM or 10 μM during 4 days (NKD-1 and NKD-2) or 6 days (NKD-3 and NKD-4). NK cytotoxic activity against K562 cells was measured at the indicated E:T ratios. Bars represent the mean percentage of lysis ± SD. **(D)** Cytotoxic activity of NK92-MI cells pre-treated for 6 days with CADO at 5 μM or 10 μM against K562 cells at the indicated **E:** T ratios. Bars represent mean percentage of lysis from three independent experiments ± SD (*P < 0.05, **P < 0.01, ***P < 0.001 calculated by unpaired t-test). Only one experiment was performed for each NK healthy donor reported in panels **(C, D)**. Experiments were performed either in duplicates or triplicates.

and proliferative capacities of NK cells isolated from four different healthy donors (NKD1, NKD2, NKD3, NKD4) and of the NK92-MI cell line. NKD1 and NKD2 were pre-treated for 4 days, whereas NKD3, NKD4, and NK92-MI were pre-treated for 6 days with CADO before co-culture with target cells at different effectors to target (E:T) ratios (30:1, 10:1, 3:1, and 1:1).

After 4 days of NK cell pre-treatment with 5 or 10 µM of CADO, we observed a decrease in NK-mediated lysis of target cells only at 3:1 and 1:1 E:T ratios (Figure 4C). After 6 days of NK cell pre-treatment with 5 or 10 µM of CADO, the impairment of NK-mediated lysis was observed at all E:T ratios tested except at the 30:1 ratio (Figure 4D). Similarly, pre-treatment of NK92-MI with 5 or 10 µM of CADO significantly impaired their cytotoxicity toward target cells at all E:T ratios tested (Figure 4E). The impairment of NK cell activity by CADO is associated with an impairment of their proliferation, as reported in Figure S3. Together, these results argue that ADO impairs the cytotoxic activity of NK cells in a time and dose-dependent manner and are in line with previous reports showing that adenosine analogs impair mouse NK cells' mediated killing (30, 31).

Concluding remarks

During the EMT process, the transcription factor SNAI1 acts as a direct repressor of E-cadherin promoter (32). In the present report we provide additional mechanistic insights showing that, similar to its role in CD47 upregulation (12), SNAI1-dependent EMT upregulates the expression of CD73 in TNBC cells. Furthermore, we show clinical evidence that such a regulation may occur in TNBC patients. Considering the encouraging but still moderate clinical responses of immune checkpoint blockades in TNBC, our results provide a rationale for further investigating the relevance of targeting EMT pathways in combination with immune checkpoint blockades to enhance the clinical benefit of immunotherapy in TNBC.

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 author.

Author contributions

Conception and design: MH, MZ, and BJ. Development of methodology: MH, BH, MX, MZ, and BJ. Acquisition of data: MH, MX, KM and MZ. Analysis and interpretation of data: MH, AO, MM, MZ. Writing, reviewing, and/or revising the manuscript: MH, MZ and BJ. Administrative, technical, or material support: MV, BH, AC, MZ, GB and BJ. Study supervision: MH, CD, JT, MZ and BJ. All authors contributed to the article and approved the submitted version.

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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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.982821/full#supplementary-material

FIGURE S1
Epithelial and mesenchymal protein expression in MDA-MB-468 and MDA-MB-231 cells. Representative Western-blot analysis of three independent experiments showing protein levels of ZEB1, Vimentin, and E-cadherin in MDA-MB-468 vs. MDA-MB-231 cells. Actin was used as a loading control.

FIGURE S2
Expression of SNAI1 and CD73 mRNA in MDA-MB-468 cells treated with increasing doses of Dox. (A) RT-qPCR measurements of SNAI1 and CD73 mRNA using increasing doses of Dox, n=4. *P < 0.05 by Mann Whitney. Means ± SEM are shown. (B) Representative FACS contour plot of three independent experiments showing intra-cellular SNAI1 and surface CD73 staining in MDA-MB-468 iSNAI1 displaying EPI, EMT, and MET phenotype conditions. (C) Expression of epithelial and EMT markers (E-Cadherin, SNAIL1, ZEB1, N-Cadherin and Vimentin) in epithelial-like Py-230 and mesenchymal-like Py-8119 cells. Actin was loaded as a control. (D) The expression of full length (FL) and deleted (Del) forms of SNAIL1 in Py-8119 cells transfected with control (CTRL) or SNAIL1 (SNAIL1) CRISPR plasmids. Actin was loaded as a control. (E) RT-qPCR measurement of Nt5e mRNA in Py-8119 described in D. Nt5e expression was calculated relative to control cells expressing FL SNAIL1. Bars represent mean from four independent experiments ± SD; *P < 0.05 calculated by Mann Whitney. (F) Flow cytometry analysis of cell surface CD73 in cells described in D. Left panel: Delta mean fluorescence intensity (MFI); middle panel: percentage of CD73-positive (CD73+) cells and left panel: representative FACS histograms of indicated cells stained with control isotype or anti-CD73 antibody. Bars represent means from three independent experiments ± SD; *P < 0.05 calculated by unpaired t test.

FIGURE S3
Ki67 quantification in NK cells isolated from healthy donors (NKD-3 and -4) and NK92-MI cells following CADO treatment. Percentage of Ki67-positive cells in NKD-3, NKD-4, and NK92-MI after treatment for 6 days with CADO at 5 and 10 μM. For NK92-MI, bars represent the mean of three independent experiments ± SEM (*P < 0.05, ** P < 0.01 calculated by unpaired t-test)Table S1 Sequence of SYBR-GREEN RT-qPCR primers used for amplification of immunoprecipitated DNA samples from ChIP assays.

TABLE S1
Sequence of SYBR-GREEN RT-qPCR primers used for amplification of immunoprecipitated DNA samples from ChIP assays.

TABLE S2
Information about the TNBC patient described in Figure 3. The table shows the METABRIC 258 TNBC patient and sample IDs; sex; ER, HER2, PR, *NT5E* status, mRNA expression of *NT5E*, CDH1, SNAI1 and VIM. NP: Not profiled.

References

1. Bianchini G, Balko JM, Mayer IA, Sanders ME, Gianni L. Triple-negative breast cancer: Challenges and opportunities of a heterogeneous disease. *Nat Rev Clin Oncol* (2016) 13(11):674–90. doi: 10.1038/nrclinonc.2016.66

2. Denkert C, Liedtke C, Tutt A, von Minckwitz G. Molecular alterations in triple-negative breast cancer-the road to new treatment strategies. *Lancet* (2017) 389(10087):2430–42. doi: 10.1016/S0140-6736(16)32454-0

3. Emens LA, Cruz C, Eder JP, Braiteh F, Chung C, Tolaney SM, et al. Long-term clinical outcomes and biomarker analyses of atezolizumab therapy for patients with metastatic triple-negative breast cancer: A phase 1 study. *JAMA Oncol* (2019) 5(1):74–82. doi: 10.1001/jamaoncol.2018.4224

4. Antonioli L, Blandizzi C, Malavasi F, Ferrari D, Hasko G. Anti-Cd73 immunotherapy: A viable way to reprogram the tumor microenvironment. *Oncoimmunology* (2016) 5(9):e1216292. doi: 10.1080/2162402X.2016.1216292

5. Antonioli L, Blandizzi C, Pacher P, Hasko G. Immunity, inflammation and cancer: A leading role for adenosine. *Nat Rev Cancer* (2013) 13(12):842–57. doi: 10.1038/nrc3613

6. Spychala J, Lazarowski E, Ostapkowicz A, Ayscue LH, Jin A, Mitchell BS. Role of estrogen receptor in the regulation of ecto-5'-Nucleotidase and adenosine in breast cancer. *Clin Cancer Res* (2004) 10(2):708–17. doi: 10.1158/1078-0432.ccr-0811-03

7. Buisseret L, Pommey S, Allard B, Garaud S, Bergeron M, Cousineau I, et al. Clinical significance of Cd73 in triple-negative breast cancer: Multiplex analysis of a phase iii clinical trial. *Ann Oncol* (2018) 29(4):1056–62. doi: 10.1093/annonc/mdx730

8. Loi S, Pommey S, Haibe-Kains B, Beavis PA, Darcy PK, Smyth MJ, et al. Cd73 promotes anthracycline resistance and poor prognosis in triple negative breast cancer. *Proc Natl Acad Sci U.S.A.* (2013) 110(27):11091–6. doi: 10.1073/pnas.1222251110

9. Samanta D, Park Y, Ni X, Li H, Zahnow CA, Gabrielson E, et al. Chemotherapy induces enrichment of Cd47(+)/Cd73(+)/Pdl1(+) immune evasive triple-negative breast cancer cells. *Proc Natl Acad Sci U.S.A.* (2018) 115 (6):E1239–E48. doi: 10.1073/pnas.1718197115

10. Synnestvedt K, Furuta GT, Comerford KM, Louis N, Karhausen J, Eltzschig HK, et al. Ecto-5'-Nucleotidase (Cd73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal epithelia. *J Clin Invest* (2002) 110 (7):993–1002. doi: 10.1172/JCI15337

11. Noman MZ, Janji B, Abdou A, Hasmim M, Terry S, Tan TZ, et al. The immune checkpoint ligand pd-L1 is upregulated in emt-activated human breast cancer cells by a mechanism involving zeb-1 and mir-200. *Oncoimmunology* (2017) 6(1):e1263412. doi: 10.1080/2162402X.2016.1263412

12. 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

13. Montserrat N, Gallardo A, Escuin D, Catasus L, Prat J, Gutierrez-Avigno FJ, et al. Repression of e-cadherin by snail, Zeb1, and twist in invasive ductal carcinomas of the breast: A cooperative effort? *Hum Pathol* (2011) 42(1):103–10. doi: 10.1016/j.humpath.2010.05.019

14. Byles V, Zhu L, Lovaas JD, Chmilewski LK, Wang J, Faller DV, et al. Sirt1 induces emt by cooperating with emt transcription factors and enhances prostate cancer cell migration and metastasis. *Oncogene* (2012) 31(43):4619–29. doi: 10.1038/onc.2011.612

15. Kandarian F, Sunga GM, Arango-Saenz D, Rossetti M. A flow cytometry-based cytotoxicity assay for the assessment of human nk cell activity. *J Vis Exp* (2017) 126). doi: 10.3791/56191

16. Davis FM, Azimi I, Faville RA, Peters AA, Jalink K, Putney JWJr., et al. Induction of epithelial-mesenchymal transition (Emt) in breast cancer cells is calcium signal dependent. *Oncogene* (2014) 33(18):2307–16. doi: 10.1038/onc.2013.187

17. Biswas T, Gu X, Yang J, Ellies LG, Sun LZ. Attenuation of tgf-beta signaling supports tumor progression of a mesenchymal-like mammary tumor cell line in a syngeneic murine model. *Cancer Lett* (2014) 346(1):129–38. doi: 10.1016/j.canlet.2013.12.018

18. Dongre A, Rashidian M, Eaton EN, Reinhardt F, Thiru P, Zagorulya M, et al. Direct and indirect regulators of epithelial-mesenchymal transition-mediated immunosuppression in breast carcinomas. *Cancer Discovery* (2021) 11(5):1286–305. doi: 10.1158/2159-8290.CD-20-0603

19. Turcotte M, Allard D, Mittal D, Bareche Y, Buisseret L, Jose V, et al. Cd73 promotes resistance to Her2/Erbb2 antibody therapy. *Cancer Res* (2017) 77 (20):5652–63. doi: 10.1158/0008-5472.CAN-17-0707

20. 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

21. Shrestha R, Bridle KR, Crawford DHG, Jayachandran A. Tnfalphamediated epithelialtomesenchymal transition regulates expression of immune checkpoint molecules in hepatocellular carcinoma. *Mol Med Rep* (2020) 21(4):1849–60. doi: 10.3892/mmr.2020.10991

22. Allard B, Allard D, Buisseret L, Stagg J. The adenosine pathway in immuno-oncology. *Nat Rev Clin Oncol* (2020) 17(10):611–29. doi: 10.1038/s41571-020-0382-2

23. Blay J, White TD, Hoskin DW. The extracellular fluid of solid carcinomas contains immunosuppressive concentrations of adenosine. *Cancer Res* (1997) 57(13):2602–5.

24. Bottcher JP, Bonavita E, Chakravarty P, Blees H, Cabeza-Cabrerizo M, Sammicheli S, et al. Nk cells stimulate recruitment of Cdc1 into the tumor microenvironment promoting cancer immune control. *Cell* (2018) 172(5):1022–37.e14. doi: 10.1016/j.cell.2018.01.004

25. Vijayan D, Young A, Teng MWL, Smyth MJ. Targeting immunosuppressive adenosine in cancer. *Nat Rev Cancer* (2017) 17(12):765. doi: 10.1038/nrc.2017.110

26. Ohta A, Sitkovsky M. Role of G-Protein-Coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. *Nature* (2001) 414(6866):916–20. doi: 10.1038/414916a

27. Deaglio S, Dwyer KM, Gao W, Friedman D, Usheva A, Erat A, et al. Adenosine generation catalyzed by Cd39 and Cd73 expressed on regulatory T cells mediates immune suppression. *J Exp Med* (2007) 204(6):1257–65. doi: 10.1084/jem.20062512

28. Lappas CM, Rieger JM, Linden J. A2a adenosine receptor induction inhibits ifn-gamma production in murine Cd4+ T cells. *J Immunol* (2005) 174(2):1073–80. doi: 10.4049/jimmunol.174.2.1073

29. Young A, Ngiow SF, Gao Y, Patch AM, Barkauskas DS, Messaoudene M, et al. A2ar adenosine signaling suppresses natural killer cell maturation in the tumor microenvironment. *Cancer Res* (2018) 78(4):1003–16. doi: 10.1158/0008-5472.CAN-17-2826

30. Beavis PA, Divisekera U, Paget C, Chow MT, John LB, Devaud C, et al. Blockade of A2a receptors potently suppresses the metastasis of Cd73+ tumors. *Proc Natl Acad Sci U.S.A.* (2013) 110(36):14711–6. doi: 10.1073/pnas.1308209110

31. Raskovalova T, Huang X, Sitkovsky M, Zacharia LC, Jackson EK, Gorelik E. Gs protein-coupled adenosine receptor signaling and lytic function of activated nk cells. *J Immunol* (2005) 175(7):4383–91. doi: 10.4049/jimmunol.175.7.4383

32. Batlle E, Sancho E, Franci C, Dominguez D, Monfar M, Baulida J, et al. The transcription factor snail is a repressor of e-cadherin gene expression in epithelial tumour cells. *Nat Cell Biol* (2000) 2(2):84–9. doi: 10.1038/35000034

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The yin-yang of immunity: Immune dysregulation in myelodysplastic syndrome with different risk stratification

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Myelodysplastic syndrome (MDS) is a heterogeneous group of myeloid clonal diseases with diverse clinical courses, and immune dysregulation plays an important role in the pathogenesis of MDS. However, immune dysregulation is complex and heterogeneous in the development of MDS. Lower-risk MDS (LR-MDS) is mainly characterized by immune hyperfunction and increased apoptosis, and the immunosuppressive therapy shows a good response. Instead, higher-risk MDS (HR-MDS) is characterized by immune suppression and immune escape, and the immune activation therapy may improve the survival of HR-MDS. Furthermore, the immune dysregulation of some MDS changes dynamically which is characterized by the coexistence and mutual transformation of immune hyperfunction and immune suppression. Taken together, the authors think that the immune dysregulation in MDS with different risk stratification can be summarized by an advanced philosophical thought "Yin-Yang theory" in ancient China, meaning that the opposing forces may actually be interdependent and interconvertible. Clarifying the mechanism of immune dysregulation in MDS with different risk stratification can provide the new basis for diagnosis and clinical treatment. This review focuses on the manifestations and roles of immune dysregulation in the different risk MDS, and summarizes the latest progress of immunotherapy in MDS.

KEYWORDS

myelodysplastic syndrome, immune dysregulation, Yin-Yang theory, different risk stratification, immunotherapy

1 Introduction

"Yin-Yang theory" is an advanced philosophical thought in ancient China, and it is also the earliest naive materialism. Yin and Yang refer to two opposite aspects of interrelated things or phenomena in the natural world, and contain the concept of unity of opposites (1). In general, everything that is active, external, ascending, warm, bright and hyperactive belongs to Yang, while everything that is quiet, internal, descending, cold, dark and hypofunction belongs to Yin. The essence of the "Yin-Yang theory" includes four aspects: opposites between Yin and Yang, mutual rooting of Yin and Yang, waxing and waning of Yin and Yang, transformation between Yin and Yang (2). "Opposites between Yin and Yang" refers to the mutual restriction and struggle between Yin and Yang. "Mutual rooting of Yin and Yang" means that Yin depends on Yang and Yang depends on Yin. Neither side can exist independently of the other. This interdependent relationship is also known as "mutual root". "Waxing and waning of Yin and Yang" means that Yin and Yang are not in a static state, but in a state of dynamic change. Waning of Yin will lead to waxing of Yang and vice versa. "Transformation between Yin and Yang" refers to either Yin or Yang may transform into its opposite side in given conditions. If we regard the waning and waxing relation between Yin and Yang is a process of quantitative change, then the inter-transformation between Yin and Yang is a qualitative change.

There seems to be some concepts in common between "Yin-Yang theory" and " Immunology ". Specifically, the view of "Yin-Yang theory" is that the immune system is a unity of opposites between Yin and Yang, and it is necessary to keep relative balance between Yin (immune suppression) and Yang (immune hyperfunction), so as to fully play the role in the normal function (3). Immune hyperfunction of body such as the hypersensitive reaction of immunocompetent cells can be defined as the disease of "Yin waning and Yang waxing" in the traditional Chinese medicine, and its typical diseases are autoimmune disorders (AD) and various allergic reactions. On the contrary, immunodeficiency such as immune cell deficiency can be regarded the disease of "Yin waxing and Yang waning" in the traditional Chinese medicine, and its typical diseases are immunodeficiency diseases and tumors. The treatment of "adjusting Yin-Yang" in traditional Chinese medicine refers to that "reducing excess" is immunosuppressive therapy, and "supplementing insufficiency" is immunopotentiation therapy.

Myelodysplastic syndrome (MDS) is a heterogeneous group of myeloid clonal diseases originated from hematopoietic stem cells, with characteristics of dysplasia in the bone marrow (BM), ineffective hematopoiesis, refractory cytopenias and a high risk of transformation to acute myeloid leukemia(AML). The pathogenesis of MDS has heterogeneity and most patients have no clear etiological and causative factors. At present, the research on its pathogenesis involves multiple aspects including genetic abnormalities of endogenous hematopoietic stem/progenitor cells, epigenetic alterations, exogenous BM microenvironment changes and immune dysregulation. The dysregulation of immune and inflammatory signaling pathways in BM microenvironment plays an important role in the occurrence and development of MDS, which is also one of the research hotspots in recent years.

Revised international prognostic scoring system (IPSS-R) is one of the gold standard of risk stratification and prognostic assessment for MDS patients. According to IPSS-R, patients with lower-risk MDS (LR-MDS) are those with very low-risk, low-risk and some subsets of intermediate-risk (≤3.5 points), and patients with higher-risk MDS (HR-MDS) are those with some subsets of intermediate-risk (> 3.5 points), high-risk and very high-risk. With the deepening of the study on the immune pathogenesis of MDS, researchers found that the immune dysregulation in MDS with different risk stratification is different, and changes dynamically in the process of disease progression. The immune abnormalities of most LR-MDS such as the significant increase of cytotoxic T lymphocyte (CTL) and helper T cell 17 (Th17), and the significant decrease of regulatory T cells (Treg) suggest that the immune system is in an activated and pro-inflammatory state (Yang), resulting in an increase of apoptosis rate of hematopoietic stem cells (HSCs) (4). In addition, many patients with LR-MDS seem to benefit from immunosuppressive therapy. On the contrary, the immune system of most HR-MDS is in an inhibitory state (Yin), which makes a massive expansion of abnormal clone in BM microenvironment (5). Immune activation therapy including immune checkpoint inhibitors and tumor vaccines may prolong the survival for these HR-MDS patients. However, we should recognize that the immune dysregulation of some MDS (possibly mainly intermediate-risk patients) may be the coexistence of immune hyperfunction and immune suppression, which changes dynamically and transforms mutually in the process of development and treatment. Specifically, there may be immune suppression at a certain stage of LR-MDS so as to promote the development of LR-MDS to HR-MDS and even AML. There also may be immune activation during the process of development and treatment of HR-MDS, which makes HR-MDS good curative effect, even transforming to LR-MDS. In conclusion, the immune dysregulation of LR-MDS and HR-MDS can be summarized by an advanced philosophical thought "Yin-Yang theory" in ancient China. The balance of immune hyperfunction (Yang) and immune suppression (Yin) is constantly changing between LR-MDS and HR-MDS (3–5), and can transform to each other under certain conditions.

It is well known that there is clear evidence of immune dysregulation in MDS patients. Many cytokines, almost all types of immune cells, immune checkpoints, immune and inflammatory signaling pathways participate in the pathogenesis of MDS (Figure 1). However, the manifestation and exact mechanism of immune dysregulation in MDS with different risk stratification are different and the immunotherapy

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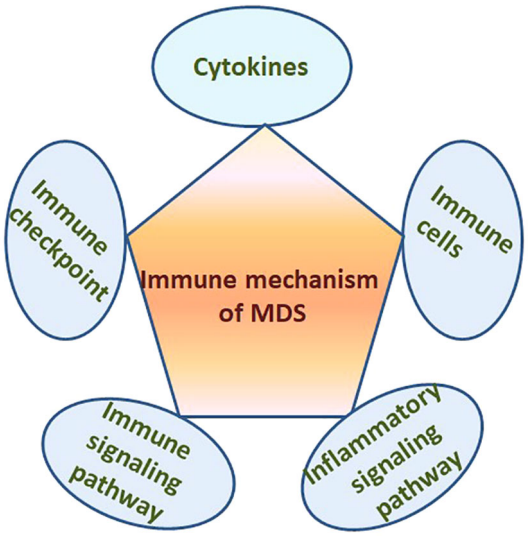

FIGURE 1
Many cytokines, immune cells, immune checkpoints, immune and inflammatory signaling pathways in the BM microenvironment participate in the pathogenesis of MDS.

plans should also be different. This review focuses on the different manifestations of immune dysregulation in MDS patients with different risk stratification, and summarizes the latest progress of relevant immunotherapy especially the emerging immunotherapy methods.

2 MDS and autoimmune disorders

Immune dysregulation and inflammatory reaction participate in the pathogenesis of MDS. Therefore, the relationship between AD which based on immune inflammatory response and MDS has also attracted the attention of the scholars. According to relevant researches, AD appears in around one third of MDS patients which was significantly higher than that of healthy people (6), while AD also increased the risk of MDS with an odds ratio (OR) from 1.5 to 3.5 (6, 7). The effect of AD on the clinical characteristics and prognosis to MDS patients is still controversial. In terms of clinical characteristics, the MDS patients with AD seems to be associated with female, lower hemoglobin levels and higher IPSS-R score (4, 7, 8) (Table 1). In terms of prognosis, most studies believe that AD has a positive or no effect on prognosis of MDS (Table 1) (8–14). Most AD associated with MDS can be efficiently managed with immune-therapeutic treatments. In conclusion, there is a clear relationship between AD and MDS, but its internal mechanism is not clear, and its potential prognostic impact is still controversial.

Because the previous reviews had shown the relationship and mutual influence between AD and MDS, this paper will not review again in detail. This paper summarizes the relevant research results in recent 5 years in Table 1 (8–14). At present, most studies believe that immune dysregulation is the common basis of the two diseases. Chronic immune stimulation may be the trigger factor for MDS, and some patients with MDS can get remission after immunosuppressive treatment, which provides evidence for this view.

TABLE 1 The studies evaluated the frequency and characteristics of MDS patients with AD in the last 5 years.

Years Authors	Country	Ratio (n/N)	Main type of AD	Clinical features	Impact on survival	Reference
2021 Dongni Jiang et al	China	27.7% 57/206	Vasculitis (19.3%, 11/57) Serum immune abnormality (17.5%, 10/57) RA (12.3%, 7/57)	Lower risk group;More MDS-MLD	Better PFSBetter OS	(9)
2021 Na Xiao et al	China	19.6% 21/107	Vasculitis (23.8%,5/21) SLE (19.0%,4/21) RA (14.3%,3/21)	More MDS-MLDMore MDS-EB1	No difference	(10)
2019 Julie Seguier et al	France	11%88/ 801	Polyarthritis (27.2%,22/81) Immune cytopenias disorder (18.5%,15/81) Vasculitis (13.6%,11/81)	More MDS-MLDMore CMML-1	Better OS	(11)
2018 Montoro Jet al	Spain	48%68/ 142	Hypothyroidism (16.2%,11/68) RA (13.2%,9/68) Polymyalgia rheumatic (8.9%,6/68)	More FemaleLower hemoglobin value	Inferior OS	(12)
2016 Mekinian A et al	France	17.9% 123/688	Vasculitis (32.0%,39/123) CTD (25%,31/123) Arthritis (23%,28/123)	More MDS-MLDMore MDS-EB1More CMML-1	No difference	(13)
2016 Komrokji RS et al	USA	27.8% 391/ 1408	Hypothyroidism (44%,171/391) ITP (12%,46/391) RA (7%,28/391)	More FemaleLower RBC transfusion dependent	Better OSLess AML transformation	(8)
2016 Lee SJ et al	Korea	33.3% 67/201	ND (35.8%,24/67) Behcet disease (14.9%,10/67) RA (13.4%,9/67)	More 5q- and +8	No difference	(14)

AD, autoimmune disease; AIM, autoimmune manifestation; MDS-MLD, Myelodysplastic syndrome-with multilineage dysplasia; PFS, free survival time; OS, overall survival; SLE, Systemic lupus erythematosus; RA, Rheumatoid arthritis; MDS-EB1, MDS with excess blasts 1; CTD, Connective tissue disease; ITP, Idiopathic thrombocytopenic purpura; RBC, Red blood cell; ND, Neutrophilic dermatosis.

3 Immune dysregulation of MDS

Immune dysregulation in BM microenvironment plays an important role in the occurrence and development of MDS (Figure 2), which can be proved by the overexpression of TLR, CD14 and other immune related genes. However, the manifestations and intrinsic mechanisms of immune dysregulation in LR-MDS and HR-MDS are different. Cytokines, immune cells, immune checkpoints, immune and inflammatory signaling pathways play different roles in immune dysregulation of MDS with different risk stratification (Figure 3), which is itemized here below.

3.1 Cytokines

The abnormal expression of cytokines, chemokine and growth factors participate in the occurrence and development of MDS especially the abnormal secretion of cytokines. The levels of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), lnterleukin-6 (IL-6), IL-8, IL-32 and granulocyte macrophage colony stimulating factor (GM-CSF) generally increase in MDS patients, and their expression levels may be related to disease outcome (15, 16). Pardanani et al. found that the levels of 19 of the 30 plasma cytokines in MDS patients changed significantly, among which the increased levels of CXCchemokineligand-10(CXCL10), IL-7 and IL-6 seemed to be predictors of lower survival (16), while the increased levels of IL-4 and CCL3 were significantly correlated with higher remission rate (5). However, abnormal secretion of cytokine is different in MDS with different risk stratification.

In LR-MDS patients, the levels of pro-inflammatory cytokines such as TNF-α、IFN-γ、IL-8、IL-12 and IL-17 increase and induce the apoptosis of normal HSCs in BM (17, 18). In HR-MDS patients, myeloid-derived suppressor cells (MDSCs) from BM secrete a large number of immunosuppressive cytokines such as IL-10, IL-1β and TGF-β, causing tumor cells to escape from immune surveillance (19). IL-6 is an important regulatory factor of immune and inflammatory response, having different biological role and expression level in immune microenvironment in LR-MDS and HR-MDS. When being at low level, IL-6 mainly participates in clonal hematopoiesis of indeterminate potential (CHIP), hemocytopenia and BM hypoplastic of LR-MDS. When being at high level, IL-6 mainly participates in the tumor invasion, metastasis and recurrence of HR-MDS. So the overexpressed IL-6 is a predictor of lower survival and poor prognosis in patients with MDS (16, 20).

In addition, immunosuppressive cytokines (Yin) may also exist in immune microenvironment of some LR-MDS (Yang), and pro-inflammatory cytokines (Yang) may also be highly expressed in immune microenvironment of some HR-MDS (Yin). There may be a process of struggle between immunosuppressive cytokines and pro-inflammatory cytokines in these patients, which ultimately determines the development and outcome of the disease. Just like the "Yin-Yang theory", two opposing immune status may also be interrelated and can transform to each other. For example, as a typical pro-apoptotic cytokine, TNF-α expression is generally increased in the peripheral blood of MDS

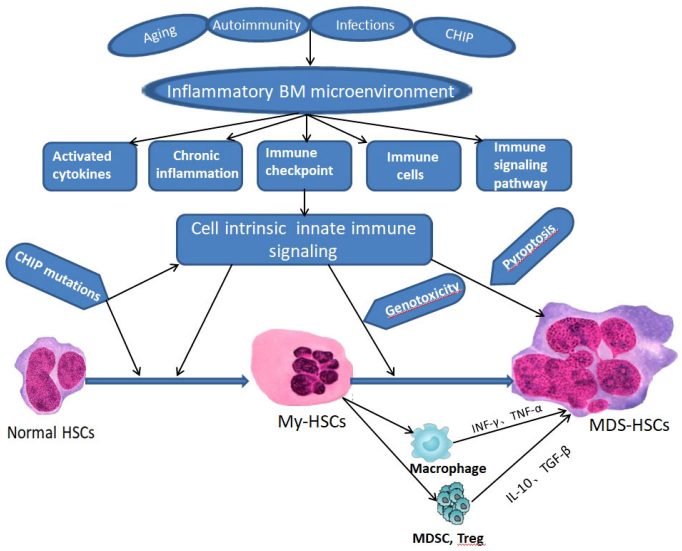

FIGURE 2
Schematic of innate immune signaling dysregulation in the pathogenesis of MDS. CHIP - clonal hematopoiesis of indeterminate potential, BM, bone marrow, HSCs - hematopoietic stem cells; My-HSCs, myeloid biased HSCs.

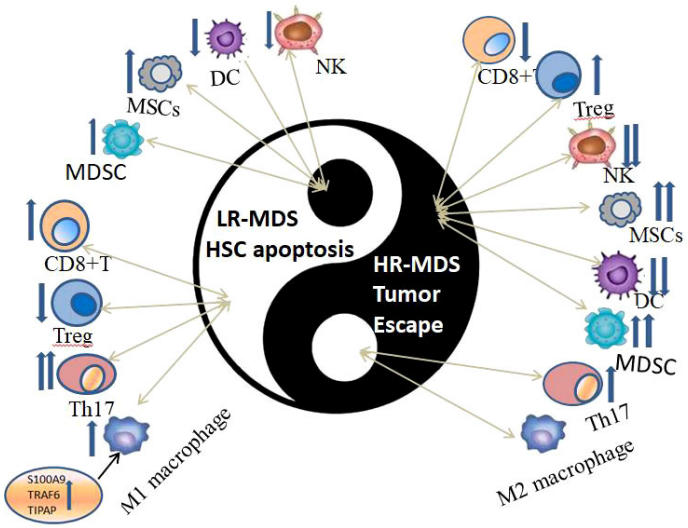

FIGURE 3
The immune dysregulation of immune cells in HR-MDS and LR-MDS can be summarized by an advanced philosophical thought "Yin-Yang theory" in ancient China, which are opposite, interrelated and can transform to each other under certain conditions.

patients and negatively correlated with IPSS-R prognostic score. So, TNF-α mainly participates in the occurrence and development of LR-MDS (18). It is worth noting that the level of TNF-α in some HR-MDS patient is also increased compared with healthy controls and may affect its disease development. The results confirms that immune activating molecules (Yang) have also increased in the immunosuppression microenvironment of HR-MDS (Yin), which is "Yin contains Yang" and "Yin generates Yang" in "Yin-Yang theory". TGF-β is a pleiotropic cytokine, which is generally increased in the BM microenvironment of MDS and participates in the pathogenesis both of LR-MDS and HR-MDS. In LR-MDS, high level of TGF-β can directly enhance the p38-MAPK signaling pathway to promote the expression of pro-inflammatory genes of downstream and the differentiation of Th17 cells, increasing the expression of IL-17 and IFN-γ and finally inducing the occurrence and development of disease (21). In HR-MDS, high level of TGF-β from mesenchymal stem cells (MSCs) inhibits the normal function of B, T and NK cells and induces the proliferation of Treg cells, so as to promote the immunosuppressive microenvironment and development of disease (22). The influence of TGF-β to the immune status of MDS depends on the specific cell and microenvironment. It can not only promote apoptosis in LR-MDS (Yang), but also play an immunosuppressive role in HR-MDS (21, 22), and it also may mediate the mutual transformation of LR-MDS and HR-MDS under some conditions, reflecting the unity of opposites and mutual transformation of Yin and Yang. In addition, there are many dysregulated cytokines in the BM immune microenvironment of MDS, but the specific regulatory mechanism and impact on prognosis are still unclear, which needs further research.

3.2 Immune cells

3.2.1 T lymphocyte subsets

T lymphocytes are the most important immune cells, and take part in both the cell-mediated immunity and humoral immunity at the same time. T lymphocytopenia is very common in MDS patients, but its manifestations are different in different risk stratification. CD8+T lymphocytes which are also called CTL are the effector T cell of anti-tumor immunity because they can directly kill tumor cells. CD8+T lymphocytes have been shown to activate and proliferate, inhibit malignant and normal HSCs hematopoiesis, and induce intramedullary apoptosis in LR-MDS patients (23). However, CD8+T lymphocytes show a significant decrease and induce the overexpression of programmed cell death protein 1/programmed cell death ligand 1 (PD-1/PD-L1) in the tumor microenvironment of HR-MDS patients, thereby enhancing the ability of tumor cells to escape host immunosurveillance (24). Treg cells are key regulators of immune system with strong immunosuppressive function and important for immune tolerance. Treg cells in BM and peripheral blood of LR-MDS patients are significantly decreased, and can be used as a prognostic factor to predict the degree of anemia, the rate of AML transformation and overall survival(OS) (25). On the contrary, the number and activity of Treg cells in HR-MDS

are increased, which promotes MDS to escape immunosurveillance and transform to AML (26).

Th17 is originated from pluripotent CD4+T cells and mainly secretes pro-inflammatory cytokine IL-17. The levels of Th17 and IL-17 in BM and peripheral blood in LR-MDS patients are significantly higher than those in HR-MDS and healthy control group, which stimulate a variety of cytokines to produce inflammatory reaction, and finally lead to increased apoptosis of BM cells and ineffective hematopoiesis (17, 27). Previous study in our research group had got similar conclusions and proved that cyclosporine A, an immunosuppressive agent, can inhibit the function of Th17 cells to improve the morbid hematopoiesis of LR-MDS, further confirming that Th17 taken part in the immune hyperfunction of LR-MDS (28). Interestingly, although the levels of Th17 and IL-17 are significantly lower in HR-MDS than those in LR-MDS, they are higher than those in healthy control group (27). It suggests that there are also high expression of pro-inflammatory cell and cytokine (Yang) in the immunosuppressive microenvironment of HR-MDS (Yin), reflecting the concept of "Yin contains Yang" and "Yin generates Yang".

Th22 cells are a newly discovered subset of helper T cells, which mainly secrete IL-22 and TNF-α. Based on the present studies, Th22 cells may have a dual immunomodulatory activity of pro-inflammatory and immunosuppression. For example, Shao et al. found that Th22 cells in peripheral blood of MDS patients increased significantly, and were significantly higher of HR-MDS than that of LR-MDS, indicating that Th22 cells may be more involved in immune escape (Yin) of MDS (29). However, another study found that number and effectors of Th22 cells in LR-MDS patients were higher than those in HR-MDS, suggesting that Th22 tends to pro-inflammatory characteristics (Yang) (30). Current studies have found that Th22 cells in MDS immune microenvironment may have both pro-inflammatory (Yang) and immunosuppressive (Yin) functions, may change dynamically, and even transform to each other under some conditions. Of course, it needs further research.

3.2.2 Natural killer cells

NK cells are the first line of defense against antitumor immunity with direct killing effect and take part in both innate immunity system and adaptive immunity system. NK cells in peripheral blood and BM of MDS patients decrease significantly and show negative correlation with IPSS-R score (31, 32). In HR-MDS, the number and function of NK cells such as cracking tumor cells, secreting cytokines and proliferation *in vitro* are significantly damaged, consequently inhibiting the normal anti-tumor immune response and promoting disease progression (31). In LR-MDS, the number of NK cells is also decreased but higher than HR-MDS, and the existing NK cells seem to have increased activity and cytotoxicity to CHIP of MDS, thereby inhibiting disease progression (32). In addition, it observed that the number of NK cells in the BM immune microenvironment of LR-MDS patients is reduced, but the existing NK cells have stronger immune function. From the perspective of "Yin-Yang theory", that is "Yang contains Yin" and "Yin contains Yang".

Activating killer immunoglobulin-like receptors (aKIRs) are the most important molecules in regulating NK cell activation and function. In recent years, it has been observed that the number and haplotype of aKIR gene have changed in MDS patients, which affects its immune monitoring and prognosis. In 2015, Daher et al. first reported that the number of aKIR gene was associated with the risk stratification of MDS, and found that the number of it in HR-MDS patients was significantly lower than that in LR-MDS patients, but both of them were lower than that in healthy volunteers (33). Stringaris et al. found that the overexpressing aKIR haplotype A was significant correlation with the higher risk of AML transformation in MDS patients by further study, and may be an independent predictor of clinical outcome in MDS patients (34).

3.2.3 Dendritic cell

DC is a important immunomodulatory factor, and the role of it in MDS has not yet been fully elucidated up to now. Current studies have shown that the number and the ability to activate T cells of mature and immature DC in MDS patients are significantly reduced, especially in HR- MDS (35), but the concrete effects of DC on the immune response for MDS patients with different risk stratification are different. The high level of pro-inflammatory factors such as IFN-γ and TNF-α in the BM immune microenvironment of LR-MDS (Yang) promote the maturation of reduced DC (Yin), and then fully mature DC which pulsed with antigens can induce the specific T cells to kill clonal MDS cells (Yang), resulting in the increased apoptosis of precursor cell (36). DC dysregulation in LR-MDS also reflects the view of "Yin-Yang theory", that is "Yang contains Yin", "Yin contains Yang", "Yang generates Yin" and "Yin generates Yang". In HR-MDS, the abnormality of DC is mainly characterized by the reduction of the number especially plasma like dendritic cells (pDCs), and the ability of DC to activate T cells is also significantly weakened (35, 37). In addition, whether HR-MDS or LR-MDS, the DC has obvious problem of differentiation and maturation. For example, the expression of some surface antigens such as CD54, CD80 and CD86 are reduced, and the ability to stimulate T cells and antigen presentation in mixed lymphocyte reaction is significantly reduced (38). In conclusion, all of the above studies illustrate the viewpoint of DC cell inefficiency in LR-MDS patients especially HR-MDS.

3.2.4 Mesenchymal stem cells

MSCs are the key component of BM microenvironment in MDS patients and play an important role in maintaining the

immune stability by down-regulating the intensity of immune response, regulating natural immunity and adaptive immunity. However, there is significant difference in the density and immune regulatory function of MSCs between LR-MDS and HR-MDS patients. The density of MSCs in HR-MDS patients is significantly higher than that in LR-MDS patients and has independent prognostic significance which is associated with lower OS and higher AML transformation rate (39, 40). MSCs of HR-MDS patients have immunosuppressive properties which are characterized by high level of TGF-β expression and the significantly enhanced ability to induce Treg and inhibit the proliferation and activation of T cells (5). There are great differences in the ability of MSCs to induce Treg between HR-MDS and LR-MDS. Compared with LR-MDS MSCs, HR-MDS MSCs can induce more Treg (40). It also reflects that immunosuppression from MSCs is more obvious in HR-MDS, although they also mildly displays immunosuppression (Yin) in the pro-inflammatory immune microenvironment of LR-MDS (Yang). In addition, the effect of MSCs on DC will also change dynamically with the disease state of MDS. The ability of MSCs to inhibit DC differentiation and maturation in HR-MDS is significantly better than LR-MDS MSCs (41). On the contrary, the ability of MSCs to inhibit DC differentiation and maturation is weak in BM immune microenvironment of LR-MDS (Yang), but there is still mild inhibition (Yin). Finally, it leads to the over activation of DCs with the strongest antigen-presenting function in LR-MDS which induce the excessive proliferation and activation of T cells in BM and then release a lot of pro-inflammatory molecules (Yang) to induce massive apoptosis of normal HSCs (40–42). In LR-MDS, the effect of MSCs on DC reflects that the "Yang contains Yin" and "Yin generates Yang" in "Yin-Yang theory". In addition, some MSCs of HR-MDS (Yin) can promote the pro-inflammatory cytokines such as TNF-α and IFN-γ secretion (Yang) which induce the increase of PD-L1/2 synthesis and secretion, and finally inhibit the activation and proliferation of CD4+T cells and promote the apoptosis of T cells (Yin) (43). It is the "Yin contains Yang" and "Yang generates Yin" in "Yin-Yang theory". In conclusion, all of these results suggest that MSCs have different immune regulation in different risk stratification MDS, which may be very important for understanding the pathogenesis of MDS and developing new immunotherapies.

3.2.5 Myeloid-derived suppressor cells

MDSCs are special immune cells which are recently found and have inhibitory effects on the body's immunity. MDSCs take part in the occurrence and development of MDS, but have different immunomodulatory effects in different risk. The number of MDSCs in LR-MDS patients is significantly lower than that in HR-MDS patients, which may be related to the immunosuppressive BM microenvironment of HR-MDS (44). In clinical practice, it is found that some LR-MDS (false Yang) will transform to HR-MDS (Yin), and the number of MDSCs will gradually increase in this process which may gradually induce the immune microenvironment of LR-MDS from activated state to inhibited state, that is "Yang generates Yin" and "Yin-Yang transformation" (45). Under the interaction of S100A9 and CD33, the BM microenvironment of HR-MDS drives the significant expansion of MDSCs and induces the immunosuppressive cytokines such as IL-10 and TGF-β overexpression. They inhibit the proliferation and function of T cells and NK cells, thus directly inhibiting normal hematopoiesis (46). MDSCs in HR-MDS can express CD155 to connect T cell immune receptor with immune checkpoint molecule T cell immunoglobulin and ITIM domain (TIGIT) and transmit inhibitory signal to NK cells, further aggravating the immunosuppressive microenvironment (46). In addition, high levels of monocytic MDSCs (M-MDSCs) in HR-MDS showed higher levels of intracellular IL-10, TGF-β and CXCR4 (45). In conclusion, MDSCs play an important role in the imbalance of immune monitoring of MDS and may be an important potential therapeutic target.

3.2.6 Other immune cells

In addition to the above immune cells, other innate immune cells in MDS patients also have abnormal regulation, such as macrophages, monocytes, neutrophils and so on. In MDS, the number of macrophages is decreased and phagocytosis of macrophages is impaired. The study found that percentage of macrophages in BM of HR-MDS is significantly lower than that of LR-MDS (47). Macrophages in LR-MDS BM microenvironment are mainly the M1 type and secrete a variety of pro-apoptotic cytokines including TNF-α, inducing BM cell apoptosis. In addition, macrophages which highly express S100A8s can hinder the normal differentiation of erythrocytes in LR-MDS microenvironment (48). In HR-MDS, M2 macrophages are dominant in quantity and function, suggesting that high level of M2 macrophages may be an early warning index for the poor prognosis of MDS (49). Monocytes in MDS have a unique phenotype and can reduce the production of matrix metalloproteinase (MMP).which is an important secretion product and can inhibit the supporting role of BM microenvironment for HSCs (50). In addition, high level of MMP significantly inhibited erythrocyte proliferation which finally caused hemocytopenia, so monocytes mainly participate in the pathogenesis of LR-MDS (51). MDS derived tumor-associated neutrophils are the product of abnormal hematopoiesis and have functional defects which may eventually lead to high mortality of infection patients in HR-MDS (52).

3.3 Immune checkpoint

Immune checkpoints have become a research hotspot in recent years because of its unique immunosuppressive role in tumor-specific immunity, and had achieved a series of results. MDS cells have been proved to have ability to utilize the immunosuppressive effect of immune checkpoints to promote their survival and proliferation. Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and PD-1/PD-L1 have been the most extensively studied immune checkpoints (53). In addition, many new immune checkpoint molecules including T cell immunoglobulin mucin-3 (Tim-3), lymphocyte activation gene-3 (LAG-3), CD47and TIGIT had also been successively proved to participate in the occurrence and development of MDS (54, 55).

As early as 2014, the University of Texas MD cancer center in the United States found that the expression of PD-1, PD-L1 and PD-L2 in CD34+ cells of MDS patients was significantly increased, which was related to the risk stratification of disease and the drug resistance mechanism of hypomethylating agent (HMA) (24). *In vitro* and animal experiments further showed that the BM microenvironment of MDS can induce the overexpression of immune checkpoint molecules such as PD-1/PD-L1 by activating MDSCs and specific cytokines (53, 56). The serum concentration of CTLA-4 in MDS patients increased too, and the HR-MDS group was significantly higher than LR-MDS. In addition, the overexpressed CTLA-4 in MDS is associated with high mortality (57). However, it can also be explained that CTLA-4, an important immunosuppressive molecule, can be used by tumor cells to induce immunosuppressive state and make tumor growth and development.

Tim-3 is a newly discovered immune regulatory molecule in recent years and combines with its ligand galectin 9 (Gal-9) to produce negative immune regulation, leading to Th1 cell apoptosis, IFN-γ release decreased and MDSCs proliferation. There are few studies on Tim-3 in MDS patients now, but the existing studies have confirmed that the expression of Tim-3 in BM of MDS patients is significantly higher than that of control group. The expression level of Tim-3 in low-risk group, medium-risk group and high-risk group increases successively, suggesting that Tim-3 may be a marker of malignant clones of MDS cells and participate in the malignant transformation of MDS (58, 59). In addition, the study found that the LAG3 expression on CD8+T and Treg cells in MDS patients was significantly higher than that in healthy controls. The overexpression of LAG3 may be the molecular basis for the low function of CD8+ effector T cells and the high function of Treg cells, so as to promote immune escape and eventually lead to disease progression (54). TIGIT is also a new immune checkpoint molecule, which is high expression on NK and T cells in MDS patients. TIGIT can directly inhibit the antitumor immune function mediated by NK and T cells and indirectly reduce the secretion of activated cytokines such as CD107a, IFN-γ and TNF-α to participate in disease progression and immune escape of MDS (55). As a new immune checkpoint molecule, CD47 on tumor cells is combined with signal-regulatory proteinα(SIRPα)to send "don't eat me" signal to immune system, playing a key role in tumor cells recognition and immune escape, and gradually becoming an effective target of tumor immunotherapy. The studies have confirmed that MDS cells significantly overexpress CD47, which is associated with higher risk and poor OS (60). In conclusion, there is sufficient evidence to indicate that immune checkpoint molecules participate in the abnormal myeloid clonal response in MDS patients, which provides a new immunotherapy for HR-MDS. In addition, HMA treatment can significantly improve the expression of several immune checkpoint molecules on MDS cells such as PD-L1, Tim-3 and CD47, providing a theoretical basis for the mechanism of HMA resistance and the combination therapy with immune checkpoint molecular inhibitors (61).

Immune checkpoints are significantly overexpressed in HR-MDS patients, promoting the formation of immunosuppressive microenvironment (Yin), which is related to lower OS and higher AML transformation rate (57–60). Moreover, the studies had proved that immune checkpoint inhibitors can improve the prognosis of some HR-MDS patients and promote the recovery or enhancement the functions of immune active cells. From the perspective of "Yin-Yang theory", it may be that HR-MDS immunosuppressive microenvironment (Yin) can also promote the production of immune active cells and molecules (Yang) and enhance the anti-tumor immune response under the certain conditions (such as immune activation treatment), that is "Yin generates Yang" and "Yin-Yang coexist". In addition, the current study confirmed that the expression of immune checkpoint molecules such as PD-1/PD-L1 and CTLA-4 in some LR-MDS was also increased (24, 53). The results further suggest that immunosuppressive molecules are also expressed in the activated immune microenvironment of LR-MDS (Yang), there is "Yang contains Yin". The expression of immune checkpoint molecules in some LR-MDS may be further increased under certain conditions such as the treatment of immunosuppressant, resulting in the gradual transformation of the activated immune microenvironment of LR-MDS to the inhibitory state ("Yin-Yang transformation"). Clinically, it is manifested as LR-MDS finally developed into HR-MDS, and immunosuppressant is ineffective in these LR-MDS patients.

3.4 Immune signaling pathway

Chronic innate immune and related inflammatory signaling pathways have been reported to play an important role in the

pathogenesis of MDS for many years, but specific evidence has not been found until recently. Next, we will focus on the role of them in the occurrence and development of MDS in different risk stratification.

3.4.1 Apoptosis signaling pathway

It is well known that abnormal apoptosis is an important factor in the pathogenesis of MDS. However, due to the heterogeneity of the disease, the different risk stratification MDS are affected by apoptosis differently, and LR-MDS is more closely related to apoptosis. Increased apoptosis was observed in LR-MDS, while apoptosis resistance was observed in HR-MDS. LR-MDS cells tend to pro-apoptotic phenotype, while HR-MDS cells changed to anti-apoptosis phenotype (62). As we all know that apoptosis is mediated by death receptor Fas and its specific ligand (Fas-L). In LR-MDS patients, TNF-α, Fas-L, TNF-Related apoptosis-inducing ligand receptor 1 (TRAIL-R1) and other pro-apoptotic cytokines are up-regulated, which promote the apoptosis of MDS clonal cells (63). The malignant clones with dysplasia in HR-MDS patients may produce resistance to pro-apoptotic effect of TNF-α, resulting in the increase of abnormal MDS clonal cells. In addition, CD34+ cells in HR-MDS show higher expression of anti-apoptotic gene Bcl-2 and lower apoptotic cell related antibody (Apo2.7), which explains why BM cells in HR-MDS are more resistant to apoptosis than those in LR-MDS.

Increased apoptosis is a unique characteristic of LR-MDS. Recent studies have found that in addition to the differential expression of apoptotic genes, a special inflammatory cell death process called pyroptosis may also contribute to apoptosis in LR-MDS (64). Reactive oxygen species produced by S100A9 and tumor necrosis factor receptor-associated factor 6 (TRAF6) can activate NLRP3 inflammasome, which eventually leads to the formation of pyroptosis in MDS patients and promotes hematopoietic failure of MDS (56). Inhibiting pyroptosis such as neutralizing S100A9, inhibiting NLRP3 and eliminating Caspase-1 have been shown to improve hematopoietic failure in MDS, providing new therapeutic prospects in MDS (65).

3.4.2 Toll-like receptor signaling

Toll-like receptor (TLR) gene encodes key promoters of innate immune signal and plays a core role in innate immune response. The study found that more than 50% of MDS patients had overexpression of TLR signaling pathway and downstream effector molecules, including TLR-1, TLR-2, TLR-4, TLR-6, TLR-7, TLR-9 and its downstream effector molecules such as MyD88 or IRAK1 and IRAK4 kinases (66, 67). The enhanced TLR signaling is particularly significant in LR-MDS and leads to increased apoptosis and ineffective hematopoietic of the disease (66). In 2013, the University of Texas MD Anderson Cancer Center detected the mRNA expression of eight TLRs (TLR1-4 and TLR6-9) in HSCs of MDS patients and found that the TLR of LR-MDS was significantly higher than that of HR-MDS, especially TLR2 and TLR4 which were associated with increased apoptosis and better OS rate (67). Except for TLR, the mRNA expression of MyD88, a downstream molecule of TLR signaling pathway, is also increased, especially in LR-MDS, and blocking MyD88 can lead to increased erythroid colony formation (68). In addition, the levels of TLR4 ligands S100A8 and S100A9 in BM and peripheral blood of MDS patients especially LR-MDS are also increased. As an endogenous damage related mode molecule (DAMP), S100A8 and S100A9 can enhance the production of inflammatory components and pro-inflammatory cytokines by binding with TLR4, so as promoting the ineffective hematopoiesis of LR-MDS (69). In conclusion, the above studies show that TLR signal enhancement is a significant feature of MDS, especially LR-MDS. On the contrary, because the immune microenvironment of HR-MDS is inhibitory (Yin), so although the activated TLR signal (Yang) also plays a certain role in the pathogenesis of HR-MDS, it may not be able to resist the whole immunosuppressive microenvironment.

4 Immunotherapy for LR-MDS

Tumor immunotherapy is known as the most promising ways to cure cancer. Immune dysregulation plays an important role in the pathogenesis of MDS, so immunotherapy should also be one of the most promising treatments for MDS patients, and has achieved good clinical efficacy.

The “Yin-Yang of immunity in LR-MDS” shows us that immune system of most LR-MDS is in an activated and pro-inflammatory state, which leads to the increase of apoptosis. We can regard these LR-MDS as a disease of “Yang excess”. Therefore, immunosuppressant and immunomodulatory treatment will be a reasonable treatment for the disease. From the perspective of “Yin-Yang theory” of traditional Chinese medicine, it is described as “damaging its excess Yang” and “enriching Yin and suppressing Yang”. But some LR-MDS may be the coexistence of immune hyperfunction and immune suppression, so immunosuppressant treatment may accelerate the progression of the disease. So we should closely monitor the changes of immune indicators for these patients and provide precise immunotherapy. Next, we will review current application and prospect of immunotherapy in LR-MDS (Figure 4).

4.1 Immunosuppressant

The immune system of LR-MDS patients is in an activated and pro-inflammatory state, so immunosuppressant can reverse these immune responses to achieve the treatment effect. In clinical practice, immunosuppressants have been successfully used in LR-MDS patients for many years, and have achieved good clinical efficacy and safety (70, 71). A recent study found

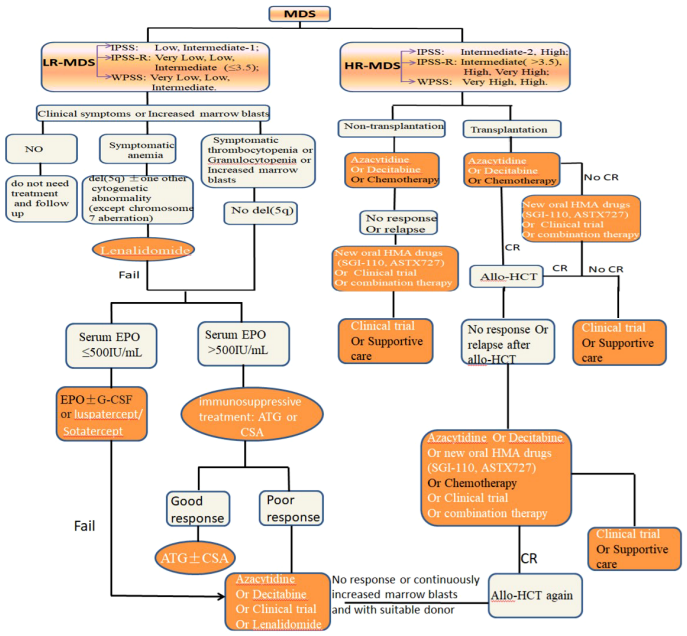

FIGURE 4
The standard treatment approach of MDS with different risk stratification, and immunotherapy is an important part (orange and white).

that the total effective rate of immunosuppressants in LR-MDS patients was 42.5%, of which the complete remission rate (CRR) was 12.5% and red blood cell transfusion independence rate was 33.4% (72). It was confirmed that immunosuppressants can successfully reduce the transfusion burden and related complications in LR-MDS patients. Antithymocyte globulin (ATG) and cyclosporin A (CSA) are the most commonly used immunosuppressants in the treatment of LR-MDS patients. ATG can reduce adaptive immunity to promote the recovery of hematopoietic function by consuming T cells and up-regulating Treg (70). CSA plays a role by inhibiting the expansion of cytotoxic T lymphocyte and inhibiting apoptosis related cytokines (71). When the ATG and CSA are combined, a long-term efficacy can be obtained (73). In addition, the use of immunosuppressants as the first-line treatment showed a better response rate than that as the third-line treatment (7). Immunosuppressants had no significant response in HR-MDS patients.

4.2 Monoclonal antibody

Some monoclonal antibodies have been proved to have therapeutic effect in LR-MDS because of their strong immunosuppressive effect, and Alemtuzumab is one of them. Alemtuzumab is a monoclonal antibody against CD52, which is mainly located on the surface of mature lymphocytes and weakens adaptive immunity by depleting lymphocytes. Therefore, Alemtuzumab can be used as an attractive alternative therapy for immunosuppressants. The latest study found that using Alemtuzumab to treat LR-MDS can achieve 68% hematological improvement or complete remission (CR), with a median remission period of 30 months (74).

Increased apoptosis is one of the characteristics of LR-MDS, so the treatment targeting for pro-apoptotic cytokines may be beneficial. TNF-α is an important pro-apoptotic cytokine in LR-MDS, so the monoclonal antibodies targeting for TNF-α such as Infliximab and Etanercept may improve prognosis in theory. However, the results of clinical trials showed that Infliximab alone had low activity and poor treatment response in LR-MDS (75). Another phase II clinical trial showed that the total effective rate of Etanercept combined with ATG in the treatment of LR-MDS was 56% (76). Anyways, the application of TNF-α inhibitors in LR-MDS patients has not achieved ideal results. But the TNF-α inhibitors have certain beneficial effects on BM inflammatory indexes, and the combination with other specific therapies may bring good news for LR-MDS patients.

TGF-β has been proved that it can participate in the occurrence of LR-MDS by promoting expression of pro-inflammatory genes, so inhibiting TGF-β is regarded as one of the potential treatments. Sotatercept (ACE-011) is a TGF-β ligand trap and has produced 49% of the total effective rate in a phase II clinical trial and can effectively improve anemia symptoms (77). Luspatecept (ACE-536) is new TGF-β Inhibitors. In a phase I/II clinical trial, 63% of LR-MDS patients has good therapeutic response and tolerability to ACE-536. Food and drug

administration (FDA) has approved it to treat adult LR-MDS patients (78). Using TGF-β inhibitors may be beneficial for these patients who have few available treatments.

