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Optimizing early-phase immunotherapy trials: the role of biomarker enrichment strategies

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Immune checkpoint inhibitors have revolutionized the treatment of solid tumors; however, their clinical efficacy remains limited to a subset of patients. Novel immunotherapy agents are being investigated in phase I clinical trials, with an increasing focus on biomarker selection strategies to optimize patient outcomes. Prior evidence suggests that biomarker-selected tumors may have better outcomes when treated with molecularly-guided therapies. However, the high complexity of tumor-host interactions and inter-patient variability indicates that a one-size-fits-all biomarker approach is unlikely to be sufficient in the immunotherapy landscape. This review highlights current biomarker-enrichment strategies in immunotherapy early drug development, addressing challenges and potential future directions for their effective implementation.

KEYWORDS

biomarker, immune checkpoint inhibitors, early-phase trials, phase 1, immunotherapy, drug response biomarkers, predictive biomarkers

1 Introduction

Immune checkpoint inhibitors (ICIs) have dramatically changed the therapeutic landscape of several tumor types, offering durable responses, prolonged survival, and even the potential for long-term remission in a small subset of patients. The approval of ipilimumab, the first anti-CTLA-4 monoclonal antibody (mAb), for the treatment of melanoma in 2011 marked the advent of a new era (1). This was followed by significant advances with the development of anti-programmed cell death 1 (anti-PD-1) or its ligand (anti-PD-L1) mAb, such as nivolumab or pembrolizumab, becoming part of the standard treatment for multiple cancer types (2–4). Despite these therapeutic milestones, nearly 80%

of patients either fail to respond to ICIs or develop resistance over time (5). Furthermore, a clinically significant proportion of patients treated with ICIs experience severe immune-related adverse events (irAEs), which lead to treatment discontinuation, and a subset may even exhibit rapid disease progression, a phenomenon known as hyperprogression (6–8). To address current limitations with classic ICIs, an expanding array of next-generation immunotherapeutic agents—including novel checkpoint inhibitors, T-cell engagers, cytokine modulators, and cell-based therapies—is being evaluated in early-phase trials, aiming to broaden the population of responders while overcoming mechanisms of intrinsic or delaying acquired resistance (9, 10). A critical priority in immunotherapy early drug development is optimizing patient selection to accelerate efficacy signals while mitigating toxicity risks, even during dose escalation phases (11).

Currently, fewer than 10% of oncology drugs entering phase 1 trials ultimately receive regulatory approval (12). This rate may be even lower for immunotherapies due to their biological and clinical complexities, underscoring a critical need, as the development of immunotherapy agents is a highly complex, time-intensive, and costly process (13). A pivotal shift in immuno-oncology (IO) has been the growing emphasis on biomarker enrichment strategies. A comprehensive analysis, which reviewed 17,368 drug development trajectories (2000–2015), found biomarker-guided patient selection significantly increased success rates in drug development (10.7% vs 1.6%) (12). Similarly, the use of biomarker-guided therapies has been associated with better outcomes in patients treated with immunotherapy in phase 1–2 trials (14). Overall, these data highlight the need to implement biomarker-driven enrichment

strategies in early-phase immunotherapy trials to refine patient selection and enhance trial efficiency. In this regard, the tumor-agnostic approval of pembrolizumab in 2017 for the treatment of microsatellite instability-high (MSI-H) or mismatch repair-deficient (dMMR) tumors represented a paradigm shift. This marked the first time the U.S. Food and Drug Administration (FDA) approved a cancer therapy based on the presence of a molecular biomarker independently of tumor histology (15, 16). Moreover, tumor mutational burden (TMB) has also emerged as another critical predictive biomarker. TMB high (TMB-H), defined by the presence of equal or more than ten mutations per megabase (Mb), has been correlated with increased neoantigen load and improved responses to ICIs across various malignancies (17, 18). Indeed, the FDA approved TMB in 2020 as a tissue-agnostic companion diagnostic for pembrolizumab in solid tumors, based on the results of the KEYNOTE-158 phase 2 trial, which showed an overall response rate (ORR) of 29% (19, 20). Furthermore, to optimize novel drug development, biomarker discovery and validation have been increasingly shifting from late-stage studies to early-phase clinical trials. However, despite substantial efforts in searching robust biomarkers, only programmed death-ligand 1 (PD-L1) expression, TMB, and dMMR/MSI-H are currently validated and used in routine clinical practice, while others remain under investigation (Table 1) (35). Integrating high-throughput technologies into early-phase clinical trial design may improve efficiency, patient selection and refine endpoint determination (36).

In this narrative review, we aimed to provide a comprehensive overview of the current landscape of biomarkers and enrichment

TABLE 1 Established and emerging biomarkers for immunotherapy in solid tumors.