4.3 Novel immune pathway inhibitors

As mentioned earlier, TLR signaling pathway plays an important role in LR-MDS and leads to ineffective hematopoietic of the disease. Therefore, the use of TLR signaling pathway inhibitory drugs can improve the prognosis of LR-MDS in theory. At present, many related drugs have being undergone preclinical trials. For example, the TLR2 inhibitor OPN-305 has passed the phase I trial of healthy subjects and is currently being tested in the phase I/II trial of MDS patients (NCT02363491) (79).

4.4 Immunomodulatory drugs

IMIDs represented by "Lenalidomide and Thalidomide" have been proved to be beneficial and safe in patients with low or medium risk, single 5q deletion (del (5q)) and transfusion dependent MDS (80). Thalidomide mainly stimulates T cells, monocytes and inhibits the pro-inflammatory cytokine expression such as TNF-α, IL-12, IL-1 and IL-6 to play an immunomodulatory role. Thalidomide can improve erythropoiesis and prolong the time of non-transfusion-dependence, but may have obvious neurotoxicity and other toxic and side effects for some LR-MDS patients (81). Therefore, Lenalidomide which was less toxic but more effect was synthesized. Lenalidomide not only has many similar immunomodulatory effects to Thalidomide, but also can induce ubiquitination of specific substrates, degrade casein kinase 1 alpha 1, selectively inhibit the del(5q) MDS cells and reverse the abnormality of karyotype (82). As early as 2006, Lenalidomide has been approved by FDA to treat anemia of low-risk or medium-risk MDS patients with transfusion-dependent and del(5q), whether with or without additional cytogenetic abnormalities.

Lenalidomide is effective to treat del (5q) MDS patients, but more and more evidence supports its sensitivity of Lenalidomide to non del (5q) LR-MDS. National Comprehensive Cancer Network (NCCN) has recognized that Lenalidomide has a certain clinical efficacy in patients with non del (5q) MDS after failure of erythropoiesis stimulating agent (ESA) treatment, with a response rate of 43%, and more than a quarter of patients have achieved blood transfusion independence (83). In addition, the latest research indicates that when Lenalidomide is used before Azacytidine, a higher rate of hematological improvement can be obtained for patients with non del (5q) MDS and fail ESA treatment (84).

4.5 Hypomethylating agent

As a specific DNA methyltransferase inhibitor, HMA can inhibit abnormal DNA methylation and have shown good curative effect for MDS patients in clinical trials and practical applications. However, in addition to the direct cytotoxicity and demethylation to cancer cells, HMA also has epigenetic regulation. HMA can promote the gene expression of anti-tumor immunity, enhance tumor immunogenicity, and stimulate a variety of immune cells including macrophages, NK cells and CD8+T cells to secrete cytokines to exert cytotoxic effects and promote tumor cells death (85). HMA can also induce autologous antitumor immune response by consuming MDSCs (86). Azacytidine can also inhibit Treg proliferation and produce a large amount of IL-17, thus playing an immunomodulatory role (87). All kinds of evidence show that HMA has the function of immune regulation by affecting epigenetic.

At present, HMA is a ideal treatment drugs for HR-MDS and AML, but based on its apparent immunomodulatory characteristics, it can also be a good choice for non del (5q) LR-MDS patients who do not respond to first-line and second-line treatment (88). In fact, HMA has been approved for the treatment of LR-MDS in the United States and Japan and got 60% hematological improvement. CC-486 is a new oral preparation of Azacytidine which produces an encouraging total effective rate of 38% in LR-MDS and has good tolerance and safety. The most common adverse events of CC-486 are neutropenia, anemia and gastrointestinal disorders (89). The authors think that LR-MDS patients who do not respond to first-line and second-line treatment may be "false LR-MDS", which is very likely to transform into HR-MDS. Therefore, HMA is effective for these patients.

In conclusion, immunosuppressive therapy and immunomodulatory therapy have a good effect to some LR-MDS patients, which can improve its hematological symptoms and morbid hematopoiesis. It accords with the activated immune microenvironment of LR-MDS. However, we should also recognize that nearly half of LR-MDS patients have poor or even ineffective effects on various immunosuppressive therapy and immunomodulatory therapy (74), while HMA drugs have a certain efficacy for these patients (90, 91). From a clinical point of view, we highly suspect that these LR-MDS tend to be "false LR-MDS", and their clinical characteristics and prognosis are more inclined to HR-MDS. It also suggests that there may also be immunosuppressive factors (Yin) in the activated BM immune microenvironment (Yang) of LR-MDS and immune activation therapy can also improve the prognosis of some LR-MDS patients, reflecting the "Yang contains Yin" and "Yang generates Yin" again.

5 Immunotherapy for HR-MDS

Different from the immune state of LR-MDS, the immune microenvironment of HR-MDS is in an inhibitory state, which makes CHIP dramatically expand and escape immune surveillance. Traditional Chinese medicine regards this kind of immune disease as "Yin excess and Yang deficiency disease", so immune activation therapy will be a reasonable treatment for HR-MDS. From the perspective of "Yin-Yang theory", it is "supplementing its deficiency" and "supporting Yang and suppressing Yin". But, there are also a few HR-MDS who may be the coexistence of immune hyperfunction and immune suppression, so immunosuppressant treatment may produce unexpected therapeutic effects for these patients. Next, we will review the current application and prospect of immunotherapy in HR-MDS (Figure 4).

5.1 HMA

From the above, we can know that HMA has immunomodulatory effect and is the main drug of the first-line treatment for HR-MDS patients. The initial response of HMA in the treatment of HR-MDS is good, but 40% of patients will become resistant to Decitabine and Azacytidine. So, there is an urgent need to explore new HMA drugs to reduce resistance. Guadecitabine (SGI-110) is a new type of HMA. The phase II clinical trial of HR-MDS patients with Azacytidine resistance showed that objective response rate was 14.3%, and the survival time of responders was significantly improved (NCT02197676) (90). ASTX727 is also a new oral HMA, which was approved by FDA in 2020 for new and secondary MDS with specific FAB subtypes (RA, MDS-RARS, MDS-RAEB and CMML) and IPSS score (middle-1, middle-2 and high risk). The studies have confirmed that oral ASTX727 and intravenous infusion of Decitabine have similar area under the concentration-time curve, safety, clinical response and lower drug resistance (91).

The mechanism of HMA resistance in MDS patients has not been fully clarified, but the up-regulated expression of immune checkpoint molecules may play a certain role. While enhancing the anti-tumor immune response, HMA also up-regulates the expression of immune checkpoint molecules, inhibits and even depletes the tumor specific T cells, resulting in tumor immune escape (61). Therefore, it seems very interesting to evaluate the efficacy of HMA combined with immune checkpoint inhibitors in HR-MDS patients, as shown below.

5.2 Immune checkpoint inhibitor

As we all know, immune escape is an important feature of HR-MDS, and one of the main mechanisms is the up-regulation of immune checkpoint molecules in BM microenvironment. Therefore, immune checkpoint inhibitor is a reasonable treatment strategy for HR-MDS. CTLA-4 and PD-1 inhibitors have been shown to play a role in HR-MDS by blocking the inhibitory signals on T cells and stimulating antitumor immune response (92–97). In addition, some new immune checkpoint inhibitors such as Tim-3 and CD47 inhibitors are also actively being conducted in various clinical trials.

5.2.1 PD-1/PD-L1 inhibitors

The expressions of PD-1, PD-L1 and PD-L2 were significantly increased in MDS patients, and were related to the risk stratification and the drug resistance mechanism of HMA (24). Therefore, PD-L1/PD-1 is an ideal therapeutic target for HR-MDS patients, and PD-L1/PD-1 inhibitors may be potential drugs for recurrent and refractory MDS. Pembrolizumab is a humanized anti-PD-1 monoclonal antibody (98). The phase 1b clinical trial show that the OS of Pembrolizumab in MDS patients with HMA refractory is 6.0 months, the 2-year total OS rate is 17% and has controllable safety and clinical activity (NCT01953692) (92). Single PD-1 inhibitors have certain efficacy in the treatment of HR-MDS patients failed by HMA, but the effect is limited. Therefore, many scholars put forward a new viewpoint of "PD-1 inhibitors combined with HMA". The latest phase 2 clinical trial showed that the objective remission rate (ORR) of Azacytidine combined with Pembrolizumab in the treatment of newly diagnosed HR-MDS patients was 76%, the CR rate was 18%. The ORR rate of Azacytidine combined with Pembrolizumab to HR- MDS patients who failed in the treatment of HMA was 25%, and the CR rate was 5% (93). In addition, a phase 1 study found that nivolumab, a PD-1 inhibitor, had good curative effect for relapsed HR-MDS after allogeneic transplantation (99). PD-1 inhibitor combined with HMA has a certain effect in HR-MDS patients, which makes some researchers focus on "PD-L1 inhibitor combined with HMA". In 2022, the latest clinical trial published on "Blood" found that the ORR of PD-L1 inhibitor Durmalumab combined with Azacytidine in the treatment of HR-MDS was 61.5%, the ORR of Azacytidine alone was 47. 6%, but Durmalumab combined with Azacytidine had more toxic than Azacytidine alone (NCT02775903) (94). Atezolizumab is a new type of PL-L1 inhibitor and Guadecitabine (SGI-110) is a new type of HMA. In the phase 2 clinical trial, the combination of them in the treatment of relapsed refractory HR-MDS can get 33% ORR and prolong the survival for some HR-MDS patients (NCT02197676) (90). In general, these clinical trials show that the PD-1/PD-L1 inhibitor may have better antitumor activity and safety in some patients. The curative effect is more obvious in primary HR-MDS patients, and it also has a certain curative effect in patients failed by HMA, which is worthy of further research. In addition, many clinical trials of PD-L1/PD-1 inhibitors in the treatment of HR-MDS are in progress (Table 2) (90, 93, 94, 99, 100), which bring hope to patients.

5.2.2 CTLA-4 inhibitor

As mentioned earlier, MDS cells have been shown to overexpress CTLA-4 which is associated with poor prognosis. Therefore, inhibiting CTLA-4 is also one of the potential treatments for HR-MDS. Lpilimumab is a humanized monoclonal antibody against CTLA-4 (101). A phase 1b clinical trial showed that Ipilimumab alone to treat HR-MDS patients failed by HMA can get 21% clinical benefit rate (clinical benefit rate is defined as disease stable condition for more than 12 months) and increase the number of effector T cells (95).

TABLE 2 Clinical trials of immune checkpoint inhibitor in MDS.

ImmuneCheckpoint	Drug	Phase	Status	YearReported	IPSS Risk Category	Outcomes	Conclusion	Clinical Trial Identifier
PD-1	Pembrolizumab	Ib	Completed	2016	Int-1 and Int-2 and High;HMA failure:28	ORR=14-25% (1PR)	Manageable safety profile and potential activity	NCT01953692
	Pembrolizumab +AZA	II	Recruiting	2019	Int-1 andInt-2 andHigh;HMA failue:20, MDSfrontline:10	HMA failue: ORR=30%, MDSfrontline: ORR = 70%	Relatively safe and well-tolerated, mayhave antitumor activity	NCT03094637
	Nivolumab	I/Ib	Active not recruiting	2020	High; post-HSCT relapse of MDS:7	ORR=43%	Moderate antitumor activity but severe GVHD and irAEs	NCT01822509
	Nivolumab +AZA	II	Completed	2018	Int-1 and Int-2 and High;MDS frontline:20	ORR=75% (CR/ CRp=50%)	Manageable safety profile and potential activity	NCT02530463
PD-L1	Durvalumab +AZA	II	Completed	2022	High;42 MDS	ORR = 61. 9%	No significant difference in safety and efficacy	NCT02775903
	Atezolizumab +Guadecitabine	I/II	Active, not recruiting	2018	Int-1 and Int-2and High;R/R MDS:9	ORR=33% (HI=22%, CR=11%)	Had an acceptable toxicity profile	NCT02935361
CTLA-4	Ipilimumab	I/Ib	Completed	2018	Int-1 and Int-2and High;HMA failue:29	ORR=7%	Safe but had limited efficacy as a monotherapy	NCT01757639
	Ipilimumab	II	Completed	2018	Int-1 and Int-2 andHigh;HMA failue:20	ORR=35%	Had limited efficacy as a monotherapy	NCT02530463
	Ipilimumab +AZA	II	Completed	2018	Int-1 and Int-2 and High;MDS frontline:21	ORR=71% (CR/ CRp=38%)	Manageable safety profile and potential activity	NCT02530463
CTLA-4+PD-1	Ipilimumab+ Nivolumab	II	Recruiting	2018	Int-1 and Int-2 and High;HMA failue:8	ORR = 29%	Clinical activity could be seen in R/ R MDS	NCT02530463
	Ipilimumab +Nivolumab +AZA	II	On Hold	2018	Int-1 and Int-2 and High;MDS frontline:6	ORR=50%(3 CR)	Had a better efficacy in frontline MDS	NCT02530463
TIM-3	MBG453+DEC	I/Ib	Recruiting	2020	High;MDS frontline:19	ORR=58%	Hada better efficacy andmanageable safety profile	NCT03066648
	MBG453 + AZA	I/Ib	Recruiting	2020	High;MDS frontline:13	ORR=70%	Had better efficacy and manageable safety profile	NCT03066648
CD-47	Magrolimab	Ib	Recruiting	Not Reported	Int-1 and Int-2 andHigh;R/R MDS:4	Not Reported	Not Reported	NCT03248479
	Magrolimab+ AZA	Ib	Recruiting	2020	Int-1 and Int-2 andHigh;MDS frontline:39	ORR= 91%	Had better efficacy and manageable safety profile	NCT03248479
	TTI-621 (SIRPαFc)	I	Recruiting	Not Reported	Not Reported	Not Reported	Not Reported	NCT02663518

ORR, overall reponse rate; Int-1, Intermediate-1; Int-2, Intermediate-2; CR, complete response; PR, partial response; CRp, complete remission with incomplete platelet recovery; CR/CRp, complete remission or complete remission with incomplete platelet recovery; AZA, Azacitidine; DEC, Decitabine; irAEs, immune-related adverse events.

Subsequently, Garcia Manero et al. reported the results of phase II clinical trials of Ipilimumab to treat patients failed by HMA and newly treated HR-MDS. It was found that Ipilimumab alone for HR-MDS patients failed by HMA can get an ORR of 35% and the combination of Ipilimumab and Azacytidine for newly treated HR-MDS patients can get an ORR of 71% (96). Above clinical trials show that the efficacy of Ipilimumab alone is limited, and the combination of HMA and Ipilimumab is better, but it still needs further research.

5.2.3 Anti-Tim-3 monoclonal antibody

Tim-3 is a newly discovered negative molecule of immune regulation in recent years. The study found that the ligand of Tim-3 is preferentially overexpressed on leukemia and MDS HSCs compared with normal HSCs (58). This discovery eventually led to the production of anti-TIM-3 monoclonal antibody, and Tim-3 has become a possible new therapy way for HR-MDS. MBG453 is a new anti-Tim-3 monoclonal antibody. The results of phase 1 clinical trial showed that MBG453 combination with Decitabine has achieved 50% CR and molecular CR (MCR) in HR-MDS patients (97). Another phase 1b clinical trial conducted by Brunner et al. has reached similar conclusions (102). In addition, the immune related adverse events of MBG453 in the above clinical trials were low, and only one patient had elevated liver enzymes (grade 3). More relevant clinical trials are also in progress, as shown in Table 2.

5.2.4 Anti-CD47 monoclonal antibody

CD47 is significantly overexpressed in MDS patients and can combine with the receptor SIRP-α to prevent macrophages from phagocytizing MDS cells (103). Therefore, CD47 has also become a new target for HR-MDS, and anti-CD47 monoclonal antibodies such as Magrolimab, TTI-621 and CC-90002 were finally introduced into the treatment. Magrolimab, also known as Hu5F9-G4, is a humanized anti-CD47 monoclonal antibody. A phase 1b trial of Magrolimab combined with Azacytidine in the treatment of HR-MDS reported 54% CR with good tolerance and safety (104). TTI-621, also known as SIRPα-IgG1 FC, is a unique SIRPαFc decoy receptor, which can target CD47 and block its activity and has been shown to be effective against recurrent or refractory hematological malignancies (105). A clinical trial to evaluate the efficacy of TTI-621 in the treatment of HR-MDS has entered phase I (NCT02663518) (105). CC-90002 is another humanized anti-CD47 monoclonal antibody. The results of phase 1 clinical trial in relapsed or refractory HR-MDS showed that it had poor efficacy and serious treatment-related side effects, which eventually led to the cessation of relevant clinical trials (106).

TIGIT, LAG3 and other immunosuppressive factors are also highly expressed in HR-MDS patients and the potential immunotherapeutic targets of HR-MDS in theory. However, there is no clinical trial using relevant monoclonal antibody now. In conclusion, the current use of immune checkpoint inhibitor in the treatment of MDS is still in its early stage, and more clinical trials are still needed to evaluate the safety, efficacy, optimal timing and potential combination therapy methods (107).

5.3 Adoptive T-cell transfer therapy

Adoptive immunotherapy is an important part of tumor immunotherapy, and adoptive T-cell transfer therapy is one of the important components. The three adoptive T-cell transfer therapy represented by chimeric antigen receptor T (CAR-T) cell therapy, Tumor infiltrating lymphocytes (TIL) therapy and T cell receptor T cells (TCR-T) therapy provide a possibility of "theoretical curative" for hematological tumors. Current studies have confirmed that T-cell transfer therapy has a good effect for lymphoma, but there are few studies on myeloid malignancies.

CAR-T cell therapy is the most common and hot adoptive T-cell transfer therapy. The clinical trials have preliminarily explored its role in MDS and achieved promising results. Finding suitable target antigen is the most critical link in CAR-T cell therapy. Natural killer type 2 receptor (NKG2D) is a positive immunomodulatory protein on NK and CD8+T cells, and is one of the ideal target antigens for CAR-T cell therapy in the treatment of HR-MDS. The phase I clinical trial of NKG2D-CAR-T cell therapy for HR-MDS is in progress (NCT02203825) and preliminarily report transient hematological improvement *in vitro* treatment, but further research is still needed (108). In 2019, Steven et al. found that the cell surface antigen CD123 was overexpressed on MDS stem cells and was related to the MDS risk stratification. So they proposed that CD123 could be used as one of the targets of CAR-T cell therapy for HR-MDS patients. It was subsequently confirmed that CD123 CAR-T cells could root out CD123+MDS stem cells *in vitro* (109). In addition, early preclinical data showed that CD123 CAR-T cell therapy can eliminate abnormal clones of MDS in derived xenotransplantation model (110).

5.4 DC-targeted immunotherapy

The ability to stimulate T cells and antigen presentation in MDS patients is significantly reduced, which makes DC cells become the new target of immunotherapy. The anti-tumor immunotherapy targeting DC cells is DC vaccine. Monocyte-Derived DC (mo DC) and leukemia derived DC (DCleu) are the main DC vaccines used for AML and MDS so far. Studies have confirmed that the Mo DC vaccine loaded with leukemia associated antigen (LAA) can effectively promote the apoptosis of HR-MDS and AML cells, and may produce good results for these patients (111). Christian

et al. found that Mo DC/DCleu vaccine can activate the innate and adaptive immune system especially leukemia specific T cells, and enhance the killing effect on leukemia cells (112). In conclusion, treatment based on DC/mo-DCC/DCleu may be a promising way for HR-MDS patients, but continuous research including animal and human trials must be carried out.

5.5 NK cell adoptive transfer therapy

With the success of adoptive cell immunotherapy in tumor immunotherapy, NK cells have attracted more and more attention in cell adoptive therapy because they do not need pre-sensitization and will not lead to graft versus host disease. Then NK cell adoptive transfer therapy appears. NK cell adoptive transfer therapy is a new research field, which has shown a certain effect in AML and HR-MDS. *In vitro* experimental studies found that NK cells whether from umbilical cord blood, paired donors or autologous collection can expand in the presence of K562 leukemia cell line and produce strong tumor cell killing effect (113). The *in vivo* experiments in 16 patients with recurrent refractory MDS or AML also found that NK cell adoptive transfer therapy had good efficacy and tolerance, which further proved that HR-MDS would respond to adoptive transfer therapy, and supported the NK cell infusion as a bridge treatment before HSCT in refractory MDS or AML (114). Although it is confirmed that targeting NK cells may be a potential and effective therapy for HR-MDS patients, there are few relevant studies on NK cell adoptive transfer therapy, and further research is still needed.

5.6 Vaccine treatment

Wilms' tumor 1(WT1) is a tumor suppressor gene located on chromosome 11p13. The immunotherapy targeting WT1 has been proved to induce the immune system to produce memory T cells and effector T cells which has the functions of immune monitoring and immune killing. Studies have confirmed that WT1 is overexpressed in CD34+MDS/AML stem cells and is associated with a higher blast cell counts and a lower OS (115). Therefore, WT1 can be used as the first target antigen for vaccine treatment for HR- MDS. The phase I clinical trial conducted by Tawara et al. found that WT1-specific T-cell receptor gene-transduced lymphocytes had some safety and persistence in the treatment of AML and HR-MDS. All patients had clonal expansion of WT1-specific T-cell (mainly CD8+T cells) at different degrees, of which 5/8 patients continuously had WT1-specific T-cell (UMIN000011519) (116). WT4869 is a synthetic peptide vaccine. Suzuki et al. evaluated the safety and efficacy of WT4869 in 25 HR-MDA/AML patients. WT1-specific T-cell was observed in 11 patients, and the median OS reached to 55.71 weeks (CTI-101374) (117).

Another tumor vaccine target in HR-MDS is PR1 peptide, which is an HLA-A2-restricted peptide targeting myeloid tumor cells. It can be recognized by CTL, and then forms PR1 specific CTL (PR1-CTL) to mediate the specific lysis of AML and HR-MDS cells. Muzaffar et al. had studied the efficacy and tolerance of PR1 peptide vaccine in HR-MDS patients. The results showed that PR1 peptide vaccine can induce specific immunity reaction and related clinical reactions in MDS patients including molecular remission and no adverse autoimmunity symptoms, which finally lead to the increase of PR1-CTL in circulating (118). In addition, in order to evaluate the effect of the combined application of PR1 and WT1 peptide vaccine in HR-MDS, 8 patients were vaccinated with both PR1 and WT1 peptide vaccines at the same time. The results showed that the number of PR1 and WT1 specific T cells increased, and stable disease lasted for more than 2 years (NCT00499772) (119).

NY-ESO-1 is another antigen with high immunogenicity and expressed in a variety of tumors. It is also another candidate target antigen in MDS and AML vaccine trials. The latest study used HLA-unrestricted NY-ESO-1 vaccine combined with Decitabine to treat HR-MDS patients, and all patients showed NY-ESO-1 gene expression and induced NY-ESO-1 specific CD4+ and CD8+T cells (120). In conclusion, the current studies suggest that tumor vaccine has a certain efficacy in HR-MDS patients, but there are few relevant clinical studies. Future studies should further expand related clinical studies and explore the efficacy and safety of vaccines combined with HMA or immune checkpoint inhibitors in the treatment of HR-MDS, so as to induce deeper and more lasting clinical response.

In summary, the immunotherapy principle of HR-MDS is to actively find various targets to restore and enhance the number and function of anti-tumor immune cells and cytokines (Yang) in the BM immunosuppressive microenvironment (Yin). The current results of studies confirm that immune activation therapy does have a certain effect on some HR-MDS and can improve its prognosis. It is further confirmed that immunosuppressive microenvironment (Yin) of HR-MDS also contains the immunocompetent cells and can promote its proliferation (Yang) under certain conditions (such as immune activation therapy), that is the "Yin contains Yang" and "Yin generates Yang".

6 Conclusions and perspectives

In the past decade, we have deeply recognized that (1): There is a close relationship between AD and MDS, and the immune dysregulation may be the common driving force for AD and MDS (2); Immune dysregulation are complex and heterogeneous in the occurrence and development of MDS. LR-MDS is immune hyperfunction and increased apoptosis, HR-MDS is immune suppression and immune escape, while some MDS change dynamically which is characterized by the coexistence and mutual transformation of immune hyperfunction and immune

suppression (3); Immune dysregulation of MDS with different risk stratification can be summarized by an advanced philosophical thought "Yin-Yang theory" in ancient China, meaning that LR-MDS and HR-MDS are opposite to each other, have a balance of waning and waxing, depend on each other and may transform into its opposite side under given conditions; (4) Immunotherapy strategy has become one of the hotspots in the treatment of MDS in recent years, and have achieved good results in clinic. The present research difficulties and challenges are that (1) The internal mechanism and mutual influence of the close relationship between AD and MDS are not yet fully clear; (2) How to identify these MDS patients who may be transformed and the concrete time and condition of transformation in the process of clinical diagnosis and treatment is important; (3) Whether these MDS patients who may be transformed can receive immunotherapy and what kind of immunotherapy they should receive, how to monitor the relevant immune changes in the process of treatment, and how to adjust the immunotherapy plan in time; (4) In addition, immunotherapy can only be said to be a promising potential treatment at present because immune dysregulation of MDS have great heterogeneity, so how to explore more reasonable combination therapy to improve the clinical response rate and OS of MDS patients is very important.

Taken together, we think that a better understanding of the mechanisms and manifestations of immune dysregulation in MDS with different risk stratification can help us to provide a new breakthrough in the area of MDS immunotherapy, and more importantly that it will provide a scientific rationale for clinical trials. Future research should further explore the immune dysregulation status of MDS with different risk stratification, and actively explore the new combination therapy ways such as immunotherapy combined with HMAs, hoping to bring new hope to patients who fail the standard therapy of MDS.

Author contributions

XP, LZ, and LL designed the paper and recommended a structure for the review. XP, LZ, LL, XZ and TD wrote the initial draft and prepared figures. FT, YLiu, XG, JB and YLi helped to revise the manuscript. All authors contributed to the article and approved the submitted version.

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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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References

1. Wang D, Qu M. Homeostasis research model based on yin-yang theory: Five examples. *Chin J Integr Med* (2021) 27(6):403–07. doi: 10.1007/s11655-021-2861-4

2. Ye X, Dong MH. A review on different English versions of an ancient classic of Chinese medicine: Huang di nei jing. *J Integr Med* (2017) 15(1):11–8. doi: 10.1016/S2095-4964(17)60310-8

3. Godson C, Perretti M. Novel pathways in the yin-yang of immunomodulation. *Curr Opin Pharmacol* (2013) 13(4):543–6. doi: 10.1016/j.coph.2013.06.010

4. Glenthoj A, Orskov AD, Hansen JW, Hadrup SR, O'Connell C, Gronbaek K. Immune mechanisms in myelodysplastic syndrome. *Int J Mol Sci* (2016) 17(6):944–56. doi: 10.3390/ijms17060944

5. Ganan-Gomez I, Wei Y, Starczynowski DT, Colla S, Yang H, Cabrero-Calvo M, et al. Deregulation of innate immune and inflammatory signaling in myelodysplastic syndromes. *Leukemia* (2015) 29(7):1458–69. doi: 10.1038/leu.2015.69

6. Fozza C, Murtas A, Caocci G, La Nasa G. Autoimmune disorders associated with myelodysplastic syndromes: clinical, prognostic and therapeutic implications. *Leuk Res* (2022) 117:106856. doi: 10.1016/j.leukres.2022.106856

7. Wang C, Yang Y, Gao S, Chen J, Yu J, Zhang H, et al. Immune dysregulation in myelodysplastic syndrome: Clinical features, pathogenesis and therapeutic strategies. *Crit Rev Oncol Hematol* (2018) 122:123–32. doi: 10.1016/j.critrevonc.2017.12.013

8. Komrokji RS, Kulasekararaj A, Al Ali NH, Kordasti S, Bart-Smith E, Craig BM, et al. Autoimmune diseases and myelodysplastic syndromes. *Am J Hematol* (2016) 91(5):E280–3. doi: 10.1002/ajh.24333

9. Jiang D, Meng F. Clinical features and prognosis of myelodysplastic syndrome complicated with autoimmune diseases. *Adv Clin Med* (2021) 11(4):9. doi: 10.12677/ACM.2021.11425 9

10. Xiao N, He X, Niu H, Yu H, Cui N, Li H, et al. Increased circulating CD4(+) CXCR5(+) cells and IgG4 levels in patients with myelodysplastic syndrome with autoimmune diseases. *J Immunol Res* (2021) 2021:4302515. doi: 10.1155/2021/4302515

11. Seguier J, Gelsi-Boyer V, Ebbo M, Hamidou Z, Charbonnier A, Bernit E, et al. Autoimmune diseases in myelodysplastic syndrome favors patients survival: A case control study and literature review. *Autoimmun Rev* (2019) 18(1):36–42. doi: 10.1016/j.autrev.2018.07.009

12. Montoro J, Gallur L, Merchan B, Molero A, Roldan E, Martinez-Valle F, et al. Autoimmune disorders are common in myelodysplastic syndrome patients and confer an adverse impact on outcomes. *Ann Hematol* (2018) 97(8):1349–56. doi: 10.1007/s00277-018-3302-0

13. Mekinian A, Grignano E, Braun T, Decaux O, Liozon E, Costedoat-Chalumeau N, et al. Systemic inflammatory and autoimmune manifestations associated with myelodysplastic syndromes and chronic myelomonocytic leukaemia: a French multicentre retrospective study. *Rheumatol (Oxf)* (2016) 55 (2):291–300. doi: 10.1093/rheumatology/kev294

14. Lee SJ, Park JK, Lee EY, Joo SH, Jung KC, Lee EB, et al. Certain autoimmune manifestations are associated with distinctive karyotypes and outcomes in patients with myelodysplastic syndrome: A retrospective cohort study. *Med (Baltimore)* (2016) 95(13):e3091. doi: 10.1097/MD.0000000000003091

15. Banerjee T, Calvi LM, Becker MW, Liesveld JL. Flaming and fanning: The spectrum of inflammatory influences in myelodysplastic syndromes. *Blood Rev* (2019) 36:57–69. doi: 10.1016/j.blre.2019.04.004

16. Pardanani A, Finke C, Lasho TL, Al-Kali A, Begna KH, Hanson CA, et al. IPSS-independent prognostic value of plasma CXCL10, IL-7 and IL-6 levels in myelodysplastic syndromes. *Leukemia* (2012) 26(4):693–9. doi: 10.1038/leu.2011.251

17. Kordasti SY, Afzali B, Lim Z, Ingram W, Hayden J, Barber L, et al. IL-17-producing CD4(+) T cells, pro-inflammatory cytokines and apoptosis are increased in low risk myelodysplastic syndrome. *Br J Haematol* (2009) 145(1):64–72. doi: 10.1111/j.1365-2141.2009.07593.x

18. Aref S, Khaled N, Al Gilany AH, Ayed M, Abouzeid T, Attia D. Impact of bone marrow natural killer cells (NK); soluble TNF-α and IL-32 levels in myelodysplastic syndrome patients. *Asian Pacific J Cancer Prev APJCP* (2020) 21 (10):2949–53. doi: 10.31557/APJCP.2020.21.10.2949

19. Teodorescu P, Pasca S, Dima D, Tomuleasa C, Ghiaur G. Targeting the microenvironment in MDS: The final frontier. *Front Pharmacol* (2020) 11:1044. doi: 10.3389/fphar.2020.01044

20. Meisel M, Hinterleitner R, Pacis A, Chen L, Earley ZM, Mayassi T, et al. Microbial signals drive pre-leukaemic myeloproliferation in a Tet2-deficient host. *Nature* (2018) 557(7706):580–84. doi: 10.1038/s41586-018-0125-z

21. Sanjabi S, Zenewicz LA, Kamanaka M, Flavell RA. Anti-inflammatory and pro-inflammatory roles of TGF-beta, IL-10, and IL-22 in immunity and autoimmunity. *Curr Opin Pharmacol* (2009) 9(4):447–53. doi: 10.1016/j.coph.2009.04.008

22. Muench DE, Ferchen K, Velu CS, Pradhan K, ChEtal K, Chen X, et al. SKI controls MDS-associated chronic TGF-β signaling, aberrant splicing, and stem cell fitness. *Blood* (2018) 132(21):e24–34. doi: 10.1182/blood-2018-06-860890

23. Zheng Z, Qianqiao Z, Qi H, Feng X, Chunkang C, Xiao L. *In vitro* deprivation of CD8(+)CD57(+)T cells promotes the malignant growth of bone marrow colony cells in patients with lower-risk myelodysplastic syndrome. *Exp Hematol* (2010) 38(8):677–84. doi: 10.1016/j.exphem.2010.04.002

24. Yang H, Bueso-Ramos C, DiNardo C, Estecio MR, Davanlou M, Geng QR, et al. Expression of PD-L1, PD-L2, PD-1 and CTLA4 in myelodysplastic syndromes is enhanced by treatment with hypomethylating agents. *Leukemia* (2014) 28 (6):1280–8. doi: 10.1038/leu.2013.355

25. Giovazzino A, Leone S, Rubino V, Palatucci AT, Cerciello G, Alfinito F, et al. Reduced regulatory T cells (Treg) in bone marrow preferentially associate with the expansion of cytotoxic T lymphocytes in low risk MDS patients. *Br J Haematol* (2019) 185(2):357–60. doi: 10.1111/bjh.15496

26. Leone P, Solimando AG, Malerba E, Fasano R, Buonavoglia A, Pappagallo F, et al. Actors on the scene: Immune cells in the myeloma niche. *Front Oncol* (2020) 10:599098. doi: 10.3389/fonc.2020.599098

27. Zhang Z, Li X, Guo J, Xu F, He Q, Zhao Y, et al. Interleukin-17 enhances the production of interferon-γ and tumour necrosis factor-α by bone marrow T lymphocytes from patients with lower risk myelodysplastic syndromes. *Eur J haematology* (2013) 90(5):375–84. doi: 10.1111/ejh.12074

28. Li L, Zhang L, Cai Y, Zeng P, Wu C, Yue L. Thalidomide combined with cyclosporine a and prednisone in the treatment of lower-risk myelodysplastic syndrome and its effect on the levels of Th17 and Th22 cells. *J Lanzhou Univ (Medical Sciences)* (2015) 41:55–60. doi: 10.13885/j.issn.1000-2812.2015.02.011

29. Shao LL, Zhang L, Hou Y, Yu S, Liu XG, Huang XY, et al. Th22 cells as well as Th17 cells expand differentially in patients with early-stage and late-stage myelodysplastic syndrome. *PloS One* (2012) 7(12):e51339. doi: 10.1371/journal.pone.0051339

30. Han X, Han L, Zhang Y. Changes and significance of Th22 cells in peripheral blood of myelodysplastic syndrome patients. *Chin J Immunol* (2013) 29(1):4. doi: 10.3969/j.issn.1000-484X. 2013.01.012

31. Xu SJ, Shao ZH, Fu R, Wang HQ, Liu H, Liu CY, et al. Subtype and functional biomarker changes of NK cells in peripheral blood of patients with myelodysplastic syndrome. *Zhongguo shi yan xue ye xue za zhi* (2017) 25(3):832–36. doi: 10.7534/j.issn.1009-2137.2017.03.036

32. Carlsten M, Järås M. Natural killer cells in myeloid malignancies: Immune surveillance, NK cell dysfunction, and pharmacological opportunities to bolster the endogenous NK cells. *Front Immunol* (2019) 10:2357. doi: 10.3389/fimmu.2019.02357

33. Daher MSC, Sobieski C, Marin D, Sekine T, Shpall E, Champlin R, et al. Association between KIR genes and risk of MDS. *J Clin Oncol Off J Am Soc Clin Oncol* (2015) 33(15_suppl):7001. doi: 10.1200/jco.2015.33.15_suppl.7001

34. Stringaris K, Marin D, Barrett AJ, Hills R, Sobieski C, Cao K, et al. KIR gene haplotype: an independent predictor of clinical outcome in MDS patients. *Blood* (2016) 128(24):2819–23. doi: 10.1182/blood-2016-05-713099

35. Saft L, Björklund E, Berg E, Hellström-Lindberg E, Porwit A. Bone marrow dendritic cells are reduced in patients with high-risk myelodysplastic syndromes. *Leuk Res* (2013) 37(3):266–73. doi: 10.1016/j.leukres.2012.10.010

36. Kerkhoff N, Bontkes HJ, Westers TM, de Gruijl TD, Kordasti S, van de Loosdrecht AA. Dendritic cells in myelodysplastic syndromes: from pathogenesis to immunotherapy. *Immunotherapy* (2013) 5(6):621–37. doi: 10.2217/imt.13.51

37. Chan A, Liu Y, Devlin S, Gao Q, Baik J, Sigler A, et al. Reduced plasmacytoid dendritic cell output is associated with high risk in low-grade myelodysplastic syndrome. *HemaSphere* (2022) 6(2):e685. doi: 10.1097/HS9.0000000000000685

38. Liu Y, Li L, Zhang L. Immune abnormalities and immunotherapy of dendritic cells in myelodysplastic syndrome–-Present state and prospects. *Clin Focus* (2016) 31(5):5. doi: 10.3969/j.issn.1004-583X.2016.05.026

39. Pang YB, Li WW, Luo JM, Ji J, Du X. [Senescent mesenchymal stem cells contribute to progression of myelodysplastic syndromes-review]. *Zhongguo shi yan xue ye xue za zhi* (2018) 26(3):942–46. doi: 10.7534/j.issn.1009-2137.2018.03.053

40. Zhao Z, Wang Z, Li Q, Li W, You Y, Zou P. The different immunoregulatory functions of mesenchymal stem cells in patients with low-risk or high-risk myelodysplastic syndromes. *PloS One* (2012) 7(9):e45675. doi: 10.1371/journal.pone.0045675

41. Wang Z, Tang X, Xu W, Cao Z, Sun L, Li W, et al. The different immunoregulatory functions on dendritic cells between mesenchymal stem cells derived from bone marrow of patients with low-risk or high-risk myelodysplastic syndromes. *PloS One* (2013) 8(3):e57470. doi: 10.1371/journal.pone.0057470

42. Itzykson R, Fenaux P. Epigenetics of myelodysplastic syndromes. *Leukemia* (2014) 28(3):497–506. doi: 10.1038/leu.2013.343

43. Davies LC, Heldring N, Kadri N, Le Blanc K. Mesenchymal stromal cell secretion of programmed death-1 ligands regulates T cell mediated immunosuppression. *Stem Cells (Dayton Ohio)* (2017) 35(3):766–76. doi: 10.1002/stem.2509

44. Jiang HJ, Fu R, Wang HQ, Li LJ, Qu W, Liang Y, et al. Increased circulating of myeloid-derived suppressor cells in myelodysplastic syndrome. *Chin Med J* (2013) 126(13):2582–4. doi: 10.3760/cma.j.issn.0366-6999.20121794

45. Kittang AO, Kordasti S, Sand KE, Costantini B, Kramer AM, Perezabellan P, et al. Expansion of myeloid derived suppressor cells correlates with number of T regulatory cells and disease progression in myelodysplastic syndrome. *Oncoimmunology* (2016) 5(2):e1062208. doi: 10.1080/2162402X.2015.1062208

46. Sarhan D, Brandt L, Felices M, Guldevall K, Lenvik T, Hinderlie P, et al. 161533 TriKE stimulates NK-cell function to overcome myeloid-derived suppressor cells in MDS. *Blood Adv* (2018) 2(12):1459–69. doi: 10.1182/bloodadvances.2017012369

47. Song D, Chang C, Guo J. Abnormal percentage of bone marrow macrophages in patients with myelodysplastic syndrome and its significance. *J Diagnostics Concepts Pract* (2018) 17(4):5. doi: 10.1016/s0145-2126(98)00037-x

48. Schneider RK, Schenone M, Ferreira MV, Kramann R, Joyce CE, Hartigan C, et al. Rps14 haploinsufficiency causes a block in erythroid differentiation mediated by S100A8 and S100A9. *Nat Med* (2016) 22(3):288–97. doi: 10.1038/nm.4047

49. Song J, Ouyang H, Dian Z. Expression of tumor associated macrophages in patients with myelodysplastic syndrome. *Chongqing Med* (2017) 46(2):3. doi: 10.3969/j.issn.1671-8348.2017.02.025

50. Iwata M, Pillai M, Ramakrishnan A, Hackman RC, Deeg HJ, Opdenakker G, et al. Reduced expression of inducible gelatinase b/matrix metalloproteinase-9 in monocytes from patients with myelodysplastic syndrome: Correlation of inducible levels with the percentage of cytogenetically marked cells and with marrow cellularity. *Blood* (2007) 109(1):85–92. doi: 10.1182/blood-2006-05-020289

51. Youn M, Huang H, Chen C, Kam S, Wilkes MC, Chae HD, et al. MMP9 inhibition increases erythropoiesis in RPS14-deficient del(5q) MDS models through suppression of TGF-β pathways. *Blood Adv* (2019) 3(18):2751–63. doi: 10.1182/bloodadvances.2019000537

52. Cao M, Shikama Y, Kimura H, Noji H, Ikeda K, Ono T, et al. Mechanisms of impaired neutrophil migration by MicroRNAs in myelodysplastic syndromes. *J Immunol (Baltimore Md 1950)* (2017) 198(5):1887–99. doi: 10.4049/jimmunol.1600622

53. Cheng P, Eksioglu EA, Chen X, Kandell W, Le Trinh T, Cen L, et al. S100A9-induced overexpression of PD-1/PD-L1 contributes to ineffective hematopoiesis in myelodysplastic syndromes. *Leukemia* (2019) 33(8):2034–46. doi: 10.1038/s41375-019-0397-9

54. Xie N, Sun YY, Jiang HJ, Liu CY, Shao ZH. Lymphocyte activation gene 3 expression on T lymphocyte cell subsets in patients with myelodysplastic syndrome. *Zhonghua Yi Xue Za Zhi* (2020) 100(24):1886–89. doi: 10.3760/cma.j.cn112137-20200217-00322

55. Meng F, Li L, Lu F, Yue J, Liu Z, Zhang W, et al. Overexpression of TIGIT in NK and T cells contributes to tumor immune escape in myelodysplastic syndromes. *Front Oncol* (2020) 10:1595. doi: 10.3389/fonc.2020.01595

56. Sallman DA, List A. The central role of inflammatory signaling in the pathogenesis of myelodysplastic syndromes. *Blood* (2019) 133(10):1039–48. doi: 10.1182/blood-2018-10-844654

57. Aref S, El Agdar M, El Sebaie A, Abouzeid T, Sabry M, Ibrahim L. Prognostic value of CD200 expression and soluble CTLA-4 concentrations in intermediate and high-risk myelodysplastic syndrome patients. *Asian Pacific J Cancer Prev APJCP* (2020) 21(8):2225–30. doi: 10.31557/APJCP.2020.21.8.2225

58. Kocibalova Z, Guzyova M, Borovska I, Messingerova L, Copakova L, Sulova Z, et al. Development of multidrug resistance in acute myeloid leukemia is associated with alterations of the LPHN1/GAL-9/TIM-3 signaling pathway. *Cancers* (2021) 13(14):3629–52. doi: 10.3390/cancers13143629

59. Tcvetkov N, Gusak A, Morozova E, Moiseev I, Baykov V, Barabanshikova M, et al. Immune checkpoints bone marrow expression as the predictor of clinical outcome in myelodysplastic syndrome. *Leukemia Res Rep* (2020) 14:100215. doi: 10.1016/j.lrr.2020.100215

60. Haddad F, Daver N. Targeting CD47/SIRPα in acute myeloid leukemia and myelodysplastic syndrome: Preclinical and clinical developments of magrolimab. *J immunotherapy Precis Oncol* (2021) 4(2):67–71. doi: 10.36401/JIPO-21-X2

61. Daver N, Boddu P, Garcia-Manero G, Yadav SS, Sharma P, Allison J, et al. Hypomethylating agents in combination with immune checkpoint inhibitors in acute myeloid leukemia and myelodysplastic syndromes. *Leukemia* (2018) 32 (5):1094–105. doi: 10.1038/s41375-018-0070-8

62. Karlic H, Herrmann H, Varga F, Thaler R, Reitermaier R, Spitzer S, et al. The role of epigenetics in the regulation of apoptosis in myelodysplastic syndromes and acute myeloid leukemia. *Crit Rev Oncol Hematol* (2014) 90(1):1–16. doi: 10.1016/j.critrevonc.2013.10.003

63. Jilg S, Reidel V, Müller-Thomas C, König J, Schauwecker J, Höckendorf U, et al. Blockade of BCL-2 proteins efficiently induces apoptosis in progenitor cells of high-risk myelodysplastic syndromes patients. *Leukemia* (2016) 30(1):112–23. doi: 10.1038/leu.2015.179

64. Langemeijer SM, Mariani N, Knops R, Gilissen C, Woestenenk R, de Witte T, et al. Apoptosis-related gene expression profiling in hematopoietic cell fractions of MDS patients. *PloS One* (2016) 11(11):e0165582. doi: 10.1371/journal.pone.0165582

65. Basiorka AA, McGraw KL, Eksioglu EA, Chen X, Johnson J, Zhang L, et al. The NLRP3 inflammasome functions as a driver of the myelodysplastic syndrome phenotype. *Blood* (2016) 128(25):2960–75. doi: 10.1182/blood-2016-07-730556

66. Paracatu LC, Schuettpelz LG. Contribution of aberrant toll like receptor signaling to the pathogenesis of myelodysplastic syndromes. *Front Immunol* (2020) 11:1236. doi: 10.3389/fimmu.2020.01236

67. Wei Y, Dimicoli S, Bueso-Ramos C, Chen R, Yang H, Neuberg D, et al. Toll-like receptor alterations in myelodysplastic syndrome. *Leukemia* (2013) 27 (9):1832–40. doi: 10.1038/leu.2013.180

68. Dimicoli S, Wei Y, Bueso-Ramos C, Yang H, Dinardo C, Jia Y, et al. Overexpression of the toll-like receptor (TLR) signaling adaptor MYD88, but lack of genetic mutation, in myelodysplastic syndromes. *PloS One* (2013) 8(8):e71120. doi: 10.1371/journal.pone.0071120

69. Giudice V, Wu Z, Kajigaya S, Fernandez Ibanez MDP, Rios O, Cheung F, et al. Circulating S100A8 and S100A9 protein levels in plasma of patients with acquired aplastic anemia and myelodysplastic syndromes. *Cytokine* (2019) 113:462–65. doi: 10.1016/j.cyto.2018.06.025

70. Lim ZY, Killick S, Germing U, Cavenagh J, Culligan D, Bacigalupo A, et al. Low IPSS score and bone marrow hypocellularity in MDS patients predict hematological responses to antithymocyte globulin. *Leukemia* (2007) 21(7):1436–41. doi: 10.1038/sj.leu.2404747

71. Chen S, Jiang B, Da W, Gong M, Guan M. Treatment of myelodysplastic syndrome with cyclosporin a. *Int J Hematol* (2007) 85(1):11–7. doi: 10.1532/IJH97.A10513

72. Stahl M, Bewersdorf JP, Giri S, Wang R, Zeidan AM. Use of immunosuppressive therapy for management of myelodysplastic syndromes: a systematic review and meta-analysis. *Haematologica* (2020) 105(1):102–11. doi: 10.3324/haematol.2019.219345

73. Haider M, Al Ali N, Padron E, Epling-Burnette P, Lancet J, List A, et al. Immunosuppressive therapy: Exploring an underutilized treatment option for myelodysplastic syndrome. *Clin lymphoma myeloma leukemia* (2016) 16 Suppl: S44–8. doi: 10.1016/j.clml.2016.02.017

74. Lai C, Ranpura V, Wu C, Olnes MJ, Parikh AR, Shenoy A, et al. Long-term outcomes in myelodysplastic syndrome patients treated with alemtuzumab. *Blood Adv* (2019) 3(7):980–83. doi: 10.1182/bloodadvances.2018030411

75. Baron F, Suciu S, Amadori S, Muus P, Zwierzina H, Denzlinger C, et al. Value of infliximab (Remicade®) in patients with low-risk myelodysplastic syndrome: final results of a randomized phase II trial (EORTC trial 06023) of the EORTC leukemia group. *Haematologica* (2012) 97(4):529–33. doi: 10.3324/haematol.2011.044347

76. Scott BL, Ramakrishnan A, Fosdal M, Storer B, Becker P, Petersdorf S, et al. Anti-thymocyte globulin plus etanercept as therapy for myelodysplastic syndromes (MDS): a phase II study. *Br J Haematol* (2010) 149(5):706–10. doi: 10.1111/j.1365-2141.2010.08145.x

77. Komrokji R, Garcia-Manero G, Ades L, Prebet T, Steensma DP, Jurcic JG, et al. Sotatercept with long-term extension for the treatment of anaemia in patients with lower-risk myelodysplastic syndromes: a phase 2, dose-ranging trial. *Lancet Haematology* (2018) 5(2):e63–72. doi: 10.1016/S2352-3026(18)30002-4

78. Platzbecker U, Germing U, Götze KS, Kiewe P, Mayer K, Chromik J, et al. Luspatercept for the treatment of anaemia in patients with lower-risk myelodysplastic syndromes (PACE-MDS): a multicentre, open-label phase 2 dose-finding study with long-term extension study. *Lancet Oncol* (2017) 18 (10):1338–47. doi: 10.1016/S1470-2045(17)30615-0

79. Zeng Q, Shu J, Hu Q, Zhou SH, Qian YM, Hu MH, et al. Apoptosis in human myelodysplastic syndrome CD34+ cells is modulated by the upregulation of TLRs and histone H4 acetylation *via* a β-arrestin 1 dependent mechanism. *Exp Cell Res* (2016) 340(1):22–31. doi: 10.1016/j.yexcr.2015.12.008

80. Fenaux P, Giagounidis A, Selleslag D, Beyne-Rauzy O, Mufti G, Mittelman M, et al. A randomized phase 3 study of lenalidomide versus placebo in RBC transfusion-dependent patients with low-/Intermediate-1-risk myelodysplastic syndromes with del5q. *Blood* (2011) 118(14):3765–76. doi: 10.1182/blood-2011-01-330126

81. Shallis RM, Chokr N, Stahl M, Pine AB, Zeidan AM. Immunosuppressive therapy in myelodysplastic syndromes: a borrowed therapy in search of the right place. *Expert Rev Hematol* (2018) 11(9):715–26. doi: 10.1080/17474086.2018.1503049

82. Gaballa MR, Besa EC. Myelodysplastic syndromes with 5q deletion: pathophysiology and role of lenalidomide. *Ann Hematol* (2014) 93(5):723–33. doi: 10.1007/s00277-014-2022-3

83. Greenberg PL, Stone RM, Al-Kali A, Barta SK, Bejar R, Bennett JM, et al. Myelodysplastic syndromes, version 2.2017, NCCN clinical practice guidelines in oncology. *J Natl Compr Cancer Network JNCCN* (2017) 15(1):60–87. doi: 10.6004/jnccn.2017.0007

84. Komrokji RS, Al Ali NH, Padron E, Cogle C, Tinsley S, Sallman D, et al. Lenalidomide and prednisone in low and intermediate-1 IPSS risk, non-Del(5q) patients with myelodysplastic syndromes: Phase 2 clinical trial. *Clin lymphoma myeloma leukemia* (2019) 19(4):251–54. doi: 10.1016/j.clml.2018.12.014

85. Dan H, Zhang S, Zhou Y, Guan Q. DNA Methyltransferase inhibitors: Catalysts for antitumour immune responses. *OncoTargets Ther* (2019) 12:10903–16. doi: 10.2147/OTT.S217767

86. Zhou J, Yao Y, Shen Q, Li G, Hu L, Zhang X. Demethylating agent decitabine disrupts tumor-induced immune tolerance by depleting myeloid-derived suppressor cells. *J Cancer Res Clin Oncol* (2017) 143(8):1371–80. doi: 10.1007/s00432-017-2394-6

87. Costantini B, Kordasti SY, Kulasekararaj AG, Jiang J, Seidl T, Abellan PP, et al. The effects of 5-azacytidine on the function and number of regulatory T cells and T-effectors in myelodysplastic syndrome. *Haematologica* (2013) 98(8):1196–205. doi: 10.3324/haematol.2012.074823

88. Jabbour EJ, Garcia-Manero G, Strati P, Mishra A, Al Ali NH, Padron E, et al. Outcome of patients with low-risk and intermediate-1-risk myelodysplastic syndrome after hypomethylating agent failure: a report on behalf of the MDS clinical research consortium. *Cancer* (2015) 121(6):876–82. doi: 10.1002/cncr.29145

89. Garcia-Manero G, Gore SD, Kambhampati S, Scott B, Tefferi A, Cogle CR, et al. Efficacy and safety of extended dosing schedules of CC-486 (oral azacitidine) in patients with lower-risk myelodysplastic syndromes. *Leukemia* (2016) 30 (4):889–96. doi: 10.1038/leu.2015.265

90. Sébert M, Renneville A, Bally C, Peterlin P, Beyne-Rauzy O, Legros L, et al. A phase II study of guadecitabine in higher-risk myelodysplastic syndrome and low blast count acute myeloid leukemia after azacitidine failure. *Haematologica* (2019) 104(8):1565–71. doi: 10.3324/haematol.2018.207118

91. Savona MR, Odenike O, Amrein PC, Steensma DP, DeZern AE, Michaelis LC, et al. An oral fixed-dose combination of decitabine and cedazuridine in myelodysplastic syndromes: a multicentre, open-label, dose-escalation, phase 1 study. *Lancet Haematology* (2019) 6(4):e194–203. doi: 10.1016/S2352-3026(19)30030-4

92. Garcia-Manero G, Ribrag V, Zhang Y, Farooqui M, Marinello P, Smith BD. Pembrolizumab for myelodysplastic syndromes after failure of hypomethylating agents in the phase 1b KEYNOTE-013 study. *Leukemia Lymphoma* (2022) 63 (7):1660–68. doi: 10.1080/10428194.2022.2034155

93. Chien KS, Kim K, Nogueras-Gonzalez GM, Borthakur G, Naqvi K, Daver NG, et al. Phase II study of azacitidine with pembrolizumab in patients with intermediate-1 or higher-risk myelodysplastic syndrome. *Br J Haematol* (2021) 195 (3):378–87. doi: 10.1111/bjh.17689

94. Zeidan AM, Boss I, Beach CL, Copeland WB, Thompson E, Fox BA, et al. A randomized phase 2 trial of azacitidine with or without durvalumab as first-line therapy for higher-risk myelodysplastic syndromes. *Blood Adv* (2022) 6(7):2207–18. doi: 10.1182/bloodadvances.2021005487

95. Zeidan AM, Knaus HA, Robinson TM, Towlerton AMH, Warren EH, Zeidner JF, et al. A multi-center phase I trial of ipilimumab in patients with myelodysplastic syndromes following hypomethylating agent failure. *Clin Cancer Res an Off J Am Assoc Cancer Res* (2018) 24(15):3519–27. doi: 10.1158/1078-0432.CCR-17-3763

96. Garcia-Manero G, Daver N, Montalban-Bravo G, Jabbour E, DiNardo C, Kornblau S, et al. A phase II study evaluating the combination of nivolumab (nivo) or ipilimumab (ipi) with azacitidine in pts with previously treated or untreated myelodysplastic syndromes (MDS). *Blood* (2016) 128(22):344. doi: 10.1182/blood.V128.22.344.344

97. Brunner A, Borate U, Esteve J, Porkka K, Knapper S, Vey N, et al. Aml-190: anti-tim-3 antibody mbg453 in combination with hypomethylating agents (hmas) in patients with high-risk myelodysplastic syndrome (hr-mds) and acute myeloid leukemia: a phase 1 study. *Clin Lymphoma Myeloma Leukemia* (2020) 20(1):188–89. doi: 10.1016/S2152-2650(20)30728-X

98. Tschernia NP, Kumar V, Moore DT, Vincent BG, Coombs CC, Van Deventer H, et al. Safety and efficacy of pembrolizumab prior to allogeneic stem cell transplantation for acute myelogenous leukemia. *Transplant Cell Ther* (2021) 27(12):1021.e1–21.e5. doi: 10.1016/j.jtct.2021.08.022

99. Davids MS, Kim HT, Costello C, Herrera AF, Locke FL, Maegawa RO, et al. A multicenter phase 1 study of nivolumab for relapsed hematologic malignancies after allogeneic transplantation. *Blood* (2020) 135(24):2182–91. doi: 10.1182/blood.2019004710

100. van Vugt MJH, Stone JA, De Greef R, Snyder ES, Lipka L, Turner DC, et al. Immunogenicity of pembrolizumab in patients with advanced tumors. *J immunotherapy Cancer* (2019) 7(1):212. doi: 10.1186/s40425-019-0663-4

101. Spallone G, Ventura A, Mazzeo M, DIR C, Lombardo P, Silvaggio D, et al. Cancer duplicity: a case of chronic lymphocytic leukemia and metastatic melanoma treated with ipilimumab. *Ital J Dermatol venereology* (2021) 156(1):112–13. doi: 10.23736/S2784-8671.20.06585-2

102. Brunner A, Esteve J, Porkka K, Knapper S, Vey N, Scholl S, et al. Efficacy and safety of sabatolimab (MBG453) in combination with hypomethylating agents (HMAs) in patients with acute myeloid leukemia (AML) and high-risk myelodysplastic syndrome (HR-MDS): Updated results from a phase 1b study. *Blood* (2021) 138:244–47. doi: 10.1182/blood-2021-146039

103. Jiang H, Fu R, Wang H, Li L, Liu H, Shao Z. CD47 is expressed abnormally on hematopoietic cells in myelodysplastic syndrome. *Leuk Res* (2013) 37(8):907–10. doi: 10.1016/j.leukres.2013.04.008

104. Sallman D, Asch A, Al Malki M, Lee D, Donnellan W, Marcucci G, et al. The first-in-Class anti-CD47 antibody magrolimab (5F9) in combination with azacitidine is effective in MDS and AML patients: Ongoing phase 1b results. *Blood* (2019) 134:569. doi: 10.1182/blood-2019-126271

105. Ansell SM, Maris MB, Lesokhin AM, Chen RW, Flinn IW, Sawas A, et al. Phase I study of the CD47 blocker TTI-621 in patients with relapsed or refractory hematologic malignancies. *Clin Cancer Res an Off J Am Assoc Cancer Res* (2021) 27 (8):2190–99. doi: 10.1158/1078-0432.CCR-20-3706

106. Zeidan AM, DeAngelo DJ, Palmer J, Seet CS, Tallman MS, Wei X, et al. Phase 1 study of anti-CD47 monoclonal antibody CC-90002 in patients with relapsed/refractory acute myeloid leukemia and high-risk myelodysplastic syndromes. *Ann Hematol* (2022) 101(3):557–69. doi: 10.1007/s00277-021-04734-2

107. Linder K, Lulla P. Myelodysplastic syndrome and immunotherapy novel to next in-line treatments. *Hum Vaccines immunotherapeutics* (2021) 17(8):2602–16. doi: 10.1080/21645515.2021.1898307

108. Baumeister SH, Murad J, Werner L, Daley H, Trebeden-Negre H, Gicobi JK, et al. Phase I trial of autologous CAR T cells targeting NKG2D ligands in patients with AML/MDS and multiple myeloma. *Cancer Immunol Res* (2019) 7 (1):100–12. doi: 10.1158/2326-6066.CIR-18-0307

109. Stevens BM, Zhang W, Pollyea DA, Winters A, Gutman J, Smith C, et al. CD123 CAR T cells for the treatment of myelodysplastic syndrome. *Exp Hematol* (2019) 74:52–63.e3. doi: 10.1016/j.exphem.2019.05.002

110. Zhang W, Stevens B, Budde E, Forman S, Jordan C, Purev E. Anti-CD123 CAR T-cell therapy for the treatment of myelodysplastic syndrome. *59th ASH Annu Meeting* (2017).

111. Van Acker HH, Versteven M, Lichtenegger FS, Roex G, Campillo-Davo D, Lion E, et al. Dendritic cell-based immunotherapy of acute myeloid leukemia. *J Clin Med* (2019) 8(5). doi: 10.3390/jcm8050579

112. Ansprenger C, Amberger DC, Schmetzer HM. Potential of immunotherapies in the mediation of antileukemic responses for patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) - with a focus on dendritic cells of leukemic origin (DC(leu)). *Clin Immunol (Orlando Fla)* (2020) 217:108467. doi: 10.1016/j.clim.2020.108467

113. Lieberman NAP, DeetGolier K, Haberthur K, Chinn H, Moyes KW, Bouchlaka MN, et al. An uncoupling of canonical phenotypic markers and functional potency of ex vivo-expanded natural killer cells. *Front Immunol* (2018) 9:150. doi: 10.3389/fimmu.2018.00150

114. Björklund AT, Carlsten M, Sohlberg E, Liu LL, Clancy T, Karimi M, et al. Complete remission with reduction of high-risk clones following haploidentical NK-cell therapy against MDS and AML. *Clin Cancer Res an Off J Am Assoc Cancer Res* (2018) 24(8):1834–44. doi: 10.1158/1078-0432.CCR-17-3196

115. Nagasaki J, Aoyama Y, Hino M, Ido K, Ichihara H, Manabe M, et al. Wilms tumor 1 (WT1) mRNA expression level at diagnosis is a significant prognostic marker in elderly patients with myelodysplastic syndrome. *Acta haematologica* (2017) 137(1):32–9. doi: 10.1159/000452732

116. Tawara I, Kageyama S, Miyahara Y, Fujiwara H, Nishida T, Akatsuka Y, et al. Safety and persistence of WT1-specific T-cell receptor gene-transduced lymphocytes in patients with AML and MDS. *Blood* (2017) 130(18):1985–94. doi: 10.1182/blood-2017-06-791202

117. Ueda Y, Ogura M, Miyakoshi S, Suzuki T, Heike Y, Tagashira S, et al. Phase 1/2 study of the WT1 peptide cancer vaccine WT4869 in patients with myelodysplastic syndrome. *Cancer Sci* (2017) 108(12):2445–53. doi: 10.1111/cas.13409

118. Qazilbash MH, Wieder E, Thall PF, Wang X, Rios R, Lu S, et al. PR1 peptide vaccine induces specific immunity with clinical responses in myeloid malignancies. *Leukemia* (2017) 31(3):697–704. doi: 10.1038/leu.2016.254

119. Rezvani K, Yong AS, Mielke S, Jafarpour B, Savani BN, Le RQ, et al. Repeated PR1 and WT1 peptide vaccination in montanide-adjuvant fails to induce sustained high-avidity, epitope-specific CD8+ T cells in myeloid malignancies. *Haematologica* (2011) 96(3):432–40. doi: 10.3324/haematol.2010.031674

120. Wong KK, Hassan R, Yaacob NS. Hypomethylating agents and immunotherapy: Therapeutic synergism in acute myeloid leukemia and myelodysplastic syndromes. *Front Oncol* (2021) 11:624742. doi: 10.3389/fonc.2021.624742

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Case report: A successful re-challenge report of GLS-010 (Zimberelimab), a novel fully humanized mAb to PD-1, in a case of recurrent endometrial cancer

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With the widespread use of immune checkpoint inhibitors (ICI), there is growing concern about reports of immune-related adverse events (irAE). In clinical practice, patients who experience severe toxicities by ICI-based therapies would require utmost caution in resuming ICI therapy because of the potential risk of serious irAEs caused by the reintroduction of immunotherapy. In this study, we report a case of recurrent endometrial cancer patient with PD-L1 positive as well as dMMR suffering from immunotherapy-associated myocarditis after first-line treatment with ICI combined with a multi-targeted anti-angiogenic agent. After symptomatic treatment, the patient was in complete remission from treatment toxicities. Subsequently, through MDT discussions, we selected a new PD-1 agent, zimberelimab, for rechallenge therapy, and the patient achieved a sustained disease remission without any treatment-related toxicities. To date, the manner and timing of the ICI re-challenge has been a subject of iterative deliberation. We believe that our experience could shed some light on ICI rechallenge therapy, and we look forward to more literatures to refine the ICI rechallenge scenarios.

KEYWORDS

GLS-010, re-challenge, immune checkpoint inhibitors, endometrial cancer, myocarditis

Introduction

With the introduction of immune checkpoint inhibitors (ICI) such as programmed cell death-1 (PD-1) and programmed cell death-ligand 1 (PD-L1) antibodies, a significant improvement of the efficacy was seen in numerous malignancies (1–3). Supported by a growing body of evidence, ICI-based therapies have gradually crossed over from backline to frontline treatments (4, 5) and even emerged in the neoadjuvant front (6). In the context of widespread use of ICIs, the number of patients developing immune-related adverse events (irAE) is also rising (7). The category and grading of irAE can be attributed to a variety of factors, including the type of ICIs, the mode of administration such as monotherapy or in combined with chemo- or radiotherapy, and the duration of ICI use, etc.

Myocarditis is a very rare but highly lethal irAE, with the reported incidence of less than 1% (8). According to irAE management guidelines, once myocarditis has occurred, restarting ICI therapy requires great caution even after complete resolution of symptoms, and the manner and timing of ICI resumption is still an open issue. Herein, we would share a case of endometrial cancer who had developed immune-associated myocarditis and was successfully treated with a re-challenge of PD-1.

Case report

A 58-year-old female patient was admitted with "intermittent vaginal bleeding for 1 month". After admission, the patient was diagnosed with endometrial cancer by hysteroscopy. On December 9, 2020, she underwent a total hysterectomy combined with pelvic and para-aortic lymph node dissection. The postoperative pathological diagnosis showed low-differentiated endometrioid adenocarcinoma, invading the outer 1/2 layer of the uterine wall, with a large number of vascular carcinoma thrombi visible, no cancerous tissue involved in the cervix and parametrium area, 1/13 metastases in the para-aortic lymph nodes and 1/33 metastases in the pelvic lymph nodes, the combined positive score (CPS) for PD-L1 (22C3) was 2 and the tumor presented as dMMR. Since January 2021, the patient received 3 cycles of adjuvant chemotherapy with liposomal paclitaxel (135 mg/m^2, iv. Q3w) in combination with carboplatin (AUC = 5, iv. Q3w), during which, after 2 cycles of chemotherapy, she underwent an adjuvant radiotherapy to the pelvic and para-aortic lymph node areas at a dose of 50.4Gy in 28 fractions. On May 12, 2021, she underwent a routine computed tomography (CT) review showing abnormally enlarged left parietal abdominal aortic lymph nodes and diagnosed as tumor progression. On May 21, 2021, she was enrolled in a clinical study of Fruqintinib in combination with Sintilimab for advanced solid tumors (registry number: CTR20190514), in which Fruquintinib (5 mg, po. d1-21, Q4w), and Sintilimab (200 mg, iv. Q3w) for 2 cycles. Fortunately, the tumor was in partial remission at the end of 2 cycles of treatment by routine review.

However, on June 30, 2021, the patient began complaining of shortness of breath after mild activity and abnormally elevated blood troponin up to 250 ng/L, along with the abnormal elevation of creatine kinase (up to 432 U/L) and lactate dehydrogenase (up to 325 U/L). No significant abnormalities were seen on the electrocardiogram, and she was diagnosed with grade 2 ICI-associated myocarditis. ICI therapy was immediately suspended and symptomatic treatment with methylprednisolone was administered. On September 2, 2020, the patient complained of increased chest tightness and shortness of breath, and blood troponin showed 47.3 ng/L. Chest CT reported interstitial pneumonia in both lungs (Figure 1A), and genetic testing of pathogenic microorganisms from alveolar lavage fluid was diagnosed as secondary *Pneumocystis jirovecii*

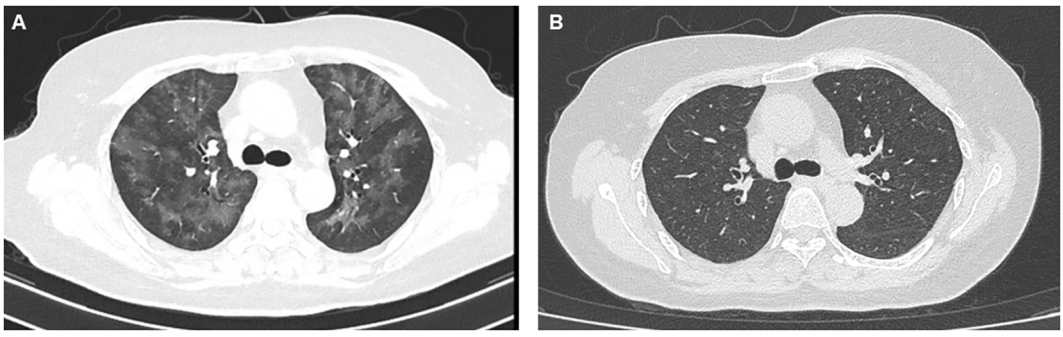

FIGURE 1
The performance of CT scan of the pulmonary infection induced by hormone use **(A)** and the result of CT image after anti-infection as well as symptomatic treatment **(B)**.

infection due to immune depression. Targeted anti-infective therapy was given, along with symptomatic treatment with methylprednisolone combined with human immunoglobulin. Until November 2, 2021, the patient's shortness of breath gradually improved, and the chest CT showed that the inflammatory manifestations of the lungs basically disappeared (Figure 1B), and the myocardial enzyme index returned to the normal range. However, unfortunately, due to the interruption of antitumor treatment for 5 months, the para-aortic lymph nodes were significantly enlarged and fuses into clusters, which was considered as tumor progression.

Given the benefit of previous immunotherapy, after a multidisciplinary treatment (MDT) discussion and a thorough risk communication with the patient, she began treatment with Zimberelimab (240 mg, iv. Q3w), a novel fully humanized mAb to PD-1, from January 25, 2022, the tumor was in partial remission after 2 cycles of treatments (Figure 2), and the disease remained in continuous remission until our latest follow-up visit, September 14, 2022. Surprisingly, the patient did not experience any elevation of cardiac enzymes or symptoms related to myocarditis throughout the immunotherapy. The entire treatment history could be seen in Figure 3.

Discussion

Based on the published experience, for those who had severe irAEs, the restart of ICI therapy can be implemented in three main scenarios: first, by switching from anti-PD-(L)1 antibodies to anti-CTLA-4 approach or vice versa, which must be initiated on the premise that both types of ICI have a definite efficacy in that disease; second, by choosing the same class of ICI but with a different agent when irAEs have largely resolved; and third, the restarting ICI should be under conditions where a secondary prevention efforts has been well-established (9).

In this case, by a multidisciplinary discussion, we chose to restart ICI therapy in the second script. A retrospective study that adopted the same class of ICI re-challenge method reported that 40 (50%) of the 80 patients would reoccur with varying degrees or types of toxicities, but only 14 (18%) cases would experience a recurrence of those initial irAEs (10). Another retrospective study including 38 NSCLCs showed that by the same type of ICI treatment, 18 (48%) patients would not experience any further irAEs and 10 (26%) patients developed new irAEs, compared with only 10 (26%) of the initial irAEs. These recurrent and new irAEs were mild and manageable (11).

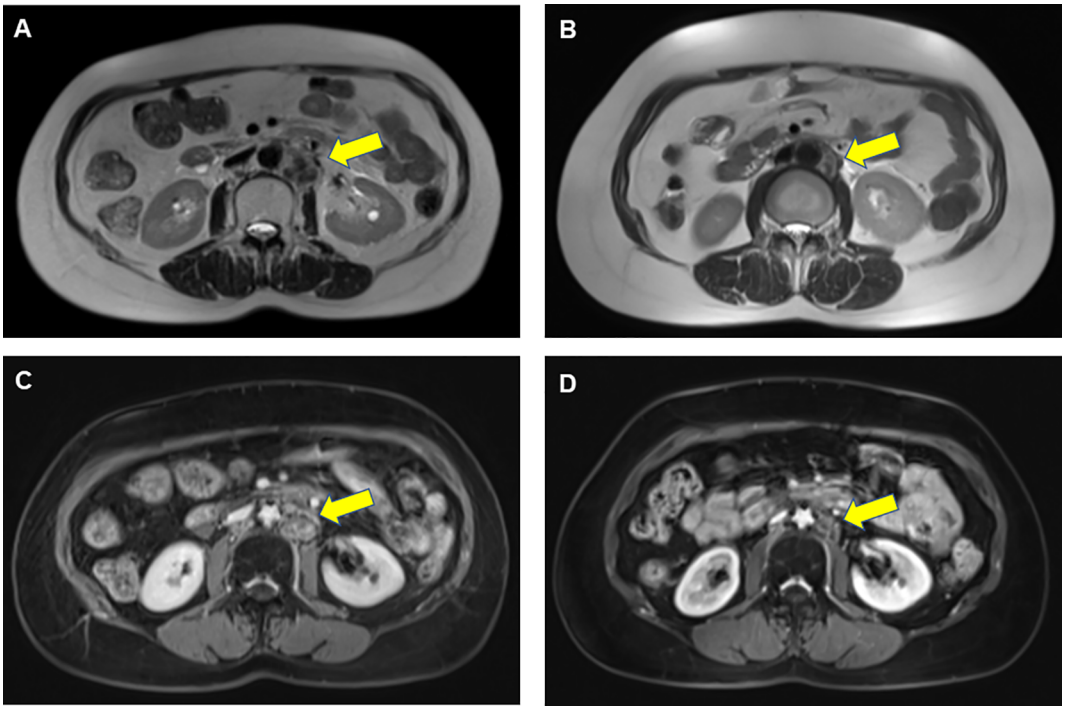

FIGURE 2
Results of abdominal MRI scans before and after ICI rechallenge treatment in a patient with endometrial cancer. Prior to PD-1 rechallenge, T2-weighted image **(A)** and T1-weighted enhanced scans **(C)** showed enlarged lymph nodes visible next to the abdominal aorta. After 2 cycles of PD-1 rechallenge, T2-weighted image **(B)** and T1-weighted enhanced sequence **(D)** indicated that those lymph nodes achieved partial remission. The yellow arrow represented tumor locations.

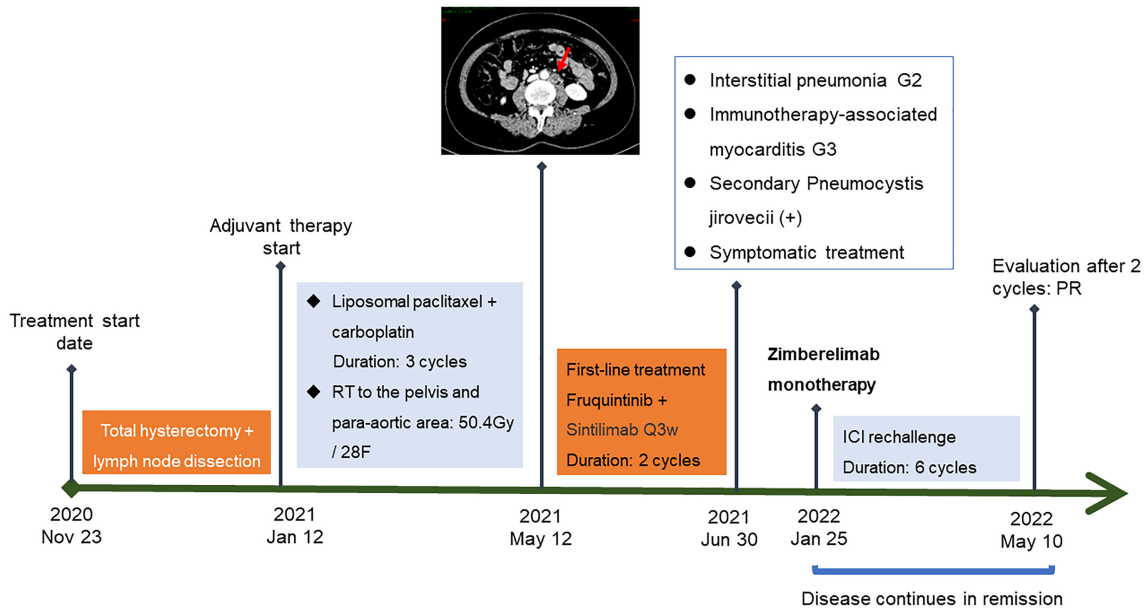

FIGURE 3
The entire treatment history of a patient with recurrent endometrial cancer treated with PD-1 rechallenge.

Accordingly, it is worthwhile to consider similar PD-(L)1 rechallenge therapy for this patient. Through 6 cycles of treatment, her disease achieved a well control. More importantly, she did not experience any further irAEs.

Targeting inhibition of vascular endothelial growth factor (VEGF)/VEGF receptor 2 (VEGFR2) could not only reduce tumor growth but also improve the vascular normalization and modulate the response to immunotherapy (12). Although the patient suffered from the immune myocarditis after first-line Fruqintinib/Sintilimab treatment, we could not deny that the modulation of the immune microenvironment by Fruqintinib underlined her long survival benefit.

It has been reported that cardiovascular toxicity is one of the major toxicities of anti-angiogenic drugs (13). However, published literature showed that the most common toxicities of the multitargeted agent, Fruqintinib, were hypertension, coagulation disorders, and thrombosis. Elevated cardiac enzymes were a very rare event. Therefore, we hypothesized that the myocardial injury in this case was largely attributed to ICI-related toxicity.

To summarize, our courage to re-challenge ICI was based on the following conditions. Her postoperative pathological results showed tumor was positive PD-L1 expression and dMMR status, which strongly predicted that she would most likely benefit from PD-1 therapy (14, 15), and previous literatures showed that the risk of initial irAEs after re-challenge treatment was not irreversible and those initial toxicities did not necessarily return. Then, Zimberelimab, as a new PD-1 antibody, has not been reported any cardiovascular events based on the current evidence. In addition, there are no superior later line options for her after failure from first-line therapy. In light of the recent study, Zimberelimab has demonstrated favorable preliminary results in the recurrent gynecologic malignancies (16). Finally, on the basis of MDT, we reduced the dose intensity of Zimberelimab (from 240mg Q2w to 240 mg Q3w) to possibly maximize the safety of the restart ICI treatment.

Patient perspective

Although ICIs-associated myocarditis is an uncommon event, it is a highly lethal toxicity. According to retrospective studies, the probability of recurrence of initial toxicities after ICIs re-challenge was less than 30%. It was confirmed by our case report that, under the framework of MDT, PD-1 re-challenge would be feasible and manageable for those who are potential benefit from ICIs.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

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

YC and JY designed and drafted the manuscript. AH, QY and GL providing suggestions for revisions and review of 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.

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References

1. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* (2012) 366:2443–54. doi: 10.1056/NEJMoa1200690

2. Larkin J, Chiarion-Sileni V, Gonzalez R. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med* (2015) 373:23–34. doi: 10.1056/NEJMc1509660

3. 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) 373:1627–39. doi: 10.1056/NEJMoa1507643

4. Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, et al. PD-1 blockade with nivolumab in relapsed or refractory hodgkin's lymphoma. *N Engl J Med* (2015) 372:311–9. doi: 10.1056/NEJMoa1411087

5. Horn L, Mansfield AS, Szczęsna A, Havel L, Krzakowski M, Hochmair MJ, et al. IMpower133 study group. first-line atezolizumab plus chemotherapy in extensive-stage small-cell lung cancer. *N Engl J Med* (2018) 379:2220–9. doi: 10.1056/NEJMoa1809064

6. Forde PM, Chaft JE, Smith KN, Anagnostou V, Cottrell TR, Hellmann MD, et al. Neoadjuvant PD-1 blockade in resectable lung cancer. *N Engl J Med* (2018) 378:1976–86. doi: 10.1056/NEJMoa1716078

7. Martins F, Sofiya L, Sykiotis GP, Lamine F, Maillard M, Fraga M, et al. Adverse effects of immune-checkpoint inhibitors: epidemiology, management and surveillance. *Nat Rev Clin Oncol* (2019) 16:563–80. doi: 10.1038/s41571-019-0218-0

8. Patel RP, Parikh R, Gunturu KS, Tariq RZ, Dani SS, Ganatra S, et al. Cardiotoxicity of immune checkpoint inhibitors. *ESMO Open* (2017) 2:e000247. doi: 10.1007/s11912-021-01070-6

9. Haanen J, Ernstoff M, Wang Y, Menzies A, Puzanov I, Grivas P, et al. Rechallenge patients with immune checkpoint inhibitors following severe immune-related adverse events: Review of the literature and suggested prophylactic strategy. *J Immunother Cancer* (2020) 8:e000604. doi: 10.1136/jitc-2020-000604

10. Pollack MH, Betof A, Dearden H, Rapazzo K, Valentine I, Brohl AS, et al. Safety of resuming anti-PD-1 in patients with immune-related adverse events (irAEs) during combined anti-CTLA-4 and anti-PD1 in metastatic melanoma. *Ann Oncol* (2018) 29(1):250–5. doi: 10.1093/annonc/mdx642

11. Santini FC, Rizvi H, Plodkowski AJ, Ni A, Lacouture ME, Gambarin-Gelwan M, et al. Safety and efficacy of re-treating with immunotherapy after immune-related adverse events in patients with NSCLC. *Cancer Immunol Res* (2018) 6:1093–9. doi: 10.1158/2326-6066.CIR-17-0755

12. Gao F, Yang C. Anti-VEGF/VEGFR2 monoclonal antibodies and their combinations with PD-1/PD-L1 inhibitors in clinic. *Curr Cancer Drug Targets* (2020) 20:3–18. doi: 10.2174/1568009619666191114110359

13. Dobbin SJH, Cameron AC, Petrie MC, Jones RJ, Touyz RM, Lang NN. Toxicity of cancer therapy: What the cardiologist needs to know about angiogenesis inhibitors. *Heart* (2018) 104:1995–2002. doi: 10.1136/heartjnl-2018-313726

14. Davis AA, Patel VG. The role of PD-L1 expression as a predictive biomarker: An analysis of all US food and drug administration (FDA) approvals of immune checkpoint inhibitors. *J Immunother Cancer* (2019) 26:7:278. doi: 10.1186/s40425-019-0768-9

15. Luchini C, Bibeau F, Ligtenberg MJL, Singh N, Nottegar A, Bosse T, et al. ESMO recommendations on microsatellite instability testing for immunotherapy in cancer, and its relationship with PD-1/PD-L1 expression and tumour mutational burden: A systematic review-based approach. *Ann Oncol* (2019) 30:1232–43. doi: 10.1093/annonc/mdz116

16. Wu X, Xia L, Zhou Q, Zhu JQ, Wang K, Chen J, et al. GLS-010 (Zimberelimab), a novel fully human anti-PD-1 mAb in chinese patients with recurrent/metastatic cervical cancer: Results from a multicenter, open-label, single-arm phase II trial. *Int J Gynecologic Cancer* (2020) 30:A147. doi: 10.1136/ijgc-2020-IGCS.307

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Preclinical and clinical studies of CAR-NK-cell therapies for malignancies

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The development of chimeric antigen receptor T (CAR-T) cell therapy, a specific type of immunotherapy, in recent decades was a fantastic breakthrough for the treatment of hematological malignancies. However, difficulties in collecting normal T cells from patients and the time cost of manufacturing CAR-T cells have limited the application of CAR-T-cell therapy. In addition, the termination of related clinical trials on universal CAR-T cell therapy has made further research more difficult. Natural killer (NK) cells have drawn great attention in recent years. Chimeric antigen receptor-NK (CAR-NK) cell therapy is a promising strategy in the treatment of malignant tumors because of its lack of potential for causing graft-versus-host disease (GVHD). In this review, we will address the advances in and achievements of CAR-NK cell therapy.

KEYWORDS

chimeric antigen receptor, T cells, natural killer cells, immunotherapy, malignancies

Introduction

In recent decades, CAR-T-cell therapy was a research focus and was thought to be a promising targeted immunotherapy, especially in the treatment of relapsed and refractory B-cell malignant tumors. To date, two CD19-CAR-T-cell therapies have been approved for the treatment of acute lymphocytic leukemia (ALL) and diffuse large B-cell lymphoma (DLBCL) (1). Studies of CAR-T cells targeting CD38 and BCMA for the treatment of multiple myeloma (MM) have been implemented in clinical trials (2). However, CAR-T cell therapy is still facing several problems. The FDA has terminated all clinical trials concerning universal CAR-T-cell therapy due to safety consideration and related increased attention on gene editing. It is also difficult to collect sufficient numbers of T lymphocytes from patients who have been heavily pretreated. Furthermore, several weeks of CAR-T-cell preparation time hinder the use of this therapy to patients with rapid disease progression (3). In addition, cytokine release syndrome (CRS) and

neurological toxicity (NT), the most common adverse events of CAR-T-cell therapy, are life-threatening (4). All of these factors may restrict further clinical applications of CAR-T-cell therapy.

In recent years, NK cells have been regarded as an alternative to T cells due to their accessibility and safety (5). Considering the short duration *in vivo*, the cytotoxicity and adverse events of CAR-NK-cell therapy are better manageable than those of CAR-T cell therapy. Moreover, the lower incidence of GVHD induced by NK cells makes them a promising immunotherapy for allogenic cell transplantation (6). CAR-NK-cell therapy has thus become a research hotspot and new strategy for malignancies.

In this review, we will discuss the similarities and differences between CAR-T cells and CAR-NK cells and focus on recent advances and preclinical studies of CAR-NK cells.

The biological characteristics of NK cells

NK cells are innate immune effectors and are found mainly in the bone marrow, peripheral blood, spleen and liver (7). NK cells possess cytotoxic features similar to those of CD8+ T cells and play important roles in tumor immunology. CD8+ T-cell-mediated cytotoxicity relies on the combination of the T-cell receptor (TCR) and an antigen presented by major histocompatibility complex-I (MHC-I). NK cells can recognize MHC-I expressed on healthy cells and avoid attacking them (8, 9). Tumor cells can down-modulate MHC-I to escape CD8+ T-cell-mediated cytotoxicity, while NK cells can be activated through the loss of MHC-I and control the proliferation and metastasis of tumors (8, 10). Thus, NK cells have more specific anti-tumor effects and are associated with fewer off-target complications (9, 11).

The activation of NK cells can be mediated through different pathways, including signals from Toll-like receptors (TLRs) recognizing pathogen-associated molecular patterns (PAMPs), cytokines such as interleukin (IL)-2 or IL-15, and interplay between activating and inhibitory receptors (7, 12, 13). Activating NK-cell receptors include members of the natural cytotoxicity receptor (NCR) family (NKp30, NKp44 and NKp46), C-type lectin-like activating receptors (NKG2C and NKG2D), activating killer immunoglobulin receptors (KIR2DS1, KIR2DS4 and KIR2DL4) and costimulatory receptor DNAX accessory molecule 1 (DNAM-1) (14). While killer cell immunoglobulin-like receptors (KIRs) and the heterodimeric C-type lectin receptor NKG2A are inhibitory receptors associated with the tolerance of NK cells to normal cells (14).

The sources of NK cells for immunotherapy

NK cells for preclinical studies and clinical therapy may be derived from a wide range of sources, such as peripheral blood (PB), cord blood (CB), hematopoietic stem cells (HSCs), induced pluripotent stem cells (iPSCs) and NK-cell lines (15–19).

The most accessible source of NK cells is peripheral blood. However, a number of issues limit the use of NK cells from peripheral blood, including the high monetary and time costs, low cell proliferation capacity and short survival time (20). The expression of genes related to the cell cycle and cell proliferation is higher in NK cells from umbilical cord blood (UCB) than in those from peripheral blood (21). Furthermore, the advantages of UCB-derived NK cells, including the convenience of collection and low associated incidence of GVHD, make UCB a better source of NK cells than PB (22, 23). In addition, human stem and progenitor cells (HSPCs) isolated from cord blood can also be derived into NK cells with the stimulation of various growth factors and cytokines, including IL-2, IL-7 and IL-15 (24). Similarly, NK cells can also be derived from iPSCs in the presence of these stimulators (25).

NK-cell lines, mostly derived from NK/T-cell lymphoma (NKTCL) patients, such as the NK-92 and KHYG-1 cell lines, may be a potential rapid and abundant source for NK cells for immunotherapy (26, 27). These cell lines are easily transduced and maintain cytotoxicity during expansion. The NK-92 cell line, obtained from a good manufacturing practice (GMP)-compliant master cell bank and treated in a GMP-compliant procedure, is the only cell line approved by the FDA for clinical use (28, 29). Since the first report of the transfusion of irradiated NK-92 cells for adoptive immunotherapy of malignancies (30) and the first CAR-NK-92 cells targeting HER-2 (31), NK-92 cells has been applied in several clinical trials, and some encouraging results have been achieved in the treatment of refractory lymphoma, multiple myeloma and other solid tumors. Several patients even achieved a complete response (CR) (32–34). NK-cell lines must be irradiated before infusion due to the risk of tumor engraftment and tumorigenicity. The short lifespan of irradiated cells may result in treatment failure or a short duration of disease remission, thus limiting their clinical application (32, 33, 35).

The similarities and differences between CAR-T cells and CAR-NK cells

CARs consist of an extracellular domain (a single-chain variable antibody fragment (scFv) or a functional domain of a specific ligand) for the identification of target antigens, a transmembrane region and an intracellular domain (36). The intracellular domain of CAR-T cells is composed of CD3ζ activation signaling (first generation of CARs) and costimulatory molecules (CD28, 4-1BB or CD134) (second or third generation of CARs) (Figure 1A). Based on NK-cell characteristics, several CAR-NK cells contain DNAX-activation protein (DAP) 10 or DAP12 as an intracellular domain

(Figure 1C). DAP12 and NKG2D are expressed on NK cells and participate in the activation of downstream signals, while DAP10 is necessary for NKG2D costimulatory signaling. These CAR-NK cells were mainly designed for the treatment of both leukemia and solid tumors and showed strong anti-tumor effects (37, 38). A lack of cytokines such as IL-2 or IL-15 may lead to the short *in vivo* lifetime of NK cells. NK cells can be engineered to both express CARs and autonomously produce IL-2 or IL-15 (fourth generation of CARs), thus enhancing their persistence and proliferation (Figure 1B) (39, 40).

Lentivirus-based vectors have been extensively used in CAR gene transduction of T cells. Compared with T cells, NK cells showed resistance to viral transfection and lower transduction efficiency, which may be due to the natural capacity of NK cells to defend against viral infection (41, 42). Other approaches, including retroviral vectors, transposon vectors and the electroporation of DNA or mRNA plasmids, are alternative ways to transfer the CAR gene into NK cells (43–48).

CAR-T cells can kill tumor cells with specific target antigens through active cell lysis and the production of cytokines,

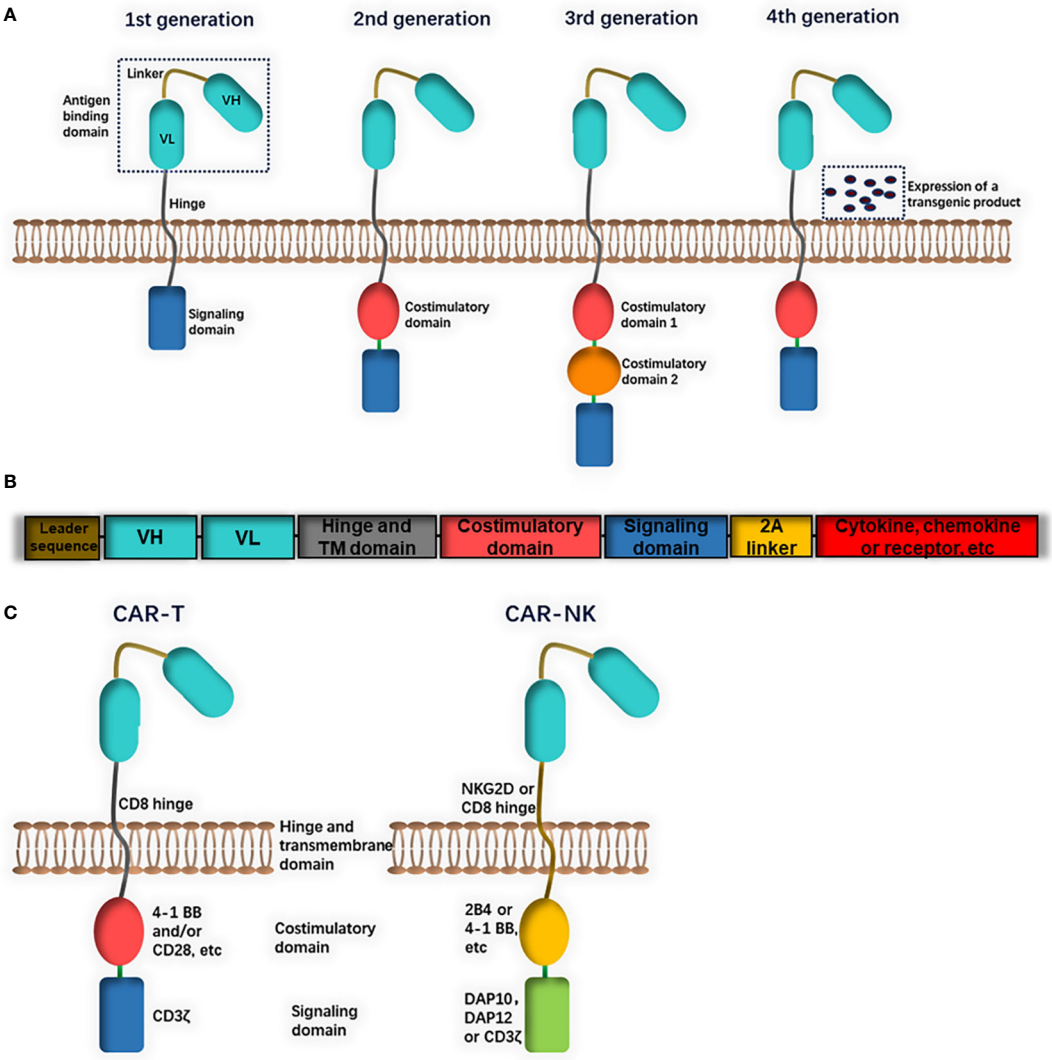

FIGURE 1
The structure of chimeric antigen receptors (CAR). **(A)** CAR consist of an extracellular antigen binding domain, a transmembrane hinge and intracellular domain. The extracellular domain could be a single chain fragment of variable region (ScFv) antibody or a functional domain of specific ligand. The intracellular domain is composed of a signaling domain (first generation) and one costimulatory domain (second generation) or two (third generation). **(B)** Fourth generation CARs include a constitutive or inducible expression of a transgenic product (cytokine, chemokine or receptor, etc.). **(C)** Differences in CAR constructs between CAR-T and CAR-NK: CAR-T cells usually contains a CD8 transmembrane domain, CD3ζ signaling domain and 4-1BB and/or CD28 costimulatory domain. CAR-NK cells may be with different domains (for example, NKG2D transmembrane domain, DAP10 or DAP12 signaling domain and 2B4 costimulatory domain).

including IL-1α, IL-2, IL-6, IL-8, IL-10, and tumor necrosis factor-α (TNF-α) (6, 49). However, these cytokines are also highly associated with CRS and severe neurotoxicity (49). CAR-NK cells secrete a different cytokine profile, such as IFN-γ and GM-CSF, which are associated with a lower risk of CRS and neurotoxicity (50). In addition, CAR-NK cells can lyse tumor cells directly by releasing cytoplasmic granules containing perforin and granzyme or inducing tumor cell apoptosis by expression of Fas ligand or TNF-related apoptosis-inducing ligand (TRAIL) (51). NK cells also participate in antibody-dependent cellular cytotoxicity (ADCC) (52). NK cells can activate and interact with other immune cells, such as T cells, dendritic cells and macrophages (53). All these features enable them to exert anti-tumor activity in pathways other than the CAR-specific pathway and reduce the risk of relapse or resistance mediated by target antigen escape (54–56).

Preclinical studies of CAR-NK cells in the treatment of hematopoietic malignancies

NK cells have been engineered to express CARs to redirect their activity against B-cell malignancies. To date, CD19 is the most common target in both preclinical and clinical studies of CAR-T-cell therapy. Similarly, a number of preclinical studies of CAR-NK therapy have focused on this target. NK-92 cells engineered with CARs recognizing CD19 showed increased cytotoxicity against B-cell malignancies (57, 58). CD19-CAR-NK cells from other cell sources, including PB, iPSCs and CB, also showed activity against B-cell malignancies *in vitro* (40, 59, 60). Other molecules, including CD20 and Flt3, were also developed as specific targets for CAR-NK immunotherapy against B-cell tumors (61, 62).

CD38 and CD138 are classic markers of plasma cells and are highly expressed in multiple myeloma (MM). Although CD38-CAR-T-cell therapy for MM and CD38-CAR-NK-cell therapy for acute myeloid leukemia (AML) have been reported in several studies (63, 64), CD38-CAR-NK cells have not been evaluated for the treatment of multiple myeloma. Jiang et al. developed CD138-targeting CAR-NK cells and demonstrated enhanced anti-tumor activity *in vitro* and in xenograft mouse models (65). B-cell maturation antigen (BCMA) is another ideal target for CAR cell therapy due to its restricted expression in B-cell lineage cells. BCMA-CAR-NK cells modified with CXCR4 significantly reduced the tumor burden and extended the survival of tumor-bearing mice (66). Signaling lymphocytic activation molecule family member 7 (SLAMF7 or CS1) is another potential target for its high expression in plasma cells and MM. Second-generation CS1-specific CAR-NK-92 cells were established by Chu et al. and showed cytotoxicity against CS1-positive MM cells and xenograft models (67).

To date, T-cell malignancies, including peripheral T-cell lymphoma and T-cell acute lymphoblastic leukemia (T-ALL), remains a refractory disease. Three CAR-NK cell therapies targeting CD3, CD5 and CD7 have been investigated for the treatment of T-cell malignancies. These modified CAR-NK-92 cells showed significant anti-tumor cytotoxicity against T-cell lymphomas and T-ALL both *in vitro* and *in vivo* (68–70).

In addition to specific tumor markers, antigens that are widely expressed in multiple malignancies have been developed as immunotherapy targets. For example, NKG2D ligands are expressed on a variety of tumor cells. MHC class I chain-related protein A (MICA), an NKG2D ligand, has been identified on some leukemia cells and solid tumor cells, such as lung, breast, ovary and colon cancer cells (71–73). NKG2D ligands have also been detected on MM cells and glioma cells (74, 75). Leivas et al. developed engineered NK cells targeting NKG2D ligands in MM (76). Data from *in vitro* tests and mouse models showed enhanced anti-tumor activity of NKG2D-CAR-NK cells compared with memory CAR-T cells (76). Du et al. generated peripheral blood-derived NK cells coexpressing NKG2D-specific CAR and IL-15 and demonstrated their activity in lysing tumor cells both *in vitro* and in a xenograft AML model (77).

Preclinical studies of CAR-NK cells in the treatment of solid tumors

Although CAR-T-cell therapies have achieved great progress in the treatment of hematological malignancies, their effect on solid malignancies has been poor. This poor efficacy may be due to the insufficient homing capacity and the immunosuppressive tumor microenvironment (78). Thus, CAR-NK cell therapies for solid tumors have become a promising immunotherapy strategy. Glioblastoma, breast cancer and ovarian cancer are the most widely researched solid tumors to determine the potential of CAR-NK-cell therapy (summarized in Table 1).

Glioblastoma

Glioblastoma is the most common malignant primary cerebral tumor in adults. Even though patients undergo surgical resection and receive radio- and/or chemotherapy, the median survival time is approximately 15 months (98). Interleukin-13 receptor α2 (IL-13Rα2), epidermal growth factor receptor (EGFR), EGFR variant III (EGFRvIII) and growth factor receptor tyrosine kinase Erb2 (HER2) have been explored as immunotherapy targets for glioblastoma. They are

TABLE 1 Preclinical studies of CAR-NK cell therapy.

Malignancy	Target	Source of NK cells	Reference
Hematological cancer			
B-cell malignancies	CD19	NK-92, PB-NK or CB-NK	(40, 57–60)
	CD20	NK-92	(61)
	Flt3	NK-92	(62)
Multiple myeloma	CD138	NK-92	(65)
	BCMA	NK-92	(66)
	CS1	NK-92	(67)
	NKG2D	PB-NK	(77)
T-cell malignancies	CD3	NK-92	(68)
	CD5	NK-92	(69)
	CD7	NK-92	(70)
AML	NKG2D	PB-NK	(77)
Solid cancer			
Glioblastoma	HER2	NK-92	(79)
	EGFR and/or EGFRvIII	NK-92, NKL, KHYG1 or YTS	(80–84)
Breast cancer	HER2	NK-92	(29, 85, 86)
	EGFR and/or EGFRvIII	NK-92 or PB-NK	(87)
	EpCAM	NK-92	(88)
	TF	NK-92	(89)
	B7-H6	NK-92	(90)
Ovarian cancer	HLA-G	PB-NK	(91)
	CD24	NK-92	(92)
	CD44	NK-92	(93)
	CD133	NK-92	(94)
	Mesothelin	iPSC-NK or NK-92	(95, 96)
	αFR	NK-92	(97)

overexpressed in 40-60% of glioblastoma patients, while these antigens are undetectable or only minimally expressed in normal brain tissue (99–102). IL-13Rα2 can enhance the invasiveness of glioblastoma (103). EGFRvIII drives tumorigenicity and mediates resistance to radiotherapy and chemotherapy (104, 105). Together, IL-13Rα2 and EGFRvIII can promote the proliferation of glioblastoma cells (103), while overexpression of HER2 contributes to malignant transformation (106).

There have been several preclinical studies of IL-13Rα2-specific CAR-T-cell therapy in the treatment of glioblastoma (107–110). Other studies demonstrated the significant cytotoxicity of CAR-T cells against EGFRvIII- or HER2-positive glioblastoma both *in vitro* and *in vivo* (111–114).

Until now, most preclinical studies of CAR-NK-cell therapy for glioblastoma were targeting EGFR, EGFRvIII and HER2. Different NK cells, including NK-92, NKL, KHYG-1 and YTS cells, engineered to target EGFR and/or EGFRvIII, showed enhanced cytotoxicity against glioblastoma both *in vitro* and *in vivo* (80–83). CAR-NK cells recognizing both EGFR and EGFRvIII showed stronger anti-tumor effects than single targeted NK cells (84). NK-92/5.28z cells, engineered HER2-specific NK cells with CD28 and CD3ζ signaling domains, have been demonstrated to have the ability to lyse HER2-positive glioblastoma cells *in vitro* and in orthotopic glioblastoma xenograft NSG mouse models (79).

Breast cancer

As a very common malignancy in female patients, breast cancer is another solid tumor that is studied for CAR-NK-cell immunotherapy. Similar to glioblastoma, HER2, EGFR and EGFRvIII are also targets for breast cancer.

The anti-tumor activity of NK-92/5.28z cells was also evaluated in HER-2-positive breast cancer. Data revealed that tumor cells expressing HER-2 enhanced the proliferation and cytokine release (such as granzyme B, IFN-γ, IL-8 and IL-10) of NK-92/5.28z cells [87]. The modified NK-92 cells displayed

significant cytotoxicity *in vitro* and in xenograft mouse models (85). NK-92 cells engineered to target HER2 developed by Liu et al. also demonstrated similar anti-tumor effects (86).

A second-generation CAR that can recognize both EGFR and EGFRvIII was constructed by Chen et al. (87). NK-92 cells transduced with this CAR showed enhanced cytotoxicity and production of IFN-γ against breast cancer cells. Xenograft mouse models of breast cancer brain metastasis were used for *in vivo* evaluation of anti-tumor activity. CAR-NK-92 cell infusion significantly suppressed tumor growth. Similarly, two EGFR-targeted CAR-NK cells were developed (87). Cytokine release and cytotoxicity assays were performed and revealed that EGFR-CAR NK cells specifically lysed triple-negative breast cancer cells *in vitro* and suppressed breast cancer cell line-derived xenograft and patient-derived xenograft (PDX) tumors in mouse models (87).

Epithelial cell adhesion molecule (EpCAM), tissue factor (TF) and B7-H6 have also been reported as targets for the treatment of breast cancer. Studies have shown the increased tumor killing ability of these CAR-NK-92 cells against breast cancer cells (88–90).

Ovarian cancer

Ovarian cancer is a highly malignant tumor with a 5-year survival rate lower than 40% (115). Several studies have focused on CAR-NK immunotherapies for the treatment of ovarian cancer.

Human leukocyte antigen G (HLA-G) is a tumor-associated antigen (TAA) that is expressed on 40-100% of solid tumors and a limited subset of immune-privileged tissues and adult tissues, such as erythroid precursors and pancreatic islets (116, 117). Jan et al. developed CAR-NK cells targeting HLA-G and evaluated the synergy of CAR-NK cells combined with low-dose chemotherapy (118). Jan et al. developed CAR-NK cells targeting HLA-G and evaluated the synergy of CAR-NK cells combined with low-dose chemotherapy (116). Their study showed that pretreatment with low-dose chemotherapy can induce the overexpression of HLA-G, thus enhancing the anti-tumor cytotoxicity of HLA-G-CAR-NK cells (91).

Since cancer stem cells (CSC) play an important role in metastatic spread and chemoresistance in solid tumors, CSC markers such as CD24, CD44 and CD133 have been explored as specific targets for ovarian cancer immunotherapy (92–94). CAR-NK-92 cells targeting CD24, CD44 or CD133 have shown significant anti-tumor effects in preclinical studies (92–94).

Mesothelin and folate receptor alpha (αFR) are alternative targets that are overexpressed in ovarian cancer. Both iPSC-derived CAR-NK cells and NK-92 cell line-derived CAR-NK cells targeting mesothelin showed robust specific anti-tumor activity both *in vitro* and *in vivo* (95, 96). Ao et al. developed αFR-targeted CAR-NK-92 cells and demonstrated not only their antigen-specific cytotoxicity and proliferation *in vitro* but also their ability to eliminate cancer cells in mouse models (97).

Clinical applications of CAR-NK cells

Since the first CAR-NK-cell clinical trials (NCT00995137, clinicaltrials.gov) started in 2009, there have been 39 studies registered in clinicaltrials.gov evaluating the feasibility, safety and efficacy of CAR-NK cells in the treatment of malignancies. Eight clinical trials sponsored by PersonGen BioTherapeutics and Asclepius Technology Company Group, including NCT02742727, NCT02839954, NCT02892695, NCT02944162, NCT03941457, NCT03931720, NCT03940820 and NCT03940833, which were estimated to be completed in 2018-2019, have been stopped updating for 3 years. It's a pity that no data of these trials were reported till now. The rest of 31 trials were summarized in Table 2.

Similar to CAR-T-cell therapies, most CAR-NK-cell trials target markers on hematopoietic malignancies, such as CD19, CD20, CD22 and BCMA. Notably, there have been eight CAR-NK-cell clinical studies have focused on solid malignancies, which are thought to poorly responsive to CAR-T cells. These CAR-NK cells may target markers such as HER2, NKG2D, mesothelin and PSMA expressed on malignancies, including brain, prostate, ovarian, pancreatic and lung cancers (Table 2).

Discussion

Studies in recent years suggest that CAR-NK-cell therapies may be equally effective as CAR-T-cell therapies. Compared with CAR-T cells, CAR-NK cells have multiple advantages for the treatment of malignancies. CAR-NK-cell therapy seldom causes severe CRS or neurotoxicity. The low associated risk of GVHD and the safety of allogeneic NK-cell infusion shorten the time of cell preparation, which greatly benefits patients with lymphopenia or rapid progression. However, several nonnegligible problems still exist. The best source of NK cells and their *in vitro* expansion strategy, and the most effective signaling domain for CAR activation still need to be elaborated. Antigen escape and tumor heterogeneity, the most common difficulties in immunotherapies, as well as *in vivo* duration, are also problems to be considered. CAR-NK-cell immunotherapy is still in its early stages. Strategies to improve the efficacy and

TABLE 2 Clinical trials for CAR-NK cell immunotherapy.

NO. NCT	Other Name/ID Numbers	States	Start Date	Phase	Disease	Target	Sponsor locations	NK source
NCT00995137	NKCD19 R01CA113482 NCI-2011-01226	Completed in May 2013.	October 2009	I	B-Lineage Acute Lymphoblastic Leukemia	CD19	St. Jude Children's Research Hospital	PB-NK
NCT01974479	NKCARCD19	Suspended for an interim review of (CAR) CD19 research strategy	September 2013	I	B-Lineage Acute Lymphoblastic Leukemia	CD20	National University Health System, Singapore	PB-NK
NCT03056339	2016-0641 NCI-2018-01221	Active, not recruiting Primary results published.(119)	June 21, 2017	I and II	B Lymphoid Malignancies	CD19	M.D. Anderson Cancer Center	UCB-NK
NCT03383978	EudraCT 2016-000225-39	Recruiting	December 1, 2017	I	Glioblastoma	HER2	Johann Wolfgang Goethe University Hospital	NK-92
NCT03415100	NRC-NK-01	Completed Results submitted in February 2021	January 2, 2018	I	Metastatic Solid Tumors	NKG2D	The Third Affiliated Hospital of Guangzhou Medical University	PB-NK
NCT03656705	CNK-101	Enrolling by invitation	September 29, 2018	I	Non-small Cell Lung Carcinoma	PD-1	Xinxiang medical university	NK-92
NCT03692663	TABP EIC-01	Recruiting	December, 2018	Early I	Castration-resistant Prostate Cancer	PSMA	Allife Medical Science and Technology Co., Ltd.	Unknown
NCT03824964	CD19/CD22 CAR NK-BJZL-01	Unknown	February 1, 2019	Early I	Relapsed or Refractory B Cell Lymphoma	CD19/ CD22	Allife Medical Science and Technology Co., Ltd.	Unknown
NCT03692767	CD22 CAR NK-BJZL-01	Unknown	March 2019	Early I	Relapsed and Refractory B Cell Lymphoma	CD22	Allife Medical Science and Technology Co., Ltd.	Unknown
NCT03690310	CD19 CAR NK-BJZL-01	Unknown	March 2019	Early I	Relapsed and Refractory B Cell Lymphoma	CD19	Allife Medical Science and Technology Co., Ltd.	Unknown
NCT03692637	Mesothelin Car NK-HNRM-01	Unknown	March 2019	Early I	Epithelial Ovarian Cancer	Mesothelin	Allife Medical Science and Technology Co., Ltd.	PB-NK
NCT04245722	FT596-101	Recruiting	March 19, 2020	I	B-Cell Lymphoma, Chronic Lymphocytic Leukemia	CD19	Fate Therapeutics	iPSC-NK
NCT04623944	NKX101-101	Recruiting	September 21, 2020	I	Adults With AML or MDS	NKG2D	Nkarta Inc.	PB-NK
NCT05215015	IBR733-T01 WX-IBR-7	Recruiting	November 30, 2020	Early I	Acute Myeloid Leukemia	CD33/ CLL1	Wuxi People's Hospital	Unknown
NCT04639739	CAR NK for NHL	Not yet recruiting	December 17, 2020	Early I	Relapsed or Refractory B Cell Non-Hodgkin Lymphoma	CD19	Xinqiao Hospital of Chongqing	Unknown
NCT04747093	ITNK-2021	Recruiting	January 29, 2021	I and II	B Cell Malignancies	CD19	Nanfang Hospital of Southern Medical University	Induced-T Cell Like NK cells
NCT04796675	CAR-NK-CD19 cells	Recruiting	April 10, 2021	I	B Lymphoid Malignancies	CD19	Wuhan Union Hospital, China	CB
NCT04887012	IR2021002168	Recruiting	May 1, 2021	I	Refractory or Relapsed B-cell Non Hodgkin Lymphoma	CD19	Second Affiliated Hospital, School of Medicine, Zhejiang University	PB-NK
NCT05020678	NKX019-101	Recruiting	August 20, 2021	I	Adults With B-cell Cancers	CD19	Nkarta Inc.	PB-NK
NCT05137275	IBR854-03	Recruiting	November 24, 2021	Early I	Locally Advanced or Metastatic Solid Tumors	5T4	Shanghai East Hospital	Unknown
NCT05008536	BCMA NK for MM	Recruiting	October 1, 2021	Early I	Relapsed or Refractory Multiple Myeloma	BCMA	Xinqiao Hospital of Chongqing	UCB-NK and CB-NK

(Continued)

TABLE 2 Continued

NO. NCT	Other Name/ID Numbers	States	Start Date	Phase	Disease	Target	Sponsor locations	NK source
NCT05247957	CARNK-001	Recruiting	October 13, 2021	I	Relapsed or Refractory Acute Myeloid Leukemia	NKG2D	Hangzhou Cheetah Cell Therapeutics Co., Ltd	UCB-NK
NCT05213195	CARNK-002	Recruiting	December 10, 2021	I	Refractory Metastatic Colorectal Cancer	NKG2D	Zhejiang University	Unknown
NCT04847466	10000096, 000096-C	Recruiting	December 14, 2021	II	Recurrent or Metastatic Gastric or Head and Neck Cancer	PD-L1	National Cancer Institute (NCI)	NK-92
NCT05008575	CD33 CAR NK-AML	Recruiting	December 23, 2021	I	Relapsed or Refractory Acute Myeloid Leukemia	CD33	Xinqiao Hospital of Chongqing	Unknown
NCT05194709	IBR854-T01, WX-IBR-8	Recruiting	December 30, 2021	Early I	Advanced Solid Tumors	5T4	Wuxi People's Hospital	Unknown
NCT05379647	NK-002 (QN-019a)	Recruiting	November 4, 2021	I	B-Cell Malignancies	CD19	Zhejiang University	iPSC-NK
NCT05182073	FT576-101	Recruiting	November 24, 2021	I	Multiple Myeloma	BCMA	Fate Therapeutics	iPSC-NK
NCT05110742	2021-0526	Not yet recruiting	June 30, 2022	I and II	Relapse or Refractory Hematological Malignances	CD5	M.D. Anderson Cancer Center	CB-NK
NCT05092451	2021-0386	Not yet recruiting	August 1, 2022	I and II	Relapse or Refractory Hematological Malignances	CD70	M.D. Anderson Cancer Center	CB-NK
NCT05336409	CNTY-101-111-01	Not yet recruiting	December 2022	I	Relapsed or Refractory CD19-Positive B-Cell Malignancies	CD19	Century Therapeutics, Inc.	iPSC-NK

Allife Medical Science and Technology has just revised the completion date of NCT03692663. As for their other clinical trials, NCT03824964, NCT03692767, NCT03690310 and NCT03692637, we are looking forward to their renewal.

safety of CAR-NK-cell immunotherapy must be further explored in the future.

Author contributions

HL: conceptualization and writing original draft. WS: writing review and editing. ZL: writing review and editing. MZ: conceptualization, supervision, and writing – review and editing. All authors contributed to the article and approved the submitted version.