Biomarker	Predictive value	Assay/Tissue type	Limitations
PD-L1 (21)	Predicts response in NSCLC, HNSCC, gastric, TNBC, cervical, urothelial	IHC (clones: 22C3, 28-8, SP142, SP263) on tumor and/or immune cells	- Heterogeneity (intra/inter-tumoral, temporal). - Assay variability. - Lack of universal cut-off. - Variability in predictive value across tumor types.
dMMR/MSI-H (22, 23)	Strong predictor of response; FDA-approved for pembrolizumab (tissue-agnostic)	IHC (MLH1, MSH2, MSH6, PMS2); PCR; NGS	- Discordant cases (IHC vs PCR/NGS) may still respond. - Biological heterogeneity (co-alterations may modulate response).
TMB-H (17, 18)	Predicts response; FDA-approved for pembrolizumab (tissue-agnostic)	Targeted panels, WES, WGS or liquid biopsy (ctDNA)	- Lack of standardization. - Variable panel sizes. - Predictive value inconsistent across tumor types.
<i>POLE/POLD1</i> mutations in EDMs (24)	Predict sensitivity (ultramutator phenotype)	NGS, Sanger sequencing, FFPE assays	- Very low prevalence (<3%). - Requires broad prescreening.
SWI/SNF complex (ARID1A, PBRM1, SMARCA4, SMARCB1) (8)	Predict potential sensitivity	NGS, molecular profiling	- Exploratory.
Resistance mutations (<i>B2M</i> , <i>JAK1/2</i> , <i>STK11/LKB1</i> , <i>KEAP1</i> , <i>EGFR</i> , <i>PTEN</i> , <i>MDM2</i>) (7, 8, 25–34)	Associated with resistance or hyperprogression	NGS, molecular profiling	- Context-dependent: not absolute (e.g., some MSI-H with <i>B2M</i>/<i>JAK1/2</i> still respond). - Remain exploratory.

NSCLC, non-small cell lung cancer; HNSCC, neck squamous cell carcinoma; TNBC, triple-negative breast cancer; IHC, immunohistochemistry; TPS, Tumor Proportion Score; CPS, Combined Positive Score; ctDNA, circulating tumor DNA; PCR, polymerase chain reaction; NGS, next-generation sequencing; WES, Whole exome sequencing; WGS, Whole genome sequencing; FFPE, specialized mutation assays using formalin-fixed paraffin-embedded; EDMs, exonuclease domains.

strategies in immunotherapy while highlighting advances, limitations, and persistent barriers associated with the broad implementation of biomarker-driven patient selection in early-phase trials.

2 Tissue-based immunotherapy biomarkers

2.1 Programmed cell death-ligand 1

PD-L1 has been extensively investigated for predicting response to ICIs (37). PD-L1 immunohistochemistry (IHC) has received regulatory approval as a companion or complementary diagnostic test for several ICIs across various tumor types, including non-small cell lung cancer (NSCLC), gastric cancer, triple-negative breast cancer (TNBC), cervical cancer, urothelial carcinoma, and head and neck squamous cell carcinoma (HNSCC) (21). Despite its initial promise, translating it into a consistently reliable biomarker for patient selection has been significantly challenged by inherent biological complexities and technical standardization (38). PD-L1 expression can be induced by cytokines such as interferon-gamma (IFN- γ), exhibiting notable intra-tumoral and inter-metastatic heterogeneity, and frequently diverges between primary tumors and metastases (39–41). For example, discordant expression was observed in 50% of advanced melanoma patients when they were longitudinally sampled (40). Also, PD-L1 expression can be modulated by prior or concurrent treatments, adding temporal variability as another layer of complexity (41–43). For instance, in a cohort of patients with metastatic NSCLC, prior ICI exposure was associated with reduced PD-L1 expression compared to treatment-naïve patients (44). This results in significant heterogeneity within and across tumor sites, making a single pre-treatment biopsy a potentially unreliable indicator of the overall immune landscape (19, 20, 45). Moreover, the predictive value of PD-L1 varies depending on whether expression is assessed on tumor cells (TCs) or immune cells (ICs). While PD-L1 expression on TCs has shown a more consistent correlation with treatment outcomes, expression on ICs has demonstrated variable and often limited associations with response to ICIs (39). Such sampling bias can lead to misclassification, limiting the ability to detect efficacy signals, which is especially critical in small, exploratory phase 1 trials. PD-L1 expression can be evaluated using different scoring systems, such as the Tumor Proportion Score (TPS) and the Combined Positive Score (CPS), each with distinct clinical cut-offs specific to the assay, tumor type, and therapeutic agent. Moreover, differential predictive value has been observed across these methods, adding further complexity to the interpretation of PD-L1 expression (21). Beyond biological complexity, the utility of PD-L1 as a reliable biomarker is further constrained by significant technical challenges. The FDA has approved multiple IHC assays for PD-L1 detection utilizing different antibody clones (Dako 22C3, Dako 28-8, Ventana SP142, and Ventana SP263), each developed in conjunction with specific PD-(L)1 inhibitors (21).

In phase 1 clinical trials, the use of different thresholds to define PD-L1 positivity has yielded variable results, underscoring the lack of standardization in the application of this biomarker. The expansion cohort of the KEYNOTE-001 reported an ORR of 45%, 16.5% and 10.7% in patients with a TPS $\geq$ 50%, 1–49% and <1%, respectively (46). These findings led to FDA approval for PD-L1 expression and provided the basis for subsequent trials such as KEYNOTE-010 (cut-off $\geq$ 1%) and KEYNOTE-024 (cut-off $\geq$ 50%) in NSCLC (4, 47). Similarly, other anti-PD-(L)1 mAbs (e.g., atezolizumab or nivolumab) demonstrated responses predominantly in patients with high PD-L1 expression (Table 2) (48, 59). However, growing evidence suggests that PD-(L)1 blockade efficacy may be independent of PD-L1 expression in specific contexts, with clinical benefits observed in PD-L1 negative tumors (2, 60). For example, in advanced renal cell carcinoma (RCC) treated with nivolumab, the median overall survival (OS) was paradoxically longer in the PD-L1<1% population (27.4 months) compared to PD-L1 $\geq$ 1% group (21.8 months), challenging the pan-cancer predictive value (61). Overall, these limitations constrain its pan-cancer application. While PD-L1 remains an established biomarker in clinical decision-making for approved ICIs in specific, well-defined contexts, its profound biological dynamism and susceptibility to technical variability render it an insufficient enrichment tool in early-phase drug development (62). Assay-dependent misclassification and arbitrary thresholds may overestimate drug activity in biomarker-selected populations or inadvertently exclude PD-L1-negative patients who could benefit from treatment. Therefore, while PD-L1 retains clinical utility as an accessible and widely implemented biomarker, particularly for patient selection in specific indications, its limitations suggest that it may be more appropriately integrated as a stratification factor, an exploratory endpoint, or as part of a more comprehensive, multi-parametric biomarker approach.