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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. Wang W, Jiang J, Wu C. CAR-NK for tumor immunotherapy: Clinical transformation and future prospects. *Cancer Lett* (2020) 472:175–80. doi: 10.1016/j.canlet.2019.11.033

2. Feng Y, Liu X, Li X, Zhou Y, Song Z, Zhang J, et al. Novel BCMA-OR-CD38 tandem-dual chimeric antigen receptor T cells robustly control multiple myeloma. *Oncoimmunology* (2021) 10:1959102. doi: 10.1080/2162402X.2021.1959102

3. Rezvani K, Rouce R, Liu E, Shpall E. Engineering natural killer cells for cancer immunotherapy. *Mol Ther* (2017) 25:1769–81. doi: 10.1016/j.ymthe.2017.06.012

4. Grigor EJM, Fergusson D, Kekre N, Montroy J, Atkins H, Seftel MD, et al. Risks and benefits of chimeric antigen receptor T-cell (CAR-T) therapy in cancer: A systematic review and meta-analysis. *Transfus Med Rev* (2019) 33:98–110. doi: 10.1016/j.tmrv.2019.01.005

5. Zhang C, Oberoi P, Oelsner S, Waldmann A, Lindner A, Tonn T, et al. Chimeric antigen receptor-engineered NK-92 cells: An off-the-Shelf cellular therapeutic for targeted elimination of cancer cells and induction of protective antitumor immunity. *Front Immunol* (2017) 8:533. doi: 10.3389/fimmu.2017.00533

6. Shifrin N, Raulet DH, Ardolino M. NK cell self tolerance, responsiveness and missing self recognition. *Semin Immunol* (2014) 26:138–44. doi: 10.1016/j.smim.2014.02.007

7. Rafei H, Daher M, Rezvani K. Chimeric antigen receptor (CAR) natural killer (NK)-cell therapy: leveraging the power of innate immunity. *Br J Haematol* (2021) 193:216–30. doi: 10.1111/bjh.17186

8. Chiossone L, Dumas PY, Vienne M, Vivier E. Natural killer cells and other innate lymphoid cells in cancer. *Nat Rev Immunol* (2018) 18:671–88. doi: 10.1038/s41577-018-0061-z

9. Guillerey C, Huntington ND, Smyth MJ. Targeting natural killer cells in cancer immunotherapy. *Nat Immunol* (2016) 17:1025–36. doi: 10.1038/ni.3518

10. Wu SY, Fu T, Jiang YZ, Shao ZM. Natural killer cells in cancer biology and therapy. *Mol Cancer* (2020) 19:120. doi: 10.1186/s12943-020-01238-x

11. 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) 12:239–52. doi: 10.1038/nri3174

12. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on toll-like receptors. *Nat Immunol* (2010) 11:373–84. doi: 10.1038/ni.1863

13. Chan CJ, Smyth MJ, Martinet L. Molecular mechanisms of natural killer cell activation in response to cellular stress. *Cell Death Differ* (2014) 21:5–14. doi: 10.1038/cdd.2013.26

14. Marofi F, Saleh MM, Rahman HS, Suksatan W, Al-Gazally ME, Abdelbasset WK, et al. CAR-engineered NK cells; a promising therapeutic option for treatment of hematological malignancies. *Stem Cell Res Ther* (2021) 12:374. doi: 10.1186/s13287-021-02462-y

15. Cooper MA, Fehniger TA, Turner SC, Chen KS, Ghaheri BA, Ghayur T, et al. Human natural killer cells: a unique innate immunoregulatory role for the CD56(bright) subset. *Blood* (2001) 97:3146–51. doi: 10.1182/blood.V97.10.3146

16. Campbell JJ, Qin S, Unutmaz D, Soler D, Murphy KE, Hodge MR, et al. Unique subpopulations of CD56+ NK and NK-T peripheral blood lymphocytes identified by chemokine receptor expression repertoire. *J Immunol* (2001) 166:6477–82. doi: 10.4049/jimmunol.166.11.6477

17. Chabannon C, Mfarrej B, Guia S, Ugolini S, Devillier R, Blaise D, et al. Manufacturing natural killer cells as medicinal products. *Front Immunol* (2016) 7:504. doi: 10.3389/fimmu.2016.00504

18. Geiger TL, Sun JC. Development and maturation of natural killer cells. *Curr Opin Immunol* (2016) 39:82–9. doi: 10.1016/j.coi.2016.01.007

19. Mehta RS, Shpall EJ, Rezvani K. Cord blood as a source of natural killer cells. *Front Med (Lausanne)* (2015) 2:93. doi: 10.3389/fmed.2015.00093

20. Martin-Antonio B, Sune G, Perez-Amill L, Castella M, Urbano-Ispizua A. Natural killer cells: Angels and devils for immunotherapy. *Int J Mol Sci* (2017) 18:1868-87. doi: 10.3390/ijms18091868

21. Li L, Chen H, Marin D, Xi Y, Miao Q, Lv J, et al. A novel immature natural killer cell subpopulation predicts relapse after cord blood transplantation. *Blood Adv* (2019) 3:4117–30. doi: 10.1182/bloodadvances.2019000835

22. Oran B, Shpall E. Umbilical cord blood transplantation: a maturing technology. *Hematol Am Soc Hematol Educ Program* (2012) 2012:215–22. doi: 10.1182/asheducation.V2012.1.215.3798291

23. Nomura A, Takada H, Jin CH, Tanaka T, Ohga S, Hara T. Functional analyses of cord blood natural killer cells and T cells: a distinctive interleukin-18 response. *Exp Hematol* (2001) 29:1169–76. doi: 10.1016/S0301-472X(01)00689-0

24. Spanholtz J, Preijers F, Tordoir M, Trilsbeek C, Paardekooper J, de Witte T, et al. Clinical-grade generation of active NK cells from cord blood hematopoietic progenitor cells for immunotherapy using a closed-system culture process. *PLoS One* (2011) 6:e20740. doi: 10.1371/journal.pone.0020740

25. Miller JS, Alley KA, McGlave P. Differentiation of natural killer (NK) cells from human primitive marrow progenitors in a stroma-based long-term culture system: identification of a CD34+7+ NK progenitor. *Blood* (1994) 83:2594–601. doi: 10.1182/blood.V83.9.2594.2594

26. Maki G, Klingemann HG, Martinson JA, Tam YK. Factors regulating the cytotoxic activity of the human natural killer cell line, NK-92. *J Hematother Stem Cell Res* (2001) 10:369–83. doi: 10.1089/152581601750288975

27. Cheng M, Zhang J, Jiang W, Chen Y, Tian Z. Natural killer cell lines in tumor immunotherapy. *Front Med* (2012) 6:56–66. doi: 10.1007/s11684-012-0177-7

28. Suck G, Odendahl M, Nowakowska P, Seidl C, Wels WS, Klingemann HG, et al. NK-92: an 'off-the-shelf therapeutic' for adoptive natural killer cell-based cancer immunotherapy. *Cancer Immunol Immunother* (2016) 65:485–92. doi: 10.1007/s00262-015-1761-x

29. Nowakowska P, Romanski A, Miller N, Odendahl M, Bonig H, Zhang C, et al. Clinical grade manufacturing of genetically modified, CAR-expressing NK-92 cells for the treatment of ErbB2-positive malignancies. *Cancer Immunol Immunother* (2018) 67:25–38. doi: 10.1007/s00262-017-2055-2

30. Tonn T, Becker S, Esser R, Schwabe D, Seifried E. Cellular immunotherapy of malignancies using the clonal natural killer cell line NK-92. *J Hematother Stem Cell Res* (2001) 10:535–44. doi: 10.1089/15258160152509145

31. Uherek C, Tonn T, Uherek B, Becker S, Schnierle B, Klingemann HG, et al. Retargeting of natural killer-cell cytolytic activity to ErbB2-expressing cancer cells results in efficient and selective tumor cell destruction. *Blood* (2002) 100:1265–73. doi: 10.1182/blood.V100.4.1265.h81602001265_1265_1273

32. Arai S, Meagher R, Swearingen M, Myint H, Rich E, Martinson J, et al. Infusion of the allogeneic cell line NK-92 in patients with advanced renal cell cancer or melanoma: a phase I trial. *Cytotherapy* (2008) 10:625–32. doi: 10.1080/14653240802301872

33. Tonn T, Schwabe D, Klingemann HG, Becker S, Esser R, Koehl U, et al. Treatment of patients with advanced cancer with the natural killer cell line NK-92. *Cytotherapy* (2013) 15:1563–70. doi: 10.1016/j.jcyt.2013.06.017

34. Williams BA, Law AD, Routy B, denHollander N, Gupta V, Wang XH, et al. A phase I trial of NK-92 cells for refractory hematological malignancies relapsing after autologous hematopoietic cell transplantation shows safety and evidence of efficacy. *Oncotarget* (2017) 8:89256–68. doi: 10.18632/oncotarget.19204

35. Boyiadzis M, Agha M, Redner RL, Sehgal A, Im A, Hou JZ, et al. Phase 1 clinical trial of adoptive immunotherapy using "off-the-shelf" activated natural killer cells in patients with refractory and relapsed acute myeloid leukemia. *Cytotherapy* (2017) 19:1225–32. doi: 10.1016/j.jcyt.2017.07.008

36. Sadelain M. Chimeric antigen receptors: driving immunology towards synthetic biology. *Curr Opin Immunol* (2016) 41:68–76. doi: 10.1016/j.coi.2016.06.004

37. Chang YH, Connolly J, Shimasaki N, Mimura K, Kono K, Campana D. A chimeric receptor with NKG2D specificity enhances natural killer cell activation and killing of tumor cells. *Cancer Res* (2013) 73:1777–86. doi: 10.1158/0008-5472.CAN-12-3558

38. Topfer K, Cartellieri M, Michen S, Wiedemuth R, Muller N, Lindemann D, et al. DAP12-based activating chimeric antigen receptor for NK cell tumor immunotherapy. *J Immunol* (2015) 194:3201–12. doi: 10.4049/jimmunol.1400330

39. Imamura M, Shook D, Kamiya T, Shimasaki N, Chai SM, Coustan-Smith E, et al. Autonomous growth and increased cytotoxicity of natural killer cells expressing membrane-bound interleukin-15. *Blood* (2014) 124:1081–8. doi: 10.1182/blood-2014-02-556837

40. Liu E, Tong Y, Dotti G, Shaim H, Savoldo B, Mukherjee M, et al. Cord blood NK cells engineered to express IL-15 and a CD19-targeted CAR show long-term persistence and potent antitumor activity. *Leukemia* (2018) 32:520–31. doi: 10.1038/leu.2017.226

41. Schmidt P, Raftery MJ, Pecher G. Engineering NK cells for CAR therapy-recent advances in gene transfer methodology. *Front Immunol* (2020) 11:611163. doi: 10.3389/fimmu.2020.611163

42. Littwitz E, Francois S, Dittmer U, Gibbert K. Distinct roles of NK cells in viral immunity during different phases of acute friend retrovirus infection. *Retrovirology* (2013) 10:127. doi: 10.1186/1742-4690-10-127

43. Matosevic S. Viral and nonviral engineering of natural killer cells as emerging adoptive cancer immunotherapies. *J Immunol Res* (2018) 2018:4054815. doi: 10.1155/2018/4054815

44. Imai C, Iwamoto S, Campana D. Genetic modification of primary natural killer cells overcomes inhibitory signals and induces specific killing of leukemic cells. *Blood* (2005) 106:376–83. doi: 10.1182/blood-2004-12-4797

45. Zayed H, Izsvak Z, Walisko O, Ivics Z. Development of hyperactive sleeping beauty transposon vectors by mutational analysis. *Mol Ther* (2004) 9:292–304. doi: 10.1016/j.ymthe.2003.11.024

46. Rostovskaya M, Fu J, Obst M, Baer I, Weidlich S, Wang H, et al. Transposon-mediated BAC transgenesis in human ES cells. *Nucleic Acids Res* (2012) 40:e150. doi: 10.1093/nar/gks643

47. Xiao L, Cen D, Gan H, Sun Y, Huang N, Xiong H, et al. Adoptive transfer of NKG2D CAR mRNA-engineered natural killer cells in colorectal cancer patients. *Mol Ther* (2019) 27:1114–25. doi: 10.1016/j.ymthe.2019.03.011

48. Heintz N, Gong S. Two-step bacterial artificial chromosome (BAC) engineering: Electroporation of competent BAC host cells with the recombinant shuttle vector. *Cold Spring Harb Protoc* (2020) 2020:098079. doi: 10.1101/pdb.prot098079

49. Hunter BD, Jacobson CA. CAR T-cell associated neurotoxicity: Mechanisms, clinicopathologic correlates, and future directions. *J Natl Cancer Inst* (2019) 111:646–54. doi: 10.1093/jnci/djz017

50. Klingemann H. Are natural killer cells superior CAR drivers? *Oncoimmunology* (2014) 3:e28147. doi: 10.4161/onci.28147

51. Screpanti V, Wallin RP, Ljunggren HG, Grandien A. A central role for death receptor-mediated apoptosis in the rejection of tumors by NK cells. *J Immunol* (2001) 167:2068–73. doi: 10.4049/jimmunol.167.4.2068

52. Wu J, Mishra HK, Walcheck B. Role of ADAM17 as a regulatory checkpoint of CD16A in NK cells and as a potential target for cancer immunotherapy. *J Leukoc Biol* (2019) 105:1297–303. doi: 10.1002/JLB.2MR1218-501R

53. Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, et al. Innate or adaptive immunity? the example of natural killer cells. *Science* (2011) 331:44–9. doi: 10.1126/science.1198687

54. Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, et al. T Cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. *Lancet* (2015) 385:517–28. doi: 10.1016/S0140-6736(14)61403-3

55. Sotillo E, Barrett DM, Black KL, Bagashev A, Oldridge D, Wu G, et al. Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. *Cancer Discovery* (2015) 5:1282–95. doi: 10.1158/2159-8290.CD-15-1020

56. Farag SS, Caligiuri MA. Human natural killer cell development and biology. *Blood Rev* (2006) 20:123–37. doi: 10.1016/j.blre.2005.10.001

57. Romanski A, Uherek C, Bug G, Seifried E, Klingemann H, Wels WS, et al. CD19-CAR engineered NK-92 cells are sufficient to overcome NK cell resistance in b-cell malignancies. *J Cell Mol Med* (2016) 20:1287–94. doi: 10.1111/jcmm.12810

58. Boissel L, Betancur M, Wels WS, Tuncer H, Klingemann H. Transfection with mRNA for CD19 specific chimeric antigen receptor restores NK cell mediated killing of CLL cells. *Leuk Res* (2009) 33:1255–9. doi: 10.1016/j.leukres.2008.11.024

59. Quintarelli C, Sivori S, Caruso S, Carlomagno S, Falco M, Boffa I, et al. Efficacy of third-party chimeric antigen receptor modified peripheral blood natural killer cells for adoptive cell therapy of b-cell precursor acute lymphoblastic leukemia. *Leukemia* (2020) 34:1102–15. doi: 10.1038/s41375-019-0613-7

60. Muller S, Bexte T, Gebel V, Kalensee F, Stolzenberg E, Hartmann J, et al. High cytotoxic efficiency of lentivirally and alpharetrovirally engineered CD19-specific chimeric antigen receptor natural killer cells against acute lymphoblastic leukemia. *Front Immunol* (2019) 10:3123. doi: 10.3389/fimmu.2019.03123

61. Muller T, Uherek C, Maki G, Chow KU, Schimpf A, Klingemann HG, et al. Expression of a CD20-specific chimeric antigen receptor enhances cytotoxic activity of NK cells and overcomes NK-resistance of lymphoma and leukemia cells. *Cancer Immunol Immunother* (2008) 57:411–23. doi: 10.1007/s00262-007-0383-3

62. Oelsner S, Waldmann A, Billmeier A, Roder J, Lindner A, Ullrich E, et al. Genetically engineered CAR NK cells display selective cytotoxicity against FLT3-positive b-ALL and inhibit in vivo leukemia growth. *Int J Cancer* (2019) 145:1935–45. doi: 10.1002/ijc.32269

63. Drent E, Groen RW, Noort WA, Themeli M, Lammerts van Bueren JJ, Parren PW, et al. Pre-clinical evaluation of CD38 chimeric antigen receptor engineered T cells for the treatment of multiple myeloma. *Haematologica* (2016) 101:616–25. doi: 10.3324/haematol.2015.137620

64. Gurney M, Stikvoort A, Nolan E, Kirkham-McCarthy L, Khoruzhenko S, Shivakumar R, et al. CD38 knockout natural killer cells expressing an affinity optimized CD38 chimeric antigen receptor successfully target acute myeloid leukemia with reduced effector cell fratricide. *Haematologica* (2022) 107:437–45. doi: 10.3324/haematol.2020.271908

65. Jiang H, Zhang W, Shang P, Zhang H, Fu W, Ye F, et al. Transfection of chimeric anti-CD138 gene enhances natural killer cell activation and killing of multiple myeloma cells. *Mol Oncol* (2014) 8:297–310. doi: 10.1016/j.molonc.2013.12.001

66. Ng YY, Du Z, Zhang X, Chng WJ, Wang S. CXCR4 and anti-BCMA CAR co-modified natural killer cells suppress multiple myeloma progression in a xenograft mouse model. *Cancer Gene Ther* (2022) 29:475–83. doi: 10.1038/s41417-021-00365-x

67. Chu J, Deng Y, Benson DM, He S, Hughes T, Zhang J, et al. CS1-specific chimeric antigen receptor (CAR)-engineered natural killer cells enhance in vitro and *in vivo* antitumor activity against human multiple myeloma. *Leukemia* (2014) 28:917–27. doi: 10.1038/leu.2013.279

68. Chen KH, Wada M, Firor AE, Pinz KG, Jares A, Liu H, et al. Novel anti-CD3 chimeric antigen receptor targeting of aggressive T cell malignancies. *Oncotarget* (2016) 7:56219–32. doi: 10.18632/oncotarget.11019

69. Chen KH, Wada M, Pinz KG, Liu H, Lin KW, Jares A, et al. Preclinical targeting of aggressive T-cell malignancies using anti-CD5 chimeric antigen receptor. *Leukemia* (2017) 31:2151–60. doi: 10.1038/leu.2017.8

70. You F, Wang Y, Jiang L, Zhu X, Chen D, Yuan L, et al. A novel CD7 chimeric antigen receptor-modified NK-92MI cell line targeting T-cell acute lymphoblastic leukemia. *Am J Cancer Res* (2019) 9:64–78.

71. Pende D, Rivera P, Marcenaro S, Chang CC, Biassoni R, Conte R, et al. Major histocompatibility complex class I-related chain a and UL16-binding protein expression on tumor cell lines of different histotypes: analysis of tumor susceptibility to NKG2D-dependent natural killer cell cytotoxicity. *Cancer Res* (2002) 62:6178–86.

72. Watson NF, Spendlove I, Madjd Z, McGilvray R, Green AR, Ellis IO, et al. Expression of the stress-related MHC class I chain-related protein MICA is an indicator of good prognosis in colorectal cancer patients. *Int J Cancer* (2006) 118:1445–52. doi: 10.1002/ijc.21510

73. Carlsten M, Bjorkstrom NK, Norell H, Bryceson Y, van Hall T, Baumann BC, et al. DNAX accessory molecule-1 mediated recognition of freshly isolated ovarian carcinoma by resting natural killer cells. *Cancer Res* (2007) 67:1317–25. doi: 10.1158/0008-5472.CAN-06-2264

74. Friese MA, Platten M, Lutz SZ, Naumann U, Aulwurm S, Bischof F, et al. MICA/NKG2D-mediated immunogene therapy of experimental gliomas. *Cancer Res* (2003) 63:8996–9006.

75. Carbone E, Neri P, Mesuraca M, Fulciniti MT, Otsuki T, Pende D, et al. HLA class I, NKG2D, and natural cytotoxicity receptors regulate multiple myeloma cell recognition by natural killer cells. *Blood* (2005) 105:251–8. doi: 10.1182/blood-2004-04-1422

76. Leivas A, Valeri A, Cordoba L, Garcia-Ortiz A, Ortiz A, Sanchez-Vega L, et al. NKG2D-CAR-transduced natural killer cells efficiently target multiple myeloma. *Blood Cancer J* (2021) 11:146. doi: 10.1038/s41408-021-00537-w

77. Du Z, Ng YY, Zha S, Wang S. piggyBac system to co-express NKG2D CAR and IL-15 to augment the *in vivo* persistence and anti-AML activity of human peripheral blood NK cells. *Mol Ther Methods Clin Dev* (2021) 23:582–96. doi: 10.1016/j.omtm.2021.10.014

78. Daher M, Melo Garcia L, Li Y, Rezvani K. CAR-NK cells: the next wave of cellular therapy for cancer. *Clin Transl Immunol* (2021) 10:e1274. doi: 10.1002/cti2.1274

79. Zhang C, Burger MC, Jennewein L, Genssler S, Schonfeld K, Zeiner P, et al. ErbB2/HER2-specific NK cells for targeted therapy of glioblastoma. *J Natl Cancer Inst* (2016) 108:djv375. doi: 10.1093/jnci/djv375

80. Han J, Chu J, Keung Chan W, Zhang J, Wang Y, Cohen JB, et al. CAR-engineered NK cells targeting wild-type EGFR and EGFRvIII enhance killing of glioblastoma and patient-derived glioblastoma stem cells. *Sci Rep* (2015) 5:11483. doi: 10.1038/srep11483

81. Murakami T, Nakazawa T, Natsume A, Nishimura F, Nakamura M, Matsuda R, et al. Novel human NK cell line carrying CAR targeting EGFRvIII induces antitumor effects in glioblastoma cells. *Anticancer Res* (2018) 38:5049–56. doi: 10.21873/anticanres.12824

82. Nakazawa T, Murakami T, Natsume A, Nishimura F, Morimoto T, Matsuda R, et al. KHYG-1 cells with EGFRvIII-specific CAR induced a pseudoprogression-like feature in subcutaneous tumors derived from glioblastoma-like cells. *Anticancer Res* (2020) 40:3231–7. doi: 10.21873/anticanres.14304

83. Muller N, Michen S, Tietze S, Topfer K, Schulte A, Lamszus K, et al. Engineering NK cells modified with an EGFRvIII-specific chimeric antigen receptor to overexpress CXCR4 improves immunotherapy of CXCL12/SDF-1alpha-secreting glioblastoma. *J Immunother* (2015) 38:197–210. doi: 10.1097/CJI.0000000000000082

84. Genssler S, Burger MC, Zhang C, Oelsner S, Mildenberger I, Wagner M, et al. Dual targeting of glioblastoma with chimeric antigen receptor-engineered natural killer cells overcomes heterogeneity of target antigen expression and enhances antitumor activity and survival. *Oncoimmunology* (2016) 5:e1119354. doi: 10.1080/2162402X.2015.1119354

85. Schonfeld K, Sahm C, Zhang C, Naundorf S, Brendel C, Odendahl M, et al. Selective inhibition of tumor growth by clonal NK cells expressing an ErbB2/HER2-specific chimeric antigen receptor. *Mol Ther* (2015) 23:330–8. doi: 10.1038/mt.2014.219

86. Liu H, Yang B, Sun T, Lin L, Hu Y, Deng M, et al. Specific growth inhibition of ErbB2expressing human breast cancer cells by genetically modified NK92 cells. *Oncol Rep* (2015) 33:95–102. doi: 10.3892/or.2014.3548

87. Liu Y, Zhou Y, Huang KH, Fang X, Li Y, Wang F, et al. Targeting epidermal growth factor-overexpressing triple-negative breast cancer by natural killer cells expressing a specific chimeric antigen receptor. *Cell Prolif* (2020) 53:e12858. doi: 10.1111/cpr.12858

88. Sahm C, Schonfeld K, Wels WS. Expression of IL-15 in NK cells results in rapid enrichment and selective cytotoxicity of gene-modified effectors that carry a tumor-specific antigen receptor. *Cancer Immunol Immunother* (2012) 61:1451–61. doi: 10.1007/s00262-012-1212-x

89. Hu Z. Tissue factor as a new target for CAR-NK cell immunotherapy of triple-negative breast cancer. *Sci Rep* (2020) 10:2815. doi: 10.1038/s41598-020-59736-3

90. Lin YZ, Lee CC, Cho DY, Wang YL, Chen CY, Weng CY, et al. Suppression of breast cancer cells resistant to a pure anti-estrogen with CAR-transduced natural killer cells. *Am J Cancer Res* (2021) 11:4455–69.

91. Jan CI, Huang SW, Canoll P, Bruce JN, Lin YC, Pan CM, et al. Targeting human leukocyte antigen G with chimeric antigen receptors of natural killer cells convert immunosuppression to ablate solid tumors. *J Immunother Cancer* (2021) 9: e003050. doi: 10.1136/jitc-2021-003050

92. Klapdor R, Wang S, Morgan M, Dork T, Hacker U, Hillemanns P, et al. Characterization of a novel third-generation anti-CD24-CAR against ovarian cancer. *Int J Mol Sci* (2019) 20:660-74. doi: 10.3390/ijms20030660

93. Klapdor R, Wang S, Morgan MA, Zimmermann K, Hachenberg J, Buning H, et al. NK cell-mediated eradication of ovarian cancer cells with a novel chimeric antigen receptor directed against CD44. *Biomedicines* (2021) 9:1339-52. doi: 10.3390/biomedicines9101339

94. Klapdor R, Wang S, Hacker U, Buning H, Morgan M, Dork T, et al. Improved killing of ovarian cancer stem cells by combining a novel chimeric antigen receptor-based immunotherapy and chemotherapy. *Hum Gene Ther* (2017) 28:886–96. doi: 10.1089/hum.2017.168

95. 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

96. Cao B, Liu M, Wang L, Liang B, Feng Y, Chen X, et al. Use of chimeric antigen receptor NK-92 cells to target mesothelin in ovarian cancer. *Biochem Biophys Res Commun* (2020) 524:96–102. doi: 10.1016/j.bbrc.2020.01.053

97. Ao X, Yang Y, Li W, Tan Y, Guo W, Ao L, et al. Anti-alphaFR CAR-engineered NK-92 cells display potent cytotoxicity against alphaFR-positive ovarian cancer. *J Immunother* (2019) 42:284–96. doi: 10.1097/CJI.0000000000000286

98. Bahr O, Herrlinger U, Weller M, Steinbach JP. Very late relapses in glioblastoma long-term survivors. *J Neurol* (2009) 256:1756–8. doi: 10.1007/s00415-009-5167-6

99. Debinski W, Gibo DM, Hulet SW, Connor JR, Gillespie GY. Receptor for interleukin 13 is a marker and therapeutic target for human high-grade gliomas. *Clin Cancer Res* (1999) 5:985–90.

100. Bigner SH, Humphrey PA, Wong AJ, Vogelstein B, Mark J, Friedman HS, et al. Characterization of the epidermal growth factor receptor in human glioma cell lines and xenografts. *Cancer Res* (1990) 50:8017–22.

101. Ekstrand AJ, Sugawa N, James CD, Collins VP. Amplified and rearranged epidermal growth factor receptor genes in human glioblastomas reveal deletions of sequences encoding portions of the n- and/or c-terminal tails. *Proc Natl Acad Sci U.S.A.* (1992) 89:4309–13. doi: 10.1073/pnas.89.10.4309

102. Koka V, Potti A, Forseen SE, Pervez H, Fraiman GN, Koch M, et al. Role of her-2/neu overexpression and clinical determinants of early mortality in glioblastoma multiforme. *Am J Clin Oncol* (2003) 26:332–5. doi: 10.1097/01.COC.0000020922.66984.E7

103. Newman JP, Wang GY, Arima K, Guan SP, Waters MR, Cavenee WK, et al. Interleukin-13 receptor alpha 2 cooperates with EGFRvIII signaling to promote glioblastoma multiforme. *Nat Commun* (2017) 8:1913. doi: 10.1038/s41467-017-01392-9

104. Nishikawa R, Ji XD, Harmon RC, Lazar CS, Gill GN, Cavenee WK, et al. A mutant epidermal growth factor receptor common in human glioma confers enhanced tumorigenicity. *Proc Natl Acad Sci U.S.A.* (1994) 91:7727–31. doi: 10.1073/pnas.91.16.7727

105. Nagane M, Levitzki A, Gazit A, Cavenee WK, Huang HJ. Drug resistance of human glioblastoma cells conferred by a tumor-specific mutant epidermal growth factor receptor through modulation of bcl-XL and caspase-3-like proteases. *Proc Natl Acad Sci U.S.A.* (1998) 95:5724–9. doi: 10.1073/pnas.95.10.5724

106. Hynes NE, MacDonald G. ErbB receptors and signaling pathways in cancer. *Curr Opin Cell Biol* (2009) 21:177–84. doi: 10.1016/j.ceb.2008.12.010

107. Krenciute G, Prinzing BL, Yi Z, Wu MF, Liu H, Dotti G, et al. Transgenic expression of IL15 improves antiglioma activity of IL13Ralpha2-CAR T cells but results in antigen loss variants. *Cancer Immunol Res* (2017) 5:571–81. doi: 10.1158/2326-6066.CIR-16-0376

108. Pituch KC, Miska J, Krenciute G, Panek WK, Li G, Rodriguez-Cruz T, et al. Adoptive transfer of IL13Ralpha2-specific chimeric antigen receptor T cells creates a pro-inflammatory environment in glioblastoma. *Mol Ther* (2018) 26:986–95. doi: 10.1016/j.ymthe.2018.02.001

109. Yin Y, Boesteanu AC, Binder ZA, Xu C, Reid RA, Rodriguez JL, et al. Checkpoint blockade reverses anergy in IL-13Ralpha2 humanized scFv-based CAR T cells to treat murine and canine gliomas. *Mol Ther Oncolytics* (2018) 11:20–38. doi: 10.1016/j.omto.2018.08.002

110. Xu C, Bai Y, An Z, Hu Y, Zhang C, Zhong X. IL-13Ralpha2 humanized scFv-based CAR-T cells exhibit therapeutic activity against glioblastoma. *Mol Ther Oncolytics* (2022) 24:443–51. doi: 10.1016/j.omto.2022.01.002

111. Thokala R, Binder ZA, Yin Y, Zhang L, Zhang JV, Zhang DY, et al. High-affinity chimeric antigen receptor with cross-reactive scFv to clinically relevant EGFR oncogenic isoforms. *Front Oncol* (2021) 11:664236. doi: 10.3389/fonc.2021.664236

112. Abbott RC, Verdon DJ, Gracey FM, Hughes-Parry HE, Iliopoulos M, Watson KA, et al. Novel high-affinity EGFRvIII-specific chimeric antigen receptor T cells effectively eliminate human glioblastoma. *Clin Transl Immunol* (2021) 10: e1283. doi: 10.1002/cti2.1283

113. Agliardi G, Liuzzi AR, Hotblack A, De Feo D, Nunez N, Stowe CL, et al. Intratumoral IL-12 delivery empowers CAR-T cell immunotherapy in a pre-clinical model of glioblastoma. *Nat Commun* (2021) 12:444. doi: 10.1038/s41467-020-20599-x

114. Ahmed N, Salsman VS, Kew Y, Shaffer D, Powell S, Zhang YJ, et al. HER2-specific T cells target primary glioblastoma stem cells and induce regression of autologous experimental tumors. *Clin Cancer Res* (2010) 16:474–85. doi: 10.1158/1078-0432.CCR-09-1322

115. Huang X, Qiu M, Wang T, Li B, Zhang S, Zhang T, et al. Carrier-free multifunctional nanomedicine for intraperitoneal disseminated ovarian cancer therapy. *J Nanobiotechnol* (2022) 20:93. doi: 10.1186/s12951-022-01300-4

116. Menier C, Rabreau M, Challier JC, Le Discorde M, Carosella ED, Rouas-Freiss N. Erythroblasts secrete the nonclassical HLA-G molecule from primitive to definitive hematopoiesis. *Blood* (2004) 104:3153–60. doi: 10.1182/blood-2004-03-0809

117. Cirulli V, Zalatan J, McMaster M, Prinsen R, Salomon DR, Ricordi C, et al. The class I HLA repertoire of pancreatic islets comprises the nonclassical class ib antigen HLA-G. *Diabetes* (2006) 55:1214–22. doi: 10.2337/db05-0731

118. Anna F, Bole-Richard E, LeMaoult J, Escande M, Lecomte M, Certoux JM, et al. First immunotherapeutic CAR-T cells against the immune checkpoint protein HLA-G. *J Immunother Cancer* (2021) 9:e001998. doi: 10.1136/jitc-2020-001998

119. 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

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Effects of gut microbiota on immune responses and immunotherapy in colorectal cancer

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Accumulating evidence suggests that gut microbial dysbiosis is implicated in colorectal cancer (CRC) initiation and progression through interaction with host immune system. Given the intimate relationship between the gut microbiota and the antitumor immune responses, the microbiota has proven to be effective targets in modulating immunotherapy responses of preclinical CRC models. However, the proposed putative mechanisms of how these bacteria affect immune responses and immunotherapy efficacy remains obscure. In this review, we summarize recent findings of clinical gut microbial dysbiosis in CRC patients, the reciprocal interactions between gut microbiota and the innate and/or the adaptive immune system, as well as the effect of gut microbiota on immunotherapy response in CRC. Increased understanding of the gut microbiota-immune system interactions will benefit the rational application of microbiota to the clinical promising biomarker or therapeutic strategy as a cancer immunotherapy adjuvant.

KEYWORDS

colorectal cancer, gut microbiota, inflammation, immune response, immunotherapy

Introduction

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death with more than 1.9 million new cases and 935,000 deaths estimated to occur in 2020 worldwide (1). Although risk factors are recognized as western dietary patterns, excess body weight, and lifestyle factors including heavy alcohol consumption and cigarette smoking, the specific underlying pathogenetic mechanisms are still not fully-elucidated.

The gut microbiota has been discussed widely over the past decades which plays an important role in all the different phases of CRC process from oncogenesis to metastasis, from treatment to prognosis prediction (2, 3). Investigators have identified specific microbial features relevant to CRC, data from cross-sectional epidemiological studies and

unbiased microbiome profiling of stools and colorectal tissues have uncovered specific bacterial taxa that contribute to CRC (4). Mechanistic insights into a microbe's contributions to carcinogenesis support that gut microbiota alterations induce genotoxin production, inflammation, metabolic regulation, and local and systemic immune response, thereby influencing the development of CRC (5). Immune escape or suppression has long been proposed to constitute a critical step in both tumor formation and progression (6). Studies have highlighted that intact mucosal immunity maintains a balanced bacterial composition in the gut, whereas disruption of this immunological circuit, either prior to, or as a consequence of tumor development, accelerates CRC initiation and progression (7, 8). In addition, some specific bacterial taxa are shown to regulate immunotherapy responses in both animal models and human cohorts (9–12). In this sense, researchers are focusing on the deep and complex relationship between microbiota and immune regulation to better understand cancer biology and to formulate novel therapeutic approach. In this review, we outline the clinical findings of changes in gut microbiota composition in CRC patients, highlight potential mechanisms of gut microbiota-modulated immune responses and the possible impacts on responses to immunotherapy in CRC.

Clinical findings of microbial changes in CRC patients

Next-generation sequencing studies have revealed the potential association of microbial compositional changes (dysbiosis) within CRC patients, including increased proportions of *Fusobacterium nucleatum*, *Bacteroides fragilis*, *Escherichia coli*, and *Streptococcus*, *Peptostreptococcus* species (13–15). These findings raised the possibilities for clinical applications using gut microbiota analysis as screening, prognostic or predictive biomarkers. Moreover, deciphering key microbiome signatures within different stages of cancer progression may offer possibilities for treatment stratification and metastasis surveillance (16). The gut microbiota changes across all stages of CRC patients were summarized in Table 1. As indicated, dynamic shifts in microbial composition in gut microbiota were observed during multistep CRC progression.

Gut microbiota elicits tumor-promoting inflammation

It is well-known that chronic inflammation is a risk for CRC initiation and development. Overall, 2.2 million new cancer cases were attributable to infections by different etiological agents, including viruses, bacteria and parasites in 2018, representing 13% of all cancer cases (28). The bacterium enterotoxigenic *Bacteroides fragilis* (ETBF) is a significant source of chronic inflammation and has been implicated as a risk factor for CRC, which can up-regulate spermine oxidase (SMO)-dependent generation of reactive oxygen species (ROS) and induce inflammation, leading to DNA damage in colonic epithelial cells (29). Diverse cytokines like tumor necrosis factor (TNF)α, interlukin (IL)-6, IL-1β, IL-23, and IL-17, can be triggered by microbes or their products, contribute to the progression of intestinal tumorigenesis (30). Upon invading the stroma, bacteria can trigger both innate responses *via* recognition through pattern recognition receptors (PRRs), eliciting secretion of a repertoire of cytokines and chemokines. *Streptococcus gallolyticus* has long been associated with colonic pathologies. Although a causal relationship to CRC is not clear, increased production of inflammatory factors, including cyclooxygenase (COX)-2, IL-1 and IL-8, in *Streptococcus gallolyticus*-bearing tumor tissue might indicate its possible contribution to tumor progression (31). *Fusobacterium nucleatum* has been reported to drive a pro-inflammatory intestinal microenvironment through metabolite receptor -dependent modulation of IL-17 expression in $Apc^{min/+}$ mice (32). *Fusobacterium nucleatum* colonization leads to increased intestinal short chain fatty acid (SCFA) levels and human CRC tissues harboring *Fusobacterium nucleatum* are primed to sense these immunomodulatory metabolites, with higher expression of the genes encoding the SCFA receptors *FFAR2* and *NIACR1*. Furthermore, in $Ffar2^{-/-}$ mice, *Fusobacterium nucleatum* failed to increase Th17 cell frequency, suggesting that *Fusobacterium nucleatum* shaped Th17 response is FFAR2-dependent (32). The inflammasome NACHT, LRR, and PYD domains-containing protein 3 (NLRP3) is a global sensor of pathogen-associated molecular pattern molecules (PAMPs) and damage-associated molecular patterns (DAMPs) and its activation leads to secretion of both IL-1β and IL-18. Studies have provided evidence to indicate that the gut microbiota serve as critical modulators of inflammasome activity and susceptibility to the development of intestinal inflammation and cancer (33). Indeed, the presence of some of the cytokines (IL-17, IL-6, IL-1β, TNFα) also correlate with poor prognosis in CRC patients (34). The chronic activation of inflammatory signals not only suppresses adaptive immune responses but simultaneously supports tumor growth, *via* mechanisms such as the increased release of growth and immunomodulatory factors (35). For example, tumor infiltrating myeloid cells release growth factors, such as epidermal growth factor (EGF), hepatocyte growth factor (HGF), and fibroblast growth factor (FGF) (36, 37). These growth factors can support cancer cell proliferation, survival, motility, and invasion, by triggering the activation of MAPK, Wnt/β-catenin or PI3K/AKT/mTOR signaling pathway, thus facilitate CRC progression (38). The continuation of inflammatory responses and tumor progression to malignancy on the one hand, changes the composition of immune cells from immune activators to immune suppressors, on the other hand, potential cytokines and chemokines facilitate the recruitment of immune cells with

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TABLE 1 Summary of microbial changes across stages of CRC.

References	Specimens	Sample size	Stage I	Stage II	Stage III	Stage IV
(15)	Stool	Healthy (n = 251) MP (n = 67) S0 (n = 73) SI/II (n = 111) SIII/IV (n = 74)	↑ *Gemella morbillorum*		↑ *Fusobacterium nucleatum*, *Colinsella aerofaciens*, *Dorea longicatena*, *Porphyromonas uenonis*, *Selenomonas sputigena*, *Streptococcus anginosus*, *Desulfovibrio vietnamensis*, *Bilophila wadsworthia*	
(16)	Tissue	Healthy (n=61) Adenoma (n=47) Carcinoma (n=52)	↑ *Fusobacterium*, *Parvimonas*, *Gemella*, *Leptotrichia* ↓ *Bacteroides*, *Blautia*, *Faecalibacterium prausnitzii*, *Sutterella*, *Collinsella aerofaciens*, *Alistipes putredinis*		—	—
(17)	Stool	Healthy (n=358) Adenoma (n=42) S0-II (n=47) SIII-IV (n=44)	↑ *Fusobacterium nucleatum*, *Peptostreptococcus stomatis*, *Porphyromonas asaccharolytica*; ↓ *Eubacterium Rectale*, *Eubacterium eligens*, *Streptococcus salivarius*		—	—
(18)	Stool	Healthy (n=45) CRC (n=53)	↑ *Enterobacteriaceae*, *Fusobacterium nucleatum*; ↓ *Lactobacillus*, *Bifidobacterium*, *Clostridium cluster* I		—	—
(19)	Tissue	SI (n=7) SII (n=37) SII (n=31)	↑ *Peptostreptococcus*, *Parvimonas*	↑ *Fusobacterium*, *Streptococcus*, *Parvimonas*, *Burkholderiales*, *Caulobacteraceae*, *Delftia*, *Oxalobacteraceae*	↑ *Fusobacterium*, *Burkholderiales*, *Caulobacteraceae*, *Oxalobacteraceae*, *Faecalibacterium*, *Sutterella*	—
(20)	Tissue Stool	S0 (n=8) SI-II (n=97) SIII-IV (n=73)	—	—	↑ *Peptoclostridiu*, *Akkermansia*; ↓ *Gelria*	
(21)	Tissue	Dysplasia (n=3) Adenocarcinoma (n=15)	—	—	↑ *Fusobacteria*, *β-Proteobacteria*	
(22)	Stool	Healthy (n=178) SIII-IV (n=74)	—	—	↑ *Fusobacterium nucleatum*, *Peptostreptococcus stomatis*, *Gemella morbillorum*, *Parvimonas micra*	
(23)	Stool	Healthy (n=187) SI-II (n=74) SIII-IV (n=63)	—	↑ *Fusobacterium nucleatum*, *Parvimonas micra*		
(24)	Tissue	SI-II (n=12) SIII-IV (n=11)	↑ *Escherichia*, *Halomonas*, *Shewanella*, *Granulicatella*, *Lactobacillus*		↑ *Bacteroides*, *Prevotella*, *Peptostreptococcus*, *Streptococcus*, *Ruminococcus*, *Fusobacterium*, *Akkermansia*; ↓ *Granulicatella*, *Lactobacillus*	
(25)	Tissue	SI-II (n=32) SIII-IV (n=48)	↑ *Dietzia*, *Paludibacter*, *Porphyromonadaceae*, *Propionibacterium*		↑ *Granulicatella*, *Coprococcus*, *Phycisphaeraceae*	
(26)	Stool	Healthy (n=30) SI-II (n=19) SIII-IV (n=23)	↑ *Peptostreptococcus*, *Collinsella* *Ruminococcus*, *Parvimonas*, *Peptostreptococcus*	↑ *Hydrogenoanaerobacterium*	↑ *Akkermansia*	↑ *Phascolarctobacterium*, *Parasutterella*, *Comamonas*, *Cloacibacillus*, *Olsenella*; ↓ *Escherichia-Shigella*, *Alistipes*, *Blautia*, *Eisenbergiella*, *Intestinimonas*, *Eggerthella*, *Anaeroglobus*
(27)	Stool	Healthy (n=91) SI-II (n=39) SIII-IV (n=32)	↑ *Klebsiella quasipneumoniae*, *Klebsiella oxytoca*, *Klebsiella variicola*		↑ *Faecalibacterium prausnitzii*, *Bacillus cereus*, *Lactococcus species*	

MP, multiple polypoid adenomas with lowgrade dysplasia; S0, intramucosal carcinoma, stage 0/pTis CRC; SI, stage I CRC; SII: stage II CRC; SIII, stage III CRC; SIV, stage IV CRC. ↑, increase in the abundance; ↓, decrease in the abundance.

immunosuppressive functions, such as myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) (39), which eventually maintains tumor cell survival and immune escape (discussed below). In addition, chronic inflammation is now accepted as a major influence in the outcome of CRC, treatment with anti-inflammatory (such as aspirin) mitigates CRC progression and extends patient survival (40).

Gut dysbiosis facilitates immune escape in CRC

Here, we outline the potential mechanisms of microbial-modulated immune escape from innate and adaptive immune perspectives and discuss dysbiosis-immune interaction during CRC progression.

Modulating innate immune surveillance against tumors

The immune system has an extraordinary capacity to recognize and respond to a range of microbial patterns and danger signals. The first cells to fight microbes are the myeloid derived innate immune cells (41). Bacterial species that translocate through the epithelial barrier induce recruitment of myeloid cells. Through their PPRs, these cells recognize microbes, thus influencing the type and intensity of innate and adaptive immunity (42). Dysbiosis can enhance gut responsiveness to bacteria and its products, however during chronic infections, it may lead to a miscoordination between inflammation and immune suppression, thus favors tumor growth. In fact, an important feature of tumors is the generation, development, and expansion of myeloid cells with special immunosuppressive properties including tumor-associated neutrophils (TANs), tumor-associated macrophages (TAMs), regulatory dendritic cells (DCs), and MDSCs (43). For example, *Fusobacterium* promotes the growth of colorectal cancer through the induction of the activity of MDSCs and TAMs (44, 45). Tumor invading pathogen bacteria also trigger the activation of neutrophils that infiltrate into tumor stroma, whereby the earliest tumor-infiltrating neutrophils may serve to inhibit expansion of colon microbiota to limit tumorigenesis and progression (46–48). However, established tumors may evolve a more pro-tumorigenic TAN phenotype and elicit the production of tumor-promoting cytokines (49). Neutrophils can also produce 'neutrophil extracellular traps' (NETs), upon activation to ensnare and neutralize pathogens. Recent studies highlight the function of NETs in cancer progression and metastasis, NETs are able to wake up dormant cancer cells promoting cancer relapse, and are able to entrap circulating cancer, thus enhancing metastasis spread (50). Bacteria derivations also play a role in escalating the tumor-associated immune suppression. Tryptophan-derived microbial metabolites activate the aryl hydrocarbon receptor in TAMs to suppress anti-tumor immunity (51). Gut microbiota tend to produce butyrate, which in turn can inhibit the DCs' antigen presentation (52, 53). Beyaz et al. showed that high fat diets (HFDs) resulted in changes in the composition of the gut microbiota (54), and in a Kras-driven mouse model of intestinal cancer, HFD-altered gut microbiome, which, in turn, resulted in reduced major histocompatibility complex (MHC) II expression on DCs and engages in immune evasion (55), suggesting that dietary in association with the gut microbiota, are critical modulators to the development of intestinal cancer. In addition to the aforementioned myeloid cells, another example is the inhibitory effect of *Fusobacterium nucleatum* on natural killer (NK) cells. *Fusobacterium nucleatum* produces the Fap2 protein, which binding to the inhibitory receptor T cell immunoglobulin and ITIM domain (TIGIT) on NK cells, thus directly inhibiting cell-killing of tumor cells (56). Yet, we have very little insight into the roles of microbiota on innate immune cell populations, further studies are needed to interrogate the precise functional contributions of gut microbe on these innate immune cell subsets.

Reprogramming adaptive anti-tumoral responses

With improved tools, recent work has suggested two broad categories of bacteria-related tumor escape of adaptive immune attack: 1. Microbes influence anti-tumor effectors directly by serving as antigens which mediate recognition by host T cells (57, 58), 2. Microbes facilitate the resistance of immune attack through the immune suppressive pathways such as inducing immune exhaustion (59, 60).

Intestinal microbiota has been proposed to induce commensal-specific memory T cells that cross-react with tumor-associated antigens. Indeed, memory responses by CD4+ and CD8+ T cells specific for *Enterococcus hirae*, *Bacteroides fragilis*, and *Akkermansia muciniphila* are associated with favorable clinical outcome in cancer patients (11, 57, 58), suggesting that microbe-specific T lymphocytes may contribute to anti-tumoral immune responses. The optimal recognition of the antigen induces a specific activation of T cells, thereby driving T cell activation and differentiation of CD4+T cell subsets into Th1, Th2, and Th17 or Tregs (61, 62). It should be noted that dysbiosis induced T cells are capable of switching their phenotypes, which in turn set the proclivity to inflammatory, immunostimulatory or immunosuppressive reactions depending on tumor context and specific bacteria. The modulation by distinctive microbiome antigens can also consequently affect the activation of cytotoxic CD8+ T cells that limit the direct lysis of cancer cells (63).

In cancer, like in chronic infection, the long exposure to the antigen leads to a dysfunction of T cells, represents the state of "exhaustion" (64, 65). In the early stage of azoxymethane (AOM)/dextran sulfate sodium (DSS) mice model, gut dysbiosis (increased *Prevotellaceae* and decreased *Anaeroplasmataceae*) promoted tumorigenesis by stimulating CD8+ T cells activation, durable hyperstimulation of CD8+ T cells resulted in T cell exhaustion, leading to increased tumor susceptibility (66). Microbiota can also provoke sustained expression of the inhibitory molecules, such as cytotoxic T lymphocyte antigen 4 (CTLA-4), T cell immunoglobulin and mucin domain-containing protein 3 (TIM3), programmed cell –death protein 1 (PD–1), or the ligand PD-L1, which are the most prominent examples of immune–checkpoint molecules underlying immune–escape mechanisms (60). The FAS/FAS ligand (FASL) apoptotic pathway is also highly relevant to immune evasion, which induces apoptosis of lymphocytes

(67). Histopathological analyses have revealed that FASL is upregulated in metastatic tumor compared the primary tumor in patients with CRC (68). Thus, simultaneous loss or downregulation of FAS and upregulation of FASL on tumor cells might contribute to tumor evasion of immune–mediated cytolysis. The microbiota-derived SCFAs, such as butyrate, can be absorbed across the intestinal epithelium and exert their influence on T cells *via* G-protein-coupled receptors (GPRs). Butyrate was shown to promote cellular metabolism, enhance memory potential of activated CD8+ T cells through promoting mitochondrial function and cellular metabolism (69). Taken together, these findings reveal a role for the microbiota in the modulation of T cell responses in CRC which may have important implications on immunotherapy.

As another important adaptive immune cell type, B cells perform immune surveillance as antigen presenting cells (APCs) or function by stimulating immunoglobulins (Ig A) and producing cytokines (IL-10, TGFβ, often terms as regulatory B cells, Bregs) (70). Changes in gut microbiota composition and a diverse role of B cells have been implicated at the mucosal interface. *Helicobacter hepaticus* colonization has been shown to relieve tumor burden in CRC mice and increase B cells maturation and infiltration (71). *Parvimonas micra* was also shown to be closely associated with the antigen-presenting HLA-DR (+) B cells in a CRC cohort (72). In another study of familial adenomatous polyposis (FAP), loss of resident memory T cells and γδ T cells, excess IgA antibody secretion and increased IgA+ peripheral B cells were found to accompany intestinal microbial dysbiosis, implicating mucosal immune dysfunction as a contributing factor in the etiology of CRC (73). Above studies highlighted the interactions between microbiota and B cells in CRC, but the underlying mechanism remains largely unresolved. Some recent reports have shed light on the microbial metabolites, SCFAs and microbial tryptophan catabolites to regulate B cell activation and antibody responses (74–76). More recently, Wang et al. found that leucine-tRNA-synthetase-2 (LARS2)-expressing B cell (LARS B) with TGF-β1-dominant feature correlates with shortened survival in CRC, mechanistically, LARS2 programmed mitochondrial nicotinamide adenine dinucleotide (NAD+) regeneration and oxidative metabolism, thus determining the regulatory feature of LARS B cells in which the NAD-dependent protein deacetylase sirtuin-1 (SIRT1) was involved (77).

Overall, the immune impact of gut microbiota on CRC partially depends on shaping innate and adaptive immune responses (e.g., suppressing immunosurveillance, inducing T cell exhaustion and apoptosis, etc.), consequently leading to immune escape (Figure 1). In future, clarification of possible role of microbiota in modulation of populations and functions of innate and adaptive immune cells, as well as the crosstalk between different types of immune cells remain important research areas.

Role of gut microbiota in shaping an immune-privileged (pre-) metastatic niche

Although studies have observed the presence of microbiota in metastatic liver or lung organs of CRC patients, the underline mechanisms by which microbiota affects CRC metastases formation are only now being uncovered. In the secondary sites, immunosuppressive cell types, such as TAMs and MDSCs populate (pre) metastatic niches, where they help direct metastatic dissemination by creating a niche that is permissive to tumor colonization (78). These cells have been shown to achieve these pro-tumoral functions by (1) generating a proinflammatory milieu (2), remodeling the matrix and creating a pro-angiogenic, pro-invasive environment (3), maintaining an immunosuppressive microenvironment, and (4) secreting growth factors that maintains the growth of metastatic cells. Pathogen *Escherichia coli* can upregulate Cathepsin K (CTSK) expression which serves as a vital mediator between the imbalance of intestinal microbiota and CRC metastasis (79). CRC-secreted CTSK stimulates CRC progression through accelerating M2 polarization of TAMs in a TLR4-mTOR-dependent pathway. Meanwhile, cytokines (IL-10, IL-17) secreted by activated M2 macrophage, in turn, promote CRC cells invasion and metastasis by activating NF-κB pathway (79). *Fusobacterium nucleatum* can boost liver metastasis by modulating liver microenvironment featured with accumulation of MDSCs, and reduction of NK and Th17 cells (80, 81). *Peptostreptococcus anaerobius* was also reported to induce chronic inflammation and modulate tumor microenvironment by recruiting MDSCs, TANs and TAMs (82). More recently, Bertocchi et al. demonstrate that *Escherichia coli* induces gut vascular barrier (GVB) disruption, which allows bacteria to reach the liver and initiate the recruitment of inflammatory cells, contributing to pre-metastatic niche maturation and favoring metastases formation (83). These results demonstrate that host microbiota acts as a key modulator during CRC metastasis by facilitating (pre-) metastatic niche formation which support cancer cells seeding in secondary organs (Figure 2). Re-education of the metastatic niche, through alterations in metastasis-related bacteria and associated pathways, may have favorable consequences for metastatic CRC therapy.

Microbiota influence response of cancer immunotherapy

Cancer immunotherapies, specifically immune checkpoint inhibitors (ICIs) PD-1/PD-L1 and CTLA-4, have become effective strategies for cancer treatment (84). The reciprocal interactions between gut microbiota and cancer immune

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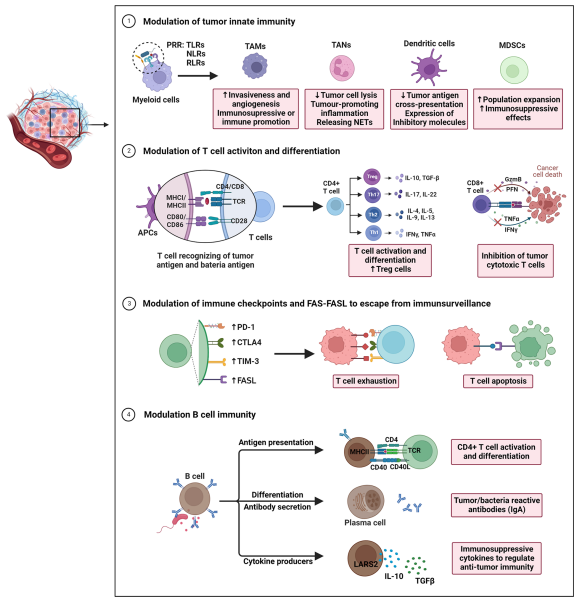

FIGURE 1
Overview of gut microbiota-modulated immune responses in tumor progression. The underlying actions and mechanisms by which the microbiota affects tumor immune escape are summarized as follows: 1) Under pathogenic conditions (dysbiosis), bacterial species that translocate through the epithelial barrier induce recruitment of myeloid cells. Myeloid cells recognize microbes *via* their pattern recognition receptors (PPR). During this process, the immunosuppressive mechanisms observed in the tumor microenvironment (TME) are activated underlie chronic infections. 2) Intestinal microbiota and intra-tumoral bacteria can be directly presented by antigen-presenting cells, thereby driving T cell activation and differentiation of CD4+T cell subsets into Th1, Th2, and Th17 or Tregs. Microbiota may also inhibit the dendritic cells' antigen presentation, consequently decreasing the CD8+T cell response. 3) By modulating T cell immune checkpoint receptor-ligand pairs (e.g., CTLA-4/CD80/CD86 and PD1/PD-L1/PD-L2), they impact T cell exhaustion, and directly impacting efficacy of immune checkpoint inhibitors. Additional mechanisms of immune escape include expression of the apoptotic proteins FAS/FASL to induce immune cell apoptosis. 4) Gut microbiota may also regulate B cell infiltration, development, and polarization. These B cells exert anti/pro-tumor immunity through acting as APCs to reshape T cell responses, secreting tumor/bacteria-specific antibodies (IgA), and producing cytokines (IL-10, TGFβ), all of which are associated with immune processes in CRC.

response raised the possibility that gut microbiota could significantly influence cancer immunotherapy response. In CRC, microbiota-based methods to enhance immunotherapy efficacy has not yet been demonstrated in human cohort. However, in animal models, specific bacterial species have been shown associated with immunotherapy response, including *Bifidobacterium* spp (9, 85). *Bacteroides fragilis* (10), *Akkermansia muciniphila* (11, 86), and *Alistipes shahii* (12). In Microsatellite Stability (MSS)-type CRC tumor-bearing mice, changes in gut microbiome affected the expression of immune-related cytokines IFN-γ and IL-2 in the tumor microenvironment, resulting in a different therapeutic effect of PD-1 antibody, and *Prevotella_sp._CAG:485* and *Akkermansia* may maintain the normal efficacy of PD-1 antibody (87). Mager et al. investigated the efficacy of ICIs therapy in both AOM/DSS induced colitis-associated cancer and MC38 tumor-bearing models, they found that *Bifidobacterium pseudolongum*, *Lactobacillus johnsonii*, and *Olsenella species*—that significantly enhanced efficacy of anti-PD-L1 and anti-CTLA-4 (88). Another study identified that tumors in antibiotic-treated or germ-free mice did not respond to CTLA-4 blockade, oral gavage of *Bacteroides thetalotaomicron*, *Bacteroides fragilis*, *Burkholderia cepacia*, or the combination of *Bacteroides fragilis* and *Burkholderia cepacian* recovered the anticancer response to CTLA-4 Ab following antibiotic treatment in mice subcutaneously engrafted with MC38 cells (10). Tanoue et al. isolated a consortium of 11 bacterial strains that is capable of enhancing therapeutic efficacy of ICIs in subcutaneous MC38 tumor models (89). Furthermore, gut microbiota was shown to impact immunotherapy efficacy related to innate responses. Researchers have observed that systemic administration of *Bifidobacterium* converts the nonresponder mice into responders to anti-CD47 immunotherapy and improves the antigen-presenting capacity of DCs (90). Song et al. found that an engineered LPS-targeting fusion protein significantly boosts anti-PD-L1 therapy against CRC tumors (91), suggesting that anti-LPS treatment may promote anti-PD-L1 immunotherapy for mouse model of CRC. Collectively, these results indicate that

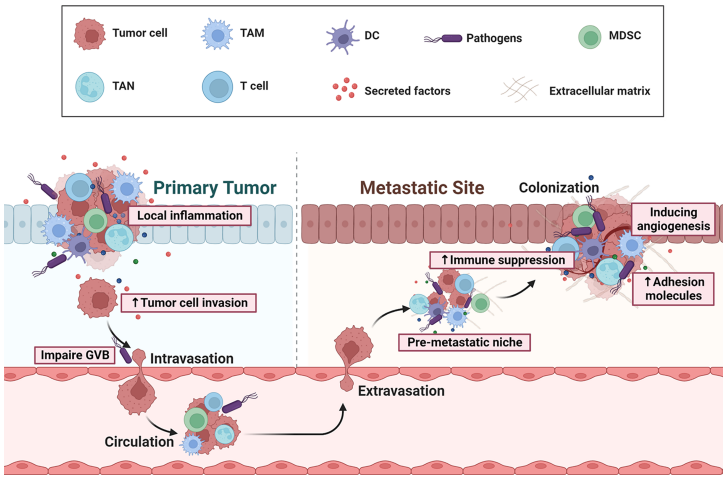

FIGURE 2
Overview of gut microbial dysbiosis on CRC initiation and metastasis. Dysbiosis can cause a chronic, pro-inflammatory milieu, by eliciting secretion of a repertoire of cytokines or growth factors, and thereby facilitates tumor cell invasion, adhesion, extravasation and survival. Some pathogens (eg: *Escherichia coli*) can induce gut vascular barrier (GVB) disruption, which allows bacteria to reach the distant organ and initiate the recruitment of immune cells with immunosuppressive functions, such as MDSCs and Tregs. Thus, the microbiota can contribute to the immune escape of distant tumors, the pre-metastatic niche maturation, adhesion, angiogenesis, and eventually the formation of metastasis.

the gut microbiota could be used to develop new therapeutic strategies to enhance CRC immunotherapy response. Recently, a human clinical trial (NCT04729322) which studies the effect of fecal microbiota transplant and re-introduction of anti-PD-1 therapy (pembrolizumab or nivolumab) for the treatment of metastatic CRC in anti-PD-1 non-responders is under recruiting. Although still under investigation, we are beginning to exploit the tremendous potential of the gut microbiota to predict immunotherapy response, and to enhance immune surveillance for a more precise immunotherapeutic intervention.

Conclusions and perspectives

It is important to decipher the specialized roles of gut microbiota in regulating the immune response in cancer, as the current landscape of the gut microbiota-host immune axis has expanded from basic research to clinical development (92). We have gained insights into the gut microbiota dysbiosis in CRC patients (Table 1). However, considerable challenges remain, for example, although multiple studies have identified specific bacteria that are associated with CRC, inconsistency across these studies exists. This may due to diverse life styles, various diet patterns and different disease stages, since gut microbiome varied substantially according to these factors (93). More studies mining of clinical large cohort data, omics, and preclinical models are needed to facilitate consensus for potential characteristics of bacterial alternation and to determine whether such changes are a cause or an effect in CRC development. Additionally, although we have deepened our view on the innate and adaptive immune responses modulated by gut microbiota in CRC (Figure 1), owing to the complex relationship between commensal and pathogenic microbes and host immunity, more detailed studies of the sophisticated network between gut microbiota and host immune system are required. In CRC, the individual heterogeneity between patients in the response to ICIs is largely associated with the gut microbiota composition, suggesting that manipulation of gut microbiota could improve immunotherapy responses (2). Therapeutics that target microbiota is explored in conjunction with cancer immunotherapies such as FMT, prebiotics, probiotics, Chinese traditional medicine, and dietary approaches (94–97). At present, microbial intervention is mainly performed in preclinical studies at the animal level, whereas it is not yet tested with large samples in the context of clinical trials and more clear mechanisms of effective microbiota to enhance immune surveillance and influence immunotherapy responses remain unknown in CRC.

In summary, substantial efforts must be devoted to pursue a deeper understanding of the mechanistic links and to exploit for clinical benefit. The insight gained into the specialized functions of the microbiota on immunity and cancer will help to apply gut microbiota-based strategies into the clinical anti-tumor adjuvant therapies, particularly in the context of conjunction with existing immunotherapies.

Author contributions

XH and LZ conceived the study. XH, ZZ, and JW screened the literatures and wrote the manuscript. LZ reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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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. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

2. Wong SH, Yu J. Gut microbiota in colorectal cancer: mechanisms of action and clinical applications. *Nat Rev Gastroenterol Hepatol* (2019) 16(11):690–704. doi: 10.1038/s41575-019-0209-8

3. Seely KD, Morgan AD, Hagenstein LD, Florey GM, Small JM. Bacterial involvement in progression and metastasis of colorectal neoplasia. *Cancers (Basel)* (2022) 14(4):1019. doi: 10.3390/cancers14041019

4. Clay SL, Fonseca-Pereira D, Garrett WS. Colorectal cancer: the facts in the case of the microbiota. *J Clin Invest* (2022) 132(4):e155101. doi: 10.1172/JCI155101

5. Tilg H, Adolph TE, Gerner RR, Moschen AR. The intestinal microbiota in colorectal cancer. *Cancer Cell* (2018) 33(6):954–64. doi: 10.1016/j.ccell.2018.03.004

6. Hanahan D. Hallmarks of cancer: New dimensions. *Cancer Discov* (2022) 12 (1):31–46. doi: 10.1158/2159-8290.CD-21-1059

7. Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. *Cell Res* (2020) 30(6):492–506. doi: 10.1038/s41422-020-0332-7

8. Yu LC. Microbiota dysbiosis and barrier dysfunction in inflammatory bowel disease and colorectal cancers: exploring a common ground hypothesis. *J BioMed Sci* (2018) 25(1):79. doi: 10.1186/s12929-018-0483-8

9. 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(6264):1084–9. doi: 10.1126/science.aac4255

10. Vetizou M, Pitt JM, Daillere R, Lepage P, Waldschmitt N, Flament C, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* (2015) 350(6264):1079–84. doi: 10.1126/science.aad1329

11. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillere R, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* (2018) 359(6371):91–7. doi: 10.1126/science.aan3706

12. 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* (2018) 359(6371):97–103. doi: 10.1126/science.aan4236

13. Gao R, Wang Z, Li H, Cao Z, Gao Z, Chen H, et al. Gut microbiota dysbiosis signature is associated with the colorectal carcinogenesis sequence and improves the diagnosis of colorectal lesions. *J Gastroenterol Hepatol* (2020) 35(12):2109–21. doi: 10.1111/jgh.15077

14. Dai Z, Coker OO, Nakatsu G, Wu WKK, Zhao L, Chen Z, et al. Multi-cohort analysis of colorectal cancer metagenome identified altered bacteria across populations and universal bacterial markers. *Microbiome* (2018) 6(1):70. doi: 10.1186/s40168-018-0451-2

15. Yachida S, Mizutani S, Shiroma H, Shiba S, Nakajima T, Sakamoto T, et al. Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes of the gut microbiota in colorectal cancer. *Nat Med* (2019) 25(6):968–76. doi: 10.1038/s41591-019-0458-7

16. Nakatsu G, Li X, Zhou H, Sheng J, Wong SH, Wu WK, et al. Gut mucosal microbiome across stages of colorectal carcinogenesis. *Nat Commun* (2015) 6:8727. doi: 10.1038/ncomms9727

17. Zeller G, Tap J, Voigt AY, Sunagawa S, Kultima JR, Costea PI, et al. Potential of fecal microbiota for early-stage detection of colorectal cancer. *Mol Syst Biol* (2014) 10:766. doi: 10.15252/msb.20145645

18. 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

19. Zhang M, Lv Y, Hou S, Liu Y, Wang Y, Wan X. Differential mucosal microbiome profiles across stages of human colorectal cancer. *Life (Basel)* (2021) 11 (8):831. doi: 10.3390/life11080831

20. Zwinsova B, Petrov VA, Hrivnakova M, Smatana S, Micenkova L, Kazdova N, et al. Colorectal tumour mucosa microbiome is enriched in oral pathogens and defines three subtypes that correlate with markers of tumour progression. *Cancers (Basel)* (2021) 13(19):4799. doi: 10.3390/cancers13194799

21. Kinross J, Mirnezami R, Alexander J, Brown R, Scott A, Galea D, et al. A prospective analysis of mucosal microbiome-metabonome interactions in colorectal cancer using a combined MAS 1HNMR and metataxonomic strategy. *Sci Rep* (2017) 7(1):8979. doi: 10.1038/s41598-017-08150-3

22. Loftus M, Hassouneh SA, Yooseph S. Bacterial community structure alterations within the colorectal cancer gut microbiome. *BMC Microbiol* (2021) 21(1):98. doi: 10.1186/s12866-021-02153-x

23. Yu J, Feng Q, Wong SH, Zhang D, Liang QY, Qin Y, et al. Metagenomic analysis of faecal microbiome as a tool towards targeted non-invasive biomarkers for colorectal cancer. *Gut* (2017) 66(1):70–8. doi: 10.1136/gutjnl-2015-309800

24. Pan HW, Du LT, Li W, Yang YM, Zhang Y, Wang CX. Biodiversity and richness shifts of mucosa-associated gut microbiota with progression of colorectal cancer. *Res Microbiol* (2020) 171(3-4):107–14. doi: 10.1016/j.resmic.2020.01.001

25. Burns MB, Montassier E, Abrahante J, Priya S, Niccum DE, Khoruts A, et al. Colorectal cancer mutational profiles correlate with defined microbial communities in the tumor microenvironment. *PLoS Genet* (2018) 14(6):e1007376. doi: 10.1371/journal.pgen.1007376

26. Sheng Q, Du H, Cheng X, Cheng X, Tang Y, Pan L, et al. Characteristics of fecal gut microbiota in patients with colorectal cancer at different stages and different sites. *Oncol Lett* (2019) 18(5):4834–44. doi: 10.3892/ol.2019.10841

27. Gao R, Zhu Y, Kong C, Xia K, Li H, Zhu Y, et al. Alterations, interactions, and diagnostic potential of gut bacteria and viruses in colorectal cancer. *Front Cell Infect Microbiol* (2021) 11:657867. doi: 10.3389/fcimb.2021.657867

28. de Martel C, Georges D, Bray F, Ferlay J, Clifford GM. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. *Lancet Glob Health* (2020) 8(2):e180–90. doi: 10.1016/S2214-109X(19)30488-7

29. Goodwin AC, Destefano Shields CE, Wu S, Huso DL, Wu X, Murray-Stewart TR, et al. Polyamine catabolism contributes to enterotoxigenic bacteroides

fragilis-induced colon tumorigenesis. *Proc Natl Acad Sci U.S.A.* (2011) 108 (37):15354–59. doi: 10.1073/pnas.1010203108

30. Lasry A, Zinger A, Ben-Neriah Y. Inflammatory networks underlying colorectal cancer. *Nat Immunol* (2016) 17(3):230–40. doi: 10.1038/ni.3384

31. Abdulamir AS, Hafidh RR, Bakar FA. Molecular detection, quantification, and isolation of streptococcus gallolyticus bacteria colonizing colorectal tumors: inflammation-driven potential of carcinogenesis *via* IL-1, COX-2, and IL-8. *Mol Cancer* (2010) 9:249. doi: 10.1186/1476-4598-9-249

32. Brennan CA, Clay SL, Lavoie SL, Bae S, Lang JK, Fonseca-Pereira D, et al. Fusobacterium nucleatum drives a pro-inflammatory intestinal microenvironment through metabolite receptor-dependent modulation of IL-17 expression. *Gut Microbes* (2021) 13(1):1987780. doi: 10.1080/19490976.2021.1987780

33. Man SM. Inflammasomes in the gastrointestinal tract: infection, cancer and gut microbiota homeostasis. *Nat Rev Gastroenterol Hepatol* (2018) 15(12):721–37. doi: 10.1038/s41575-018-0054-1

34. Mlecnik B, Bindea G, Angell HK, Maby P, Angelova M, Tougeron D, et al. Integrative analyses of colorectal cancer show immunoscore is a stronger predictor of patient survival than microsatellite instability. *Immunity* (2016) 44(3):698–711. doi: 10.1016/j.immuni.2016.02.025

35. Trinchieri G. Cancer immunity: Lessons from infectious diseases. *J Infect Dis* (2015) 212 Suppl 1:S67–73. doi: 10.1093/infdis/jiv070

36. Guc E, Pollard JW. Redefining macrophage and neutrophil biology in the metastatic cascade. *Immunity* (2021) 54(5):885–902. doi: 10.1016/j.immuni.2021.03.022

37. Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. *Cancer Res* (2006) 66(2):605–12. doi: 10.1158/0008-5472.CAN-05-4005

38. Stefani C, Miricescu D, Stanescu-Spinu II, Nica RI, Greabu M, Totan AR, et al. Growth factors, PI3K/AKT/mTOR and MAPK signaling pathways in colorectal cancer pathogenesis: Where are we now? *Int J Mol Sci* (2021) 22 (19):10260. doi: 10.3390/ijms221910260

39. Barnet MB, Blinman P, Cooper W, Boyer MJ, Kao S, Goodnow CC. Understanding immune tolerance of cancer: Re-purposing insights from fetal allografts and microbes. *Bioessays* (2018) 40(8):e1800050. doi: 10.1002/bies.201800050

40. Chan AT, Ogino S, Fuchs CS. Aspirin use and survival after diagnosis of colorectal cancer. *JAMA* (2009) 302(6):649–58. doi: 10.1001/jama.2009.1112

41. Alvarez R, Oliver L, Valdes A, Mesa C. Cancer-induced systemic myeloid dysfunction: Implications for treatment and a novel nanoparticle approach for its correction. *Semin Oncol* (2018) 45(1-2):84–94. doi: 10.1053/j.seminoncol.2018.05.001

42. Schwabe RF, Jobin C. The microbiome and cancer. *Nat Rev Cancer* (2013) 13(11):800–12. doi: 10.1038/nrc3610

43. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol* (2013) 14(10):1014–22. doi: 10.1038/ni.2703

44. Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, et al. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* (2013) 14(2):207–15. doi: 10.1053/j.gastro.2018.04.001

45. Sethi V, Kurtom S, Tarique M, Lavania S, Malchiodi Z, Hellmund L, et al. Gut microbiota promotes tumor growth in mice by modulating immune response. *Gastroenterology* (2018) 155(1):33–7.e36. doi: 10.1053/j.gastro.2018.04.001

46. Zhang D, Chen G, Manwani D, Mortha A, Xu C, Faith JJ, et al. Neutrophil ageing is regulated by the microbiome. *Nature* (2015) 525(7570):528–32. doi: 10.1038/nm.2278

47. Yang XD, Ai W, Asfaha S, Bhagat G, Friedman RA, Jin G, et al. Histamine deficiency promotes inflammation-associated carcinogenesis through reduced myeloid maturation and accumulation of CD11b+Ly6G+ immature myeloid cells. *Nat Med* (2011) 17(1):87–95. doi: 10.1038/nm.2278

48. Coffelt SB, Wellenstein MD, de Visser KE. Neutrophils in cancer: neutral no more. *Nat Rev Cancer* (2016) 16(7):431–46. doi: 10.1038/nrc.2016.52

49. Mizuno R, Kawada K, Itatani Y, Ogawa R, Kiyasu Y, Sakai Y. The role of tumor-associated neutrophils in colorectal cancer. *Int J Mol Sci* (2019) 20(3):529. doi: 10.3390/ijms20030529

50. Masucci MT, Minopoli M, Del Vecchio S, Carriero MV. The emerging role of neutrophil extracellular traps (NETs) in tumor progression and metastasis. *Front Immunol* (2020) 11:1749. doi: 10.3389/fimmu.2020.01749

51. Hezaveh K, Shinde RS, Klotgen A, Halaby MJ, Lamorte S, Ciudad MT, et al. Tryptophan-derived microbial metabolites activate the aryl hydrocarbon receptor in tumor-associated macrophages to suppress anti-tumor immunity. *Immunity* (2022) 55(2):324–40.e328. doi: 10.1016/j.immuni.2022.01.006

52. Kaisar MMM, Pelgrom LR, van der Ham AJ, Yazdanbakhsh M, Everts B. Butyrate conditions human dendritic cells to prime type 1 regulatory T cells *via* both histone deacetylase inhibition and G protein-coupled receptor 109A signaling. *Front Immunol* (2017) 8:1429. doi: 10.3389/fimmu.2017.01429

53. Liu L, Li L, Min J, Wang J, Wu H, Zeng Y, et al. Butyrate interferes with the differentiation and function of human monocyte-derived dendritic cells. *Cell Immunol* (2012) 277(1-2):66–73. doi: 10.1016/j.cellimm.2012.05.011

54. Beyaz S, Chung C, Mou H, Bauer-Rowe KE, Xifaras ME, Ergin I, et al. Dietary suppression of MHC class II expression in intestinal epithelial cells enhances intestinal tumorigenesis. *Cell Stem Cell* (2021) 28(11):1922–35.e1925. doi: 10.1016/j.stem.2021.08.007

55. Schulz MD, Atay C, Heringer J, Romrig FK, Schwitalla S, Aydin B, et al. High-fat-diet-mediated dysbiosis promotes intestinal carcinogenesis independently of obesity. *Nature* (2014) 514(7523):508–12. doi: 10.1038/nature13398

56. Gur C, Ibrahim Y, Isaacson B, Yamin R, Abed J, Gamliel M, et al. Binding of the Fap2 protein of fusobacterium nucleatum to human inhibitory receptor TIGIT protects tumors from immune cell attack. *Immunity* (2015) 42(2):344–55. doi: 10.1016/j.immuni.2015.01.010

57. Zitvogel L, Kroemer G. Cross-reactivity between microbial and tumor antigens. *Curr Opin Immunol* (2022) 75:102171. doi: 10.1016/j.coi.2022.102171

58. Fluckiger A, Daillère R, Sassi M, Sixt BS, Liu P, Loos F, et al. Cross-reactivity between tumor MHC class I-restricted antigens and an enterococcal bacteriophage. *Science* (2020) 369(6506):936–42. doi: 10.1126/science.aax0701

59. Cullin N, Azevedo Antunes C, Straussman R, Stein-Thoeringer CK, Elinav E. Microbiome and cancer. *Cancer Cell* (2021) 39(10):1317–41. doi: 10.1016/j.ccell.2021.08.006

60. Zitvogel L, Ayyoub M, Routy B, Kroemer G. Microbiome and anticancer immunosurveillance. *Cell* (2016) 165(2):276–87. doi: 10.1016/j.cell.2016.03.001

61. De Almeida CV, de Camargo MR, Russo E, Amedei A. Role of diet and gut microbiota on colorectal cancer immunomodulation. *World J Gastroenterol* (2019) 25(2):151–62. doi: 10.3748/wjg.v25.i2.151

62. Geuking MB, Burkhard R. Microbial modulation of intestinal T helper cell responses and implications for disease and therapy. *Mucosal Immunol* (2020) 13 (6):855–66. doi: 10.1038/s41385-020-00335-w

63. Oster P, Vaillant L, Riva E, McMillan B, Begka C, Truntzer C, et al. Helicobacter pylori infection has a detrimental impact on the efficacy of cancer immunotherapies. *Gut* (2022) 71(3):457–66. doi: 10.1136/gutjnl-2020-323392

64. Botticelli A, Zizzari I, Mazzuca F, Ascierto PA, Putignani L, Marchetti L, et al. Cross-talk between microbiota and immune fitness to steer and control response to anti PD-1/PDL-1 treatment. *Oncotarget* (2017) 8(5):8890–9. doi: 10.18632/oncotarget.12985

65. Crespo J, Sun H, Welling TH, Tian Z, Zou W. T Cell anergy, exhaustion, senescence, and stemness in the tumor microenvironment. *Curr Opin Immunol* (2013) 25(2):214–21. doi: 10.1016/j.coi.2012.12.003

66. Yu AI, Zhao L, Eaton KA, Ho S, Chen J, Poe S, et al. Gut microbiota modulate CD8 T cell responses to influence colitis-associated tumorigenesis. *Cell Rep* (2020) 31(1):107471. doi: 10.1016/j.celrep.2020.03.035

67. Guégan JP, Ginestier C, Charafe-Jauffret E, Ducret T, Quignard JF, Vacher P, et al. CD95/Fas and metastatic disease: What does not kill you makes you stronger. *Semin Cancer Biol* (2020) 60:121–31. doi: 10.1016/j.semcancer.2019.06.004

68. Nozoe T, Yasuda M, Honda M, Inutsuka S, Korenaga D. Fas ligand expression is correlated with metastasis in colorectal carcinoma. *Oncology* (2003) 65(1):83–8. doi: 10.1159/000071208

69. Bachem A, Makhlouf C, Binger KJ, de Souza DP, Tull D, Hochheiser K, et al. Microbiota-derived short-chain fatty acids promote the memory potential of antigen-activated CD8+ T cells. *Immunity* (2019) 51(2):285–97.e5. doi: 10.1016/j.immuni.2019.06.002

70. Liu X, Chen Y, Zhang S, Dong L. Gut microbiota-mediated immunomodulation in tumor. *J Exp Clin Cancer Res* (2021) 40(1):221. doi: 10.1186/s13046-021-01983-x

71. Overacre-Delgoffe AE, Bumgarner HJ, Cillo AR, Burr AHP, Tometich JT, Bhattacharjee A, et al. Microbiota-specific T follicular helper cells drive tertiary lymphoid structures and anti-tumor immunity against colorectal cancer. *Immunity* (2021) 54(12):2812–24.e2814. doi: 10.1016/j.immuni.2021.11.003

72. Lowenmark T, Li X, Lofgren-Burstrom A, Zingmark C, Ling A, Kellgren TG, et al. Parvimonas micra is associated with tumour immune profiles in molecular subtypes of colorectal cancer. *Cancer Immunol Immunother* (2022) 71 (10):2565–75. doi: 10.1007/s00262-022-03179-4

73. Noble A, Durant L, Dilke SM, Man R, Martin I, Patel R, et al. Altered mucosal immune-microbiota interactions in familial adenomatous polyposis. *Clin Transl Gastroenterol* (2022) 13(7):e00428. doi: 10.14309/ctg.0000000000000428

74. Rosser EC, Piper CJM, Matei DE, Blair PA, Rendeiro AF, Orford M, et al. Microbiota-derived metabolites suppress arthritis by amplifying aryl-hydrocarbon receptor activation in regulatory b cells. *Cell Metab* (2020) 31(4):837–51.e810. doi: 10.1016/j.cmet.2020.03.003

75. Yang W, Cong Y. Gut microbiota-derived metabolites in the regulation of host immune responses and immune-related inflammatory diseases. *Cell Mol Immunol* (2021) 18(4):866–77. doi: 10.1038/s41423-021-00661-4

76. Honda K, Littman DR. The microbiota in adaptive immune homeostasis and disease. *Nature* (2016) 535(7610):75–84. doi: 10.1038/nature18848

77. Wang Z, Lu Z, Lin S, Xia J, Zhong Z, Xie Z, et al. Leucine-tRNA-synthase-2-expressing b cells contribute to colorectal cancer immunoevasion. *Immunity* (2022) 55(6):1067–81.e8. doi: 10.1016/j.immuni.2022.04.017

78. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. *Nat Med* (2013) 19(11):1423–37. doi: 10.1038/nm.3394

79. Li R, Zhou R, Wang H, Li W, Pan M, Yao X, et al. Gut microbiota-stimulated cathepsin K secretion mediates TLR4-dependent M2 macrophage polarization and promotes tumor metastasis in colorectal cancer. *Cell Death Differ* (2019) 26(11):2447–63. doi: 10.1038/s41418-019-0312-y

80. Yin H, Miao Z, Wang L, Su B, Liu C, Jin Y, et al. Fusobacterium nucleatum promotes liver metastasis in colorectal cancer by regulating the hepatic immune niche and altering gut microbiota. *Aging* (2022) 14(4):1941–58. doi: 10.18632/aging.203914

81. Sakamoto Y, Mima K, Ishimoto T, Ogata Y, Imai K, Miyamoto Y, et al. Relationship between fusobacterium nucleatum and antitumor immunity in colorectal cancer liver metastasis. *Cancer Sci* (2021) 112(11):4470–7. doi: 10.1111/cas.15126

82. Long X, Wong CC, Tong L, Chu ESH, Ho Szeto C, Go MYY, et al. Peptostreptococcus anaerobius promotes colorectal carcinogenesis and modulates tumour immunity. *Nat Microbiol* (2019) 4(12):2319–30. doi: 10.1038/s41564-019-0541-3

83. Bertocchi A, Carloni S, Ravenda PS, Bertalot G, Spadoni I, Lo Cascio A, et al. Gut vascular barrier impairment leads to intestinal bacteria dissemination and colorectal cancer metastasis to liver. *Cancer Cell* (2021) 39(5):708–24.e711. doi: 10.1016/j.ccell.2021.03.004

84. Zhang Y, Zhang Z. The history and advances in cancer immunotherapy: understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications. *Cell Mol Immunol* (2020) 17(8):807–21. doi: 10.1038/s41423-020-0488-6

85. Matson V, Fessler J, Bao R, Chongsuwat T, Zha Y, Alegre ML, et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science* (2018) 359(6371):104–8. doi: 10.1126/science.aao3290

86. Gharaibeh RZ, Jobin C. Microbiota and cancer immunotherapy: in search of microbial signals. *Gut* (2019) 68(3):385–8. doi: 10.1136/gutjnl-2018-317220

87. Xu X, Lv J, Guo F, Li J, Jia Y, Jiang D, et al. Gut microbiome influences the efficacy of PD-1 antibody immunotherapy on MSS-type colorectal cancer *via* metabolic pathway. *Front Microbiol* (2020) 11:814. doi: 10.3389/fmicb.2020.00814

88. Mager LF, Burkhard R, Pett N, Cooke NCA, Brown K, Ramay H, et al. Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. *Science* (2020) 369(6510):1481–9. doi: 10.1126/science.abc3421

89. Tanoue T, Morita S, Plichta DR, Skelly AN, Suda W, Sugiura Y, et al. A defined commensal consortium elicits CD8 T cells and anti-cancer immunity. *Nature* (2019) 565(7741):600–5. doi: 10.1038/s41586-019-0878-z

90. Fu Y-X, Weichselbaum RR, Chang EB, Lin W, Xue L, Ni K, et al. Intratumoral accumulation of gut microbiota facilitates CD47-based immunotherapy *via* STING signaling. *J Exp Med* (2020) 217(5):e20192282. doi: 10.1084/jem.20192282

91. Song W, Tiruthani K, Wang Y, Shen L, Hu M, Dorosheva O, et al. Trapping of lipopolysaccharide to promote immunotherapy against colorectal cancer and attenuate liver metastasis. *Adv Mater* (2018) 30(52):e1805007. doi: 10.1002/adma.201805007

92. 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

93. Song M, Chan AT. Environmental factors, gut microbiota, and colorectal cancer prevention. *Clin Gastroenterol Hepatol* (2019) 17(2):275–89. doi: 10.1016/j.cgh.2018.07.012

94. Zhou CB, Zhou YL, Fang JY. Gut microbiota in cancer immune response and immunotherapy. *Trends Cancer* (2021) 7(7):647–60. doi: 10.1016/j.trecan.2021.01.010

95. Matson V, Chervin CS, Gajewski TF. Cancer and the microbiome-influence of the commensal microbiota on cancer, immune responses, and immunotherapy. *Gastroenterology* (2021) 160(2):600–13. doi: 10.1053/j.gastro.2020.11.041

96. Xu B, Wang X, Wang H, Cao L, Ge Y, Yuan B, et al. Efficacy and safety of herbal formulas with the function of gut microbiota regulation for gastric and colorectal cancer: A systematic review and meta-analysis. *Front Cell Infect Microbiol* (2022) 12:875225. doi: 10.3389/fcimb.2022.875225

97. McQuade JL, Daniel CR, Helmink BA, Wargo JA. Modulating the microbiome to improve therapeutic response in cancer. *Lancet Oncol* (2019) 20 (2):e77–91. doi: 10.1016/S1470-2045(18)30952-5

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The mechanisms on evasion of anti-tumor immune responses in gastric cancer

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The immune system and the tumor have been at each other's throats for so long that the neoplasm has learned to avoid detection and avoid being attacked, which is called immune evasion. Malignant tumors, such as gastric cancer (GC), share the ability to evade the body's immune system as a defining feature. Immune evasion includes alterations to tumor-associated antigens (TAAs), antigen presentation mechanisms (APMs), and the tumor microenvironment (TME). While TAA and APM are simpler in nature, they both involve mutations or epigenetic regulation of genes. The TME is comprised of numerous cell types, cytokines, chemokines and extracellular matrix, any one of which might be altered to have an effect on the surrounding ecosystem. The NF-kB, MAPK, PI3K/AKT, JAK/STAT, Wnt/β-catenin, Notch, Hippo and TGF-β/Smad signaling pathways are all associated with gastric cancer tumor immune evasion. In this review, we will delineate the functions of these pathways in immune evasion.

KEYWORDS

gastric cancer, immune evasion, tumor associated antigen, antigen presentation, tumormicroenvironment, signaling pathway

Abbreviations: APCs, antigen-presenting cells; APM, antigen presentation mechanism; CAFs, cancer-associated fibroblasts; CCL, C-C motif chemokine ligand; CCR, C-C motif chemokine receptor; CD, cluster of differentiation; CTLs, cytotoxic T lymphocytes; CTLA-4, cytotoxic T lymphocyte associated protein 4; CXCL, C-X-C motif chemokine ligand; IDO 1, indoleamine 2,3-dioxygenase 1; MDSCs, Myeloid-derived suppressor cells; MHC, major histocompatibility complex; TAA, tumor-associated antigen; TAMs, tumor-associated macrophages; TAP, transporter associated with antigen processing; Teff, effector T cell; TGF-β, transforming growth factor-β; Th cell, helper T cell; TME, tumor microenvironment; TNF-α, tumor necrosis factor-α; Treg, regulatory T cell.

1 Introduction

Gastric cancer (GC) was fifth most frequently occurring and fourth most lethal among malignant tumors worldwide as of the year 2020 (1). GC is a malignant disease characterized by a convoluted immune response, in particular to persistent inflammation. Therefore, the immune system is pivotal in cancer initiation and progression (2). As our understanding of gastric cancer's immune-related research grows, we find that gastric cancer's immune escape mechanism is distinct from that of other malignancies. This could point immunotherapy against GC in a novel route. In 2002, Gavin P. Dunn and Robert D. Schreiber developed the notion of tumor immunoediting, which divides the process into three stages: elimination, equilibrium and evasion (3). The preclinical tumor is killed during the elimination stage of the immune response to neoplasms. To survive the immune system's destruction during the equilibrium phase, tumor cells may constantly mutate, for example. There is a dynamic equilibrium between the breakdown of the immune system and the growth of tumor cells. Malignant tumors, such as GC, are characterized by immune evasion, the ability of the neoplasm to elude the monitoring and attack of the immune system (4). Antigen loss or variation, a deficiency in class I Major Histocompatibility Complex (MHC I) molecules, the production of immune-suppressing cytokines, a lack of co-stimulators and other immune-suppression mechanisms are all biological processes that contribute to immune evasion (5). We classify them as follows: loss or alterations in tumor-associated antigen (TAA), damage to the antigen presentation mechanism (APM), and immunosuppression by the tumor microenvironment (TME). The immune system plays a crucial role in tumor development, hence researchers are looking into using immunologic techniques to increase the longevity of GC patients. Despite the fact that tumor immunotherapy (especially immune checkpoint inhibitor) has had a lot of achievements, many patients do not respond to treatment and many cases become resistant to treatment. For this reason, it is essential to always be on the lookout for cutting-edge therapies. Multiple signaling pathways have emerged as possible contributors to immune evasion. Inhibitors of signaling pathways may therefore form part of future immunotherapies. Consequently, the signaling pathway is highlighted as a crucial component of GC's immune evasion strategy in this paper.

2 The mechanism of immune evasion

In order to escape immune monitoring and eradication, tumor cells modify their TAA and APM in a number of ways, then entering the final evasion stage (5). Tumor cells that have evaded the immune system are able to survive and have an effect on the tumor microenvironment (TME) through a number of signaling pathways, dampening the anti-tumor immune response (6).

2.1 Escaping surveillance by invalid TAA

To a certain extent, TAA can be divided into three categories (7): first, TAA presented on the surface of tumor cells by major histocompatibility complex (MHC) molecules or antigen presenting cells (APCs) and recognized by autoantibody or heteroantibody; second, target molecules or ligands recognized by natural killer (NK) cell receptors; and third, TAA presented on the surface of tumor cells by MHC molecules or APCs and recognized by autoantibody or heteroantibody (APCs). Correspondingly, TAA's modulation can also be divided into three groups: 1) the up-regulation of immunosuppressive antigen; 2) the loss of original recognized antigen; 3) the generation of unrecognizable antigens (4). Antigen expression is known to be influenced by epigenetic regulation, as well as mutations in genes (8). A genetic mutation is any alteration to the gene's base pair sequence or makeup. Epigenetic regulation is the heritable alteration of gene expression that does not involve a change in nucleotide sequence and includes DNA methylation, histone modification, and regulation by non-coding RNA (ncRNA). Gene silencing occurs when DNA is methylated at the C-terminus of 5'-CpG-3' by DNA methyltransferases (DNMTs) to produce 5-methylcytosine (5-mC) (9). Enzymes collaborate to modify histones in various ways, including methylation, acetylation, phosphorylation, ubiquitination and ADP ribosylation (10). Complexity of the regulation mechanisms of ncRNAs in immune evasion will be detailed in the later section.

Carcinoembryonic antigen (CEA), HER2, carbohydrate antigen 19-9 (CA19-9) and CA72-4 are some of the most common GC autoantigens. They are highly expressed, but their immunological impact is quite modest (11). The main function executor is a member of the CEA family called CEA-related cell adhesion molecule-1 (CEACAM1). Evidence suggests that CEACAM1 inhibits NKG2D ligand (NKG2DL) expression in tumor cells (11). To make tumor cells more susceptible to NK cell-mediated cytotoxicity, Chen et al. (11) found that silencing CEACAM1 in mice and human tumor cells increases surface NKG2DL expression. Since HER2 belongs to a member of the EGFR family, it can also activate the downstream PI3K/AKT and ERK pathways, which in turn control cell proliferation, invasion and migration (12). Previous studies have shown that CD8+ cytotoxic T lymphocytes (CTLs) can recognize HER2, which aids in the immune system's fight against tumors (12). A recent study by Wu et al. (13) demonstrates, however, that HER2 protects cancer cells from STING-mediated innate antitumor immunity by activating AKT1, suggesting that HER2 recruits AKT1 to lower STING

signal, hence restricting anti-virus defense and anti-tumor immunity. Nevertheless, the mechanism by which CA19-9 and CA72-4 play a role in immune escape remains unexplored at this time and warrants further investigation.

The main activating receptor expressed by NK cells, NK group 2D (NKG2D), binds to ligands such as MHC class I peptide related sequence A (MICA), MICB and six UL16 binding proteins (ULBPs) (14). The primary ligand on the surface of tumors is a variant of the traditional MHC protein called MIC A/B. Both β2-microglobulin (β2m) and antigen are inaccessible to MIC A/B (14). Gene promoter hypermethylation, histone deacetylation and protein shedding contribute to reduced MIC A/B expression on tumor surfaces (15, 16), hence dampening NK cell-mediated innate immunity.

Immune checkpoints (ICPs) and co-inhibitory molecules (CIMs) consist of ligands and their respective receptors. Particularly, these ICPs are focusing on the PD-1/PD-L1 field. PD-L1 on the tumor surface interacts to PD-1 on the T cell surface, leading to T cell depletion (17). Studies show that PD-L1 is highly expressed on the surface of tumors, and its induction process is complex. Helper T (Th) cells, cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells that have been activated can all generate interferon (IFN), which can then activate the JAK/STAT pathway and lead to PD-L1 expression. In the meantime, IL-10 can boost PD-L1 expression (12, 18). Moreover, the C > G variant of the rs4143815 SNP in the 3'-UTR of the PD-L1 gene increases PD-L1 expression and may increase cancer risk (19). Tumor-associated macrophage (TAM) production of tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) positively regulated PD-L1 (20). High expression of MHC II in GC cells can be partially explained by the lack of traditional co-stimulatory proteins CD80 and CD86 in the tumor, which limit MHC class II recognition (21). In addition, researchers discovered a decreased expression of co-stimulatory molecules such as 4-1BBL (tumor necrosis factor receptor superfamily member 9 ligands), B7-1 and CD40 on the tumor surface (22–24). ICPs inhibitors paired with co-stimulatory molecular agonists may be a viable way for tumor treatment, according to these studies. CD47, also called integrin-related proteins, is a cell surface glycoprotein of 50-kDa that inhibits APM induced by macrophages through the "don't eat me" signaling CD47/SIRP pathway (25). Macrophage-mediated innate immune and APM inactivation is aided by the CD47/SIRP (signal-regulatory protein) axis, which inhibits phagocytosis by downregulating integrin signal activation from the interior of macrophages (26). The MYC mutation causes CD47 upregulation and contributes to PD-L1 overexpression in a similar fashion (27). The work by Yoshida K et al. (25) found that GC that express the surface marker CD47 proliferated strongly in both vitro and *in vivo*.

Factor-associated suicide (Fas) is a member of the tumor necrosis factor (TNF) family of type II transmembrane proteins. It is also known as CD95 or apoptosis antigen-1 (Apo-1). This protein has the ability to connect with its ligand, set off the apoptosis cascade, and maintain a pro-apoptotic environment (FasL) (28). Activated T and NK cells are the most common sources of FasL. Wang et al. found that low levels of Fas expression on the surface of GC cells were associated with a poor prognosis *in vitro* studies (29). The rs2234767 G> A polymorphism in the Fas promoter region may be associated with susceptibility to GC (30), which may be associated with SNP-induced down-regulation of Fas. Similarly, epigenetic changes can affect Fas expression. Fas expression was downregulated due to hypermethylation of its promoter region (31).

2.2 Escaping surveillance by damaged APM

Antigen presentation involves antigen processing and degradation by APCs such macrophages and dendritic cells (DCs), followed by presentation of the antigen peptide/major histocompatibility complex (MHC) complex to T lymphocytes (32). APCs deliver antigen polypeptides by joining them with processed MHC class II for specific recognition by CD4+ T lymphocytes (33). CD8+ T lymphocytes, and in particular CTLs, are capable of direct MHC class I detection (34).

The major histocompatibility complex (MHC) family is a group of membrane proteins responsible for presenting antigens on cell surfaces, where they can be recognized by T lymphocytes, which then kill the cell (35). The human MHC locus, also known as the human leukocyte antigen locus, is found on chromosome 6 and contains around 200 genes (HLA) (35). Many malignant tumors include aberrant expression of class I and class II molecules, the primary types responsible for presenting antigens to T lymphocytes (36). HLA class I molecules consist of the heavy chains (HLA-A, -B, -C, -E, -F and -G) and the β2m (37). Changes in epigenetic regulation and a mutation in the HLA gene, called β2m, are primarily responsible for the dramatic reduction in class I HLA expression (38–40). Down-regulation of HLA class I is caused by hypermethylation of the promoters of the HLA-A, -B and -C genes, which is a hallmark of GC (39, 40). An example is the finding by Ye et al. that promoter methylation is linked to reduced HLA-A expression in BGC-823 cells (40). Recent research has indicated that HLA-G is overexpressed, leading researchers to hypothesize that non-canonical HLA class I may have a deleterious effect in GC due to unidentified antigen (41). EZH2 (enhancer of zeste homolog 2) is a major component of Polycomb inhibitor complex 2, which catalyzes histone H3 lysine 27 trimethylation (H3K27me3) (42). Activation of EZH2 in tumors results in H3K27 methylation, which in turn silences key immune genes including HLA class I (42). The HLA class II trans-activator promoter is associated with epigenetic control of HLA class II (CIITA) (43). Decoy receptor 3 for interferon beta and tumor necrosis factor (TNF) inhibits HLA class II (mostly HLA-DR

gene) expression by hypermethylation and histone deacetylation of CIITA-Promoter IV (CIITA-PIV), which is activated by STAT1 and requires histone deacetylases (HDACs) (DCR3) (41). HLA class II antigen presentation stimulates Th cells activation without co-stimulatory molecule, and its upregulation has been described in some forms of GC (41). Antigen presentation can be improved by binding TAA to HLA, but this cannot happen without TAP (transporter associated with antigen processing) and tapasin (TAP binding protein) (44). A decrease in histone H3 acetylation and TAP1 expression is caused by the decreased binding of histone acetyltransferases (HATs) to gene promoters, which in turn decreases the accessibility/transcription of the RNA polymerase II complex (27). When EZH2 is turned on in a tumor, TAP1 and TAP2 are also suppressed (42). The results of these studies provide evidence that inhibiting key enzymes that regulate epigenetics may be an effective treatment for GC.

APM impairment impacts the TME as a result of a combination of fewer invading APCs and their malfunction, which means that tumor cells are able to evade immune monitoring and clearance due to the combined effects of inefficient TAA and defective APM (45). Those tumor cells that are able to avoid being eliminated by the immune system join with other local cells and cytokines to create an immunosuppressive microenvironment that aids in the growth and survival of the tumor. (Figure 1)

2.3 Signaling pathways involved in immune evasion

The term tumor microenvironment (TME) encompasses everything from other cells to their secretions to the metabolites they create. The signal route of tumor infiltrating lymphocytes (TILs) can be further regulated and inhibited by inflammatory substances secreted by tumor cells (Table 1). Tumor cells are able to evade immune surveillance when the early microenvironment regulates many signaling pathways, resulting in a diminished anti-tumor immune response and, ultimately, immune suppression (92).

2.3.1 NF-kB signaling pathway

The nuclear factor kappa B (NF-kB) pathway plays a crucial role in modifying the immune response to infection, especially in chronic inflammation. This pathway is composed of two main subgroups: 1) NF-kB1/NF-kB2 (p50/p52); 2) Rel A (p65), Rel B and c-Rel (93). Toll-like receptors (TLR), EGF, PI3K, IL-1 and TNF can all activate the NF-kB signaling pathway (93). Interleukin-6 (IL-6), tumor necrosis factor (TNF), T helper 2 (Th2) cells, regulatory T cells (Treg), type 2 (N2) neutrophils, myeloid-derived suppressor cells (MDSCs) and mesenchymal stem cells (MSCs) are all up-regulated when the NF-kB p65 or c-Rel pathway is active (46, 47, 50, 83, 94). Recently, O'Reilly found that NF-kB1 has anticancer qualities since activating the STAT1 pathway increased GC growth in NF-kB1$^{-/-}$ mice by decreasing TAP gene expression and inhibiting innate immunity (79). Furthermore, deleting NF-kB1 also increased the expression of CTLA-4 and PD-1 in lymphocytes and the expression of programmed death ligand-1 (PD-L1) in myeloid and gastric epithelial cells (79). Similarly, HLA class II was up-regulated in GC epithelial cells from NF-kB1$^{-/-}$ mice (79).

Tumor-induced MSCs interact with neighboring cells in the tumor microenvironment (TME) to promote tumor progression (48). The exosomes secreted by GC cells modulate the immunomodulatory activity of MSCs *via* the NF-kB signaling pathway, thereby boosting MSCs' capacity to activate immune cells, sustaining an inflammatory milieu and promoting tumor growth (49). In addition to regulating angiogenesis and morphogenesis, mesenchymal stromal cells (MSCs) have been demonstrated to recruit cancer-associated fibroblasts (CAFs), IL-6 and M2macrophage, all of which have been linked to cancer progression (46, 49, 83). Examples include MSC-derived M2 macrophages, which express vascular endothelial growth factor (VEGF) in an NF-kB p65-dependent manner (93), MSC-derived IL-6 activating neutrophils, which in turn increases angiogenesis and tumor spread (84), and tumor-derived factor being able to polarize neutrophils to the N2 phenotype. N2-polarized neutrophils support metastasis and inhibit the immune system (95). Different kinds of circulating neutrophils include high-density neutrophils (HDN) and low-density neutrophils (LDN) (96). According to research by Sagiv JY et al. (96), LDN is associated with cancer and is induced by HDN through activation of transforming growth factor (TGF), which promotes tumor progression. The TME of GC also inhibits apoptosis in neutrophils and promotes the production of inflammatory molecules like IL-1 and TNF-α, dampening the immune response (97). Furthermore, TNF-α also increases CD47 expression *via* the NF-kB signaling pathway at the transcriptional level (98). Since MSCs have the ability to regenerate, they are a promising tool in the fight against cancer (51). CAFs are derived from bone marrow-derived stem cells, pericytes and normal gastric fibroblasts stimulated by TGF–β. A possible contributor to the development and spread of cancer is the accumulation of CAFs in GC tissue (51). Also, CXC motif chemokine ligands 1/2 (CXCL1/2) can be induced by TNF in an NF-kB-dependent manner in stromal cells and endothelial cells (51). On the one hand, IL-8 produced by CAFs increases cisplatin resistance in GC *via* activating NF-kB p65 and binding CXCR1/2 (46, 53). On the other one hand, IL-17 can heighten the inflammatory response by stimulating NF-kB p65 and MAPK, which in turn increases IL-8 secretion (83). These cytokines cause the production of S100A8/9, a calcium-binding protein with a small molecular weight. High levels of S100A8/9 are found in inflammatory conditions; these conditions are related with a decrease in DCs and an increase in MDSCs (90). Also, S100A8 can stimulate the expression of

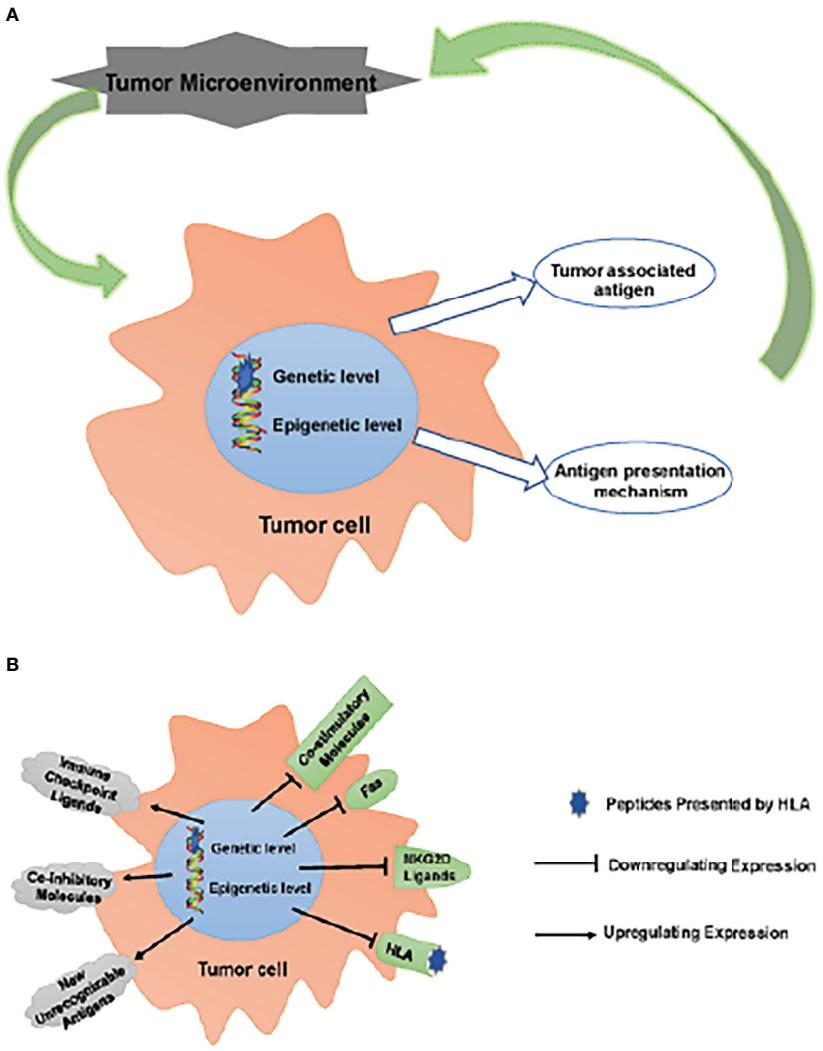

FIGURE 1
Escaping immune surveillance by changing TAA and APM. **(A)** Tumors have altered self-antigen expression through genetic mutations or epigenetic regulation, resulting in tumor cells escaping immune surveillance and clearance. Tumor cells that have escaped clearance survive to form an immunosuppressive microenvironment together with surrounding cells/cytokines, which further promotes tumor survival. **(B)** These changes up-regulate inhibitory molecules, such as ICPs (PD-1, CTLA-4, LAG-3 and TIM-3) ligands, CD44 and CD47; meanwhile, new unrecognizable antigens appear. On the other hand, the expression of co-stimulatory molecules, CD40, Fas, NKG2D ligands (MICA/B, ULBPs) and HLA is down-regulated. These changes lead to the obstacle of TAA and APM, which makes tumor cells escape immune surveillance.

PD-L1 and the polarization of TAMs from M1 to M2 (90). In contrast to M1 macrophages, which suppress antitumor immunity, M2 macrophages have been shown to have an Immunosuppressive effect. Furthermore, Probst et al. (99) discovered that immature DCs can enhance CD8+ T cell tolerance through the PD-1 and CTLA-4 molecules.

MDSCs, immature cells derived from bone marrow that can grow into dendritic cells, macrophages and granulocytes once enlarged, recruited and activated. In turn, an increased number of MDSCs can suppress DC maturation, lead to the production of Tregs, and ultimately dampen the immune response (51). Additionally, the presence of FasL on activated T lymphocytes mediates the activation of the Fas signal in tumor cells (51). It induces prostaglandin E2 (PGE2) secretion from tumor cells, which in turn increases tumor cells' potential to entice MDSCs (51). By regulating arginine and tryptophan metabolism with the help of arginase, inducible nitric oxide synthase, and indoleamine-2, 3-dioxygenase 1 (IDO1), MDSCs are also able to inhibit the activation and proliferation of T cells and NK cells (51). IL-33, a member of the IL-1 family, was found to increase the immunosuppressive capacity of MDSCs by stimulating the up-regulation of arginase-1 and blocking the death of MDSCs by enlisting MSCs (53). On the other hand, IL-33 expression was up-regulated in MDSCs following NF-kB activation. While IL-

TABLE 1 The main component and function of TME.

component	function	ref
MSCs	recruiting CAFs, M2 macrophage and IL-6; maintaining an inflammatory milieu	(46–49)
MDSCs	limiting DC maturation and inducing Treg generation due to its accumulation; suppressing T cell and NK cell activation	(21, 50–53)
M1 macrophage	secreting pro-inflammatory factors through IFN-γ and LPS activation in the early stage of inflammation	(46, 47, 49, 52, 54, 55)
M2 macrophage	inhibiting inflammatory reaction through IL-4, IL-13 activation	(46, 47, 49, 52, 56, 57)
CAFs	secreting IL-6, IL-8, VEGF, CXCL9 and TGF-β to inhibit T cell function	(49, 51, 58–60)
Th1 cell	promoting inflammatory response viasecreting IL-2, IFN-γand TNF-αin the early stage of inflammation	(61–64)
Th2 cell	inhibiting Th1 cell proliferation and inflammatory reaction	(53, 61, 63, 65)
Th17 cell	promoting inflammatory response *via* secreting IL-17, IL-22;participating in maintaining Treg/Th17 cells balance	(66–69)
Treg	regulating the inflammatory response to be chronic; secreting inhibitory cytokines to inhibit immune response; inhibiting the proliferation and activation of Teff cells and Th1 cell	(65, 66, 68, 70, 71)
DCs	antigen presentation	(72–77)
Fas/FasL	inducing apoptosis of activated Teffcells to escape immunerecognition andelimination	(28–30, 51, 62, 78)
PD-1/PD-L1	exhausting Teff cells	(18, 66, 79–82)
IL-6	mainly secreted byTh2 cells,CAFs and tumor cells;activating STAT3 andNF-kBpathwaytochange its phenotype to variants	(20, 68, 83–85)
IL-8	mainly secreted by MSCs andCAFs;stimulatingPD-L1expression viaSTAT3andmTORpathway	(46, 86)
IL-10	mainly secreted byTh2cells andTreg;inhibiting the proliferation and activation ofTeff;increasing infiltration ofTAMs, MDSCs, Th2 cells andTreg;stimulatingPD-L1expression	(18, 65, 87, 88)
IL-17	intensifying inflammatoryresponse *via* activatingNF-kB and MAPK pathway; promoting the activation ofT cells and the secretion of IL-6,IL-8 and GM-CSF	(66, 67, 83, 89)
IL-33	activating NF-kB and MAPK pathway; promoting inflammation and the secretion of GM-CSF	(53, 90)
CXCL12/ CXCR4	promotingEMTandpreventtumor cell death;promotingMDSCs accumulation;regulating PI3K/mTORpathway	(51, 91)

12 expression was down-regulated, M2 macrophage and Th2 cell polarization was enhanced (53). A novel and effective treatment may be IL-33 therapy in combination with NF-kB inhibitors.CD8+ CTLs, an HLA class I co-stimulatory molecule, are a major effector cell for eliminating tumor cells (90). Unlike the theoretical effect, IFN-γ generated by CTLs can also cause MDSCs to clump together, inhibiting the activation and proliferation of T and NK cells (90). IFN-γ can also up-regulate the expression of IDO1 from the transcriptional level (99). In addition, an active NF-kB pathway can increase CD36 transcription and fatty acid (FA) absorption activity, decreasing DC numbers, by directly modifying the s468 and t470 sites of CD36 (100, 101). CD36 prevents Treg cells from committing apoptosis and boosts Treg cell activity in specific settings (102). In addition, a rise in Treg cells occurs when NF-kB c-Rel is activated (94). Interestingly, vasoactive intestinal peptide (VIP) generated by GC cells increases responsiveness of Th2 cells, lowers proliferation of Th1 cells, interferes with the formation of B cells and suppresses the activity of NK cells by down-regulating NF-kB p65 (61). Th2 cells, on the other hand, often boost and repress the activation of effort T cells, which is counter to the antitumor effect of Th1 cells. There is a direct correlation between NF-kB pathway activation and the suppression of Fas expression on tumor surfaces (62), which in turn leads to a decrease in tumor cell death and an endless proliferation of tumor cells. Because of its significance in the immune response, inhibiting the NF-kB pathway may be helpful.

2.3.2 MAPK cascade

The mitogen-activated protein kinase (MAPK) cascade controls a wide variety of physiological and pathological processes, including cell proliferation, differentiation, stress and inflammation (103). After cells were stimulated by receptor protein tyrosine kinases (RPTKs) like growth factors (GFs), chemokines, or other stimuli, MAPK was activated by increased phosphorylation (66, 103). The MAPK pathway is separated into three branches: p38 MAPK, ERK and JNK. Extracellular regulated protein kinase (ERK) is largely activated by EGF (104). c-Jun N-terminal kinase (JNK) and p38 MAPK signaling are triggered by numerous stress stimuli, including ROS and inflammatory cytokines (such as TNF–α, IL-1β and IL-18), causing inflammation and apoptosis (70, 104, 105).

Both immune-suppressing cells (MDSCs and Treg) and immune-promoting cells (Th17 cells) can proliferate in response to GFs, chemokines and Ras mutations, which activate the ERK cascade (66). The pro-inflammatory cytokines IL-17 and IL-22 are produced mostly by Th17 cells, which evolved from Th0 cells (67). Differentiation of Th17 cells is encouraged by TGF-β, IL-1, IL-6, IL-21 and IL-23 (67), but is

inhibited by IFN-γ, IL-2 and IL-4. Treg and Th17, both CD4+ T cells, make up a balance system, and their breakdown is intimately linked to inflammatory immunosuppression in cancer (66). TGF-β is a bidirectional cytokine that, in the late stages of cancer, induces Treg and Th17 cell development from naive T cells to protect tissues against an overactive immune response (68). This functional change of TGF-β is interesting to investigate because it may offer a novel explanation for the degradation of the microenvironment. Inducing Treg cell differentiation and suppressing Th17 cell proliferation are two ways in which GC generated MSCs have recently been shown to reduce antitumor immune responses (69). C-X-C motif chemokine ligand 8 (CXCL8, IL-8) increases the malignant phenotype of GC cells, yet IL-17, which is produced by GC, can stimulate the transition of normal fibroblasts into CAFs by stimulating NF-kB signaling (106). Whether IL-17 produced by GC cells or Th17 cells serves the same purpose is currently unknown. Therefore, the role of Th17 cells in the immunological response to GC is yet to be investigated. Surprisingly, the route also increases the expression of ICPs (such as PD-1, LAG3 and CLTA4) on T cell surfaces, depleting effector T cells (Teff). PD-L1 and PD-L2 can be up-regulated at the transcriptional level by oncogenic mutations of Ras or EGFR (66). Besides, inflammatory chemicals, especially IFN-γ, often upregulate PD-L1 expression, a phenomenon known as adaptive immune resistance (81). Even though IFN-γ exerts anti-neoplastic effects in the early stages of tumor development, it has been shown that chronic IFN-γ activation of tumor cells suppresses T cells and leads to the accumulation of MDSCs (107). If we can better understand how IFN produces MDSCs, we may be able to employ interferon more effectively to treat tumors. Apoptosis in CD3+ T cells, the most abundant group of T cells, is an indication of T cell dysfunction and weakening. Apoptosis of CD3+ T cells, the main subgroups of T cells, can be induced *via* the PD-1/PD-L1 axis, when KRAS mutations increase PD-L1 (81). When the MAPK pathway is in charge of IL-10's activity, it can decrease CD8+ T cells and promote Treg-mediated immunological tolerance to cancer (70, 87). Tumor immunosuppressive cells including M2 macrophages, MDSCs and Treg can multiply while effector CD4+ and CD8+ T lymphocytes are inhibited from doing so by IL-10 and TGF-β (66). Tumor-derived cytokines such as PGE2, IL-10, IL-1, TGF-β and VEGF may induce the differentiation of immature myeloid cells (CD33+ cells) into MDSCs (87, 108). In addition, IL-1, IL-6 and IL-17 are believed to increase production of CXCL12, which can recruit MDSCs (89, 91). CXCL12 is produced in the stomach mucosa in response to inflammation, and this helps CXCR4+ MSCs and CAFs migrate. The elevated levels of CXCL12 promote EMT and inhibit tumor cell death by upregulating CXCR4 and CXCR7 in a positive feedback loop (59). Additionally, PGE2 can promote MDSC recruitment by stabilizing CXCR12 and activating chemokines including CXCL12 and CXCR4 (51). These results suggest that a combination of DC immunization and measures to decrease MDSCs accumulation is an effective way to treat tumors.

A MAPK cascade is useful for dampening the anti-tumor immune response (65). A decrease in the expression of DCs-related molecules such CD40, CD80, CD86 and IL-12 was observed, while an increase in IL-10 secretion was observed (72, 85). IL-12 is a critical cytokine for T cell activation and DC maturation and survival (73). It has been established that TGF-β, IL-6, IL-10 and VEGF all work together to produce tolerant DCs, which in turn promotes the growth of Th2 cells and Tregs (65). For instance, Marigo et al. demonstrated that IL-10 can transform naive T cells into Treg *in vivo* and *in vitro*, facilitating immune evasion (66, 71). Another need for CD8+ T cell death is active p38 MAPK (109). Expression of TNF-α, IL-6 granulocyte macrophage colony-stimulating factor (GM-CSF) are all controlled by p38 MAPK (110). Previous research has shown that the cytokines GM-CSF and IL-6 can rapidly produce MDSCs from bone marrow progenitor cells in both mice and humans (111). The therapeutic efficacy of a tumor vaccination can be diminished by the presence of tumor-derived GM-CSF, which suppresses apoptosis in MDSCs that are linked with tumors. The up-regulation of arginase-1 that is induced by IL-33 is another way in which this autocrine GM-CSF signal of MDSCs is amplified (53). Moreover, oxidative stress may activate the p38 MAPK pathway to down-regulate NKG2DL, including MICA/B and ULBP1-4 (11). In addition to its role in tumor evasion, the JNK pathway is essential for its maintenance. For instance, aberrant tumor glycolysis promotes JNK pathway expression, which in turn promotes PD-L1 expression (112, 113). Besides, IL-18 promotes tumor cell adhesion, migration, invasion and angiogenesis *via* the JNK pathway, which leads to an increase in thrombospondin 1 (TSP-1) (105). Besides, Kim and his team demonstrated that down-regulating Fas expression on the tumor surface *in vitro* by activating the JNK/p38 MAPK signaling pathway (78). Hence, MAPK inhibitors could be utilized to treat patients by decreasing the number of MDSCs and immature DCs that have accumulated in the body.