2.2 Mismatch repair deficiency

Tumors harboring deficiencies in the DNA mismatch repair (MMR) pathway characteristically accumulate an exceptionally high number of somatic mutations, a state known as MSI-H. This high mutational load makes dMMR/MSI-H tumors particularly rich in immunogenic neoantigens, providing a strong rationale for their generally higher sensitivity to ICIs (22, 23). The prevalence of dMMR/MSI-H tumors varies across cancer types, ranging from approximately 30% of the cases in endometrial cancer (EC), 10–15% in colorectal cancer (CRC), 5–8% in gastric cancers, and under 5% in many other solid tumors (63). Detection methods include IHC for MMR proteins (MLH1, MSH2, MSH6, PMS2), as well as molecular techniques such as polymerase chain reaction (PCR) and next-generation sequencing (NGS) to assess MSI status (Table 1) (64). While NGS and PCR concordance is generally high, with a reported rate of approximately 98.8%, historical discordance rates between IHC and molecular methods (NGS/PCR) have ranged from 1–10% (64, 65). More recent data using improved methodologies suggest a lower discordance rate (0.3–

TABLE 2 Main diagnostic assays and thresholds enabling ICI use in common solid tumors.

Tumor type	Approved biomarker	Assay/Clone	FDA-approved threshold for ICI use	Reference trial
NSCLC	PD-L1 (TPS)	IHC (22C3, 28-8, SP263)	TPS $\geq$ 50%: pembrolizumab (4) TPS $\geq$ 1%: pembrolizumab (47) or nivolumab plus ipilimumab (48)	KEYNOTE-024 (4), KEYNOTE-010 (47), CheckMate-227 (48)
HNSCC	PD-L1 (CPS)	IHC (22C3)	CPS $\geq$ 1: pembrolizumab (49)	KEYNOTE-048 (49)
Gastric/GEJ	PD-L1 (CPS)	IHC (22C3)	CPS $\geq$ 5: nivolumab (50) CPS $\geq$ 10: pembrolizumab (51)	CheckMate 649 (50) KEYNOTE-859 (51)
Colorectal Cancer	dMMR/MSI-H	IHC (MLH1, MSH2, MSH6, PMS2); PCR; NGS	dMMR/MSI-H	KEYNOTE-177 (52), CheckMate-8HW (53)
Cervical cancer	PD-L1 (CPS)	IHC (22C3)	CPS $\geq$ 1: pembrolizumab (54)	KEYNOTE-826 (54)
Urothelial carcinoma	PD-L1 (CPS or IC %)	IHC (22C3, SP142)	CPS $\geq$ 10: pembrolizumab (55) IC $\geq$ 5%: atezolizumab (56)	IMvigor210 (56), KEYNOTE-052 (55)
Tissue-agnostic	dMMR/MSI-H	IHC (MLH1, MSH2, MSH6, PMS2); PCR; NGS	dMMR/MSI-H (any tumor)	KEYNOTE-016 (57), KEYNOTE-158 (58)
Tissue-agnostic	TMB-H	NGS (FoundationOne CDx)	$\geq$ 10 mut/Mb	KEYNOTE-158 (58)

NSCLC, non-small cell lung cancer; IHC, immunohistochemistry; TPS, Tumor Proportion Score; HNSCC, neck squamous cell carcinoma; CPS, Combined Positive Score; GEJ, gastroesophageal junction; IC, immune cells; NGS, next-generation sequencing; Mb, megabase.

1.6%) (Supplementary Table S1) (66, 67). However, each technique offers unique strengths and limitations. While IHC offers shorter turnaround times and cost-effectiveness, it may fail to identify dMMR in cases of missense mutations where non-functional proteins retain antigenicity. Conversely, NGS provides superior sensitivity, detecting MSI-H tumors that are missed by IHC (e.g., those with atypical gene involvement as MLH1, PMS1, or co-occurring *POLE* mutations), but it often entails longer turnaround time and higher costs (68, 69). Importantly, discordant cases such as pMMR/MSI-H tumors may still harbor a high mutational burden and consequently respond favorably to ICIs (17, 67). Therefore, the choice of detection method in early-phase trials may be significant, as it can influence the characterization of the enriched patient population and the subsequent interpretation of efficacy signals (63).

The clinical utility of dMMR/MSI-H as a predictive biomarker for ICIs was first demonstrated across multiple studies. KEYNOTE-016, a phase 2 study, reported an ORR of 40% and 71% with pembrolizumab in advanced dMMR/MSI-H CRC and dMMR/MSI-H non-CRC, respectively (57). Subsequently, the phase 2 multicohort trial, KEYNOTE-158, showed an ORR of 34.3% with a median progression-free survival (PFS) of 4.1 months and OS of 23.5 months, in pre-treated patients with various dMMR/MSI-H non-CRC solid tumors (58). These findings culminated in the 2017 FDA accelerated approval of pembrolizumab for dMMR/MSI-H solid tumors, establishing it as the first tissue-agnostic biomarker for cancer therapy (Table 2) (70). More recently, phase 3 trials, such as CheckMate 8HW and KEYNOTE-177, have confirmed the benefit of ICIs in the first-line treatment of dMMR/MSI-H mCRC compared with standard chemotherapy combinations (52, 53).