2.3.3 PI3K/AKT signaling pathway

Protein kinase B (AKT) is triggered in response to phosphatidylinositol 3-kinase (PI3K) activation, and once in the nucleus, it affects cell proliferation, invasion, metabolic reprogramming, migration, autophagy, senescence and carcinogenesis. Besides, the non-classical NF-kB signaling pathway can also be activated by AKT (82). When the phosphatase and tension homolog deleted on chromosome 10 (PTEN) gene is lost or mutated, a negative regulator of AKT is turned off, leading to PD-L1 overexpression in cancer (82, 114). T cell proliferation and effector function are inhibited by PD-1

and PD-L1 or PD-L2 interaction, which also induces apoptosis and encourages the conversion of CD4+ T cells into Foxp3+ Treg cells (88). In contrast, PD-1 up-regulates FasL and increases IL-10 production, which further suppresses the immune response (88). For example, TGF-β signaling enhances Treg cell activity by upregulating Foxp3 expression under the chronic inflammation (115). Inducer of the epithelial-mesenchymal transition (EMT) that can upregulate pro-inflammatory cytokines including IL-1, IL-6 and IL-8 to improve immune cell chemotaxis and migration is SNAIL (115), whose gene transcription is promoted by AKT activating NF-kB (115). By the way, IL-8 from MSCs increases PD-L1 expression in GC cells *via* the c-MYC signaling, which is regulated by the STAT3 and mTOR signaling pathways (86). In addition, AKT-supported immune evasion enhances the activity of immunosuppressive Treg cells by making them more resistant to CD8+ T cell-mediated death (115). AKT activation of NF-kB increases the migration of Th17 cells to TME, which are primarily regulated by C-C motif chemokines ligands 20 (CCL20) (91). C-C motif chemokine 20 (CCL20) has been found to have a crucial role in cancer as a mediator by interacting with C-C motif chemokine receptor 6 (CCR6) (116). Moreover, the presence of both CXCL12 and CXCR4 in gastric adenocarcinoma promotes GC invasion by up-regulating the PI3K/mTOR pathway and the MET process (91). Finally, through modulating Treg differentiation and PD-1/PD-L1 expression, the PI3K/AKT signaling pathway facilitates immune evasion. T cells, B cells and NK cells can all have their activation and proliferation suppressed by CD4+ Treg and CD4+ Treg can also attract MDSCs in the tumor stroma (117). For instance, stopping CD8+ T lymphocytes from being recruited to malignancies can be achieved by stimulating the PI3K/AKT/mTOR signal in M2 macrophage (118). High frequencies of Treg cells and low numbers of Teff were found to be characteristic of GC, as revealed by Kumagai's research (119). While glucose deprivation is lethal to CD8+ T cells and conventional CD4+ T cells, RhoA Y2-mutation increases the PI3K/AKT/mTOR signaling pathway, increasing the quantity of free fatty acids (FFA) in the TME, allowing Treg cells to survive and operate under FFA metabolism, which demonstrates that RhoAY42-mutant GC is not a promising candidate for PD-1 blocking monotherapy (119). Still, Targeted PI3K and PD-1 inhibitor combo therapy still outperforms PD-1 inhibitor therapy alone.

2.3.4 JAK/STAT signaling pathway

Janus kinase (JAK) rapidly recruits and catalyzes the tyrosine phosphorylation of signal transducers and tyrosine activators (STAT) situated on the receptor after receiving a signal from upstream receptor molecules. As soon as these receptors are activated, STAT proteins bind to them *via* the SH2 domain and translocate to the nucleus, where they control the transcription of specific genes (120). Interestingly, if JAK stimulates SHP-2, it may enter the MAPK cascade, and if PI3K is active, the PI3K/AKT pathway is initiated (120). Conversely, the p38 MAPK cascade can activate downstream STAT1. In addition to promoting carcinogenesis, STAT3 activation can block STAT1-mediated tumor suppression (121). In spite of this, STAT1 is typically regarded as a tumor suppressor. Intriguingly, Gabrilovich discovered that inducible nitric oxide synthase (iNOS) and arginase-1 overexpression in TAMs suppressed T cells *via* activation of STAT1 (122). Similar to what we see with PD-L1 expression *in vitro*, O'Reilly discovered that activating the STAT1 pathway may also enhance GC formation in NF-kB1$^{-/-}$mice (79).

The JAK/STAT cascade was first discovered in the IFN-α, IFN-γ and IL-6-mediated signaling pathways (123). What's more, IL-8, IL-17, IL-22, TGF-β, GM-CSF and EGF all stimulate the JAK/STAT pathway as well (124, 125). Activation of STAT3 results in increased expression of the genes encoding for Th17, M2 macrophage, MDSCs, Th2, Treg, PD-L1 and IDO 1 (86, 120, 121, 126). The expression of IL-6 is triggered by the aromatic hydrocarbon receptor (AHR) being activated by indoleamine IDO1 (82). At the same time, IDO1 activity can keep its expression continuing *via* the autocrine Kyn/AhR/IL-6/STAT3 signal loop (126). STAT3 and NF-kB were also activated by the Ras/Raf/MEK pathway, which led to the expression of IL-1, IL-6, IL-10, TNF and VEGF (127). This is because cytokines belonging to the IL-10 family block APCs, which in turn impedes CLT function and promotes Treg formation (127). Foxp3 expression by Tregs is dependent on STAT5 activation, which in turn is required for the production of GM-CSF-stimulated T cells (124, 127). Studies showed that immunosuppressive cytokines and cells accumulated in the TME due to the JAK/STAT cascade's primary role in this process. Given these results, we believe that STAT inhibitor is a potential drug and may one day be used to treat GC by enhancing positive TME.

2.3.5 Wnt signaling pathway

The Wnt signaling pathway is essential for the maintenance of pluripotency in stem cells, the regulation of embryogenesis, homeostasis, regeneration, the formation of malignant tumors and more (128). The primary objective of immunotherapy is to induce an immunocompetent response within the tumor microenvironment in order to improve recognition of the tumor, destruction of tumor cells, and responsiveness to treatment. Recent years have revealed a number of Wnt signaling pathways to be involved in immune evasion and immunological control of cancer (129).

The Wnt/β-catenin pathway relies heavily on β-catenin as a signaling molecule. Abnormal activation of Wnt/β-catenin was linked to an increase in Th2 cells, Tregs, tolerant DCs and PD-L1 (63, 74). Meanwhile, CD8+ T cell infiltration and IFN-γ release were also both suppressed by the abnormal activation of the Wnt pathway (63, 130). Furthermore, previous studies demonstrated that CD4+CD25+β-catenin+Treg cells were more robust and competitive than control Treg cells *in vivo* (131). The β-catenin/TCF4 signaling pathway induces the production of immature DCs and Treg cells phenotypes through metabolizing vitamin A to produce retinoic acid (132). Similarly, the immunosuppressive effect of over-activated Wnt/β-catenin pathway on DCs and CTLs in human melanoma has been shown by Yaguchi's team (133). Ample evidence indicates that abnormally activated Wnt/β-catenin pathway up-regulated the expression of PD-1, thereby inhibiting the infiltration of T cells in the immune environment (134). The loss of Teff cell infiltration was linked to improperly active Wnt signal transduction, and this relationship was frequently accompanied by gene mutation and abnormal methylation, according to a large-scale genomic analysis of tumor samples (135). A recent bioinformatics analysis found that the abnormal activation of tumor cells intrinsic Wnt/β-catenin signaling is critically important in non-T cells infiltration tumors (136), which is of great significance for the treatment of immune desert tumors. For example, Wang et al. demonstrated that the β-catenin/TCF inhibitor iCRT14 significantly suppressed tumor growth *via* effectively enhancing the infiltration of T and NK cellsin anexperimental model of T cell deficiency (137). However, it is more important to determine the sequence of Wnt pathway's abnormal activation and T cell infiltration in immune desert tumors. Besides, its abnormal activation also prevented CD4+ T cells from developing into Th1 and Th17 cells (63). In addition, the Wnt pathway regulates multiple immune cell functions, including those of MDSCs and NK cells, that plays a crucial role in cancer immune editing (138). Studies have demonstrated that the Wnt pathway is primarily responsible for cell cycle progression and the production of aberrant proteins that might cause cancer in cells. The Wnt pathway's mechanism has recently been refined thanks to studies in the field of immunology. There is a stronger infiltration of Treg cells, immature DCs and lower Teff cells after activation of the Wnt pathway (139, 140).

2.3.6 TGF-β signaling pathway

Differentiation, apoptosis, migration and other cellular activities are all controlled by the TGF-β signaling pathway, which is produced in the TME and secreted by tumor cells and stromal cells (141). Therefore, this pathway and its interruption play an important role in tumor suppression or promotion (142). The function of TGF-β mainly involves two pathways, namely the canonical pathway (Smad-dependent) and the non-canonical pathway (Smad-independent). When it comes to canonical pathway, the drosophila mothers against decapentaplegic protein (Smad) are essential. Activated R-Smads join with Smad4 to create a heteromeric Smad complex, then entering cell nuclear. It regulates gene expression by binding transcription factors and transcription co-regulators (143). Restoring tumor immunity *in vivo* may be as simple as blocking the TGF-β signaling pathway in CD8+ T cells, as discovered by Thomas et al. (144). TGF-β/Smad pathway also aided in the infiltration of Treg cells (145). These findings support the hypothesis that the TGF-β/Smad signaling pathway, by suppressing immune responses, promotes cancer. In addition, the tumor cells activate TGF-β signaling, which alters a major component of the TME known as CAFs, which in turn alters the extracellular matrix (ECM) in a way that rejects immune cells and may affect immunotherapy responses (146). Besides, TGF-β also operates on both ends of the NKG2D axis, and studies have demonstrated that it substantially inhibits NKG2D-mediated tumor killing (147).

2.3.7 Other signaling pathways

Notch and Hippo pathways are also engaged in immune evasion; high levels of Notch receptor expression correspond with the presence of immature DCs, M2 macrophages, N2 neutrophils and CD4+ T cells in GC tissue (75). Similarly, high expression of Notch3 is associated with low infiltration of activated CD8+ T cells in TME (140). To decrease tumor growth, Hippo signaling has been studied extensively. Depletion of CD8+ CTLs and elevation of FoxP3+ Treg have both been linked to interference with the Hippo pathway (148). However, Hippo pathway inactivation can activate EGFR, which then activates the PI3K/mTOR and Ras/Raf pathways (149). As a result, one therapeutic strategy involves focusing on molecules that set off the Hippo pathway. In short, these intricate pathways work together to keep the surrounding tissue hospitable to tumor growth (Figure 2).

3 Impact of ncRNAs on immune evasion

As we will see, ncRNAs are an integral part of the epigenetic regulatory process, and their interference with gene transcription and translation has an effect on TAA, AMP and TME. Epstein-Barr virus (EBV) and Helicobacter pylori (Hp) infection in GC entail a more sophisticated process of ncRNAs, and this review will not cover it.

Micro RNAs (miRNAs) adversely regulate gene expression by interacting with mRNA 3'-UTR targets, resulting in polyadenylation, decreased mRNA stability, and translational inhibition (150). On the other hand, miRNAs can influence transcription by binding to certain genes in a targeted manner (150). Inhibiting PD-L1 protein translation by binding to the 3'-

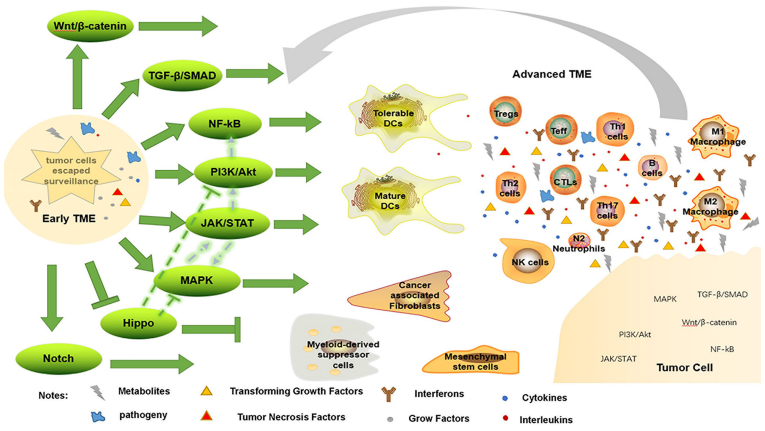

FIGURE 2
Immune evasion by changing TME. When tumor cells escape immune surveillance, they form early TME, enhancing immunosuppressive environment, weakening anti-tumor response, and further promote tumorigenesis through a variety of signaling pathways. Conversely, this advanced TME promotes tumor progression through these pathways.

UTR of PD-L1 mRNA, miR-200c enhances the anti-tumor response by decreasing PD-L1 expression (151). MiR-16-5p, miR-152, miR-375 and miR-570 are other micro RNAs that can inhibit PD-L1 expression (54, 60, 152–154). In particular, miR-152 is down-regulated by TGF-β and can improve immunological recognition by targeting the 3'-UTR of HLA-G and PD-L1 mRNA (60, 152). By inactivating JAK2, a common upstream inhibitor of STAT3, miR-375 suppresses the JAK2/STAT3 pathway to down-regulate PD-L1 (153). Also, miR-588, miR-29a-3p, miR-34a and miR-30c increase anti-tumor immune response by promoting CD8+ T cell, M1 macrophage, B cells, GZMB and IFN-γ infiltration (55, 155–157). It has been shown that CAFs infiltration can be suppressed by miR-141-3p, which can directly target STAT4 to inhibit its expression and restrict the Wnt/β-catenin pathway (158). On the contrary, miR-1920 and miR-675-3p could up-regulate the expression of PD-1/PD-L1 (159, 160). There is a drop in CD8+ T cells and NK cells in response to miR-494, miR-1269a and miR-17-5p, but an increase in MDSCs, M2 macrophages, tolerance DCs and Treg infiltration (56, 64, 76). A protein called monocyte chemoattractant protein-1 (ZC3H12A) is encoded by this gene, which has anti-tumor effects because it suppresses chronic inflammation. Mir-425-3p can directly target this gene to boost the inflammatory response and facilitate immune evasion (161).

Sponging with miRNAs to interfere with its function, influencing downstream targets, is also relevant for long noncoding RNAs (lncRNAs) and circular RNAs (circRNAs). For instance, linc00936 works together with miR-425-3p to enhance the body's natural anti-inflammatory response by increasing ZC3H12A expression (161). Oppositely, lncRNA POU3F3, HOTAIR, MALAT1, H19, MIR100HG and linc00963 favored immunological escape by down-regulating IL-21R, Treg, M2 macrophages and TGF-β and up-regulating the infiltration of mature DCs, CD8+ T cells, M1 macrophages and IFN-γ (57, 77, 162–166). Additionally, PD-L1 expression was boosted by SNHG15 and UCA1 through spongingmiR-141, miR-193a and miR-214, respectively (167, 168). There has been little exploration into the role of circRNAs in GC immune escape, but this could be an exciting new field of study. Table 2 summarizes this paper's discussion of the role of ncRNAs as regulatory mechanisms in GC immune evasion.

4 Perspectives

Despite progress in gene sequencing technology and the promise of precision medicine, there are still too many examples when treatment causes more harm than good. The primary goals of modern immunotherapy are Teff function restoration and ICPs inhibition (169). In recent years, however, monotherapy has been found to have drawbacks; For example, RhoA Y42 mutant GC is not a viable candidate for PD-1 blocking monotherapy (119). Furthermore, several signaling pathway components, such as EGFR, HER2 and VEGF, have become effective therapeutic targets because of their crucial involvement in GC (170). Specially, the HER2 inhibitor, Trastuzumab, can reduce the activity of the PI3K/AKT pathway, which is responsible for the uncontrolled growth of tumor cells, and so restore innate antitumor immunity (12, 13, 170). Tumor cell proliferation, invasion, migration, and the development of an immunosuppressive TME can all be stifled by inhibiting these signaling pathways. In addition, ncRNAs are treated in two major ways, either as an alternative

TABLE 2 Impact of epigenetic regulation on immune evasion in GC.

	Mechanism	Effect	Ref
miR-200c	down-regulating PD-L1 expression	anti-oncogene	(151)
miR-16-5p	down-regulating PD-L1 expression; increasing Teff infiltration	anti-oncogene	(54)
miR-152	down-regulating HLA-G and PD-L1 expression	anti-oncogene	(60, 152)
miR-375	inactivating JAK2/STAT3 pathway to decrease PD-L1 expression	anti-oncogene	(153)
miR-570	down-regulating PD-L1 expression	anti-oncogene	(154)
miR-588	increasing CD8+ T cellsinfiltration by up-regulating CXCL5/9/10	anti-oncogene	(155)
miR-29a-3p	increasing M1 macrophage and B cell infiltration by targeting COL1A2	anti-oncogene	(156)
miR-34a	reducing lactic acid accumulation in T cells; increasing Teff, IFN-γ and GZMB infiltration	anti-oncogene	(157)
miR-30c	promoting M1 macrophage polarization	anti-oncogene	(55)
miR-141-3p	inhibitingSTAT4/Wnt/β-catenin pathway to decrease CAFs	anti-oncogene	(158)
miR-1290	up-regulating PD-1 expression via Ghl2/ZEB1 axis	oncogene	(159)
miR-675-3p	up-regulating PD-L1 expression via CXXC4/MAPK axis	oncogene	(160)
miR-494	increasing MDSCs infiltration by PTEN/PI3K/Akt axis	oncogene	(64)
miR-1269a	inhibiting CXCL9 expression to increase MDSCs, M2 macrophage and decrease CD8+, CD4+ T, NK and B cells	oncogene	(56)
miR-17-5p	inhibiting DCs endocytosis; promoting Treg differentiation;decreasingTNF-α, IL-12 and increasing IL-10 infiltration	oncogene	(76)
miR-425-3p	amplifying inflammation by targeting ZC3H12A	oncogene	(161)
linc-00936	amplifying anti-inflammatory response *via* sponging miR-425-3p	anti-oncogene	(162)
linc-POU3F3	recruiting TGF-β to activate TGF-β/SMAD2/3 pathway; promoting Treg differentiation	oncogene	(163)
linc-00963	inhibiting DCsmaturationvia miR-612/CDC5L axis	oncogene	(77)
lncRNA HOTAIR	up-regulating COL5A1 to decrease the infiltration of CD8+ T cell, M1 macrophage, neutrophils and mature DCs by sponging miR-1277-5p; up-regulating CXCR4 by sponging miR-126	oncogene	(164, 165)
lncRNA MALAT1	up-regulating IL-21R/JAK2/STAT3 *via* sponging miR-125a	oncogene	(57)
lncRNA H19	attenuating Teff(especially Th1 andCD8+ T cells), NK cell functionand increasing M2 macrophage number by activating LDHA	oncogene	(166)
lncRNA MIR100HG	decreasing Teffand IFN-γviaactivating ERK1/2	oncogene	(167)
lncRNA SNHG15	up-regulating PD-L1 expression *via* sponging miR-141	oncogene	(168)
lncRNA UCA1	up-regulating PD-L1 expression *via* sponging miR-193a, miR-214	oncogene	(150)

therapy or an inhibitory therapy, each of which could be used as a therapeutic target (171). Hence, research into immunotherapies that use molecularly targeted drugs in tandem with conventional ones has great potential in the future.

5 Conclusion

Tumor immune evasion, which includes TAA insufficiency, APM abnormalities, TME composition changes, etc., is a significant research field. Tumor cells' immunogenicity can be changed by even a little change in the antigen, allowing them to evade immune detection. Tumor formation and spread into the TME are aided by the early microenvironment once the tumor escapes immune monitoring. We present a brief overview of the immune evasion pathways associated with GC that can be used as immunotherapy targets. While the immune evasion process may be complicated, it must be deciphered in order to provide targeted care. More research and clinical trials are needed to better understand immune evasion, particularly in relation to the prognosis of GC and the development of new therapeutic options for the many distinct subtypes of the disease.

Author contributions

JW, TL and TH wrote the draft review, JW was responsible for responding to the reviewer's questions and revising the paper, MS was involved in literature search and curation, XW was involved in original idea and critical revision of the manuscript. All authors contributed to the article and approved the submitted version.

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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. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

2. Wang M, Zhao J, Zhang L, Wei F, Lian Y, Wu Y, et al. Role of tumor microenvironment in tumorigenesis. *J Cancer* (2017) 8(5):761–73. doi: 10.7150/jca.17648

3. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: From immunosurveillance to tumor escape. *Nat Immunol* (2002) 3(11):991–8. doi: 10.1038/ni1102-991

4. Schreiber R, Old L, Smyth M. Cancer immunoediting: Integrating immunity's roles in cancer suppression and promotion. *Sci (New York NY)* (2011) 331 (6024):1565–70. doi: 10.1126/science.1203486

5. Schiavoni G, Gabriele L, Mattei F. The tumor microenvironment: A pitch for multiple players. *Front Oncol* (2013) 3:90. doi: 10.3389/fonc.2013.00090

6. Scully C, Georgakopoulou E, Hassona Y. The immune system: Basis of so much health and disease: 4. immunocytes. *Dental Update* (2017) 44(5):436–8. doi: 10.12968/denu.2017.44.5.436

7. Kuroki M, Hachimine K, Huang J, Shibaguchi H, Kinugasa T, Maekawa S, et al. Re-targeting of cytotoxic T lymphocytes and/or natural killer cells to cea-expressing tumor cells with anti-cea antibody activity. *Anticancer Res* (2005) 25:3725–32.

8. Herceg Z, Vaissière T. Epigenetic mechanisms and cancer: An interface between the environment and the genome. *Epigenetics* (2011) 6(7):804–19. doi: 10.4161/epi.6.7.16262

9. Jaenisch R, Bird A. Epigenetic regulation of gene expression: How the genome integrates intrinsic and environmental signals. *Nat Genet* (2003) 33:245–54. doi: 10.1038/ng1089

10. Adams-Cioaba M, Min J. Structure and function of histone methylation binding proteins. *Biochem Cell Biol* (2009) 87(1):93–105. doi: 10.1139/O08-129

11. Chen Z, Chen L, Baker K, Olszak T, Zeissig S, Huang Y, et al. Ceacam1 dampens antitumor immunity by down-regulating Nkg2d ligand expression on tumor cells. *J Exp Med* (2011) 208(13):2633–40. doi: 10.1084/jem.20102575

12. Kiessling R, Wei W, Herrmann F, Lindencrona J, Choudhury A, Kono K, et al. Cellular immunity to the her-2/Neu protooncogene. *Adv Cancer Res* (2002) 85:101–44. doi: 10.1016/S0065-230X(02)85004-7

13. Wu S, Zhang Q, Zhang F, Meng F, Liu S, Zhou R, et al. Her2 recruits Akt1 to disrupt sting signalling and suppress antiviral defence and antitumour immunity. *Nat Cell Biol* (2019) 21(8):1027–40. doi: 10.1038/s41556-019-0352-z

14. Xing S, Ferrari de Andrade L. Nkg2d and Mica/B shedding: A 'Tag game' between nk cells and malignant cells. *Clin Transl Immunol* (2020) 9(12):e1230. doi: 10.1002/cti2.1230

15. Baragaño Raneros A, Martín-Palanco V, Fernandez A, Rodriguez R, Fraga M, Lopez-Larrea C, et al. Methylation of Nkg2d ligands contributes to immune system evasion in acute myeloid leukemia. *Genes Immun* (2015) 16(1):71–82. doi: 10.1038/gene.2014.58

16. Wu J, Atteridge C, Wang X, Seya T, Plymate S. Obstructing shedding of the immunostimulatory mhc class I chain-related gene b prevents tumor formation. *Clin Cancer Res* (2009) 15(2):632–40. doi: 10.1158/1078-0432.Ccr-08-1305

17. Yao S, Chen L. Reviving exhausted T lymphocytes during chronic virus infection by B7-H1 blockade. *Trends Mol Med* (2006) 12(6):244–6. doi: 10.1016/j.molmed.2006.04.007

18. Zhang Y, Wang F, Sun H, Huang Y, Gao J, Huang H. Apatinib combined with pd-L1 blockade synergistically enhances antitumor immune responses and promotes hev formation in gastric cancer. *J Cancer Res Clin Oncol* (2021) 147 (8):2209–22. doi: 10.1007/s00432-021-03633-3

19. Zou J, Wu D, Li T, Wang X, Liu Y, Tan S. Association of pd-L1 gene Rs4143815 C>G polymorphism and human cancer susceptibility: A systematic review and meta-analysis. *Pathology Res Pract* (2019) 215(2):229–34. doi: 10.1016/j.prp.2018.12.002

20. Ju X, Zhang H, Zhou Z, Chen M, Wang Q. Tumor-associated macrophages induce pd-L1 expression in gastric cancer cells through il-6 and tnf-α signaling. *Exp Cell Res* (2020) 396(2):112315. doi: 10.1016/j.yexcr.2020.112315

21. Gunaydin G, Kesikli S, Guc D. Cancer associated fibroblasts have phenotypic and functional characteristics similar to the fibrocytes that represent a novel mdsc subset. *Oncoimmunology* (2015) 4(9):e1034918. doi: 10.1080/2162402x.2015.1034918

22. Neo S, Yang Y, Record J, Ma R, Chen X, Chen Z, et al. Cd73 immune checkpoint defines regulatory nk cells within the tumor microenvironment. *J Clin Invest* (2020) 130(3):1185–98. doi: 10.1172/jci128895

23. Li D, Xiang S, Shen J, Xiao M, Zhao Y, Wu X, et al. Comprehensive understanding of B7 family in gastric cancer: Expression profile, association with clinicopathological parameters and downstream targets. *Int J Biol Sci* (2020) 16 (4):568–82. doi: 10.7150/ijbs.39769

24. Kuhn N, Purdon T, van Leeuwen D, Lopez A, Curran K, Daniyan A, et al. Cd40 ligand-modified chimeric antigen receptor T cells enhance antitumor function by eliciting an endogenous antitumor response. *Cancer Cell* (2019) 35 (3):473–88.e6. doi: 10.1016/j.ccell.2019.02.006

25. Yoshida K, Tsujimoto H, Matsumura K, Kinoshita M, Takahata R, Matsumoto Y, et al. Cd47 is an adverse prognostic factor and a therapeutic target in gastric cancer. *Cancer Med* (2015) 4(9):1322–33. doi: 10.1002/cam4.478

26. Morrissey M, Kern N, Vale R. Cd47 ligation repositions the inhibitory receptor sirpa to suppress integrin activation and phagocytosis. *Immunity* (2020) 53(2):290–302.e6. doi: 10.1016/j.immuni.2020.07.008

27. Liu M, Zhou J, Chen Z, Cheng A. Understanding the epigenetic regulation of tumours and their microenvironments: Opportunities and problems for epigenetic therapy. *J Pathol* (2017) 241(1):10–24. doi: 10.1002/path.4832

28. Olimón-Andalón V, Aguilar-Lemarroy A, Ratkovich-González S, Uribe-López A, Mariscal-Ramírez I, Delgadillo-Cristerna R, et al. Proapoptotic Cd95l levels in normal human serum and sera of breast cancer patients. *Tumour Biol* (2015) 36(5):3669–78. doi: 10.1007/s13277-014-3005-7

29. Wang X, Fu Z, Chen Y, Liu L. Fas expression is downregulated in gastric cancer. *Mol Med Rep* (2017) 15(2):627–34. doi: 10.3892/mmr.2016.6037

30. Xu Y, He B, Li R, Pan Y, Gao T, Deng Q, et al. Association of the polymorphisms in the Fas/Fasl promoter regions with cancer susceptibility: A systematic review and meta-analysis of 52 studies. *PloS One* (2014) 9(3):e90090. doi: 10.1371/journal.pone.0090090

31. Li W, Xia D, Wang Y, Li Y, Xue Y, Wu X, et al. Relationship between aberrant methylation of FAS promoter and biological behavior of bladder urothelial carcinoma. *J Huazhong Univ Sci Technology(Medical Sciences)* (2011) 31(06):794–8. doi: 10.1007/s11596-011-0679-6

32. Balan S, Saxena M, Bhardwaj N. Dendritic cell subsets and locations. *Int Rev Cell Mol Biol* (2019) 348:1–68. doi: 10.1016/bs.ircmb.2019.07.004

33. 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 Discovery* (2017) 7(12):1420–35. doi: 10.1158/2159-8290.Cd-17-0593

34. Du Y, Zhang T, Dai L, Zheng X, Gorin A, Oishi J, et al. Effects of mutations on replicative fitness and major histocompatibility complex class I binding affinity are among the determinants underlying cytotoxic-T-Lymphocyte escape of hiv-1 gag epitopes. *mBio* (2017) 8(6):e01050-17. doi: 10.1128/mBio.01050-17

35. *Cellular and Molecular Immunology*. Elsevier Inc (2018).

36. Rey-Jurado E, Riedel CA, González PA, Bueno SM, Kalergis AM. Contribution of autophagy to antiviral immunity. *FEBS Lett* (2015) 589 (22):3461–70. doi: 10.1016/j.febslet.2015.07.047

37. Powell A, Horgan P, Edwards J. The bodies fight against cancer: Is human leucocyte antigen (Hla) class 1 the key? *J Cancer Res Clin Oncol* (2012) 138(5):723–8. doi: 10.1007/s00432-012-1192-4

38. del Campo A, Kyte J, Carretero J, Zinchencko S, Méndez R, González-Aseguinolaza G, et al. Immune escape of cancer cells with Beta2-microglobulin loss over the course of metastatic melanoma. *Int J Cancer* (2014) 134(1):102–13. doi: 10.1002/ijc.28338

39. Hirata T, Yamamoto H, Taniguchi H, Horiuchi S, Oki M, Adachi Y, et al. Characterization of the immune escape phenotype of human gastric cancers with and without high-frequency microsatellite instability. *J Pathol* (2007) 211(5):516–23. doi: 10.1002/path.2142

40. Ye Q, Shen Y, Wang X, Yang J, Miao F, Shen C, et al. Hypermethylation of hla class I gene is associated with hla class I down-regulation in human gastric cancer. *Tissue Antigens* (2010) 75(1):30–9. doi: 10.1111/j.1399-0039.2009.01390.x

41. Florea ID, Karaoulani C. Epigenetic changes of the immune system with role in tumor development. cancer epigenetics for precision medicine. *Methods Mol Biol* (2018) 203–18. doi: 10.1007/978-1-4939-8751-1_11

42. Zingg D, Arenas-Ramirez N, Sahin D, Rosalia R, Antunes AT, Haeusel J, et al. The histone methyltransferase Ezh2 controls mechanisms of adaptive resistance to tumor immunotherapy. *Cell Rep* (2017) 20(4):854–67. doi: 10.1016/j.celrep.2017.07.007

43. Klopp Ann H, et al. Concise review: Dissecting a discrepancy in the literature: do mesenchymal stem cells support or suppress tumor growth? *Stem Cells (Dayton Ohio)* (2011) 29(1):11–9. doi: 10.1002/stem.559

44. Siddle H, Kreiss A, Tovar C, Yuen C, Cheng Y, Belov K, et al. Reversible epigenetic down-regulation of mhc molecules by devil facial tumour disease illustrates immune escape by a contagious cancer. *Proc Natl Acad Sci United States America* (2013) 110(13):5103–8. doi: 10.1073/pnas.1219920110

45. Mei ZI, Huang J, Qiao B, King-yin Lam A. Immune checkpoint pathways in immunotherapy for head and neck squamous cell carcinoma. *Int J Oral Sci* (2020) 12(02):114–22. doi: 10.1038/s41368-020-0084-8

46. Zhai J, Shen J, Xie G, Wu J, He M, Gao L, et al. Cancer-associated fibroblasts-derived il-8 mediates resistance to cisplatin in human gastric cancer. *Cancer Lett* (2019) 454:37–43. doi: 10.1016/j.canlet.2019.04.002

47. Zhang X, Shi H, Yuan X, Jiang P, Qian H, Xu W. Tumor-derived exosomes induce N2 polarization of neutrophils to promote gastric cancer cell migration. *Mol Cancer* (2018) 17(1). doi: 10.1186/s12943-018-0898-6

48. Whiteside T. Exosome and mesenchymal stem cell cross-talk in the tumor microenvironment. *Semin Immunol* (2018) 35:69–79. doi: 10.1016/j.smim.2017.12.003

49. Shen Y, Xue C, Li X, Ba L, Gu J, Sun Z, et al. Effects of gastric cancer cell-derived exosomes on the immune regulation of mesenchymal stem cells by the nf-kb signaling pathway. *Stem Cells Dev* (2019) 28(7):464–76. doi: 10.1089/scd.2018.0125

50. Jin X, Ding D, Yan Y, Li H, Wang B, Ma L, et al. Phosphorylated Rb promotes cancer immunity by inhibiting nf-kappab activation and pd-L1 expression. *Mol Cell* (2019) 73(1):22–35.e6. doi: 10.1016/j.molcel.2018.10.034

51. Yang F, Wei Y, Cai Z, Yu L, Jiang L, Zhang C, et al. Activated cytotoxic lymphocytes promote tumor progression by increasing the ability of 3ll tumor cells to mediate mdsc chemoattraction *Via* fas signaling. *Cell Mol Immunol* (2015) 12 (1):66–76. doi: 10.1038/cmi.2014.21

52. Li Z, Wang J, Zhang X, Liu P, Zhang X, Wang J, et al. Proinflammatory S100a8 induces pd-L1 expression in macrophages, mediating tumor immune escape. *J Immunol* (2020) 204(9):2589–99. doi: 10.4049/jimmunol.1900753

53. Xiao P, Wan X, Cui B, Liu Y, Qiu C, Rong J, et al. Interleukin 33 in tumor microenvironment is crucial for the accumulation and function of myeloid-derived suppressor cells. *Oncoimmunology* (2016) 5(1):e1063772. doi: 10.1080/2162402x.2015.1063772

54. Li Z, Suo B, Long G, Gao Y, Song J, Zhang M, et al. Exosomal mirna-16-5p derived from M1 macrophages enhances T cell-dependent immune response by regulating pd-L1 in gastric cancer. *Front Cell Dev Biol* (2020) 8:572689. doi: 10.3389/fcell.2020.572689

55. Zhihua Y, Yulin T, Yibo W, Wei D, Yin C, Jiahao X, et al. Hypoxia decreases macrophage glycolysis and M1 percentage by targeting microrna-30c and mtor in human gastric cancer. *Cancer Sci* (2019) 110(8):2368–77. doi: 10.1111/cas.14110

56. Zhang K, Zhang L, Mi Y, Tang Y, Ren F, Liu B, et al. A cerna network and a potential regulatory axis in gastric cancer with different degrees of immune cell infiltration. *Cancer Sci* (2020) 111(11):4041–50. doi: 10.1111/cas.14634

57. Sun L, Li J, Yan W, Yao Z, Wang R, Zhou X, et al. H19 promotes aerobic glycolysis, proliferation, and immune escape of gastric cancer cells through the microrna-519d-3p/Lactate dehydrogenase a axis. *Cancer Sci* (2021) 112(6):2245–59. doi: 10.1111/cas.14896

58. Wang J, Hu W, Wu X, Wang K, Yu J, Luo B, et al. Cxcr1 promotes malignant behavior of gastric cancer cells *in vitro* and *in vivo* in akt and Erk1/2 phosphorylation. *Int J Oncol* (2016) 48(5):2184–96. doi: 10.3892/ijo.2016.3428

59. Domanska U, Kruizinga R, Nagengast W, Timmer-Bosscha H, Huls G, de Vries E, et al. A review on Cxcr4/Cxcl12 axis in oncology: No place to hide. *Eur J Cancer (Oxford England: 1990)* (2013) 49(1):219–30. doi: 10.1016/j.ejca.2012.05.005

60. Guan Z, Song B, Liu F, Sun D, Wang K, Qu H. TGF-B induces hla-G expression through inhibiting mir-152 in gastric cancer cells. *J Biomed Sci* (2015) 22:107. doi: 10.1186/s12929-015-0177-4

61. Wang C, Zhou XJ, Li YY, Wan J, Yang LY, Li GH. Effect of vasoactive intestinal peptide (Vip) on Nkg2d signal pathway and its contribution to immune escape of Mkn45 cells. *ScientificWorld J* (2013) 2013:429545. doi: 10.1155/2013/429545

62. Shang H, Cao Z, Zhao J, Guan J, Liu J, Peng J, et al. Babao Dan induces gastric cancer cell apoptosis *Via* regulating mapk and nf-Kb signaling pathways. *J Int Med Res* (2019) 47(10):5106–19. doi: 10.1177/0300060519867502

63. Li X, Xiang Y, Li F, Yin C, Li B, Ke X. Wnt/β-catenin signaling pathway regulating T cell-inflammation in the tumor microenvironment. *Front Immunol* (2019) 10:2293. doi: 10.3389/fimmu.2019.02293

64. Moaaz M, Lotfy H, Elsherbini B, Motawea M, Fadali G. TGF-B enhances the anti-inflammatory effect of tumor- infiltrating Cd33+11b+Hla-Dr myeloid-derived suppressor cells in gastric cancer: A possible relation to microrna-494. *Asian Pacific J Cancer prevention: APJCP* (2020) 21(11):3393–403. doi: 10.31557/apjcp.2020.21.11.3393

65. Zhao F, Falk C, Osen W, Kato M, Schadendorf D, Umansky V. Activation of P38 mitogen-activated protein kinase drives dendritic cells to become tolerogenic in ret transgenic mice spontaneously developing melanoma. *Clin Cancer Res* (2009) 15(13):4382–90. doi: 10.1158/1078-0432.CCR-09-0399

66. Salaroglio IC, Mungo E, Gazzano E, Kopecka J, Riganti C. Erk is a pivotal player of chemo-Immune-Resistance in cancer. *Int J Mol Sci* (2019) 20(10). doi: 10.3390/ijms20102505

67. Martin-Orozco N, Muranski P, Chung Y, Yang X, Yamazaki T, Lu S, et al. T Helper 17 cells promote cytotoxic T cell activation in tumor immunity. *Immunity* (2009) 31(5):787–98. doi: 10.1016/j.immuni.2009.09.014

68. Yuan X, Chen L, Zhang T, Ma Y, Zhou Y, Zhao Y, et al. Gastric cancer cells induce human Cd4+Foxp3+ regulatory T cells through the production of TGF-B1. *World J Gastroenterol* (2011) 17(15):2019–27. doi: 10.3748/wjg.v17.i15.2019

69. Wang M, Chen B, Sun XX, Zhao XD, Zhao YY, Sun L, et al. Gastric cancer tissue-derived mesenchymal stem cells impact peripheral blood mononuclear cells

Via disruption of Treg/Th17 balance to promote gastric cancer progression. *Exp Cell Res* (2017) 361(1):19–29. doi: 10.1016/j.yexcr.2017.09.036

70. Peluso I, Yarla NS, Ambra R, Pastore G, Perry G. Mapk signalling pathway in cancers: Olive products as cancer preventive and therapeutic agents. *Semin Cancer Biol* (2019) 56:185–95. doi: 10.1016/j.semcancer.2017.09.002

71. Zdanov S, Mandapathil M, Abu Eid R, Adamson-Fadeyi S, Wilson W, Qian J, et al. Mutant kras conversion of conventional T cells into regulatory T cells. *Cancer Immunol Res* (2016) 4(4):354–65. doi: 10.1158/2326-6066.Cir-15-0241

72. Wang S, Yang J, Qian J, Wezeman M, Kwak LW, Yi Q. Tumor evasion of the immune system: Inhibiting P38 mapk signaling restores the function of dendritic cells in multiple myeloma. *Blood* (2006) 107(6):2432–9. doi: 10.1182/blood-2005-06-2486

73. Lippitz B. Cytokine patterns in patients with cancer: A systematic review. *Lancet Oncol* (2013) 14(6):e218–28. doi: 10.1016/s1470-2045(12)70582-x

74. Deng R, Zuo C, Li Y, Xue B, Xun Z, Guo Y, et al. The innate immune effector Isg12a promotes cancer immunity by suppressing the canonical Wnt/Beta-catenin signaling pathway. *Cell Mol Immunol* (2020) 17(11):1163–79. doi: 10.1038/s41423-020-00549-9

75. Hu J, Yu J, Gan J, Song N, Shi L, Liu J, et al. Notch1/2/3/4 are prognostic biomarker and correlated with immune infiltrates in gastric cancer. *Aging* (2020) 12(3):2595–609. doi: 10.18632/aging.102764

76. Cui Z, Xie X, Qi W, Yang Y, Bai Y, Han J, et al. Cell-free mir-17-5p as a diagnostic biomarker for gastric cancer inhibits dendritic cell maturation. *OncoTargets Ther* (2019) 12:2661–75. doi: 10.2147/ottS197682

77. Wei Z, Chen L, Meng L, Han W, Huang L, Xu A. Lncrna hotair promotes the growth and metastasis of gastric cancer by sponging mir-1277-5p and upregulating Col5a1. *Gastric Cancer* (2020) 23(6):1018–32. doi: 10.1007/s10120-020-01091-3

78. Kim H, Lee J, Park H, Lee G, Kim H, Ha K, et al. Schizandra chinensis extracts induce apoptosis in human gastric cancer cells *Via* Jnk/P38 mapk activation and the ros-Mediated/Mitochondria-Dependent pathway. *Pharm Biol* (2015) 53(2):212–9. doi: 10.3109/13880209.2014.913297

79. O'Reilly LA, Putoczki TL, Mielke LA, Low JT, Lin A, Preaudet A, et al. Loss of nf-Kb1 causes gastric cancer with aberrant inflammation and expression of immune checkpoint regulators in a stat-1-Dependent manner. *Immunity* (2018) 48 (3):570–83.e8. doi: 10.1016/j.immuni.2018.03.003

80. Zhang S, Zeng Z, Liu Y, Huang J, Long J, Wang Y, et al. Prognostic landscape of tumor-infiltrating immune cells and immune-related genes in the tumor microenvironment of gastric cancer.

81. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1 and IFN-Γ up-regulate PD-L1 in nasopharyngeal carcinoma: Implications for oncotargeted therapy. *Oncotarget* (2014) 5(23):12189–202. doi: 10.18632/oncotarget.2608

82. Chen J, Jiang C, Jin L, Zhang X. Regulation of pd-L1: A novel role of pro-survival signalling in cancer. *Ann Oncol* (2016) 27(3):409–16. doi: 10.1093/annonc/mdv615

83. Sokolova O, Naumann M. Nf-Kb signaling in gastric cancer. *Toxins* (2017) 9 (4). doi: 10.3390/toxins9040119

84. Zhu Q, Zhang X, Zhang L, Li W, Wu H, Yuan X, et al. The il-6-Stat3 axis mediates a reciprocal crosstalk between cancer-derived mesenchymal stem cells and neutrophils to synergistically prompt gastric cancer progression. *Cell Death Dis* (2014) 5:e1295. doi: 10.1038/cddis.2014.263

85. Bernal C, Zorro M, Sierra J, Gilchrist K, Botero J, Baena A, et al. Encephalitozoon intestinalis inhibits dendritic cell differentiation through an il-6-Dependent mechanism. *Front Cell infection Microbiol* (2016) 6:4. doi: 10.3389/fcimb.2016.00004

86. Sun L, Wang Q, Chen B, Zhao Y, Shen B, Wang H, et al. Gastric cancer mesenchymal stem cells derived il-8 induces pd-L1 expression in gastric cancer cells *Via* Stat3/Mtor-C-Myc signal axis. *Cell Death Dis* (2018) 9(9):928. doi: 10.1038/s41419-018-0988-9

87. Ostrand-Rosenberg S, Sinha P. Myeloid-derived suppressor cells: Linking inflammation and cancer. *J Immunol (Baltimore Md: 1950)* (2009) 182(8):4499–506. doi: 10.4049/jimmunol.0802740

88. Wolchok J, Kluger H, Callahan M, Postow M, Rizvi N, Lesokhin A, et al. Nivolumab plus ipilimumab in advanced melanoma. *New Engl J Med* (2013) 369 (2):122–33. doi: 10.1056/NEJMoa1302369

89. Zhang F, Cui J, Gao H, Yu H, Gao F, Chen J, et al. Cancer-associated fibroblasts induce epithelial-mesenchymal transition and cisplatin resistance in ovarian cancer *Via* Cxcl12/Cxcr4 axis. *Future Oncol (London England)* (2020) 16 (32):2619–33. doi: 10.2217/fon-2020-0095

90. Hosoi A, Matsushita H, Shimizu K, Fujii S, Ueha S, Abe J, et al. Adoptive cytotoxic T lymphocyte therapy triggers a counter-regulatory immunosuppressive mechanism *Via* recruitment of myeloid-derived suppressor cells. *Int J Cancer* (2014) 134(8):1810–22. doi: 10.1002/ijc.28506

91. Daniel SK, Seo YD, Pillarisetty VG. The Cxcl12-Cxcr4/Cxcr7 axis as a mechanism of immune resistance in gastrointestinal malignancies. *Semin Cancer Biol* (2020) 65:176–88. doi: 10.1016/j.semcancer.2019.12.007

92. Yang P, Markowitz GJ, Wang XF. The hepatitis B virus-associated tumor microenvironment in hepatocellular carcinoma. *Natl Sci Rev* (2014) 1(03):396–412. doi: 10.1093/nsr/nwu038

93. Zinatizadeh M, Schock B, Chalbatani G, Zarandi P, Jalali S, Miri S, et al. Nuclear factor kappa b (Nf-kb) signaling in cancer development and immune diseases. *Genes Dis* (2021) 8(3):287–97. doi: 10.1016/j.gendis.2020.06.005

94. Grinberg-Bleyer Y, Oh H, Desrichard A, Bhatt DM, Caron R, Chan TA, et al. Nf-Kb c-rel is crucial for the regulatory T cell immune checkpoint in cancer. *Cell* (2017) 170(6):1096–108.e13. doi: 10.1016/j.cell.2017.08.004

95. Vazquez Rodriguez G, Abrahamsson A, Jensen L, Dabrosin C. Estradiol promotes breast cancer cell migration *Via* recruitment and activation of neutrophils. *Cancer Immunol Res* (2017) 5(3):234–47. doi: 10.1158/2326-6066.Cir-16-0150

96. Sagiv J, Michaeli J, Assi S, Mishalian I, Kisos H, Levy L, et al. Phenotypic diversity and plasticity in circulating neutrophil subpopulations in cancer. *Cell Rep* (2015) 10(4):562–73. doi: 10.1016/j.celrep.2014.12.039

97. Bhattacharya A, Wei Q, Shin J, Abdel Fattah E, Bonilla D, Xiang Q, et al. Autophagy is required for neutrophil-mediated inflammation. *Cell Rep* (2015) 12 (11):1731–9. doi: 10.1016/j.celrep.2015.08.019

98. Betancur PA, Abraham BJ, Yiu YY, Willingham SB, Khameneh F, Zarnegar M, et al. A Cd47-associated super-enhancer links pro-inflammatory signalling to Cd47 upregulation in breast cancer. *Nat Commun* (2017) 8:14802. doi: 10.1038/ncomms14802

99. Muller AJ, DuHadaway JB, Donover PS, Sutanto-Ward E, Prendergast GC. Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy. *Nat Med* (2005) 11(3):312–9. doi: 10.1038/nm1196

100. Tiwary S, Berzofsky JA, Terabe M. Altered lipid tumor environment and its potential effects on nkt cell function in tumor immunity. *Front Immunol* (2019) 10:2187. doi: 10.3389/fimmu.2019.02187

101. Jiang M, Wu N, Xu B, Chu Y, Li X, Su S, et al. Fatty acid-induced Cd36 expression *Via* O-glcnacylation drives gastric cancer metastasis. *Theranostics* (2019) 9(18):5359–73. doi: 10.7150/thno.34024

102. Wang H, Franco F, Tsui Y, Xie X, Trefny M, Zappasodi R, et al. Cd36-mediated metabolic adaptation supports regulatory T cell survival and function in tumors. *Nat Immunol* (2020) 21(3):298–308. doi: 10.1038/s41590-019-0589-5

103. Kyriakis J, Avruch J. Mammalian mapk signal transduction pathways activated by stress and inflammation: A 10-year update. *Physiol Rev* (2012) 92 (2):689–737. doi: 10.1152/physrev.00028.2011

104. Ma G, Liang Y, Chen Y, Wang L, Li D, Liang Z, et al. Glutamine deprivation induces pd-L1 expression *Via* activation of Egfr/Erk/C-jun signaling in renal cancer. *Mol Cancer Res* (2020) 18(2):324–39. doi: 10.1158/1541-7786.MCR-19-0517

105. Kim J, Kim C, Kim TS, Bang SI, Yang Y, Park H, et al. Il-18 enhances thrombospondin-1 production in human gastric cancer *Via* jnk pathway. *Biochem Biophys Res Commun* (2006) 344(4):1284–9. doi: 10.1016/j.bbrc.2006.04.016

106. Cao XK, Xie B, Shao Y, Lin J. Cytokine-driven positive feedback loop organizes fibroblast transformation and facilitates gastric cancer progression. *Clin Trans Oncol* (2022). doi: 10.1007/s12094-022-02777-z

107. Song C, Piva M, Sun L, Hong A, Moriceau G, Kong X, et al. Recurrent tumor cell-intrinsic and -extrinsic alterations during mapki-induced melanoma regression and early adaptation. *Cancer Discovery* (2017) 7(11):1248–65. doi: 10.1158/2159-8290.Cd-17-0401

108. Lechner MG, Liebertz DJ, Epstein AL. Characterization of cytokine-induced myeloid-derived suppressor cells from normal human peripheral blood mononuclear cells. *J Immunol* (2010) 185(4):2273–84. doi: 10.4049/jimmunol.1000901

109. Liu X, Wu X, Cao S, Harrington S, Yin P, Mansfield A, et al. B7-H1 antibodies lose antitumor activity due to activation of P38 mapk that leads to apoptosis of tumor-reactive Cd8 T cells. *Sci Rep* (2016) 6:36722. doi: 10.1038/srep36722

110. Gnanaprakasam JNR, Estrada-Muniz E, Vega L. The anacardic 6-pentadecyl salicylic acid induces macrophage activation *Via* the phosphorylation of Erk1/2, jnk, P38 kinases and nf-kappab. *Int Immunopharmacol* (2015) 29 (2):808–17. doi: 10.1016/j.intimp.2015.08.038

111. Marigo I, Bosio E, Solito S, Mesa C, Fernandez A, Dolcetti L, et al. Tumor-induced tolerance and immune suppression depend on the C/Ebpbeta transcription factor. *Immunity* (2010) 32(6):790–802. doi: 10.1016/j.immuni.2010.05.010

112. Yu Y, Liang Y, Li D, Wang L, Liang Z, Chen Y, et al. Glucose metabolism involved in pd-L1-Mediated immune escape in the malignant kidney tumour

microenvironment. *Cell Death Discovery* (2021) 7(1):15. doi: 10.1038/s41420-021-00401-7

113. Ho PC, Liu PS. Metabolic communication in tumors: A new layer of immunoregulation for immune evasion. *J Immunother Cancer* (2016) 4:4. doi: 10.1186/s40425-016-0109-1

114. Wang J, Lv X, Guo X, Dong Y, Peng P, Huang F, et al. Feedback activation of Stat3 limits the response to Pi3k/Akt/Mtor inhibitors in pten-deficient cancer cells. *Oncogenesis* (2021) 10(1):8. doi: 10.1038/s41389-020-00292-w

115. Xue G, Zippelius A, Wicki A, Mandala M, Tang F, Massi D, et al. Integrated Akt/Pkb signaling in immunomodulation and its potential role in cancer immunotherapy. *J Natl Cancer Inst* (2015) 107(7). doi: 10.1093/jnci/djv171

116. Rubie C, Oliveira-Frick V, Rau B, Schilling M, Wagner M. Chemokine receptor Ccr6 expression in colorectal liver metastasis. *J Clin Oncol* (2006) 24 (32):5173–4. doi: 10.1200/jco.2006.07.9095

117. 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). doi: 10.3390/cancers11091318

118. Kaneda MM, Messer KS, Ralainirina N, Li H, Leem CJ, Gorjestani S, et al. Pi3kgamma is a molecular switch that controls immune suppression. *Nature* (2016) 539(7629):437–42. doi: 10.1038/nature19834

119. Kumagai S, Togashi Y, Sakai C, Kawazoe A, Kawazu M, Ueno T, et al. An oncogenic alteration creates a microenvironment that promotes tumor progression by conferring a metabolic advantage to regulatory T cells. *Immunity* (2020) 53 (1):187–203.e8. doi: 10.1016/j.immuni.2020.06.016

120. Owen KL, Brockwell NK, Parker BS. Jak-stat signaling: A double-edged sword of immune regulation and cancer progression. *Cancers (Basel)* (2019) 11 (12). doi: 10.3390/cancers11122002

121. Verdeil G, Lawrence T, Schmitt-Verhulst AM, Auphan-Anezin N. Targeting Stat3 and Stat5 in tumor-associated immune cells to improve immunotherapy. *Cancers (Basel)* (2019) 11(12). doi: 10.3390/cancers11121832

122. Kusmartsev S, Gabrilovich D. Stat1 signaling regulates tumor-associated macrophage-mediated T cell deletion. *J Immunol (Baltimore Md: 1950)* (2005) 174 (8):4880–91. doi: 10.4049/jimmunol.174.8.4880

123. Danziger O, Pupko T, Bacharach E, Ehrlich M. Viainterleukin-6 and interferon-A signaling Jak1-stat differentially regulate oncolytic versus cytoprotective antiviral states. *Front Immunol* (2018) 9:94. doi: 10.3389/fimmu.2018.00094

124. Wang T, Zhao Y, Peng L, Chen N, Chen W, Lv Y, et al. Tumour-activated neutrophils in gastric cancer foster immune suppression and disease progression throughGm-Csf-Pd-L1 pathway. *Gut* (2017) 66(11):1900–11. doi: 10.1136/gutjnl-2016-313075

125. Dong J, Li J, Liu SM, Feng XY, Chen S, Chen YB, et al. Cd33(+)/P-Stat1(+) double-positive cell as a prognostic factor for stage iiia gastric cancer. *Med Oncol* (2013) 30(1):442. doi: 10.1007/s12032-012-0442-2

126. Li F, Zhang R, Li S, Liu J. Ido1: An important immunotherapy target in cancer treatment. *Int Immunopharmacol* (2017) 47:70–7. doi: 10.1016/j.intimp.2017.03.024

127. Hsu P, Santner-Nanan B, Hu M, Skarratt K, Lee C, Stormon M, et al. Il-10 potentiates differentiation of human induced regulatory T cells *Via* Stat3 and Foxo1. *J Immunol (Baltimore Md: 1950)* (2015) 195(8):3665–74. doi: 10.4049/jimmunol.1402898

128. Nusse R, Clevers H. Wnt/β-catenin signaling, disease, and emerging therapeutic modalities. *Cell* (2017) 169(6):985–99. doi: 10.1016/j.cell.2017.05.016

129. Goldsberry WN, et al. A review of the role of wnt in CancerImmunomodulation. *Cancers (Basel)* (2019) 11(6). doi: 10.3390/cancers11060771

130. Ganesh S, Shui X, Craig K, Park J, Wang W, Brown B, et al. Rnai-mediated B-catenin inhibition promotes T cell infiltration and antitumor activity in combination with immune checkpoint blockade. *Mol Ther* (2018) 26(11):2567–79. doi: 10.1016/j.ymthe.2018.09.005

131. Ding Y, Shen S, Lino AC, Curotto de Lafaille MA, Lafaille JJ. Beta-catenin stabilization extends regulatory T cell survival and induces anergy in nonregulatory T cells. *Nat Med* (2008) 14(2):162–9. doi: 10.1038/nm1707

132. Hong Y, Manoharan I, Suryawanshi A, Majumdar T, Angus-Hill ML, Koni PA, et al. β-catenin promotes regulatory T-cell responses in tumors by inducing vitamin a metabolism in dendritic cells. *Cancer Res* (2015) 75(4):656–65. doi: 10.1158/0008-5472.Can-14-2377

133. Yaguchi T, Goto Y, Kido K, Mochimaru H, Sakurai T, Tsukamoto N, et al. Immune suppression and resistance mediated by constitutive activation of wnt/β-catenin signaling in human melanoma cells.