Despite robust evidence, there is a critical need for further understanding of dMMR/MSI-H tumors. Not all dMMR/MSI-H

tumors respond equally, suggesting biological heterogeneity that could guide future enrichment strategies. For example, the presence of co-occurring oncogenic alterations in the *PI3K-AKT* signaling pathway (e.g., *PTEN* loss, *AKT1* mutations) has been implicated in primary resistance to ICIs. Such alterations are hypothesized to contribute to an immune-excluded phenotype by modulating cytokine dysregulation, reducing T-cell infiltration, and impairing antigen presentation (71). Interestingly, loss of expression of both *MLH1* and *PMS2* has been associated with stronger responses due to greater neoantigen load (71). Additionally, discordant profiles, such as pMMR/MSI-H tumors, may still exhibit TMB-H and respond favorably to ICIs (17, 67). These data suggest that further patient stratification within the dMMR/MSI-H population may be required. Early-phase trials could be designed to identify these “hyper-responders” subgroups or rationally design combination therapies aimed at overcoming these potentially resistant pathways.

2.3 Tumor mutational burden

TMB is defined as the total number of non-synonymous mutations per Mb of the tumor genome and can be considered as a surrogate marker of overall tumor immunogenicity, emerging as a potential pan-cancer predictive biomarker for response to ICIs. TMB can be estimated using NGS approaches, ranging from whole-genome/exome sequencing (WGS/WES) of paired tumor and normal DNA to targeted gene panels based on tumor tissue or liquid biopsies (68, 69). However, its clinical application is hindered by a lack of standardization across testing platforms and analytical methodologies. We summarized common assays used in clinical practice in Supplementary Table S2. WES-based TMB calculations typically focus on missense mutations, but TMB values are also

highly influenced by pre-analytical factors such as tumor purity, with low purity potentially leading to TMB underestimation (72, 73). Moreover, variability in panel size, sequencing depth, and mutation filtering criteria further complicates cross-platform comparability and limits the utility of TMB for prospective patient selection in early-phase trials (74). International initiatives such as Friends of Cancer Research (Friends) and the Quality in Pathology (QuIP) have developed frameworks aimed at standardizing and harmonizing TMB assessment across platforms and centers globally (75, 76).

Based on the results of the single-arm phase 2 KEYNOTE-158 trial showing an ORR of 29% across tumor histologies, the FDA granted the tumor-agnostic approval of pembrolizumab for patients with unresectable or metastatic solid tumors exhibiting TMB ≥ 10 mutations/Mb, as determined by a specific assay (FoundationOne CDx) (Tables 1, 2) (19). Further evidence has emerged from clinical trials in gastric cancer (such as KEYNOTE-061), HNSCC (CONDOR and HAWK), or hepatocellular carcinoma (EPOC1704), which have contributed to this evidence base, particularly for combination immunotherapy strategies (77–82). Another study found that higher log-transformed TMB values were associated with improved response rates. Notably, some viral-related cancers (anal, cervical, and hepatocellular) exhibited better responses than expected based solely on TMB, suggesting that additional factors may influence ICI responses (83). While this approval marked a significant step forward in precision IO, major concerns still exist around the clinical application of TMB. For instance, the predictive value of TMB is not consistent across tumor types. Despite the presence of TMB-H in certain tumors such as breast, prostate cancer, and gliomas, TMB has not been definitively associated with increased immunotherapy sensitivity. This suggests that a TMB-H is not necessarily indicative of effective immune recognition, potentially due to factors such as a lack of functional neoantigens or insufficient CD8⁺ T-cell infiltration (84). Importantly, the predictive value is not universal, so its utility depends significantly on tumor-intrinsic and patient-specific characteristics. For instance, a retrospective study analyzing survival outcomes in over 8000 patients with solid tumors treated with ICIs showed that TMB-H was significantly associated with longer OS in the pan-cancer setting, including the CRC subgroup. Nevertheless, among patients with MSS CRC, no differences were observed according to TMB status, suggesting that this benefit may be driven by the dMMR/MSI-H sub-population (85).

Moreover, in a cohort of 1,662 patients analyzed using the MSK-IMPACT targeted NGS panel, higher somatic TMB (defined as the top 20% within each histologic subtype) was associated with improved OS with ICIs across cancer types. However, specific TMB cut-offs defining this “high” stratum varied markedly between histologies, suggesting a universal TMB threshold is unlikely to be optimal (86). Commonly used assays for TMB evaluation are presented in Supplementary Table S2. Consequently, applying fixed TMB thresholds for patient enrichment in early drug development, particularly across heterogeneous pan-tumor cohorts, risks oversimplification. This could compromise sensitivity by excluding potential responders or reduce specificity

by including non-responsive TMB-H individuals. Notably, a lack of concordance has been observed between TMB and other biomarkers such as PD-L1 expression. For instance, in CheckMate-026 and CheckMate-227, patients with NSCLC who had TMB-H experienced prolonged PFS with ICI treatment, regardless of PD-L1 status (87, 88). This supports the idea that TMB may define a distinct subset of immunotherapy-responsive patients, underscoring the need for integrative, multi-parametric biomarker strategies in early-phase settings. Recognizing the limitations of tissue-based TMB (tTMB) assessment, plasma-based TMB (blood TMB or bTMB) offers a less invasive alternative. Clinical trials, such as MYSTIC and BFAST, in NSCLC have explored bTMB, demonstrating its feasibility, as well as the challenges, including assay failure due to insufficient circulating tumor DNA (ctDNA) (25–30% in MYSTIC) and only moderate concordance (around 50–60%) with tTMB (89, 90). Currently, the clinical implementation of bTMB remains constrained by technical and biological limitations. These include but are not limited to low ctDNA abundance in early-stage disease, low-shedding tumors, and the resulting suboptimal sensitivity and concordance with tTMB (91). In summary, although TMB shows potential as a predictive biomarker for ICIs response, its clinical utility remains constrained by methodological variability and the need for integration with complementary biomarkers.

2.4 Genomic determinants of immunotherapy sensitivity and resistance

Specific genomic alterations may indicate increased sensitivity or inherent resistance to immunotherapy and may have a role in guiding patient stratification and rational combination approaches in early-phase clinical trials. Pathogenic mutations in the exonuclease domains (EDMs) of DNA polymerases epsilon (POLE) and delta 1 (POLD1) are emerging as predictive biomarkers of sensitivity to ICIs. These mutations impair proofreading, leading to an “ultramutator” phenotype characterized by a high frequency of base substitution mutations, distinct from the indel-driven frameshifts common in MSI-H tumors (Table 1) (24). Somatic or germline *POLE/POLD1* mutations in EDMs occur at low frequencies; somatic *POLE* and *POLD1* mutations were estimated at 2.79% and 1.37%, respectively (92, 93). Notably, *POLE* EDMs and dMMR/MSI-H can co-occur, potentially creating an exceptionally immunogenic neoantigen landscape enriched by MSI-H-driven indels, which could enhance T-cell infiltration (93). Promising clinical evidence supports their predictive value. For instance, a phase 2 trial evaluating the anti-PD-1 agent, toripalimab, in advanced solid tumors, reported an ORR of 21.4%, with a notably higher response rate among patients harboring *POLE* EDMs (66.7%) compared to those with non-EDM *POLE/POLD1* variants (9.1%) (94). Similarly, the KEYNOTE-028 study reported durable responses to pembrolizumab in patients with *POLE*-mutated EC (95). Prospective studies are ongoing (e.g., NCT05103969, NCT02693535, NCT03491345, and NCT06118658) (96–98). Despite their distinct biology and potential for profound

ICI sensitivity, their low pan-cancer incidence poses a practical hurdle for patient enrichment in early-phase trials, often requiring extensive molecular prescreening programs or a strategic focus on tumor types with already known higher prevalence, like EC. Alterations in chromatin remodeling complexes, particularly components of the SWI/SNF complex (e.g., ARID1A, PBRM1, SMARCA4, SMARCB1), have also emerged as potential biomarkers of ICI sensitivity, outlined in Table 1 (8). These mutations can lead to increased mutational burden, an inflamed tumor microenvironment (TME), and enhanced immune infiltration, suggesting their utility in identifying responsive subgroups across various tumor types (8, 99–101).

Conversely, multiple genomic alterations have been implicated in both primary and acquired resistance to ICIs. Distinct but functionally convergent mechanisms of immune resistance include alterations in β 2-microglobulin (B2M), which impair antigen presentation by disrupting MHC-I; inactivating mutations in *JAK1/2*, key mediators of IFN- γ signaling; and chromosomal losses like 9p21.3, which encompass *CDKN2A/B* and potentially *JAK2*, leading to immune exclusion and reduced CD8+ T cell infiltration (8, 25, 26, 102). The close genomic proximity of *CDKN2A* and *JAK2* on chromosome 9p facilitates frequent co-deletions, which have been observed across multiple tumor types, including melanoma (75%), lung squamous cell carcinoma (90.5%), and bladder urothelial carcinoma (80%) (103). These co-deletions are associated with impaired IFN- γ signaling, diminished immune infiltration, and further diminishing ICI sensitivity (26, 102–106). Similarly, loss-of-function mutations in *B2M* or *JAK1/2* have been identified in patients with melanoma that progresses on anti-PD-1 therapy, suggesting acquired resistance, Table 1 (8, 25, 26). However, emerging evidence indicates that the presence of these mutations does not uniformly predict treatment failure. For instance, among patients with dMMR/MSI-H CRC, the presence of *B2M* or *JAK1/2* mutations associated with clinically meaningful responses to PD-1 blockade, challenging the assumption that these alterations universally mediate resistance (107). Inactivation of the tumor suppressor *Serine/Threonine kinase 11* (*STK11/LKB1*), frequently observed in *KRAS*-mutant NSCLC, is strongly associated with primary resistance to ICIs, even in tumors with TMB-H (27, 28). This alteration contributes to an immunologically “cold” TME, characterized by poor T-cell infiltration and low PD-L1 expression (27). Similarly, loss-of-function mutations in *KEAP1*, which lead to constitutive activation of the NRF2 pathway, are associated with immune exclusion and ICI resistance (29). *KEAP1* mutations occur in approximately 2.7% of all cancers and are particularly enriched in NSCLC (15.8%) (8). *EGFR* mutations, also common in NSCLC, may promote immune escape through the upregulation of PD-(L)1 and CTLA-4, and *EGFR* amplifications have been implicated in hyperprogression following ICI therapy. However, supporting data remain inconsistent (7). In addition, *PTEN* loss fosters an immunosuppressive TME via activation of the *PI3K-AKT* signaling pathway, while *MDM2* amplification, potentially through NFATc2 degradation, has also been associated with hyperprogression and resistance (30–34). Emerging genomic alterations that mediate resistance or hyperprogression under ICI

therapy are summarized in Table 1. Together, these alterations outline a molecular landscape of intrinsic resistance to immunotherapy. Understanding this landscape of resistance mutations is critical for refining patient selection in early-phase clinical trials, potentially by excluding patients unlikely to benefit from certain monotherapies or by identifying candidates for rational combination approaches designed to overcome these specific resistance mechanisms.