134. Ruiz de Galarreta M, Bresnahan E, Molina-Sánchez P, Lindblad KE, Maier B, Sia D, et al. β-catenin activation promotes immune escape and resistance to anti-PD-1 therapy in hepatocellular carcinoma. *Cancer Discovery* (2019) 9(8):1124–41. doi: 10.1158/2159-8290.Cd-19-0074

135. Grasso CS, Giannakis M, Wells DK, Hamada T, Mu XJ, Quist M, et al. Genetic mechanisms of immune evasion in colorectal cancer. *Cancer Discovery* (2018) 8(6):730–49. doi: 10.1158/2159-8290.Cd-17-1327

136. Luke JJ, Bao R, Sweis RF, Spranger S, Gajewski TF. WNT/β-catenin pathway activation correlates with immune exclusion across human cancers. *Clin Cancer Res* (2019) 25(10):3074–83. doi: 10.1158/1078-0432.Ccr-18-1942

137. Wang C, Yan J, Yin P, Gui L, Ji L, Ma B, et al. β-catenin inhibition shapes tumor immunity and synergizes with immunotherapy in colorectal cancer. *Oncoimmunology* (2020) 9(1):1809947. doi: 10.1080/2162402x.2020.1809947

138. Galluzzi L, Spranger S, Fuchs E, López-Soto A. Wnt signaling in cancer immunosurveillance. *Trends Cell Biol* (2019) 29(1):44–65. doi: 10.1016/j.tcb.2018.08.005

139. Yang L, Zhao KL, Qin L, Ji DX, Zhang B, Zheng PF, et al. Notch signaling pathway regulates Cd4(+)Cd25(+)Cd127(Dim/-) regulatory T cells and T helper 17 cells function in gastric cancer patients. *Biosci Rep* (2019) 39(5). doi: 10.1042/BSR20182044

140. Cui Y, Li Q, Li W, Wang Y, Lv F, Shi X, et al. Notch3 is a prognostic factor and is correlated with immune tolerance in gastric cancer. *Front Oncol* (2020) 10:574937. doi: 10.3389/fonc.2020.574937

141. Morikawa M, Derynck R, Miyazono K. TGF-beta and the TGF-beta family: Context-dependent roles in cell and tissue physiology. *Cold Spring Harb Perspect Biol* (2016) 8. doi: 10.1101/cshperspect.a021873

142. Derynck R, Akhurst RJ, Balmain A. TGF-beta signaling in tumor suppression and cancer progression. *Nat Genet* (2001) 29:117–29. doi: 10.1038/ng1001-117

143. Miyazawa K, Shinozaki M, Hara T, Furuya T, Miyazono K. Two major smad path-ways in TGF-beta superfamily signalling. *Genes Cells* (2002) 7 (12):1191–204. doi: 10.1046/j.1365-2443.2002.00599.x

144. Thomas DA, Massague J. TGF-beta directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. *Cancer Cell* (2005) 8 (5):369–80. doi: 10.1016/j.ccr.2005.10.012

145. Hossain DM, Panda AK, Chakrabarty S, Bhattacharjee P, Kajal K, Mohanty S, et al. Mek inhibition prevents tumour-shed transforming growth factor-Beta-Induced T-regulatory cell augmentation in tumour milieu. *Immunology* (2015) 144(4):561–73. doi: 10.1111/imm.12397

146. Ghahremanifard P, Chanda A, Bonni S, Bose P. TGF-β mediated immune evasion in cancer-spotlight on cancer-associated fibroblasts. *Cancers (Basel)*. (2020) 12(12):3650. doi: 10.3390/cancers12123650

147. Lazarova M, Steinle A. Impairment of NKG2D-mediated tumor immunity by TGF-β. *Front Immunol* (2019) 10:2689. doi: 10.3389/fimmu.2019.02689

148. Guo Y, Liu X, Xu D, Huang C, Wang Z, Xia X, et al. Role of Lats1/2 in prognosis of advanced gastric cancer and its relationship with the tumor immune microenvironment. *Front Oncol* (2020) 10:1406. doi: 10.3389/fonc.2020.01406

149. Qiao Y, Li T, Zheng S, Wang H. The hippo pathway as a drug target in gastric cancer. *Cancer Lett* (2018) 420:14–25. doi: 10.1016/j.canlet.2018.01.062

150. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi P. A cerna hypothesis: The Rosetta stone of a hidden rna language? *Cell* (2011) 146(3):353–8. doi: 10.1016/j.cell.2011.07.014

151. Qian L, Liu F, Chu Y, Zhai Q, Wei X, Shao J, et al. MicroRNA-200c nanoparticles sensitized gastric cancer cells to radiotherapy by regulating PD-L1 expression and EMT. *Cancer Manage Res* (2020) 12:12215–23. doi: 10.2147/cmarS279978

152. Wang Y, Wang D, Xie G, Yin Y, Zhao E, Tao K, et al. Microrna-152 regulates immune response *Via* targeting B7-H1 in gastric carcinoma. *Oncotarget* (2017) 8(17):28125–34. doi: 10.18632/oncotarget.15924

153. Yan X, Luo Q, Zhou S, Pan W, Zhang L, Yang D, et al. Microrna-375 reverses the expression of pd-L1 by inactivating the Jak2/Stat3 signaling pathways in gastric cancer. *Clinics Res Hepatol Gastroenterol* (2020) 101574. doi: 10.1016/j.clinre.2020.10.015

154. Wang W, Sun J, Li F, Li R, Gu Y, Liu C, et al. A frequent somatic mutation in Cd274 3'-utr leads to protein over-expression in gastric cancer by disrupting mir-570 binding. *Hum Mutat* (2012) 33(3):480–4. doi: 10.1002/humu.22014

155. Chen Y, Zhang J, Gong W, Dai W, Xu X, Xu S. Mir-588 is a prognostic marker in gastric cancer. *Aging* (2020) 13(2):2101–17. doi: 10.18632/aging.202212

156. Nie K, Zheng Z, Wen Y, Pan J, Liu Y, Jiang X, et al. A novel cerna axis involves in regulating immune infiltrates and macrophage polarization in gastric cancer. *Int Immunopharmacol* (2020) 87:106845. doi: 10.1016/j.intimp.2020.106845

157. Ping W, Senyan H, Li G, Yan C, Long L. Increased lactate in gastric cancer tumor-infiltrating lymphocytes is related to impaired T cell function due to mir-34a deregulated lactate dehydrogenase a. *Cell Physiol Biochem* (2018) 49(2):828–36. doi: 10.1159/000493110

158. Zhou Y, Zhong J, Gong F, Xiao J. Mir-141-3p suppresses gastric cancer induced transition of normal fibroblast and bmsc to cancer-associated fibroblasts *Via* targeting Stat4. *Exp Mol Pathol* (2019) 107:85–94. doi: 10.1016/j.yexmp.2018.11.014

159. Liang Y, Liu Y, Zhang Q, Zhang H, Du J. Tumor-derived extracellular vesicles containing microrna-1290 promote immune escape of cancer cells through the Grhl2/Zeb1/Pd-L1 axis in gastric cancer. *Trans Res* (2021) 231:102–12. doi: 10.1016/j.trsl.2020.12.003

160. Li P, Luo X, Xie Y, Li P, Hu F, Chu J, et al. Gc-derived EVs enriched with microRNA-675-3p contribute to the MAPK/PD-L1-Mediated tumor immune escape by targeting CXXC4. *Mol Ther Nucleic Acids* (2020) 22:615–26. doi: 10.1016/j.omtn.2020.08.020

161. Li H, Zhao C, Zhao H, Liu G, Mao H, Liu Y. Elevated Linc00936 or silenced microrna-425-3p inhibits immune escape of gastric cancer cells *Via* elevation of Zc3h12a. *Int Immunopharmacol* (2021) 95:107559. doi: 10.1016/j.intimp.2021.107559

162. Xiong G, Yang L, Chen Y, Fan Z. Linc-Pou3f3 promotes cell proliferation in gastric cancer *Via* increasing T-reg distribution. *Am J Trans Res* (2015) 7 (11):2262–9.

163. Zhu H, Tang J, Zhang S, Qian J, Ling X, Wu X, et al. Long noncoding rna Linc00963 promotes Cdc5l-mediated malignant progression in gastric cancer. *OncoTargets Ther* (2020) 13:12999–3013. doi: 10.2147/ottS274708

164. Xiao J, Lai H, Wei S, Ye Z, Gong F, Chen L. Lncrna hotair promotes gastric cancer proliferation and metastasis *Via* targeting mir-126 to active Cxcr4 and rhoa signaling pathway. *Cancer Med* (2019) 8(15):6768–79. doi: 10.1002/cam4.1302

165. Yan L, Zhang J, Guo D, Ma J, Shui S, Han X. Il-21r functions as an oncogenic factor and is regulated by the lncrna Malat1/Mir-125a-3p axis in gastric cancer. *Int J Oncol* (2019) 54(1):7–16. doi: 10.3892/ijo.2018.4612

166. Li P, Ge D, Li P, Hu F, Chu J, Chen X, et al. Cxxc finger protein 4 inhibits the Cdk18-Erk1/2 axis to suppress the immune escape of gastric cancer cells with involvement of Elk1/Mir100hg pathway. *J Cell Mol Med* (2020) 24(17):10151–65. doi: 10.1111/jcmm.15625

167. Dang S, Malik A, Chen J, Qu J, Yin K, Cui L, et al. Lncrna Snhg15 contributes to immuno-escape of gastric cancer through targeting Mir141/Pd-L1. *OncoTargets Ther* (2020) 13:8547–56. doi: 10.2147/ottS251625

168. Wang C, Zhu C, Xu J, Wang M, Zhao W, Liu Q, et al. The lncrna Uca1 promotes proliferation, migration, immune escape and inhibits apoptosis in gastric cancer by sponging anti-tumor mirnas. *Mol Cancer* (2019) 18(1):115. doi: 10.1186/s12943-019-1032-0

169. Olnes M, Martinson H. Recent advances in immune therapies for gastric cancer. *Cancer Gene Ther* (2021). doi: 10.1038/s41417-021-00310-y

170. 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

171. Ning B, Yu D, Yu A. Advances and challenges in studying noncoding RNA regulation of drug metabolism and development of RNA therapeutics. *Biochem Pharmacol* (2019) 169:113638. doi: 10.1016/j.bcp.2019.113638

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Characterization of immune checkpoint inhibitor-associated fulminant type 1 diabetes associated with autoantibody status and ethnic origin

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Objective: Fulminant type 1 diabetes may uniquely occur as a fatal adverse event during immune checkpoint inhibitor (ICI) therapy. We investigated the clinical and immunological characteristics of ICI-associated fulminant type 1 diabetes (IFD).

Research design and methods: We enrolled 80 patients with IFD (77 cases from the literature), 56 patients with ICI-associated type 1 diabetes (IT1D) (55 cases from the literature), 45 patients with traditional fulminant type 1 diabetes (TFD), and 43 patients with acute-onset type 1 diabetes for comprehensive analysis including islet autoantibodies and subgroup analysis based on ethnic origin.

Results: Patients with IFD accounted for 58.8% (80/136) of patients with ICI-related diabetes. IFD had a more rapid onset than IT1D after ICI therapy (90.5 days *vs.* 120 days, $p < 0.05$). The onset time and number of infusions after ICI therapy initiation were lower in the antibody-positive IFD group than that in the antibody-negative IFD group (both $p < 0.001$). IFD had a more rapid onset and more serious among Caucasians than that among Asians ($p < 0.01$, $p < 0.05$, respectively), and the prevalence of islet autoantibody positivity in the Caucasian IFD were prominently higher than those in the Asian IFD ($p < 0.05$). Onset age and plasma glucose levels were significantly higher in the IFD group than those in the TFD and acute-onset type 1 diabetes groups. HbA1c levels were slightly higher in patients with IFD than those with TFD.

Conclusions: IFD is relatively common in Caucasian population where TFD is very rare or almost absent. IFD occurrence is significantly related to islet autoantibody status and ethnic origin.

KEYWORDS

fulminant type 1 diabetes, immune checkpoint inhibitors, side effects, clinical characteristics, cancer immune checkpoint therapy

Introduction

Immune checkpoint inhibitors (ICIs), which are the most popular means of tumor immunotherapy, have been increasingly used to treat solid tumors. ICIs have an anticancer effect by removing a negative regulatory signal for T cell activation from the tumor microenvironment. Common ICIs include programmed death 1 (PD-1) inhibitors, programmed death-ligand 1 (PD-L1) inhibitors, and cytotoxic T lymphocyte antigen 4 (CTLA-4) inhibitors. Many studies have confirmed that the application of these ICIs is associated with immune-related adverse events involved in multiple organs and systems. Endocrine dysfunctions are among the common adverse events that have been reported in clinical trials with ICIs, including insulin-dependent diabetes. ICI-associated diabetes is characterized by acute onset of hyperglycemia with insulin deficiency and occurrence following exposure to ICIs. According to the literature, there are two subtypes of insulin-dependent diabetes, namely, ICI-associated type 1 diabetes (IT1D) and ICI-associated fulminant type 1 diabetes (IFD). There has been an increasing number of reports of patients presenting with IT1D and IFD due to the increase of tumor immunotherapy (1–5). If not promptly recognized, IT1D and IFD can be life threatening.

Traditional fulminant type 1 diabetes (TFD) is a rare subtype of type 1 diabetes that differs from acute-onset type 1 diabetes with a distinct entity and unique clinical characteristics, and it may be mediated by multiple factors, including viral infection and pregnancy (6). TFD is characterized by the following symptoms: 1) a remarkably abrupt onset of ketosis or ketoacidosis; 2) a low glycosylated hemoglobin (HbA1c) value despite a high plasma glucose level; and 3) an absence of insulin secretion capacity (7). It remains unclear whether differences exist in clinical phenotypes and immunological characteristics between IFD and TFD. The prevalence and risk of developing IFD following the use of ICIs regimens are also unknown. Furthermore, all clinicians need to be more aware of IFD to prevent deaths due to diabetic ketoacidosis and failure of timely intervention. Therefore, further understanding of the characteristics of IFD patients is needed for improved prognostic and diagnostic application to reduce overall morbidity for this already at-risk population.

Anti-PD-1 agents (nivolumab, pembrolizumab, cemiplimab, sintilimab, and camrelizumab), anti-PD-L1 agents (atezolizumab, avelumab, and durvalumab), and an anti-CTLA-4 monoclonal antibody (ipilimumab) have been reported to cause type 1 diabetes. According to the safety database of a Japanese pharmaceutical company, the incidences of IT1D and IFD were 0.19% and 0.13%, respectively, from July 2014 to August 2017 among 20,600 patients who received nivolumab treatment. Among 3603 patients who received pembrolizumab from December 2016 to August 2017, the incidences of IT1D and IFD were 0.11% and 0.03%, respectively (8). Stamatouli et al. reported that the estimated incidence of type 1 diabetes in a large American medical center was 0.9% (9). In a recent study, Tsang et al. reported that among 538 patients with metastatic melanoma who received anti-PD-1 immunotherapy, 1.9% patients developed type 1 diabetes (10). The World Health Organization (WHO) Safety Report database shows that the number of ICI-related type 1 diabetes patients is increasing (11), which may be related to the increased use of anti-PD-1 and anti-PD-L1 therapies in various cancers. Additionally, combination therapy with CTLA-4 and PD-1 inhibitors may also increase the incidence of IT1D, IFD, and other immune-related adverse events (12). In these cases, the increment of IFD brings challenges to the clinical diagnosis and treatment management of diabetes.

TFD is common in Asians, including Japanese, Koreans, and Chinese, but is rare in Caucasians from the Americas and Europe (13). Patients with IFD have been sporadically reported in China and other Asian countries (14). However, there is increasing number of reported cases of IFD in Caucasians. Thus, it is worth exploring whether there are differences between IFD in Asians and IFD in Caucasians.

Therefore, the present study investigated the clinical and immunological features of IFD by comparing important clinical

Abbreviations: GADA, Glutamic acid decarboxylase autoantibodies; IA-2A, Protein tyrosine phosphatase autoantibodies; ICA, Islet cell autoantibodies; ICI, Immune checkpoint inhibitor; IFD ICI-associated fulminant type 1 diabetes; IT1D ICI-associated type 1 diabetes; TFD, Traditional fulminant type 1 diabetes; ZnT8A, Zinc transporter 8 autoantibodies.

indexes among four groups of diabetes, namely, IFD, IT1D, TFD, and acute-onset type 1 diabetes. We enrolled 80 patients with IFD (77 cases from the literature), 56 patients with IT1D (55 cases from the literature), 45 patients with TFD, and 43 patients with acute-onset type 1 diabetes for comprehensive analysis, including analysis of islet autoantibodies, and subgroup analysis based on ethnic origin. The present study will provided precise data on the risk of patients with IFD receiving ICI regimens and demonstrated that patients with islet autoantibody positivity or Caucasian ethnic origin are at an increased high risk of IFD.

Research design and methods

Patient inclusion and data collection

IFD was defined as fulminant type 1 diabetes induced by exposure to ICIs. TFD was defined as fulminant type 1 diabetes generally associated with viral infection or pregnancy but not associated with ICI. IT1D was defined as type 1 diabetes induced by exposure to ICIs but does not meet the diagnostic criteria for fulminant type 1 diabetes. Acute-onset type 1 diabetes was defined as typical insulin-dependent type 1 diabe with a duration of hyperglycemic symptoms less than 6 months but does not meet the diagnostic criteria for fulminant type 1 diabetes. ICI-associated diabetes included IFD and IT1D. It should be noted that ICI-associated diabetes excluded pre-existing type 2 diabetes or patients with history of diabetes prior to the use of ICI in the present study.

A total of 224 subjects were enrolled, including 80 patients with IFD, 56 patients with IT1D, 45 patients with TFD, and 43 patients with acute-onset type 1 diabetes. Among all subjects, 132 patients were reported from the literature, including 77 cases of IFD and 55 cases of IT1D. Three cases of IFD, one case of IT1D, 45 cases of TFD, and 43 cases of acute-onset type 1 diabetes were enrolled from the existing database of our diabetes center (Figure 1).

We conducted a systematic search of the literature to identify clinical case reports or articles on the use of ICIs that reported diabetes adverse events. A literature search was used to collect data from patients who developed IFD and IT1D. We gathered data on the clinical characteristics of these cases from literature in online databases, including the CNKI database (Chinese), Wanfang Medical Database (Chinese), and PubMed database. The following keywords were used: “type 1 diabetes mellitus”, “nivolumab”, “pembrolizumab”, “sintilimab”, “toripalimab”, “camrelizumab”, “ipilimumab”, “tremelimumab”, “avelumab”, “durvalumab”, “atezolizumab”, “PD-1”, “PD-L1”, “CTLA-4”, and “immune checkpoint inhibitors”. The CTLA-4 inhibitors included ipilimumab and tremelimumab, and the other inhibitors were PD-1 or PD-L1 inhibitors. The database was searched for articles published on or before December 31, 2021. The search focused on type 1 diabetes related to different ICI regimens in patients with advanced solid tumors. The exclusion criterion was duplication of data. Case reports/series of individuals previously diagnosed with type 2 diabetes prior to the start of ICI therapy and case reports of individuals without confirmed diagnosis of diabetes type were also excluded. Two researchers read and evaluated the literature independently. A third individual was consulted to reach a consensus in cases when both researchers differed on the inclusion or exclusion decision. Ultimately, 99 articles and 132 patients, including 77 patients with IFD and 55 patients with IT1D, between January 2014 and December 2021 were enrolled.

Fulminant type 1 diabetes met the following diagnostic criteria of the Committee of the Japan Diabetes Society in 2012: 1) diabetic ketosis or ketoacidosis occurred soon after the onset of hyperglycemic symptoms; 2) patient presented with plasma glucose ≥16.0 mmol/L and HbA1c <8.7% at the first visit; and 3) patient had urinary C-peptide excretion <10 μg/day,

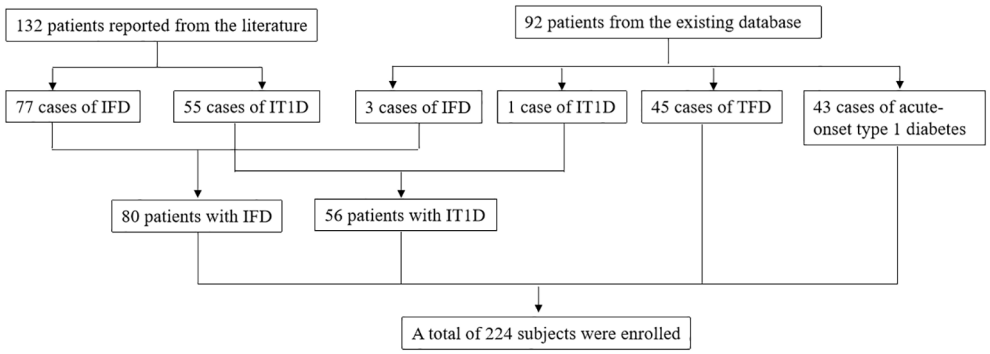

FIGURE 1
Flow diagram of study participants selection. ICI, Immune checkpoint inhibitor; TFD, Traditional fulminant type 1 diabetes; IFD, ICI-associated fulminant type 1 diabetes; IT1D ICI-associated type 1 diabetes.

fasting serum C-peptide level <0.10 nmol/L, or postprandial serum C-peptide <0.17 nmol/L at onset (15).

Data on the following parameters were recorded for each patient: demographic data (including sex, onset age, and body mass index (BMI)), tumor types, past history, family history, date of diabetes onset, hyperglycemic symptoms, number of ICI therapy infusions, and types of ICI therapy. The following laboratory data were recorded at onset: plasma glucose, electrolytes, blood gas analysis results, HbA1c, type 1 diabetes-associated autoantibody status, and human leukocyte antigen (HLA) class II alleles or genotypes if available. HLA typing was performed in a subset of the published IFD or IT1D cases. We determined whether this allele or genotype belonged to the susceptibility of spontaneous type 1 diabetes according to the corresponding literature as a reference. For example, DR3-DQ2 and DR4-DQ8 confer increased risk for Caucasian population, while DR4-DQ4 and DR9-DQ9 confer high risk for Asian populations. The onset date was defined as the day of diagnosis and commencement of treatment for diabetes. The unit of C-peptide was uniformly converted into ng/ml, and the unit of blood glucose was uniformly converted into mg/dl. The detection methods of islet autoantibodies, HbA1c, and HLA typing from most cases reported in the literature were unavailable.

Statistical analysis

All statistical analyses were performed using SPSS version 19.0 (IBM Corporation, Chicago, IL, USA). Continuous variables that were normally distributed are presented as means ± standard deviations (SDs), and continuous variables that were not normally distributed are described as the median and interquartile range (IQR). Differences between groups were analyzed using independent sample t tests, rank sum tests, or variance analysis as appropriate. The chi-squared test was used for correlation analysis of categorical variables. Other clinical variables of interest were evaluated descriptively. According to the comparison results of IFD and IT1D, a logistic regression model was used to incorporate antibody, race, and HLA susceptibility alleles to analyze the predictors of IFD after ICI treatment. For all computational analyses, $p < 0.05$ was considered statistically significant.

Results

Background of patients with IFD

Patients with IFD accounted for 58.8%(80/136) of patients with ICI-associated diabetes. The onset age of patients with IFD was 60.7 ± 12.6 years, and the BMI was 22.0±5.1 kg/m^2. Both IFD and IT1D mainly involved with anti-PD-1 and anti-PD-L1 agents was associated with the treatment of various malignancies and various ICI drugs. Regarding the profile of primary cancers (Figure 2A), lung cancer, melanoma, and renal cancer accounted for 38.8%, 30.0%, and 8.8%, respectively, while the remaining 22.2% was attributed to other cancer types for IFD. Similarly, lung cancer (25.0%) and melanoma (25.0%) accounted for the highest proportion for IT1D (Figure 2C). As shown in Figures 2B, D, nivolumab (32.5%) and pembrolizumab (30.0%) were the most common tumor immunotherapy regimens in IFD patients. Similarly, pembrolizumab (39.3%) and nivolumab (37.5%) were the most common ICI regimens for IT1D. A summary of the case reports for IFD and IT1D is shown in Supplemental Tables 1, 2, respectively.

IFD was reported in 17 countries on a global scale, including UK, Greece, Belgium, France, Australia, Portugal, Austria, Canada, Italy, Norway, USA, Brazil, Ireland, Spain, Japan, Korea, and China. IFD patients were diagnosed with a median of 90.5 days (IQR, 36.5-150 days), and they a median of five infusions (IQR, 2.3-8 infusions) after initiation of ICI therapy. The duration of symptoms at onset in patients with IFD was 5 days (IQR, 3-8 days). All patients had marked hyperglycemia (638.77±244.29 mg/dl), low C-peptide levels [0.06 (0.01-0.16) ng/ml], and low HbA1c levels (7.36±0.75). All patients with IFD exhibited abrupt onset of ketosis or ketoacidosis (arterial pH: 7.18 ± 0.16, HCO_3^-: 12.07±6.94 mmol/L), and 80.3% of patients presented with diabetic ketoacidosis. All patients with IFD were insulin dependent.

Islet autoantibodies and HLA typing

Sera collected at diabetes onset were tested for type 1 diabetes-related autoantibodies. Islet autoantibodies testing results were available for 77 of 80 patients with IFD, among which the percentage of patients who were positive for at least one autoantibody was 26.0% (20/77) (Supplemental Table 1). The autoantibody with the highest positive rate was glutamic acid decarboxylase antibody (GADA), which was found in 21.1% (15/71) of patients, followed by protein tyrosine phosphate antibody (IA-2A) (17.5%, 7/40), insulin autoantibody (IAA) (8.3%, 3/36), islet cell autoantibody (ICA) (0.04%, 1/25), and zinc transporter 8 autoantibody (ZnT8A) (6.3%, 1/16).

In total, 36 patients with IFD underwent HLA typing, and 58.3% of these patients had the high-risk HLA genotype for type 1 diabetes. As shown in Table 1, the proportions of type 1 diabetes HLA susceptibility alleles were not statistically different between IFD and IT1D. The proportion of type 1 diabetes HLA susceptibility alleles was higher in the antibody-positive IFD group compared to the antibody-negative IFD group, but there was no statistically significant difference when comparing the different ethnic IFD subgroups.

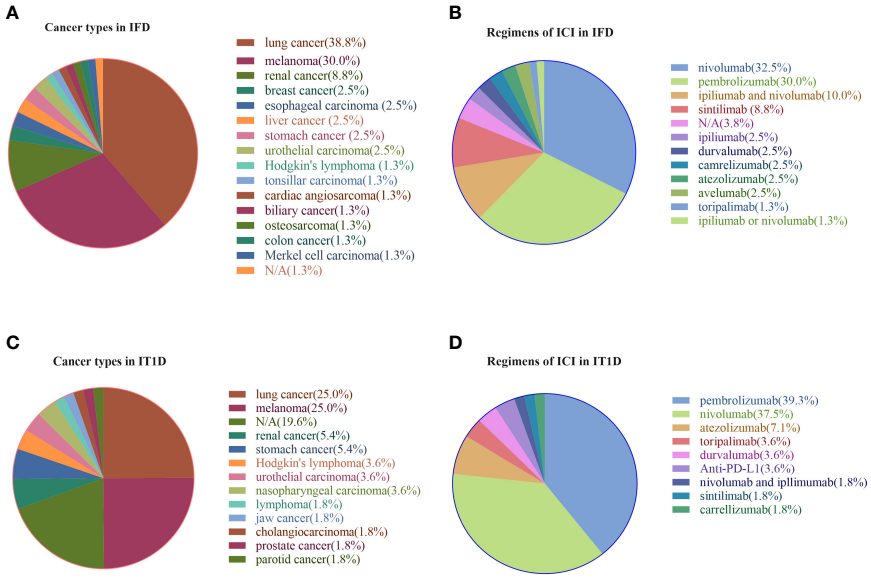

FIGURE 2
Profile of primary cancer types and ICI regimens in patients with IFD and IT1D. **(A)** Cancer types in IFD, **(B)** Regimens of ICI in IFD, **(C)** Cancer types in IT1D, **(D)** Regimens of ICI in IT1D. IFD, ICI-associated fulminant type 1 diabetes; IT1D, ICI-associated type 1 diabetes; N/A, Not Available.

Comparison of clinical characteristics between IFD and IT1D

To further understand whether the clinical features of IFD are specific, we compared the clinical and biological characteristics of IFD and IT1D. Compared to IT1D, the percentage of men was lower in IFD ($p < 0.05$), whereas the percentage of patients with diabetic ketoacidosis was significantly higher in IFD ($p < 0.05$) (Table 1). The time from initiation of ICI therapy to onset of diabetes in patients with IFD

TABLE 1 The clinical and biological characteristics of different ICI-associated diabetes.

	IFD	IT1D	P
N	80	56	
Onset age (years)	60.7 ± 12.6	63.4 ± 13.0	NS
Sex (male %)	55.7	76.1	0.023
BMI (kg/m^2)	22.0±5.1	28.1 ± 7.4	0.003
Family history of diabetes (%)	15	23.5	NS
Time from initiation of therapy to onset of diabetes (days)	90.5 (36.5, 150)	120 (63, 270)	0.017
Number of courses before diabetes onset	5.0 (2.3, 8.0)	5 (3.0, 10.5)	NS
Diabetic ketoacidosis (%)	80.3	58.0	0.007
Plasma glucose (mg/dl)	638.77 ± 244.29	585.34 ± 235.71	NS
HbA1c (%)	7.36 ± 0.75	9.05 ± 1.32	0.000
Arterial PH	7.18 ± 0.16	7.22 ± 0.16	NS
HCO_3^-(mmol/l)	12.07± 6.94	15.27 ± 7.35	NS
Serum C-peptide (ng/ml)	0.06 (0.01, 0.16)	0.46 (0.20, 0.99)	0.000
Islet autoantibodies (%)	26.0	46.0	0.017
[&]Proportion of HLA susceptibility alleles for type 1 diabetes (%)	58.3 (21/36)	75(12/16)	NS

Values are expressed as the mean ± standard deviation or median (first quartile–third quartile).
IFD, Immune checkpoint inhibitor associated fulminant type 1 diabetes; IT1D, Immune checkpoint inhibitor associated type 1 diabetes but not fulminant type 1 diabetes; NS, no significance.
[&] HLA typing was performed in a subset of the published IFD or IT1D cases. We determine whether this allele or genotype belongs to the susceptibility of spontaneous type 1 diabetes on their own according to the corresponding literature as a reference. For example, DR3-DQ2 and DR4-DQ8 confer increased risk for Caucasian population, while DR4-DQ4 and DR9-DQ9 confer high risk for Asian populations.

was significantly less than that in patients with IT1D (90.5 days vs. 120 days, $p < 0.05$). However, no statistically difference was observed for the median number of ICI infusions before diabetes onset (5 infusions vs. 5 infusions). Patients with IFD displayed a lower BMI, lower HbA1c levels, and lower serum C-peptide levels than those with IT1D (all $p < 0.01$). Compared to IT1D, the prevalence of islet autoantibodies in IFD also had a significant decreasing trend ($p = 0.017$). There was no significant difference between IFD and IT1D with respect to onset age, family history of diabetes, plasma glucose, arterial PH, and $HCO3^-$ levels.

According to the comparison results, there was a difference in the positive ratio of islet autoantibodies between the IFD and IT1D groups. In the present study, correlation analysis showed that autoantibody status in patients with IFD was associated with ethnicity (Table 2). Therefore, a logistic regression model, including three variables (antibody, race, and susceptibility genotype) was used to evaluate the predictors of IFD after ICI treatment (Supplemental Table 3). Unexpectedly, we were unable to identify any interactions or predictive risk factors for IFD and IT1D (all $p > 0.05$).

Clinical characteristics of different subgroups of IFD

Subgroup analysis was performed for patients with IFD according to their autoantibody status and ethnic origin. As shown in Table 3, the median number of infusions and time from initiation of ICI therapy to onset of diabetes in the antibody-positive IFD group were significantly lower than those in the antibody-negative IFD group (2 infusions vs. 6 infusions, $p < 0.001$; 28.5 days vs. 114 days, $p < 0.001$).

In the present study, 45.0% (36/80) of the patients were Caucasians from Belgium, Italy, Greece, and 13 other countries, whereas 55.0% (44/80) of the patients were Asians from the UK (South-East Asian origin), Japan, Korea, and China (Supplemental Table 1). As shown in Table 4, Caucasian patients with IFD had a lower median number of infusions and a more rapid onset than Asian patients with IFD (3 infusions vs. 6 infusions, $p < 0.05$; 40 days vs. 110 days, $p < 0.01$), and the proportions of diabetic ketoacidosis and positive rate of autoantibodies in Caucasian patients with IFD were significantly higher than those in Asian patients with IFD

TABLE 2 Correlation analysis of autoantibody and ethnic origin in IFD.

	Ethnic origin		Total	c^2	r	P value
	Asian	Caucasian				
Autoantibody -positive	4	16	20	11.19	0.380	0.001
Autoantibody -negative	38	19	57			

IFD, Immune checkpoint inhibitor associated fulminant type 1 diabetes.

TABLE 3 The clinical and biological characteristics of IFD in different autoantibody status.

	Autoantibody-positive IFD	Autoantibody-negative IFD	P
N	20	57	
Age (years)	57.4 ± 17.6	61.2 ± 10.3	NS
Sex (male %)	36.8	63.2	0.039
BMI (kg/m^2)	24.7 ± 7.3	20.8 ± 3.3	NS
Time from initiation of therapy to onset of diabetes (days)	28.5 (20.3, 40.3)	114 (71, 168)	0.000
Number of courses before diabetes onset	2 (1, 3.5)	6 (4, 9)	0.000
Diabetic ketoacidosis (%)	89.5	75.9	NS
Plasma glucose (mg/dl)	662.51 ± 262.49	619.83 ± 239.74	NS
HbA1c (%)	7.14 ± 0.77	7.39 ± 0.71	NS
Arterial PH	7.19 ± 0.16	7.18 ± 0.16	NS
HCO_3^-	11.33 ± 5.06	12.63 ± 7.52	NS
Serum C-peptide (ng/ml)	0.1 (0.02, 0.1)	0.05 (0.1, 0.17)	NS
Proportion of HLA susceptibility alleles for type 1 diabetes (%)	88.9 (8/9)	44.0 (11/25)	0.01

Values are expressed as the mean ± standard deviation or median (first quartile–third quartile).
IFD, Immune checkpoint inhibitor associated fulminant type 1 diabetes; NS, no significance.

TABLE 4 The clinical and biological characteristics of IFD in different ethnic origin.

	Asian origin	Caucasian origin	P
N	44	36	
Age (years)	62.9 ± 11.2	58.0 ±13.9	NS
Sex (male %)	54.5	57.1	NS
BMI (kg/m2)	20.2 ± 3.5	24.3 ± 6.0	NS
Time from initiation of therapy to onset of diabetes (days)	110 (72, 171)	40 (28, 128)	0.007
Number of courses before diabetes onset	6 (4.5, 8)	3 (2, 9)	0.033
Diabetic ketoacidosis (%)	71.4	91.2	0.029
Plasma glucose (mg/dl)	629.23 ± 260.75	650.42 ± 225.65	NS
HbA1c (%)	7.37 ± 0.75	7.36 ± 0.76	NS
Arterial PH	7.19 ± 0.17	7.16 ± 0.16	NS
HCO_3^-	13.13 ± 7.36	10.95 ± 6.45	NS
Serum C-peptide (ng/ml)	0.03 (0.01, 0.1)	0.10 (0.02, 0.19)	NS
Islet autoantibodies (%)	9.5 (4/42)	45.7 (16/35)	0.001
Proportion of HLA susceptibility alleles for type 1 diabetes (%)	68.4 (13/19)	47.1 (8/17)	NS

Values are expressed as the mean ± standard deviation or median (first quartile–third quartile).
IFD, Immune checkpoint inhibitor associated fulminant type 1 diabetes; NS, no significance.

(91.2% vs. 71.4%, $p < 0.05$; 45.7% vs. 9.5%, $p < 0.01$). Correlation analysis showed that autoantibody status in patients with IFD may be associated with ethnicity of patients with IFD (Table 2).

Comparison of clinical characteristics among IFD, TFD, and acute-onset type 1 diabetes

To eliminate the influence of racial differences, we selected 20 patients with IFD (17 cases from the literature and 3 case from our existing database), 45 patients with TFD, and 43 patients with acute-onset type 1 diabetes for comparison of clinical characteristics, and all of these patients were Chinese. As shown in Table 5, the onset age, plasma glucose levels, and proportion of patients with diabetic ketoacidosis at onset in the IFD group were significantly higher than those in the acute-onset type 1 diabetes group (all $p<0.001$). The duration of symptoms, fasting C-peptide levels, mean HbA1c levels, and prevalence of positive autoantibody in the IFD groups were significantly lower than those in the acute-onset type 1 diabetes group (all $p<0.001$). The onset age, plasma glucose levels, and

TABLE 5 Comparison of the clinical and biological characteristics among IFD, TFD, and acute-onset T1D in Chinese patients.

	TFD	IFD	acute-onset T1D
N	45	20	43
Onset Age, years	31.0 ± 13.9*	58.0 ± 9.3*#	24.2 ± 17.0
BMI (kg/m2)	22.0 ± 3.7	21.2 ± 3.2	19.4 ± 3.7
Male (%)	57.8	75.0	60.5
Duration of symptoms (days)	3 (2, 5)*	6 (3, 7)*	30 (10, 34)
Diabetic ketoacidosis (%)	77.8*	85.0*	48.8
Plasma glucose (mg/dl)	557.90 ± 198.73*	680.22 ± 283.83*#	449.33 ± 118.53
Arterial PH	7.14 ± 0.28	7.17 ± 0.13	7.25 ± 0.15
HCO_3^-	10.14 ± 5.82	11.77 ± 4.09	12.98 ± 6.95
Serum C-peptide (ng/ml)	0.05 (0.03, 0.15)*	0.01 (0.01, 0.05)*	0.32 (0.14, 0.54)
HbA1c (%)	6.81 ± 0.86*	7.68 ± 0.60*#	12.41 ± 2.59
Islet autoantibodies (%)	14.0 (6/43)*	10.5 (2/19)*	72.1 (31/43)

Values are expressed as the mean ± standard deviation or median (first quartile–third quartile).
T1D, type 1 diabetes.
TFD, Traditional fulminant type 1 diabetes.
IFD, Immune checkpoint inhibitor associated fulminant type 1 diabetes.
*P <0.05, vs. acute-onset T1D; #P<0.05, vs. TFD.

HbA1c levels in patients with IFD were significantly higher than those in patients with TFD (58.0 ± 9.3 years vs. 31.0 ± 13.9 years, 680.22 ± 283.83 mg/dl vs. 557.90 ± 198.73 mg/dl, and 7.68% ± 0.60% vs. 6.81% ± 0.86%, respectively, all $p<0.001$).

Discussion

ICIs, especially PD-1 inhibitors, can cause type 1 diabetes as an immune adverse event, which is usually accompanied with severe complications, such as diabetic ketoacidosis. The present study comprised the largest sample size of IFD patients to date. The present results indicated that IFD was not uncommon in patients receiving ICI treatment, especially among Caucasians. Nivolumab and pembrolizumab were the most common ICIs leading to diabetes, and the most common tumor types were melanoma and lung cancer. The median HbA1c was low, suggesting abrupt onset of diabetes. The proportion of IFD patients with positive autoantibodies was 26.0%, and GADA was the most prevalent diabetes-associated autoantibody. Autoantibody-positive IFD patients showed faster onset due to a lower median number of infusions and time from initiation of ICI therapy to onset of diabetes. Interestingly, Caucasians with IFD had a more rapid onset and more serious disease than Asians with IFD. At the same time, the antibody-positive rate of the Caucasian population was higher than that of the Asian population. However, it remains unknown whether the high proportion of autoantibodies in the Caucasian population causes different conditions and disease progression from those in the Asian population, thereby additional studies are warranted.

The increased number of reports of ICI-related diabetes, which are mainly related to the use of PD-1 or PDL-1 inhibitors, has provoked widespread concern. The exact mechanisms of these cases of acute insulin-dependent diabetes are currently unknown. The PD-1/PDL-1 axis affects islet autoimmunity through different mechanisms involving innate and adaptive immune cells, and these affects occur in draining lymph nodes and pancreatic tissue (16). The rarity of these secondary diabetes events makes them challenging to characterize. However, if severe hyperglycemia is not detected and treated in time, the patient is likely to die from diabetic ketoacidosis rather than the malignant tumor. Therefore, it is necessary to summarize the IFD patients reported all over the world and conduct comprehensive analyses to identify early prediction risk factors.

Recent studies have found that islet autoantibodies, especially GADA, which is considered the main autoantibody in patients with type 1 diabetes, are related to IFD. However, GADA negativity does not indicate that other islet autoantibodies are negative, suggesting that other antibodies may be positive. In the present study, the prevalence of islet autoantibodies was 26.0% and 46.0% in IFD and IT1D, respectively. Similar to this study, Clotman et al. found that 56% of patients with ICI related diabetes are positive for islet autoantibodies, including GADA (17). De Filette et al. reported that at least one autoantibody is positive in 53% of ICI related diabetes patients with GADA having the highest positive rate (51%) (18). It has been reported that an antibody-positive ICI related diabetes group has a more rapid onset and higher incidence of diabetic ketoacidosis compared to an antibody-negative ICI related diabetes group (19). The median time from ICI treatment to the diagnosis of type 1 diabetes is 5 weeks for GADA-positive cases and 9 weeks for GADA-negative cases (17). GADA-positive patients use ICIs for a median of 3.1 cycles, while GADA-negative patients use ICIs for 5.9 cycles (18). In line with this, the present study demonstrated that patients in the autoantibody-positive IFD group had a significantly lower number of median infusions and time from initiation of ICI therapy to onset of diabetes compared to patients in the autoantibody-negative IFD group. These findings provide evidence supporting the importance of detection of islet autoantibodies for patients before and after using ICI therapy.

Studies have determined that islet autoantibodies are not directly involved in disease pathogenesis (20) but that they precede and predict the development of clinical diabetes (21). In some patients, islet autoantibodies may be present prior to type 1 diabetes (22, 23), whereas in other patients who develop type 1 diabetes, seroconversion may occur after the initiation of ICI therapy (24, 25). Some researchers have suggested that baseline autoimmune antibodies may not be particularly useful as biomarkers to predict individual susceptibility to ICI related diabetes (26), whereas others have suggested that the presence of islet autoantibodies prior to treatment may predispose patients to the development of autoimmune diabetes (23). In our opinion, the absence of diabetes-related autoantibodies cannot rule out the occurrence of IFD, but in such cases, the onset will be slower than that of cases with positive autoantibodies. Autoantibodies are usually considered a biomarker of islet cell destruction. A previous prospective study with a 3-year follow-up has shown that islet autoantibodies may accelerate the decline in β cell function (27). Therefore, we suspect that ICIs inhibit immune tolerance, leading to T cell activation and loss of immune tolerance to B cells, producing islet autoantibodies. However, B cells are not necessarily involved in all patients, that is, not all patients have islet autoantibodies. If B cells are involved, the onset of type 1 diabetes will occur sooner. In the present study, most autoantibodies were measured at the onset of diabetes, and only one patient had antibody data before the onset of diabetes. Interestingly, a frozen blood sample obtained prior to treatment with nivolumab has been shown to be positive for islet autoantibodies despite no prior history of diabetes and no family history of diabetes (23). ICI may have simply accelerated a pre-existing autoimmune process that ultimately led to the development of type 1 diabetes in this patient. If the autoantibody is found to be positive at first, timely intervention will reduce the chance of ketoacidosis at the onset of the disease. In

addition, the presence of both GADA and IA-2A in the first degree relatives of patients with type 1 diabetes has been shown to confer a 61% risk of developing type 1 diabetes in 10 years (28). However, the incidence of type 1 diabetes is low in Asian populations. The prevalence of islet-specific autoantibodies has been reported to be lower in Asians compared to Caucasians (29). In the present study, the prevalence of autoantibodies in Caucasian patients with IFD was also higher than that in Asian patients with IFD. Thus, if islet autoantibodies can be a predictor of IFD, it may not work as well in Asian populations as in Caucasian populations.

The HLA class II gene is the most important susceptibility gene for type 1 diabetes. The most common allele in patients with IT1D is HLA-DR4 (9, 18). A previous study has shown that the DRB1*0405-DQB1*0401 and DRB1*0901-DQB1*0303 haplotypes contribute to the susceptibility to fulminant type 1 diabetes (30). Unfortunately, no available detailed haplotypes could be analyzed in most of the IFD cases in the present study. On a different basis, we determined that the proportion pof type 1 diabetes HLA susceptibility alleles was high regardless if the patient had IFD or IT1D, which suggested that type 1 diabetes HLA susceptibility alleles may be predictors of IFD. However, the effect of HLA susceptibility genes on IFD remains unknown. In the future, large sample size case-control studies are needed to evaluate the correlation between HLA susceptibility gene and IFD.

TFD is a rare subtype of type 1 diabetes that is especially prevalent in east Asians and rare in western Caucasians. In the present study, we found an interesting phenomenon that IFD was not infrequent in the Americas and Europe with a proportion of 45.0% in the present dataset. According to previous reports, the rate of islet autoantibody positivity may be relatively lower among Japanese individuals with ICI related diabetes than that among Caucasians (4.76% vs. 56%) (8). In the present study, the rate of autoantibody positivity in Asian IFD patients was also lower than that in Caucasian IFD patients. Compared to Asians with IFD, Caucasians with IFD had a more rapid onset and a higher proportion of diabetic ketoacidosis, which may be attributed to their higher rate of autoantibody positivity. In the present study, the correlation analysis supported this conjecture that autoantibody status in patients with IFD is associated with ethnic origin. More prospective studies are needed in the future for confirmation of these results.

The global increase in ICI use across cancer types highlights the importance of early monitoring and identification of IFD as well as increasing awareness for clinicians. The Japanese Diabetes Association recommends that patients receiving ICI therapy should have their blood glucose levels checked at each visit (every 2-3 weeks). The American Society of Clinical Oncology recommends that blood glucose levels should be measured during each course of treatment for 12 weeks during the induction period and every 3-6 weeks after ICI therapy (31). The present findings indicated that IFD can occur at various time points during the use of ICIs. According to the present study, the currently advocated responses may be insufficient to detect IFD early and reduce its severity at onset because IFD often presents with diabetic ketoacidosis within a week of diabetes symptoms. For early diagnosis and treatment of IFD, we propose the following procedures: 1) blood glucose should be monitored every week during ICI treatment; 2) diabetes-associated autoantibodies should be tested before or during treatment with ICIs, and if the autoantibodies are positive, it is necessary to pay close attention to the symptoms of hyperglycemia and strengthen the frequency of blood glucose monitoring to protect against the occurrence of diabetic ketoacidosis; 3) before initiation of ICI treatment, the patient should be informed of the rare possibility of insulin-dependent diabetes and the corresponding countermeasures; and 4) when conditions permit, the detection of type 1 diabetes susceptibility gene should be considered.

The present study had important strengths. Although obtaining IFD cases is difficult because IFD is rare, the novel design of the present study allowed collection of detailed case information by searching the literature to obtain a considerable sample size for analysis and research. We focused on fulminant type 1 diabetes as an adverse event of ICI therapy and found several interesting results. These results highlighted that the occurrence of IFD is related to autoantibody status and ethnic differences as IFD patients with positive islet autoantibodies or patients with Caucasian ethnicity have a more rapid onset.

The present study had several limitations. Most cases were obtained from existing literature, which led to incomplete clinical data, such as lack of pancreatic enzymes and daily dose of insulin. The consistency of the detection methods of some important parameters, such as islet autoantibodies and HbA1c, was not guaranteed, resulting in potential confounding factors in statistical analysis. There was also a lack of sufficient comparable HLA genotype information. Clearly, if we had conducted HLA typing in the form of case-control study, it would have allowed greater insight into the IFD risk associations. In addition, the analysis in the present study was cross-sectional, allowing the predictive potential of autoantibodies to be inferred from the clinical state only at the time of observation. Additional longitudinal cohort studies are required to demonstrate the clinical usefulness of autoantibodies. Finally, larger studies will improve the IFD risk prediction associated with autoantibodies or susceptibility genes.

In summary, IFD is relatively common in the Caucasian population, in which TFD is rare or almost absent. The present data suggested that IFD occurrence may be significantly related to autoantibody status and ethnic differences. Patients with positive islet autoantibodies or Caucasians have a more rapid and more serious onset. Prospective studies are needed to identify more effective risk prediction methods for developing ICI-induced diabetes. Due to the rapid onset of IFD, all acutely unwell patients on ICI should have their blood glucose checked and a full work-up for diabetes ketoacidosis if necessary.

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

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The patients/participants provided their written informed consent to participate in this study. Our study (2022 Scientific Research Ethics Review No. 31) was approved by the ethics review board of National Clinical Research Center for Metabolic Diseases at the Second Xiangya Hospital of Central South University.

Author contributions

JQ collected and researched data and wrote manuscript, SL researched data, edited the manuscript, and contributed to discussion, ZZ reviewed manuscript and contributed to discussion, WY and KG collected data and contributed to discussion, YX, XL, and ZL reviewed the manuscript and contributed discussion. All authors approved the final manuscript. SL and ZZ are the guarantor of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the article and approved the submitted version.

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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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.968798/full#supplementary-material

References

1. Usui Y, Udagawa H, Matsumoto S, Imai K, Ohashi K, Ishibashi M, et al. Association of serum anti-GAD antibody and HLA haplotypes with type 1 diabetes mellitus triggered by nivolumab in patients with non-small cell lung cancer. *J Thorac Oncol* (2017) 12(5):e41-e3. doi: 10.1016/j.jtho.2016.12.015

2. Hughes J, Vudattu N, Sznol M, Gettinger S, Kluger H, Lupsa B, et al. Precipitation of autoimmune diabetes with anti-PD-1 immunotherapy. *Diabetes Care* (2015) 38(4):e55-7. doi: 10.2337/dc14-2349

3. Gaudy C, Clévy C, Monestier S, Dubois N, Préau Y, Mallet S, et al. Anti-PD1 pembrolizumab can induce exceptional fulminant type 1 diabetes. *Diabetes Care* (2015) 38(11):e182–3. doi: 10.2337/dc15-1331

4. Teló GH, Carvalhal GF, Cauduro CGS, Webber VS, Barrios CH, Fay AP. Fulminant type 1 diabetes caused by dual immune checkpoint blockade in metastatic renal cell carcinoma. *Ann Oncol* (2017) 28(1):191–2. doi: 10.1093/annonc/mdw447

5. Saito D, Oikawa Y, Yano Y, Ikegami Y, Satomura A, Isshiki M, et al. Detailed time course of decline in serum c-peptide levels in anti-programmed cell death-1 therapy-induced fulminant type 1 diabetes. *Diabetes Care* (2019) 42(3):e40-e1. doi: 10.2337/dc18-1673

6. Zheng C, Zhou Z, Yang L, Lin J, Huang G, Li X, et al. Fulminant type 1 diabetes mellitus exhibits distinct clinical and autoimmunity features from classical type 1 diabetes mellitus in Chinese. *Diabetes Metab Res Rev* (2011) 27(1):70–8. doi: 10.1002/dmrr.1148

7. Imagawa A, Hanafusa T. Fulminant type 1 diabetes–an important subtype in East Asia. *Diabetes Metab Res Rev* (2011) 27(8):959–64. doi: 10.1002/dmrr.1236

8. Baden MY, Imagawa A, Abiru N, Awata T, Ikegami H, Uchigata Y, et al. Characteristics and clinical course of type 1 diabetes mellitus related to anti-programmed cell death-1 therapy. *Diabetol Int* (2019) 10(1):58–66. doi: 10.1007/s13340-018-0362-2

9. Stamatouli AM, Quandt Z, Perdigoto AL, Clark PL, Kluger H, Weiss SA, et al. Collateral damage: Insulin-dependent diabetes induced with checkpoint inhibitors. *Diabetes* (2018) 67(8):1471–80. doi: 10.2337/dbi18-0002

10. Tsang VHM, McGrath RT, Clifton-Bligh RJ, Scolyer RA, Jakrot V, Guminski AD, et al. Checkpoint inhibitor-associated autoimmune diabetes is distinct from type 1 diabetes. *J Clin Endocrinol Metab* (2019) 104(11):5499–506. doi: 10.1210/jc.2019-00423

11. Wright JJ, Salem JE, Johnson DB, Lebrun-Vignes B, Stamatouli A, Thomas JW, et al. Increased reporting of immune checkpoint inhibitor-associated diabetes. *Diabetes Care* (2018) 41(12):e150–e1. doi: 10.2337/dc18-1465

12. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob JJ, Cowey CL, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med* (2017) 377(14):1345–56. doi: 10.1056/NEJMoa1709684

13. Luo S, Zhang Z, Li X, Yang L, Lin J, Yan X, et al. Fulminant type 1 diabetes: a collaborative clinical cases investigation in China. *Acta Diabetol* (2013) 50(1):53–9. doi: 10.1007/s00592-011-0362-1

14. Li S, Zhang Y, Sun Z, Hu J, Fang C. Anti-PD-1 pembrolizumab induced autoimmune diabetes in Chinese patient: A case report. *Med (Baltimore).* (2018) 97 (45):e12907. doi: 10.1097/MD.0000000000012907

15. Imagawa A, Hanafusa T, Awata T, Ikegami H, Uchigata Y, Osawa H, et al. Report of the committee of the Japan diabetes society on the research of fulminant and acute-onset type 1 diabetes mellitus: New diagnostic criteria of fulminant type 1 diabetes mellitus (2012). *J Diabetes Investig* (2012) 3(6):536–9. doi: 10.1111/jdi.12024

16. Falcone M, Fousteri G. Role of the PD-1/PD-L1 dyad in the maintenance of pancreatic immune tolerance for prevention of type 1 diabetes. *Front Endocrinol (Lausanne).* (2020) 11:569. doi: 10.3389/fendo.2020.00569

17. Clotman K, Janssens K, Specenier P, Weets I, De Block CEM. Programmed cell death-1 inhibitor-induced type 1 diabetes mellitus. *J Clin Endocrinol Metab* (2018) 103(9):3144–54. doi: 10.1210/jc.2018-00728

18. de Filette JMK, Pen JJ, Decoster L, Vissers T, Bravenboer B, van der Auwera BJ, et al. Immune checkpoint inhibitors and type 1 diabetes mellitus: A case report and systematic review. *Eur J Endocrinol* (2019) 181(3):363–74. doi: 10.1530/EJE-19-0291

19. Akturk HK, Kahramangil D, Sarwal A, Hoffecker L, Murad MH, Michels AW. Immune checkpoint inhibitor-induced type 1 diabetes: A systematic review and meta-analysis. *Diabetes Med* (2019) 36(9):1075–81. doi: 10.1111/dme.14050

20. Bluestone JA, Buckner JH, Herold KC. Immunotherapy: Building a bridge to a cure for type 1 diabetes. *Science* (2021) 373(6554):510–6. doi: 10.1126/science.abh1654

21. Insel RA, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, et al. Staging presymptomatic type 1 diabetes: A scientific statement of JDRF, the endocrine society, and the American diabetes association. *Diabetes Care* (2015) 38(10):1964–74. doi: 10.2337/dc15-1419

22. Gauci ML, Laly P, Vidal-Trecan T, Baroudjian B, Gottlieb J, Madjlessi-Ezra N, et al. Autoimmune diabetes induced by PD-1 inhibitor-retrospective analysis and pathogenesis: A case report and literature review. *Cancer Immunol Immunother* (2017) 66(11):1399–410. doi: 10.1007/s00262-017-2033-8

23. Godwin JL, Jaggi S, Sirisena I, Sharda P, Rao AD, Mehra R, et al. Nivolumab-induced autoimmune diabetes mellitus presenting as diabetic ketoacidosis in a patient with metastatic lung cancer. *J Immunother Cancer.* (2017) 5:40. doi: 10.1186/s40425-017-0245-2

24. Shiba M, Inaba H, Ariyasu H, Kawai S, Inagaki Y, Matsuno S, et al. Fulminant type 1 diabetes mellitus accompanied by positive conversion of anti-insulin antibody after the administration of anti-CTLA-4 antibody following the discontinuation of anti-PD-1 antibody. *Intern Med* (2018) 57(14):2029–34. doi: 10.2169/internalmedicine.9518-17

25. Lowe JR, Perry DJ, Salama AK, Mathews CE, Moss LG, Hanks BA. Genetic risk analysis of a patient with fulminant autoimmune type 1 diabetes mellitus secondary to combination ipilimumab and nivolumab immunotherapy. *J Immunother Cancer* (2016) 4:89. doi: 10.1186/s40425-016-0196-z

26. Chang LS, Barroso-Sousa R, Tolaney SM, Hodi FS, Kaiser UB, Min L. Endocrine toxicity of cancer immunotherapy targeting immune checkpoints. *Endocr Rev* (2019) 40(1):17–65. doi: 10.1210/er.2018-00006

27. Huang G, Yin M, Xiang Y, Li X, Shen W, Luo S, et al. Persistence of glutamic acid decarboxylase antibody (GADA) is associated with clinical characteristics of latent autoimmune diabetes in adults: A prospective study with 3-year follow-up. *Diabetes Metab Res Rev* (2016) 32(6):615–22. doi: 10.1002/dmrr.2779

28. Achenbach P, Warncke K, Reiter J, Naserke HE, Williams AJ, Bingley PJ, et al. Stratification of type 1 diabetes risk on the basis of islet autoantibody characteristics. *Diabetes* (2004) 53(2):384–92. doi: 10.2337/diabetes.53.2.384

29. Park Y. Why is type 1 diabetes uncommon in Asia? *Ann N Y Acad Sci* (2006) 1079:31–40. doi: 10.1196/annals.1375.005

30. Kochupurakkal NM, Kruger AJ, Tripathi S, Zhu B, Adams LT, Rainbow DB, et al. Blockade of the programmed death-1 (PD1) pathway undermines potent genetic protection from type 1 diabetes. *PLoS One* (2014) 9(2):e89561. doi: 10.1371/journal.pone.0089561

31. Brahmer JR, Lacchetti C, Schneider BJ, Atkins MB, Brassil KJ, Caterino JM, et al. Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American society of clinical oncology clinical practice guideline. *J Clin Oncol* (2018) 36(17):1714–68. doi: 10.1200/JCO.2017.77.6385

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The β-carboline Harmine improves the therapeutic benefit of anti-PD1 in melanoma by increasing the MHC-I-dependent antigen presentation

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Harmine is a dual-specificity tyrosine-regulated kinase 1A (DYRK1A) inhibitor that displays a number of biological and pharmacological properties. Also referred to as ACB1801 molecule, we have previously reported that harmine increases the presentation of major histocompatibility complex (MHC)-I-dependent antigen on melanoma cells. Here, we show that ACB1801 upregulates the mRNA expression of several proteins of the MHC-I such as Transporter Associated with antigen Processing TAP1 and 2, Tapasin and Lmp2 (hereafter referred to as MHC-I signature) in melanoma cells. Treatment of mice bearing melanoma B16-F10 with ACB1801 inhibits the growth and weight of tumors and induces a profound modification of the tumor immune landscape. Strikingly, combining ACB1801 with anti-PD1 significantly improves its therapeutic benefit in B16-F10 melanoma-bearing mice. These results suggest that, by increasing the MHC-I, ACB1801 can be combined with anti-PD1/PD-L1 therapy to improve the survival benefit in cancer patients displaying a defect in MHC-I expression. This is further supported by data showing that *i)* high expression levels of TAP1, Tapasin and Lmp2 was observed in melanoma patients that respond to anti-PD1; *ii)* the survival is significantly improved in melanoma patients who express high MHC-I signature relative to those expressing low MHC-I signature; and *iii)* high expression of MHC-I

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signature in melanoma patients was correlated with increased expression of CD8 and NK cell markers and overexpression of proinflammatory chemokines involved in the recruitment of CD8+ T cells.

KEYWORDS

MHC-I antigen presentation, harmine, DYRK1A, anti-PD1 Immunotherapy, T lymphocyte and NK cells infiltration, melanoma, inflammatory chemokines

Introduction

Immune escape represents a major obstacle to successful cancer treatment based on immune checkpoint inhibitors (ICIs) (1). To escape CD8 T lymphocyte recognition, tumor cells lose their antigenicity through loss of immunogenic tumor antigens or defects in the antigen presentation machinery mediated by MHC-I (2). Moreover, malignant cells can gain additional aggressive properties by releasing factors that are responsible for the establishment of an immunosuppressive tumor microenvironment and the expression of immune checkpoint ligands (3).

MHC-I antigen presentation is the mechanism responsible for presenting "foreign" proteins on the surface of APC (antigen presenting cells) or cancer cells, thus allowing their recognition by CD8 T cells. Endogenously synthesized proteins are subjected to continuous degradation by the immunoproteasomes which is composed of several proteins, such the proteasome activator complexes 28α and β (PA28α and β) and low-molecular-weight proteins 2 and 10 (LMP2 and LMP10). Such degradation process is needed for the generation of a majority of MHC-I-presented peptides. Some peptides produced by the immunoproteasomes (containing 9 to 13 residues) are transferred into the lumen of the endoplasmic reticulum (ER) by the Transporter Associated with antigen Processing (TAP) complex, which is composed of two different subunits (TAP1 and TAP2). The heavy and light β2-microglobulin (β2M) chains of MHC-I molecules are co-transported into the ER where they fold into the MHC-I heterodimer.

Upon transport into the lumen of the ER, peptides are in the vicinity of newly assembling MHC I molecules. The complex is stabilized through interactions with chaperones such as calreticulin. Additional component of this complex includes the peptide "editor" Tapasin which helps in maintaining peptide-empty MHC I molecules in the ER. Assisted by the peptide-editors Tapasin, MHC-I molecules can bind peptides displaying the right length and sequences. Stable peptide-MHC I complexes are finally released from the ER to be exposed to the plasma membrane and displayed to CD8 T cells by the exocytic pathway (reviewed in (4)).

Despite the success of anti-PD-1 therapy, many patients experience intrinsic or acquired resistance involving several non-mutually exclusive mechanisms (5). In addition to the low mutational burden, the most straightforward cause of the lack of responsiveness to anti-PD-1/PD-L1 is defects in the recognition of tumor cells by T cells, which can be related to the absence of tumor antigens or defects in the antigen presentation mechanism by MHC (6). Therefore, improving tumor antigen presentation by cancer cells is an attractive clinical approach to restore anti-tumor immunity and improve anti-PD-1 therapy.

The beta-carboline alkaloid harmine inhibits members of the dual-specificity tyrosine-regulated kinases (DYRK), including DYRK1A, DYRK1B, DYRK2, and DYRK4, with highest affinity for DYRK1A (7). In addition to its wide range of pharmacological activities, harmine displays anti-tumor properties by suppressing cell proliferation and inducing cell death in breast, lung, and ovarian cancers (8–11) and sensitizing pancreatic cancer cells to gemcitabine (12). Harmine plays a role in the actin cytoskeleton-dependent tumor reversion process (13), a key element in the formation of immunological synapses between T-cell receptors (TCRs) and MHC-I expressing tumor cells (14).

In this study we assessed the impact of the beta-carboline derivative ACB-1801 on the expression of proteins of the MHC-I and evaluate the functional significance on the improvement of anti-PD-1 therapy

Here, we report that, in B16-F10 melanoma cells, ACB1801 upregulates the expression of proteins involved in the MHC-I peptide-loading complex, such as transporter associated with antigen processing proteins 1 and 2 (TAP1 and TAP1), Tapasin and low-molecular-weight proteins 2 (Lmp2). The therapeutic value of ACB1801-dependent increase of TAP1, TAP2, Tapasin and Lmp2 is underscored by clinical data showing that high expression levels of these proteins was observed in melanoma patients that respond to anti-PD-1 and associated with an improved survival of melanoma patients. Using B16-F10 as melanoma mouse model, we show that ACB1801 treatment induces a profound modification of the tumor immune landscape and significantly improves the therapeutic benefit of anti-PD-1.

Materials and methods

Cells and reagents

B16-F10, GEMM, A375, and CT26 cell lines were purchased from ATCC and cultured as described in the data sheet of ATCC

and a previous report (15). U87 and U251 cells were kindly provided by Dr. Anna Golebiewska (NorLux laboratory, LIH, Luxembourg) and cultured in Dulbecco's modified Eagle's medium-F12 (DMEM/F12, Gibco) supplemented with 10% fetal bovine serum (Gibco), 50-U/ml penicillin, and 250-µg/ml streptomycin. All cells were cultured in an incubator at 37°C with 5% CO2. All cell lines were frequently checked for whether they were free of mycoplasma using a Mycoalert kit (Lonza). ACB1801 was provided by AC Bioscience (Lausanne, Switzerland), mouse Tap1 monoclonal antibody (3D4), goat anti-mouse Phycoerythrin conjugated IgG secondary antibody and mouse IgG1 Isotype control (11711) were obtained from Novus, and MHC-class I H-2K^{b} antibody was obtained from Invitrogen.

RNA extraction and SYBR Green real-time (RT)-qPCR

As reported previously (16), total RNA was extracted using TRIzol solution (Invitrogen) according to manufacturer's instructions. 1 µg of total RNA was treated with DNase I and converted into cDNA using TaqMan Reverse Transcription Reagent (Applied Biosystems). The mRNA expression levels were quantified by the SYBR-GREEN qPCR method (Applied Biosystems). Relative expression was calculated using a comparative Ct method (2-ΔCt). The primer sequences are available upon request.

In vivo study approval

Animal experiments were conducted according to the European Union guidelines. The *in vivo* experimentation protocols were approved by the LIH ethical committee, Animal Welfare Society, and Luxembourg Ministry of Agriculture, Viticulture and Rural Development (agreements n. LECR-2018-12).

In vivo tumor growth and mouse treatments

C57BL/6 mice (7 weeks old) were purchased from Janvier and housed in pathogen-free conditions for one week before experiments. The mice were injected subcutaneously in the right flank with cell lines diluted in 100 µl of PBS. ACB1801 was administered to them with doses of 50, 20, and 10 mg/kg by oral gavage (per os) or 1 mg/kg by an i.p. route. Vehicle treatment was performed using methyl cellulose.

InVivoMab anti-mouse PD-1 (CD279) (BE0273) and InVivoMab rat IgG2a isotype control (BE0089) were purchased from BioXCell (Lebanon, USA), diluted in InVivoPure pH 7.0 Dilution Buffer (IP0070), and administered as indicated in the corresponding figures. Tumor volume (V) was measured using caliper every other day and estimated as follows: V (cm^3) = ½ (Length × Width^2). Mice were excluded if they did not develop tumors or developed tumors larger than the threshold defined in the approved experimentation protocols (volume > 2000 mm^3), as previously reported (15).

Tumor immune phenotyping and flow cytometry analysis

As previously reported (15), tumors were harvested, mechanically dissociated into fragments (<4 mm), and enzymatically digested using a mouse tumor dissociation kit (Miltenyi Biotec) for 45 min at 37°C. Single-cell suspensions were prepared, and red blood cells were lysed using Ammonium-Chloride-Potassium (ACK) lysis buffer (10-548E, Lonza). Live/Dead dye was used to select only live cells which were then counted using a Countess Automated Cell Counter (Invitrogen) and blocked for 30 minutes on ice with Fc block (TruStain fcX^{TM} (anti-mouse CD16/32) Antibody 101320 Biolegend). Samples were stained for surface markers for lymphoid and myeloid immune populations. For FoxP3 and intracellular staining, True-Nuclear^{TM} Transcription Factor Buffer Set 424401 Biolegend was used according to the manufacturer's recommended protocol. CD45$^+$ CD3$^-$ NK1.1$^+$ cells were defined as NK cells. Lymphocytes were defined as the CD3$^+$ subpopulation of the CD45$^+$ NK1.1$^-$ gate. CD4+ and CD8$^+$ T lymphocytes were derived from the CD3$^+$ subpopulation. Tregs were subdivided from CD4$^+$ T lymphocytes and defined as Foxp3$^+$ and CD4$^+$ Foxp3$^-$ cells were considered as CD4$^+$ T effector cells population. CD45$^+$ CD11b$^+$ cells were defined as a subset of live myeloid cells. DC were defined as the CD11c$^+$ sub-population of the CD45$^+$ CD11b$^+$ subset. Polymorphonuclear MDSCs (PMN-MDSCs) were defined as the Ly6G$^+$ Ly6C^{low} subpopulation of CD45$^+$ CD11b$^+$ subset. Total macrophages were defined as the F480$^+$ subpopulation of the CD45$^+$ CD11b$^+$ subset. Inflammatory anti-tumoral macrophages (M1) were defined as F4/80$^+$ CD206$^-$, and protumoral macrophages (M2) were defined as F4/80$^+$ CD206$^+$ subpopulations of the F480$^+$ CD45$^+$ CD11b$^+$ cells. The percentages of the different immune cell populations described above were calculated by reporting back to the total CD45$^+$ live cells.

For flow cytometry, cells were harvested in 10 mM EDTA (Invitrogen). Surface staining was done at 4°C for 30 min using appropriate antibodies according to according to the manufacturer's protocol. Dead cells were excluded using Live/Dead staining Kits (L34976; Thermo Fischer Scientific) or BD Via-Probe^{TM} Cell Viability Solution (555815; Becton Dickinson). Samples were processed on a CytoFLEX flow cytometer and analyzed using CytExpert software.

The following antibodies were purchased from Biolegend: FITC anti-mouse CD45, Brilliant Violet 785 anti-mouse CD3, APC anti-mouse CD8a, APC/Fire 750 anti-mouse CD4, PE/Cy7

anti-mouse CD49b (pan-NK cells), PE/Cy7 anti-mouse NK-1.1 antibody, Brilliant Violet 605 anti-mouse CD69, PE/Cy5 anti-mouse CD25, Brilliant Violet 421 anti-mouse FOXP3, PE/Dazzle 594 anti-mouse CD279 (PD-1), Brilliant Violet 785 anti-mouse/human CD11b, APC anti-mouse F4/80, PE/Cy5 anti-mouse CD11c, PE/Cy7 anti-mouse Ly-6G, APC/Fire 750 anti-mouse Ly-6C, Brilliant Violet 605 anti-mouse CD206 (MMR), and Brilliant Violet 421 anti-mouse CD274 (B7-H1, PD-L1). A LIVE/DEAD Fixable Blue Dead Cell Stain Kit (ThermoFisher Scientific) was used for viability dying. For compensation controls, single dye stains were performed and the fluorescence spread was checked using Fluorescence Minus One (FMO) controls. The levels of non-specific binding was evaluated using isotype controls.

Melanoma patient data mining

RNA expression reported as FPKM (Fragments Per Kilobase Million) values of anti-PD-1 treated melanoma patients from GEO (GSE78220) were retrieved and clinical data were downloaded from the corresponding published paper (17) for all the individual patients reported as responders or no responders to anti-PD-1. The FPKM value of MHC-I signature [TAP1, TAP2, TAPBP (Tapasin) and PSMB9 (Lmp2)] gene was compared between the two groups (Responders vs Non-responders). Mann Whitney U test was used to compute statistical significant difference using Graphpad Prism 8 software. Data from the TCGA skin cutaneous melanoma (SKCM) cohort (448 patients) were downloaded from cBioPortal (http://www.cbioportal.org/). IDs of patients displaying high and low TAP1, TAP2, TAPBP (Tapasin) and PSMB9 (Lmp2) mRNA expression (z-score relative to all samples) were extracted. Each patient's vital status and survival values (overall survival and disease-specific survival) were downloaded from the TCGA database. In patients displaying high and low MHC-I signature, the log2 mRNA expression levels (batch normalized from Illumina HiSeq_RNASeqV2) of markers for NK (NCR1 and NCR3), CD8 (CD8A, CD8B, KLRG1), Cytotoxicity [Granzyme B (GZMB), Perforin (PER), TNF alpha (TNF) and Interferon gamma (IFNg)] were identified. The expression of inflammatory chemokines (CCL2, CCL4, CCL5, CCL19, CCL21, CXCL9, CXCL10, CXCL11, CXCL13, XCL2) was extracted from patients displaying low and high levels of MHC-I signature, NK markers and CD8 markers. The differential expression of genes of interest was found using GraphPad software. The median survival and the p-value were calculated using the log-rank (Mantel-Cox) test in GraphPad software.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 8. An unpaired two-tailed t-test was used to determine p-values between indicated groups. Results are represented as the mean ± standard error of the mean (SEM). A p-value < 0.05 was considered statistically significant ($p \leq 0.05$ = *; $p \leq 0.01$ or ≤ 0.05 = **; $p \leq 0.001$ or ≤ 0.005 = ***; $p > 0.05$ = not significant, ns).

Results

ACB1801 increases the expression of several proteins of the MHC-I in various murine and human cancer cells

We assessed the impact of ACB1801 on the expression of antigen presentation genes (TAP1, TAP2, Tapasin, b2m, Lmp2, Lmp10, PA28α and PA26β) in murine B16-F10 melanoma. B16 cells are poorly immunogenic because they express low levels of MHC-I. This deficiency is attributed to the downregulation or loss in expression of multiple components of the MHC-I antigen-processing machinery (18). Cells treated with the culture medium alone were used as a control to evaluate the basal expression levels. We showed that ACB1801 upregulates the mRNA expression of TAP1, TAP2, Tapasin and Lmp2 genes involved in the antigen presentation in B16-F10 in a dose-dependent manner (Figure 1A).

The increased expression of TAP1, TAP2, Tapasin was also observed in colorectal CT26 cancer cells treated with 10 µM ACB-1801 (Figure 1B). Furthermore, the overexpression of TAP1, as a representative protein of the MHC-I, was detected in human melanoma cells A375, and colorectal HCT166 cells (Figure 1C) as well as in glioblastoma (U87 and U251) cells (Supplementary Figure 1). The increase of TAP1 mRNA by ACB1801 was translated into an increase of the protein expression of TAP1 in a dose-dependent manner (Figure 1D).

Initially named H−2, murine MHC-I comprises three gene loci: H−2K, H−2D, and H−2L. Several allotypes of these have been described, including H-2K^b (19). B16-F10 melanoma cells express low to undetectable levels of H-2K^b (20) which is critical for peptide-binding of murine MHC-I (21). We have previously reported that treatment of B16-F10 cells with ACB1801 increases H-2K^b bound OVA (SIINFEKL) peptide presentation by MHC-I (22). We believe that this could be related to an increase in the expression of the H-2K^b variant on the surface of B16-F10 cells following treatment with ACB1801, as shown in Figure 1D.

ACB1801 inhibits B16-F10 tumor growth and improves the therapeutic benefit of anti-PD1

The efficacy of anti-PD-1 therapy relies on the effectiveness of neoantigen presentation by MHC-I on the

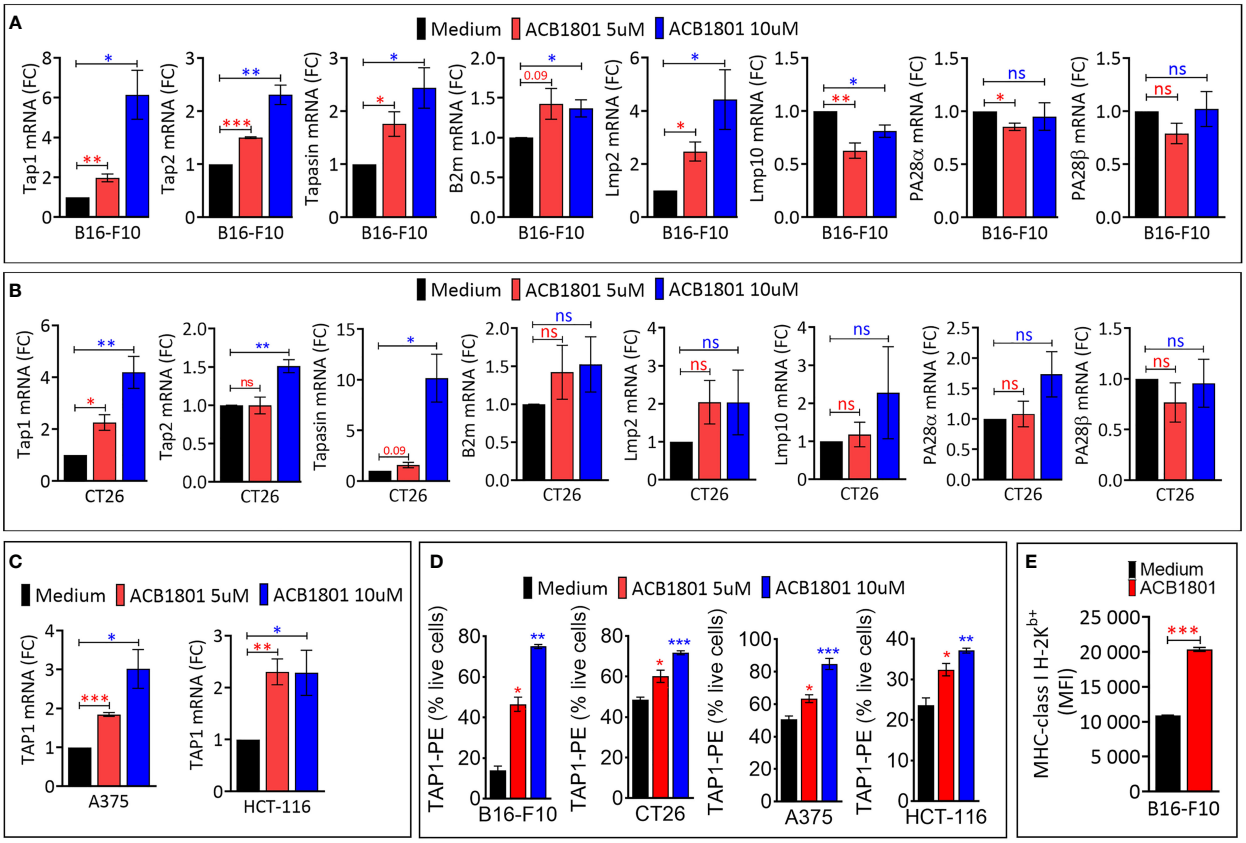

FIGURE 1
ACB1801 upregulates the expression of TAP1 in various murine and human tumor cells **(A, B)**. The mRNA expression of Tap1, Tap2, Tapasin, B2m, Lmp2, Lmp10, PA28α, PA28β, in mouse melanoma B16-F10 **(A)** and colorectal CT26 **(B)** cancer cells. **(C)** The mRNA expression of TAP1 in human melanoma A375 and colorectal HCT-116 cells. B16-F10, A375, CT26, and HCT-116 cells were treated for 24 h with culture medium (control) or with two concentrations of ACB1801 (5 and 10 uM). Results are reported as fold change (FC) relative to control cells treated with medium (black bars). **(D)** Flow cytometry analysis of the expression of TAP1 protein in B16-F10, CT26, A375, and HCT-116 cells treated for 24 h with culture medium (control) or two concentrations of ACB1801 (5 and 10 uM). Results are reported as % of positive cells relative to live cells. **(E)** Flow cytometry analysis of the expression of MHC-class I H-2K^b allotype on the cell surface of B16-F10 cells treated for 24 h with culture medium (control) or ACB1801 (5 uM). Results in **(A–E)** represent the averages of three independent experiments and are shown as mean ± SEM (error bars). Statistically significant differences were calculated relative to control conditions using an unpaired two-tailed student's t-test (ns, not significant, * =p< 0.05, ** =p< 0.005, and ***=p<0.0005).

surfaces of cancer cells (23). Melanoma patients displaying a low expression level of MHC-I are unlikely to benefit from anti-PD-1 (24). Based on these data, we assessed the impact of combining ACB1801 on the therapeutic benefit of anti-PD-1. We used a B16-F10 tumors since they are poorly immunogenic (18) and they do not respond to anti-PD-1 (15). The treatment schedule is shown in Figure 2A. Our results demonstrate that treatment with ACB1801 alone (10 mg/kg per os) decreased the tumor growth and weight of B16-F10 tumors and prologue the survival of tumor-bearing mice (Figures 2B, C, left panels, D). This effect is not restricted to B16-F10 tumors but is also observed in genetically engineered mouse melanoma (GEMM) tumors (Supplementary Figure 2) harboring genetic alterations (Braf$^{V600E/wt}$ Pten$^{-/-}$ Cdkn2$^{-/-}$) found in human melanomas.

We also showed that anti-PD-1 monotherapy had no effect on B16-F10 tumor growth, tumor weight and mice survival, as expected (Figures 2B, C, middle panels, and D). However, combining ACB1801 with anti-PD-1 remarkably improved the therapeutic benefit compared to anti-PD-L1 monotherapy (Figures 2B, C, right panels and D). Our results depicted in Figures 2E, F further indicate that ACB-1801 inhibits the growth of B16-F10 tumors in a doses dependent manner.

We next assessed whether lower doses of ABC1801 are still able to inhibit B16-F10 melanoma tumor growth and improve the therapeutic benefit of anti-PD-1. We found that ACB1801 at 1 mg/kg i.p. significantly improves the therapeutic benefit of anti-PD-1 (Figures 2G–I). Our results provide strong evidence that treatment with ACB1801 makes non-responder B16-F10 tumors strong responders to anti-PD1.

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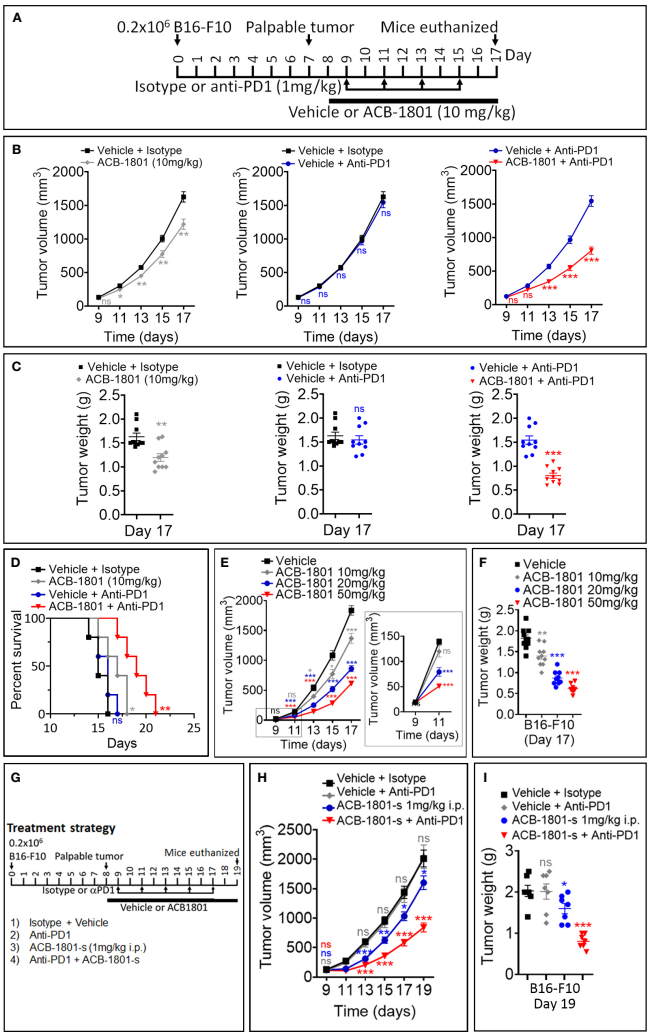

FIGURE 2
ACB1801 inhibits B16-F10 melanoma tumor growth and improves the therapeutic benefit of anti-PD-1. **(A)** Experimental schedule of B16-F10 melanoma treatment with mono and combination therapies of ACB1801 and/or anti-PD-1. B16-F10 cells (0.2 x 10^6 cells) were injected subcutaneously in the right flank of C57BL/6 mice at day 0. Palpable tumors were observed at day 8. Treatment with ACB1801 (10 mg/kg) or vehicle was started at day 8 to day 17 and delivered daily per os. Treatment with anti-PD-1 (αPD-1, 1 mg/kg) was delivered i.p. at days 9, 11, 13, and 15. Mice were euthanized at day 17. **(B–D)** Tumor growth curves **(B)**, weight (g) at day 17 **(C)**, and mice survival **(D)** of B16-F10 melanoma in mice treated with vehicle and isotype (vehicle + isotype), ACB1801 (10 mg/kg) and isotype (ACB-1801 10 mg/kg), vehicle and anti-PD-1 (vehicle + anti-PD-1), or ACB1801 (10 mg/kg) and anti-PD-1 (ACB1801 + anti-PD-1). Results are reported as the average of 10 mice per group and shown as mean ± SEM (error bars). Statistically significant differences are calculated using an unpaired two-tailed student's t-test (ns= not significant, * =p< 0.05, ** =p< 0.005, and ***=p<0.0005). Mice survival curves (5 mice per group) were generated from B16-F10 tumor-bearing mice. Lack of survival was defined as death or tumor size >1000 mm^3. Mice survival percentage was determined using Graph Pad Prism, and p-values were calculated using the log-rank (Mantel-Cox) test (* = p ≤ 0.05, ** = p ≤ 0.01). **(E, F)** Tumor growth curves **(E)** and weight (g) at day 17 **(F)** of B16-F10 melanoma in mice treated with vehicle or ACB1801 at 10, 20, and 50 mg/kg. Results are reported as the average of 10 mice per group. Enlargement of the tumor growth at days 9 and 11 is shown in the right of panel **(E)** Results are shown as mean ± SEM (error bars). Statistically significant differences are calculated using an unpaired two-tailed student's t-test (ns, not significant, and ***=p<0.0005). **(G–I)** Experimental schedule **(G)**, tumor growth **(H)**, and tumor weight **(I)** of B16-F10 melanoma treatment with mono and combination therapies of ACB1801 and/or anti-PD-1. B16-F10 cells (0.2 x 10^6 cells) were injected subcutaneously in the right flank of C57BL/6 mice at day 0. Palpable tumors were observed at day 8. Treatment with ACB1801 (1 mg/kg) or vehicle was started at day 8 to day 17 and delivered daily by i.p. injection. Treatment with anti-PD-1 (anti-PD1, 1 mg/kg) was delivered i.p. at days 9, 11, 13, and 15. Mice were euthanized at day 17. Tumor growth curves **(H)** and weight in g at day 17 **(I)** of B16-F10 melanoma in mice treated with vehicle and isotype (vehicle + isotype), ACB1801 and isotype (ACB1801-s 1 mg/kg), vehicle and anti-PD-1 (anti-PD1), or ACB1801 and anti-PD-1 (ACB-1801-s + anti-PD1). Results are reported as the average of 7 mice per group as mean ± SEM (error bars). Statistically significant differences are calculated using an unpaired two-tailed student's t-test (ns, not significant, * =p< 0.05, ** =p< 0.005, and ***=p<0.0005).

ACB1801 modifies the immune landscape of B16-10 tumors and enhances the infiltration of various anti-tumor immune effector cells

We have previously reported that ACB1801 treatment had no effect on B16-F10 tumor-bearing immunodeficient NOD scid gamma mice (NSG) lacking mature B, T, and NK cells (22). These results indicate that ACB1801-dependent inhibition of B16-F10 melanoma tumor growth involves the immune system. To evaluate whether ACB-1801 impacts the infiltration of immune cells into the tumor microenvironment, we performed a comprehensive analysis of the immune landscape of ACB1801-treated tumors using the gating strategies for lymphoid and myeloid immune phenotyping, which we have defined previously (15).

We showed a significant increase in the infiltration of NK cells, CD4 T effector (eff) cells, and $CD8^+$ T cells. This was associated with a significant decrease in the infiltration of immunosuppressive Treg cells in ACB1801-treated B16-F10 tumors compared to vehicle-treated controls (Figures 3A). We therefore found that the ratio CD8/Treg was increase in ACB-1801-treated tumors compared to controls (Figures 3B). By analyzing the infiltration of myeloid immune cells, we showed that there is no significant difference was observed in the infiltration of total ($CD11b^+$) myeloid cells, total ($F4/80^+$) macrophages, M1 ($CD206^-$) macrophages, M2 ($CD206^+$), and polymorphonuclear myeloid derived suppressor cells (PMN-MDSCs), but a significant increase was detected in the infiltration of $CD11c^+$ dendritic cells (DCs) in ACB1801-treated B16-F10 tumors compared to vehicle-treated controls (Figure 3C). To evaluate the functional status NK cells, CD4 T effector (eff) cells, and $CD8^+$ T cells infiltrating ACB-1801-treated tumors, we evaluate the expression of the activation marker CD69 and the early exhaustion marker PD-1. Our data showed a significant increase in the expression of CD69 and PD-1 markers on CD4 T effector (eff) cells and $CD8^+$ T cells, but not on NK cells, infiltrating ACB1801-treated B16-F10 tumors (Figures 3D, E).

High expression level of MHC-I signature is associated with improved survival benefit, overexpression of CD8 and NK markers as well as high expression of chemokines associated with CD8+ T cell recruitment in melanoma patients

Based on our *in vitro* data, we have defined TAP1, TAP2, TAPASIN (TAPBP) and LMP2 as an MHC-I signature which is regulated by ACB-1801.

To evaluate the therapeutic value of the MHC-I signature overexpression, we used clinical and RNA-seq data of patients treated with either pembrolizumab or nivolumab as the anti-PD-1 therapy for their metastatic melanoma (17). We showed that melanoma patients who were responsive to anti-PD-1 expressed significantly higher levels of TAP1, TAPASIN, LMP2, but not TAP2, compared to those who were not responsive to this therapy (Figure 4A). We next assessed the survival and expression of NK cell markers (NCR1 and NCR3) and CD8 T cell markers (CD8A and CD8B) in 448 patients with skin cutaneous melanoma reported in the TCGA database. The data mining workflow is shown in Figure 4B. Our results show that the overall survival (OS) and disease specific survival (DSS) are significantly higher in melanoma patients expressing high MHC-I signature than those expressing low MHC-I signature (Figure 4C). We also found that improved survival in patients expressing high MHC-I signature is associated with higher expression of NK and CD8 T cell markers (Figures 4D, E). Furthermore, we showed that the expression of the cytotoxic markers GZMB, PRF1, TNF, IFNg are significantly increased in patients displaying high MHC-I signature compared to those having low MHC-I signature (Supplementary Figure 3).

Our data suggest that melanoma patients expressing high MHC-I signature are more infiltrated by CD8 T cells compared to those expressing low MHC-I signature. We believe that the infiltration of CD8 T cells and NK cells is presumably due to the over-expression of chemokines involved in driving these cells in the tumor microenvironment. This assumption was supported by our data in Figure 4F showing high expression of the chemokine signature involved in the CD8 T cell recruitment (25) in patients displaying high MHC-I signature, CD8 and NK cells compared to those having low MHC-I signature, CD8 and NK cells. Interestingly, among patients with high MHC-I signature, 65% of them express high levels of effector CD8 T-cell markers ($CD8a^+$ $CD8b^+$ $KLRG1^+$), while 50% of them express high levels of Treg markers ($CD4^+$ $Foxp3^+$ $ISG20^+$) (Supplementary Figure 4A). Moreover, we also found that 46% of patients with high MHC-I signature express increased M2 markers $ADGRE1^+$ (Adhesion G Protein-Coupled Receptor E1) and $MRC1^+$ (Mannose Receptor C-Type 1) which are almost all positive for CD274 (PD-L1), but not for ARG1 (Arginase 1) (Supplementary Figure 4B). These results suggest that, in addition to cytotoxic effector cells, immunosuppressive cells and M2 macrophages could also be present in the tumor microenvironment of tumors expressing high MHC-I signature. Nevertheless, the function of immunosuppressive cells needs to be deeply investigated under these conditions.

Information about the TCGA melanoma patients is shown in Supplementary Tables 1–6. Collectively, these data highlight the therapeutic value of increasing the expression levels of TAP1, TAP2, TAPBP and PSMB9 in melanoma.

Discussion

Numerous studies are currently ongoing to understand the resistance mechanisms to immune checkpoint blockades and

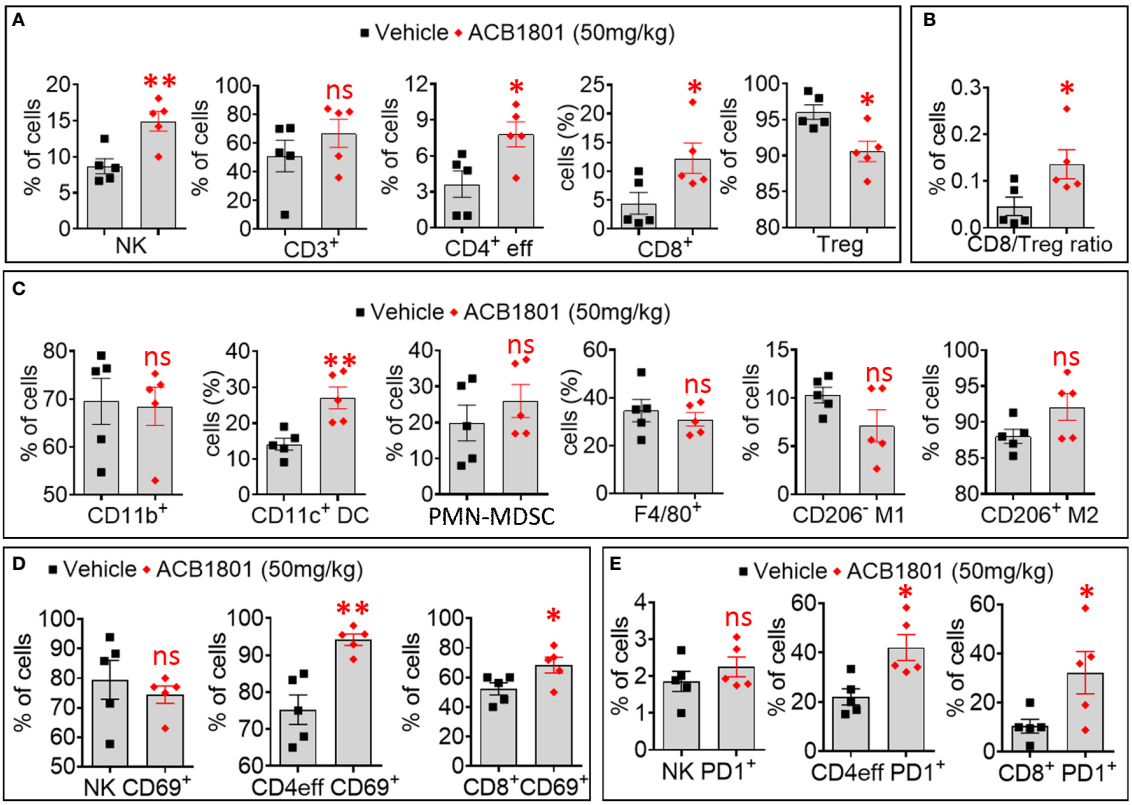

FIGURE 3
Treatment of B16-F10 tumor-bearing mice with ACB-1801 increases the infiltration of cytotoxic immune cells into the tumor microenvironment. **(A)** Flow cytometry quantification of the percent (%) live natural killer (NK) cells, CD3+, CD4+ effector T cells, CD8+ T cells, and regulatory T lymphocytes (Treg) infiltrating B16-F10 tumors treated treated with vehicle or 50 mg/kg of ACB-1801. **(B)** The ratio of CD8/Treg reported as percent (%) of cells infiltrating B16-F10 tumors treated as described in **(A)**. **(C)** Flow cytometry quantification of the percent (%) of live myeloid cells (CD11b+), dendritic cells (DC), polymorphonuclear myeloid derived suppressor cells (PMN-MDSC), total macrophages (F4/80), M1 macrophages ($CD206^-$ M1), and M2 macrophages ($CD206^+$ M2) infiltrating B16-F10 tumors treated as described in **(A)**. **(D, E)** Flow cytometry quantification of the percent (%) of live $CD69^+$ **(D)**, PD-1^+ **(E)** NK cells, CD4+ effector T cells, and CD8+ T cells infiltrating B16-F10 tumors treated as described in (A). All quantifications were performed on well-established tumors harvested at day 17. The immune cell populations were gated and quantified in live CD45+ cells. Each dot represents one tumor. Data are reported as the average of 5 mice per group as mean ± SEM (error bars). Statistically significant differences are calculated in comparison to vehicle-treated tumors using an unpaired two-tailed student's t-test (ns, not significant, * =p< 0.05, and ** =p< 0.005).

explore novel combinatorial approaches. We have shown that the β-carboline derivative ACB1801 potentiates the therapeutic benefit of anti-PD-1 in a B16-F10 melanoma mouse model, reported to resist to anti-PD-1 therapy (26, 27) and to express low levels of MHC-I (18). B16-F10 is, therefore, an appropriate mouse model for investigating the properties of molecules regulating MHC-I and assessing strategies to overcome the resistance to anti-PD-1/PD-L1.

Our *in vitro* results showed that ACB1801 increases the expression of several proteins of the MHC-I such as TAP1, TAP2, TAPBP, and the low-molecular-weight protein 2 (LMP2). Therefore, it is tempting to speculate that the effect of ACB-1801 on the increase of the antigen presentation in B16-F10 cells, previously reported by us (22), could be the result of the upregulation of several proteins involved in the MHC-I.

The precise mechanism by which ACB-1801 increases the antigen presentation to MHC-I is still not fully understood. However, we believe that the mode of action of ACB-1801 relies on its ability to remodel the actin cytoskeleton through inhibiting DYRK1A. DYRK1A is a negative regulator of the actin-related protein 2/3 (Arp2/3), which is involved in the actin polymerization process through Wiskott–Aldrich syndrome protein (WASP) phosphorylation (7, 13, 28). Therefore, we argue that inhibiting DYRK1A by ACB1801 would enhance the reorganization of the actin cytoskeleton, a prerequisite process for TCR/MHC immune synapse stabilization between T cells and APC (29–31).

Our *in vivo* data showed that, even without combination with anti-PD-1, monotherapy with ACB-1801 alone significantly inhibits the growth of B16-F10 tumors.

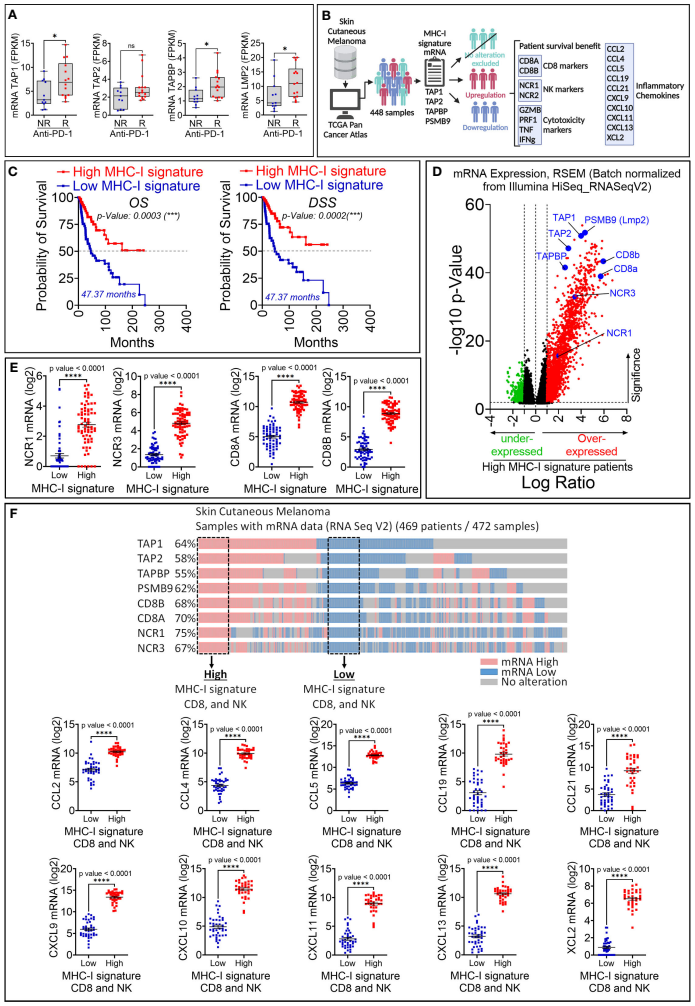

FIGURE 4
Retrospective analysis of the therapeutic value of MHC-I signature (TAP1, TAP2, TAPBP and LMP2) upregulation in melanoma patient cohorts. **(A)** The expression of MHC-I signature (TAP1, TAP2, TAPBP and LMP2) reported as FPKM in metastatic melanoma patients who are not responsive (NR) or responsive (R) to anti-PD-1. Statistically significant difference was determined using Mann Whitney U test in Graphpad Prism 9 software. **(B)** Workflow used for analyzing melanoma patient data in TCGA database. **(C)** Kaplan-Meier overall survival (OS, left panels) and disease-specific survival (DSS, right panels) curves of melanoma patients expressing high and low mRNA of MHC-I signature. Patients displaying high MHC-I signature have significantly improved OS and DSS compared to those with low MHC-I signature. The p-value of each curve was determined using the log-rank (Mantel-Cox) test. **(D)** Volcano plot of differentially expressed genes in melanoma patients with high and low mRNA expression of MHC-I signature. Scattered points represent genes. The x-axis shows the log2 fold change for the ratio of high compared to low expression of MHC-I signature. The y-axis shows significance by -log10 transformed p-value value. Red dots in the right of the dashed vertical line at +1 value represent genes that are significantly over-expressed in patients with high MHC-I signature. Green dots on left of the dashed vertical line at -1 value represent genes that are significantly under-expressed in in patients with high MHC-I signature. A gene is considered significantly differentially expressed if $|\log(FC)| \geq 0.1$ and $|\log10\ (p\text{-value})| \leq 0.01$. TAP1, TAP2, TAPBP, PSMB9, CD8 markers (CD8A and CD8B) and NK (NCR1 and NCR2) are shown in blue. **(E)** The mRNA expression of NK markers (NCR1 and NCR3) and CD8 markers (CD8A and CD8B) reported as log2 in melanoma patients displaying high and low MHC-I signature. Results are shown as mean ± SEM (error bars). Statistically significant differences of high MHC-I signature are calculated compared to patients with low MHC-I signature using an unpaired two-tailed student's t-test (**** = p<0.0001). **(F)** *Upper panel:* Strategy used to extract melanoma patient data in TCGA database expressing low and high TAP1, TAP2, Tapasin (TAPBP), Lmp2 (PSMB9), CD8A, CD8A and NCR1 and NCR3 genes. Doted boxes define patients that we have considered to assess the expression of CCL2, CCL4, CCL5, CCL19, CCL21, CXCL9, CXCL10, CXCL11, CXCL13 and XCL2 chemokines. *Lower panel:* The mRNA expression of CCL2, CCL4, CCL5, CCL19, CCL21, CXCL9, CXCL10, CXCL11, CXCL13 and XCL2 in patients defined in the upper panel. The differential expression of genes of interest was defined using GraphPad software. Results are represented as the mean ± standard error of the mean (SEM). Statistically significant differences are calculated using an unpaired two-tailed student's t-test (**** = p<0.0001).

Consistence with these data, it cannot ruled out that, through remodeling the actin cytoskeleton, ACB-1801 induces a reversion of the aggressive phenotype of the B16-F10 cells, thereby making them more prone to immune recognition and killing. Indeed, accumulating evidence suggests that tumor reversion refers to a process where cancer cell lose their malignant phenotype (also termed bad or tumor escape phenotype) and gain a good phenotype (also called tumor rejected phenotype) due to extensive genetic reprogramming (32, 33). Tumor cells with bad phenotype are derived from established tumors after escaping T-cell-mediated immune surveillance (34). From these evidences, we suspect that ACB-1801 increases tumor antigen presentation most likely through its potent property to inhibit DYRK1A and revert tumor malignant phenotype. Although a direct experimental evidence of such a mechanism is still needed, this concept is supported by evidences showing that: i) several kinases negatively regulate MHC-I expression and antigen presentation machinery in multiple cancers (35); and ii) one of the major characteristics of the malignant phenotype is the impairment of tumor antigen presentation due to genetic aberrations that provide growth and survival benefit to tumors (36). Therefore, the reversion of the malignant phenotype has been proposed to result in the unmasking of tumor cells, which would mainly occur through the rescue of tumor antigen presentation (4). Nevertheless, it is unlikely that the anti-tumor effect of ACB1801 results from exclusive action on tumor cells.

In addition to increasing the tumor antigen presentation, ACB1801 induces a deep modification of the immune landscape of B16-F10 tumors characterized by an increase of NK, CD4, and CD8 T cells and decrease of Tregs infiltration in the tumor microenvironment. Although the mechanism(s) underlying the tumor immune landscape modification by ACB1801 is still under investigation, we cannot exclude that one of these mechanisms relies on the regulation of the cytokine/chemokine repertoire in tumor cells, which are subjected to the phenotypic reversion. This statement is supported by previous studies showing that tumor cells undergoing phenotypic switch can regulate the release of cytokine/chemokine repertoire, thereby modifying the tumor immune landscape (37, 38). Schematic representation of the proposed role of ACB-1801 is provided in Figure 5.

Soluble factors released in the tumor microenvironment may act *via* an autocrine mechanism on tumor cells themselves or by a paracrine mechanism on other cells present in the tumor microenvironment, including immune cells. Therefore, it is tempting to speculate that the increased expression of CD69 and PD-1 on both CD4 effectors and CD8 T cells resulted from yet undefined soluble factors released by tumor cells. It should be highlighted that the overexpression of PD-1 on tumor-specific T cells should not only predict the exhaustion status, but can also be considered as a marker of activated tumor-reactive T cells (39, 40).

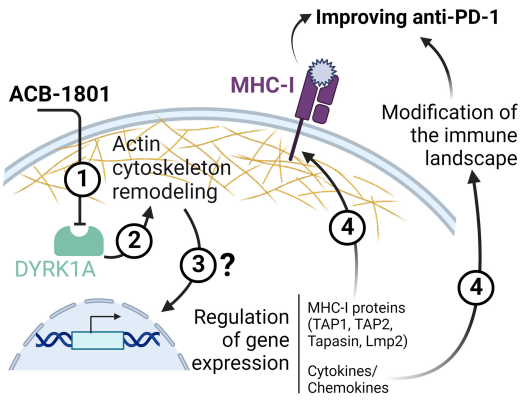

FIGURE 5
Schematic representation of the proposed role of ACB-1801. By inhibiting DYRK1A (1), ACB-1801 induces actin cytoskeleton remodeling (2) and phenotypic switch of cancer cells. Such phenotypic switch can regulate the expression of several genes (3) of the MHC-I and various cytokines and chemokines by mechanism(s) which are not fully understood. The regulation of several genes of MHC-I (TAP1, TAP2, Tapasin and Lmp2) leads to an overexpression of MHC-I (4). Whereas, the regulation of cytokine/chemokines genes induces a modification of the tumor immune landscape (4).

Nevertheless, further investigations must be carried out to profile the secretome of tumor cells treated with ACB1801 and understand its impact on the anti-tumor activity of different immune cells infiltrating treated tumors. In line with this work, previous study suggested that Harmine enhances the differentiation of Treg cells and strongly inhibits Th17 cell differentiation with minimal impact on Th1 responses *in vitro* (41). Based on our preclinical data showing an increased infiltration of cytotoxic immune cells in the tumor microenvironment of ACB-1801-treated tumors, which are further supported by clinical results, our results provides a framework for rational combination immunotherapy development of ACB-1801 and anti-PD-1.

The relevance of our study is underscored by clinical data showing that about 50% of cancer patients displayed an abnormal antigen presentation. Therefore, combining ACB1801 could substantially increase the number of cancer patients that would benefit from the impressive therapeutic value of ICI. Overall, our data can contribute to the emergence of a new wave of combination immunotherapy that would provide durable clinical outcomes and create support for immunotherapy.

Data availability statement

Patient data that support the findings of this study were derived from the following resources available in the public

domain: https://www.cbioportal.org/. Further inquiries can be directed to the corresponding author.

Ethics statement

Animal experiments were conducted according to the European Union guidelines. The in vivo experimentation protocols were approved by the Luxembourg Institute of Health ethical committee, Animal Welfare Society, and Luxembourg Ministry of Agriculture, Viticulture and Rural Development (agreements n. LECR-2018-12).

Author contributions

Study concept and experimental design: MZN, CA, and BJ; conducting experiments: MZN, KVM, IAB and MB; data acquisition: MZN, KVM, MB and AK; data analysis and interpretation: MZN, KVM, MB, AK, and BJ; study supervision: CA, GB, MK, GB, and BJ; writing the manuscript: BJ, CA, and MK. All authors contributed to the article and approved the submitted version.

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Conflict of interest

MK and CA are employees at AC Biotech and AC Bioscience, respectively. 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.980704/full#supplementary-material

References

1. Kim JM, Chen DS. Immune escape to pd-L1/Pd-1 blockade: Seven steps to success (or failure). *Ann Oncol* (2016) 27(8):1492–504. doi: 10.1093/annonc/mdw217

2. Beatty GL, Gladney WL. Immune escape mechanisms as a guide for cancer immunotherapy. *Clin Cancer Res* (2015) 21(4):687–92. doi: 10.1158/1078-0432.CCR-14-1860

3. Labani-Motlagh A, Ashja-Mahdavi M, Loskog A. The tumor microenvironment: A milieu hindering and obstructing antitumor immune responses. *Front Immunol* (2020) 11:940. doi: 10.3389/fimmu.2020.00940

4. 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

5. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell* (2017) 168(4):707–23. doi: 10.1016/j.cell.2017.01.017

6. Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature* (2014) 515(7528):577–81. doi: 10.1038/nature13988

7. Ogawa Y, Nonaka Y, Goto T, Ohnishi E, Hiramatsu T, Kii I, et al. Development of a novel selective inhibitor of the down syndrome-related kinase Dyrk1a. *Nat Commun* (2010) 1:86. doi: 10.1038/ncomms1090

8. Yochum ZA, Cades J, Wang H, Chatterjee S, Simons BW, O'Brien JP, et al. Targeting the emt transcription factor Twist1 overcomes resistance to egfr inhibitors in egfr-mutant non-Small-Cell lung cancer. *Oncogene* (2019) 38(5):656–70. doi: 10.1038/s41388-018-0482-y

9. Li YL, Ding K, Hu X, Wu LW, Zhou DM, Rao MJ, et al. Dyrk1a inhibition suppresses Stat3/Egfr/Met signalling and sensitizes egfr wild-type nsclc cells to Azd9291. *J Cell Mol Med* (2019) 23(11):7427–37. doi: 10.1111/jcmm.14609

10. Ding Y, He J, Huang J, Yu T, Shi X, Zhang T, et al. Harmine induces anticancer activity in breast cancer cells *Via* targeting taz. *Int J Oncol* (2019) 54(6):1995–2004. doi: 10.3892/ijo.2019.4777

11. Gao J, Zhu H, Wan H, Zou X, Ma X, Gao G. Harmine suppresses the proliferation and migration of human ovarian cancer cells through inhibiting Erk/Creb pathway. *Oncol Rep* (2017) 38(5):2927–34. doi: 10.3892/or.2017.5952

12. Wu LW, Zhang JK, Rao M, Zhang ZY, Zhu HJ, Zhang C. Harmine suppresses the proliferation of pancreatic cancer cells and sensitizes pancreatic cancer to gemcitabine treatment. *Onco Targets Ther* (2019) 12:4585–93. doi: 10.2147/OTT.S205097

13. Le Moigne R, Subra F, Karam M, Auclair C. The beta-carboline harmine induces actin dynamic remodeling and abrogates the malignant phenotype in tumorigenic cells. *Cells* (2020) 9(5):1168. doi: 10.3390/cells9051168

14. Roy NH, Burkhardt JK. The actin cytoskeleton: A mechanical intermediate for signal integration at the immunological synapse. *Front Cell Dev Biol* (2018) 6:116. doi: 10.3389/fcell.2018.00116

15. 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

16. Berchem G, Noman MZ, Bosseler M, Paggetti J, Baconnais S, Le Cam E, et al. Hypoxic tumor-derived microvesicles negatively regulate nk cell function by a mechanism involving tgf-beta and Mir23a transfer. *Oncoimmunology* (2016) 5(4): e1062968. doi: 10.1080/2162402X.2015.1062968

17. 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* (2016) 165(1):35–44. doi: 10.1016/j.cell.2016.02.065

18. Seliger B, Wollscheid U, Momburg F, Blankenstein T, Huber C. Characterization of the major histocompatibility complex class I deficiencies in B16 melanoma cells. *Cancer Res* (2001) 61(3):1095–9.

19. Pease LR, Schulze DH, Pfaffenbach GM, Nathenson SG. Spontaneous h-2 mutants provide evidence that a copy mechanism analogous to gene conversion generates polymorphism in the major histocompatibility complex. *Proc Natl Acad Sci U.S.A.* (1983) 80(1):242–6. doi: 10.1073/pnas.80.1.242

20. Calorini L, Marozzi A, Byers HR, Waneck GL, Lee KW, Isselbacher KJ, et al. Expression of a transfected h-2kb gene in B16 cells correlates with suppression of liver metastases in triple immunodeficient mice. *Cancer Res* (1992) 52(14):4036–41.

21. Molano A, Erdjument-Bromage H, Fremont DH, Messaoudi I, Tempst P, Nikolic-Zugic J. Peptide selection by an mhc h-2kb class I molecule devoid of the central anchor ("C") pocket. *J Immunol* (1998) 160(6):2815–23.

22. Schlapfer A, Auclair C, Janji B, Karam M, Zaeem Noman M. The 'Holy grail' in immuno-oncology: Ac bioscience sa is aiming to potentiate anti-Pd-1 therapy efficacy through tumor cell conditioning strategy. *Chimia (Aarau)* (2020) 74 (10):771–5. doi: 10.2533/chimia.2020.771

23. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science* (2018) 359(6382):1350–5. doi: 10.1126/science.aar4060

24. Shklovskaya E, Lee JH, Lim SY, Stewart A, Pedersen B, Ferguson P, et al. Tumor mhc expression guides first-line immunotherapy selection in melanoma. *Cancers (Basel)* (2020) 12(11):3374. doi: 10.3390/cancers12113374

25. 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

26. Wculek SK, Amores-Iniesta J, Conde-Garrosa R, Khouili SC, Melero I, Sancho D. Effective cancer immunotherapy by natural mouse conventional type-1 dendritic cells bearing dead tumor antigen. *J Immunother Cancer* (2019) 7(1):100. doi: 10.1186/s40425-019-0565-5

27. Ueha S, Yokochi S, Ishiwata Y, Ogiwara H, Chand K, Nakajima T, et al. Robust antitumor effects of combined anti-Cd4-Depleting antibody and anti-Pd-1/Pd-L1 immune checkpoint antibody treatment in mice. *Cancer Immunol Res* (2015) 3(6):631–40. doi: 10.1158/2326-6066.CIR-14-0190

28. Park J, Sung JY, Park J, Song WJ, Chang S, Chung KC. Dyrk1a negatively regulates the actin cytoskeleton through threonine phosphorylation of n-wasp. *J Cell Sci* (2012) 125(Pt 1):67–80. doi: 10.1242/jcs.086124

29. Calvo V, Izquierdo M. Role of actin cytoskeleton reorganization in polarized secretory traffic at the immunological synapse. *Front Cell Dev Biol* (2021) 9:629097. doi: 10.3389/fcell.2021.629097

30. Dustin ML, Cooper JA. The immunological synapse and the actin cytoskeleton: Molecular hardware for T cell signaling. *Nat Immunol* (2000) 1 (1):23–9. doi: 10.1038/76877

31. Billadeau DD, Nolz JC, Gomez TS. Regulation of T-cell activation by the cytoskeleton. *Nat Rev Immunol* (2007) 7(2):131–43. doi: 10.1038/nri2021

32. Powers S, Pollack RE. Inducing stable reversion to achieve cancer control. *Nat Rev Cancer* (2016) 16(4):266–70. doi: 10.1038/nrc.2016.12

33. Garrido F, Aptsiauri N, Doorduijn EM, Garcia Lora AM, van Hall T. The urgent need to recover mhc class I in cancers for effective immunotherapy. *Curr Opin Immunol* (2016) 39:44–51. doi: 10.1016/j.coi.2015.12.007

34. Wang S, He Z, Wang X, Li H, Liu XS. Antigen presentation and tumor immunogenicity in cancer immunotherapy response prediction. *Elife* (2019) 8. doi: 10.7554/eLife.49020

35. Brea EJ, Oh CY, Manchado E, Budhu S, Gejman RS, Mo G, et al. Kinase regulation of human mhc class I molecule expression on cancer cells. *Cancer Immunol Res* (2016) 4(11):936–47. doi: 10.1158/2326-6066.CIR-16-0177

36. Mizuno S, Yamaguchi R, Hasegawa T, Hayashi S, Fujita M, Zhang F, et al. Immunogenomic pan-cancer landscape reveals immune escape mechanisms and immunoediting histories. *Sci Rep* (2021) 11(1):15713. doi: 10.1038/s41598-021-95287-x

37. Chockley PJ, Keshamouni VG. Immunological consequences of epithelial-mesenchymal transition in tumor progression. *J Immunol* (2016) 197(3):691–8. doi: 10.4049/jimmunol.1600458

38. Suarez-Carmona M, Lesage J, Cataldo D, Gilles C. Emt and inflammation: Inseparable actors of cancer progression. *Mol Oncol* (2017) 11(7):805–23. doi: 10.1002/1878-0261.12095

39. Simon S, Labarriere N. Pd-1 expression on tumor-specific T cells: Friend or foe for immunotherapy? *Oncoimmunology* (2017) 7(1):e1364828. doi: 10.1080/2162402X.2017.1364828

40. Sharpe AH, Pauken KE. The diverse functions of the Pd1 inhibitory pathway. *Nat Rev Immunol* (2018) 18(3):153–67. doi: 10.1038/nri.2017.108

41. Khor B, Gagnon JD, Goel G, Roche MI, Conway KL, Tran K, et al. The kinase Dyrk1a reciprocally regulates the differentiation of Th17 and regulatory T cells. *Elife* (2015) 4. doi: 10.7554/eLife.05920

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