3 Non-invasive immunotherapy biomarkers

3.1 Circulating tumor DNA

While profiling specific genomic alterations and other tissue-based biomarkers provides critical biological insights, the static nature of single biopsy timepoints limits this approach. ctDNA has emerged as a minimally invasive biomarker offering dynamic insights into tumor burden, mutational landscapes and therapeutic response. ctDNA encompasses fragmented DNA released into the bloodstream from apoptotic or necrotic tumor cells, circulating tumor cells, and tumor-derived exosomes. Its potential to revolutionize early-phase immunotherapy trials lies in its ability to provide real-time molecular data, overcoming many limitations of traditional tissue-based tumor biopsies. Several studies support the prognostic and monitoring utility of ctDNA in patients receiving ICIs. Persistently detectable ctDNA levels during anti-PD-(L)1 treatment have been associated with worse clinical outcomes (108–110). For example, a prospective phase 2 trial (NCT02644369) evaluated patients with advanced solid tumors treated with pembrolizumab. The study found that a decrease in ctDNA levels from baseline was associated with better outcomes under immune checkpoint blockade (111). These findings have been supported by additional pieces of data across multiple tumor types, highlighting the potential of ctDNA early dynamics to serve as a prompt surrogate endpoint for ICI efficacy outcomes (112).

Compared to conventional tissue-based molecular profiling, ctDNA offers distinct advantages. For instance, it is minimally invasive, facilitating repeated sampling for longitudinal monitoring; it may more accurately reflect spatial and temporal tumor heterogeneity; and it may be able to detect earlier response or resistance compared to standard imaging (113, 114). These attributes are particularly valuable in early-phase immunotherapy trials, where timely and dynamic genomic insights can inform patient selection, cohort stratification, and early assessment of treatment response (115). Importantly, ctDNA also enables the non-invasive evaluation of key immunotherapy biomarkers such as TMB and MSI. Several commercial and academic platforms have demonstrated the feasibility of estimating bTMB and MSI status from ctDNA (116). However, as discussed previously, limitations in analytical sensitivity, particularly in patients with low tumor burden or low ctDNA shedding, remain a challenge (117). Substantial heterogeneity currently exists across ctDNA testing platforms. Differences in laboratory-developed protocols, sequencing

technologies, bioinformatic pipelines, and variant calling thresholds can lead to discordant findings, posing challenges for assay reproducibility and clinical interpretation (117). Successful integration of ctDNA into routine clinical trials and practice would benefit from standardized methodologies and harmonized assay performance. Given the variability in ctDNA quantification, which is affected by tumor type, stage, anatomical location, tumor burden, prior treatment lines and response to therapy among others, the FDA advises manufacturers developing ctDNA-based molecular residual disease (MRD) assays for solid tumors and incorporation of ctDNA endpoints into prospective, randomized trials to support evidence generation and eventual regulatory approval (113). This lack of harmonization is a critical barrier to the broader validation and adoption of ctDNA as a decision-making tool. Therefore, ctDNA should be interpreted cautiously in early-phase trials, especially in contexts where under-detection could obscure early efficacy signals or lead to the premature termination of promising agents (118). To facilitate the clinical implementation of ctDNA as a reliable early endpoint, initiatives like the Friends of Cancer Research ctDNA for Monitoring Treatment Response (ctMoniTR) project represent important steps forward. By analyzing harmonized patient-level data from five clinical trials involving over 200 patients with advanced NSCLC treated with PD-L1 inhibitors, the ctMoniTR initiative demonstrated that on-treatment reductions in ctDNA levels were strongly associated with improved OS and PFS (119). Additionally, dynamic changes in variant allele frequency (VAF) were predictive across multiple clinical endpoints (119). These findings underscore the potential of ctDNA dynamics as an early indicator of therapeutic benefit. To advance the field, key priorities identified through such collaborative efforts include standardizing analytical methodologies and data reporting across assays, ensuring methodological transparency, systematically collecting clinicopathological data (e.g., tumor type, prior treatment), and defining minimum intervals between diagnosis and sampling (113). In conclusion, ctDNA offers a transformative, minimally invasive approach for dynamic tumor assessment in IO, with strong potential to refine patient selection and accelerate decision-making in early-phase trials. Realizing this potential critically hinges on overcoming current challenges in analytical validation and methodological standardization.

3.2 Serum biomarkers

Beyond ctDNA, several circulating biomarkers detectable in serum or plasma offer a minimally invasive approach to assess systemic inflammation, host immune function and tumor dynamics in patients undergoing ICI therapy. Baseline hematological parameters have been explored (120, 121). For instance, baseline absolute lymphocyte count (ALC) has been proposed as a surrogate marker of immune competence. Given the central role of lymphocytes in antitumor immunity, low pretreatment ALC may indicate impaired immune readiness and has been associated with inferior clinical outcomes in patients with NSCLC and melanoma

(120, 121). Moreover, dynamic changes in lymphocyte counts following ICI initiation are complex, but their direct link to intra-tumoral immune infiltration and ICI sensitivity remains to be fully elucidated (122, 123). More consistently, derived ratios from peripheral blood counts such as the neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), and platelet-to-lymphocyte ratio (PLR), have demonstrated prognostic value across multiple tumor types (124–131). A high baseline NLR, indicative of systemic inflammation and relative lymphopenia, is consistently associated with worse outcomes with ICIs (132). Derived NLR (dNLR) has been used as an alternative to NLR and is associated with immunotherapy outcomes (133, 134). Both the dNLR and the systemic immune-inflammation index (SII), which incorporates platelet counts, have been associated with worse prognosis (132).

Markers of systemic inflammation and tumor burden, such as C-reactive protein (CRP) and lactate dehydrogenase (LDH), may also provide prognostic information. Elevated LDH at baseline often reflects increased tumor burden, tumor cell turnover, hypoxia, and anaerobic glycolysis, which contribute to an acidic and immunosuppressive TME (135). Consequently, higher LDH levels at baseline have been consistently associated with worse survival in ICI-pretreated patients (135). Interestingly, high LDH can help to identify patients with aggressive disease phenotypes less likely to benefit from immunotherapy monotherapy, guiding towards combination strategies. Similarly, elevated CRP, driven by inflammatory cytokines such as IL-6, can indicate an immunosuppressive TME, with expanded regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and inhibitory cytokines (136). Elevated baseline CRP levels have been associated with adverse PFS and OS (137). An emerging phenomenon, the “CRP flare-response”, a transient rise in CRP within the first month of ICI therapy followed by a drop below baseline, has been linked to favorable outcomes, possibly indicating an effective antitumor immune activation (138). The phase 3 OAK trial, which compared atezolizumab with docetaxel in NSCLC, the CRP flare was predictive of improved survival only in the immunotherapy arm, supporting its potential as a tumor-agnostic, immunotherapy-specific marker (139). Beyond LDH and CRP, various other circulating soluble factors (e.g., sCD25, immunomodulatory cytokines, angiogenic molecules) have been linked to reduced response rates to ICIs. However, their current utility is largely exploratory due to assay variability and lack of standardization (140–147). In summary, while several serum biomarkers are readily available and inexpensive, their main limitation is the lack of specificity. Their clinical utility is often limited by a non-specific and incomplete understanding of their precise biological roles in the context of immunotherapies and multiple factors, including infections, concurrent medications, or underlying comorbidities, can influence these markers. Consequently, while they might serve as useful stratification factors or contribute to multifactorial prognostic scores, their utility for definitive patient enrichment is limited due to a high risk of misclassifying patients based on non-specific inflammatory states (148, 149).

4 Additional enrichment strategies

To fully optimize patient selection, molecular markers may be integrated with established, real-world clinical factors that define a patient's overall disease state and fitness for therapy. Furthermore, clinical factors may serve as surrogates for underlying tumor immunogenicity or immune fitness, potentially improving the detection of efficacy signals. However, their application requires careful consideration of complexities and trade-offs. With increasing ICI exposure in phase 1 populations, prior immunotherapy response becomes a relevant consideration for patient selection. ICI-naïve patients generally show better outcomes, suggesting immune exhaustion or resistance in the ICI-exposed population (150). However, a history of clinical benefit from ICIs may represent a subset with intrinsically more immunogenic tumors and, subsequently, more likely to benefit from ICI rechallenge or novel immunotherapies, as demonstrated by several retrospective studies (151–153). Nevertheless, this approach risks excluding patients with primary resistance to ICIs who might respond to different combinations, especially since some novel agents (e.g., tebentafusp) may even show enhanced effects post-ICI (154–156).

The number of previous lines of therapy may also influence outcomes. Heavily pre-treated patients, common in phase 1 trials, may have a detrimentally altered TME and exhausted systemic immunity, potentially lowering the response probability to novel agents, including adoptive cell therapy (157–160). Evidence suggests earlier ICI administration often enhances efficacy (161, 162). Hence, limiting inclusion to patients with fewer prior lines might reduce heterogeneity and improve signal detection. However, this enrichment strategy raises ethical considerations regarding patient access and may limit the generalizability of early phase clinical trial results. Furthermore, the impact of treatment lines can be tumor-specific (e.g., MSS metastatic CRC showing minimal ICI benefit regardless of line) and may be outweighed by factors like performance status or overall tumor burden, emphasizing the need for integrated decision-making (163).

Baseline tumor burden and pattern of metastatic spread are also key considerations. Higher tumor burden at baseline has been associated with worse ICI outcomes (164). Specific metastatic sites, such as the liver, have been consistently associated with poorer ICI responses across multiple tumor types due to their association with a uniquely immunosuppressive TME (e.g., reduced CD8⁺ T-cell infiltration, increased T-cell apoptosis, systemic immune tolerance induction) (165–168). Brain metastases also present distinct challenges associated with the central nervous system (CNS) microenvironment, which is influenced by the blood-brain barrier and comprises TILs, regulatory T-cells, and glial-derived immunomodulatory cytokines, such as TGF- β (169, 170). Nevertheless, ICI combinations have shown intracranial activity in melanoma and NSCLC (169, 171–174). Inclusion of metastatic patterns as selection criteria may be balanced against the need to understand drug activity in these high-need sub-populations and would require careful integration with assessment of overall tumor burden and tumor type.

Moreover, a history of prior irAEs during prior ICI therapy presents further considerations. While irAEs during an initial ICI course have been linked to better outcomes in different retrospective studies, their predictive value for subsequent immunotherapy benefit is largely unproven (175–177). A large meta-analysis showed only weak correlation between previous irAEs and OS (178). Excluding patients with severe prior irAEs is a common safety measure, supported by recurrence rates estimated at 30% when re-treating (179).

In conclusion, clinically oriented factors like prior ICI response, treatment history, metastatic patterns, and irAEs history significantly contribute to patient heterogeneity in early-phase IO trials, complicating the interpretation of efficacy signals. Leveraging these factors for enrichment strategies requires a nuanced approach. While such strategies may offer clearer efficacy signals in selected subgroups and potentially de-risk development, they must be carefully balanced against the goals of ensuring broad patient applicability, maintaining ethical access to trials, and the critical need for prospective validation to confirm their utility in truly optimizing trial design.

5 Challenges and future directions

Over the past two decades, biomarker-driven clinical trials have significantly transformed oncology drug development by enabling early go/no-go decisions, real-time monitoring, and the co-development of companion diagnostics (180). Nevertheless, many current “all-comer” trial designs still fail to incorporate predictive biomarkers, resulting in potential over- or under-treatment. Addressing this limitation, while simultaneously reducing the redundancy of “me-too” drug development, will be essential to refine patient selection strategies and enhance immunotherapy efficacy across tumor types and indications, ultimately moving the field beyond its current plateau (181). Rapid advancements in NGS and multi-omics technologies have further fueled the identification of novel biomarkers, contributing to recent FDA approvals that mark a paradigm shift in precision oncology (182). As outlined in this review, these advances have driven a swift evolution in trial strategies from the foundational tissue-based markers, such as PD-L1, dMMR/MSI-H, and TMB, toward a more precise understanding of specific genomic drivers and the advent of dynamic, plasma-based monitoring. This evolution underscores a fundamental shift, where the imperative is replacing the pursuit of a single biomarker with the development of an integrated, multi-modal framework capable of capturing tumor and immune system complexity to guide the next generation of personalized treatments.

5.1 Challenges in trial design and biomarker validation

A central challenge remains the distinction between prognostic and predictive biomarkers. Prognostic markers provide information about clinical outcomes regardless of the therapy received, reflecting

tumor or host factors that influence disease progression and survival (8, 132). Therefore, they can support clinical trial design by enabling risk stratification. In contrast, predictive biomarkers indicate the likelihood of response or resistance to a specific therapy and require formal interaction analyses to confirm the association of biomarker positivity and a differential response to a given immunotherapy versus a control regimen (183, 184). While some biomarkers may have both prognostic and predictive roles, this distinction is critical in the context of biomarker-driven clinical trial design. Concrete examples from early-phase immunotherapy trials illustrate this distinction. MSI-H/dMMR status, demonstrated in the KEYNOTE-016 phase II trial, represents a robust predictive marker of ICI benefit (57). Similarly, in the KEYNOTE-001 trial, a PD-L1 tumor proportion score (TPS) $\geq 50\%$ was associated with improved ORR and PFS, supporting its role as a predictive biomarker (46). In contrast, elevated baseline LDH has consistently been a negative prognostic factor in phase I melanoma studies, reflecting tumor burden rather than treatment-specific effects (132).

As described, many widely used immunotherapy biomarkers, such as elevated baseline LDH or ctDNA levels, may function primarily as prognostic indicators or surrogates of tumor burden rather than as specific predictors of response to ICI therapy (132). This challenge is magnified in early-phase, often single-arm trials, where the absence of a control arm makes it statistically challenging to distinguish between true predictive value and prognostic influence. The implementation of narrow, biomarker-driven eligibility criteria lacking rigorous predictive validation risks over selection, which can severely limit trial enrollment and reduce the external validity of the trial's findings to a broader, real-world patient population (185, 186). This approach carries the risk of systematically excluding subgroups of patients who might have derived benefit from treatment. Therefore, robust statistical validation, including formal interaction testing within the trial design, and a strong biological rationale are imperative before any biomarker is used for stringent patient enrichment (132, 186).

5.2 Operational, analytical and technological frontiers

As highlighted for established markers like PD-L1 or TMB, the path from biomarker discovery to clinical implementation is limited by significant operational barriers that further complicate the integration of novel biomarkers into early-phase immunotherapy trials. High costs, lengthy assay turnaround times, lack of harmonized testing platforms, and complex regulatory environments all contribute to delays in trial activation and limit feasibility across institutions (187). For instance, with the implementation of the new *in-vitro* diagnostic device (IVD) regulation in Europe, a 6- to 12-month delay in clinical trial activation has been estimated for clinical trial protocols using IVDs (188). These challenges are particularly pronounced for complex assays beyond standard sequencing, such as deep proteomic or spatial profiling, which face persistent hurdles

related to data variability, standardization, reproducibility, and the need for robust bioinformatics infrastructure and expertise (189). Initiatives like the Clinical Proteomic Tumor Analysis Consortium (CPTAC) and transcriptomic tools such as Oncotype DX and MammaPrint have underscored the clinical value of multi-omic integration (190, 191). However, balancing the scientific advantages of biomarker-driven design with the need for broad applicability and operational efficiency remains a persistent dilemma, and the routine implementation of such approaches in early-phase trials continues to be scarce. Ultimately, clinical response to immunotherapy is rarely dictated by a single marker; instead, it emerges from the complex interplay between the tumor and the host immune system within a dynamic and heterogeneous TME. Acknowledging this complexity is the critical first step toward developing more effective biomarker strategies, compelling a move away from linear measurements. The path forward will likely require a multidimensional approach that concurrently integrates genomic data on resistance mechanisms, molecular and immune contexture profiling, and patient-specific characteristics to guide patient enrichment and support rational combination strategies in early-phase trials.

This vision is being enabled by a technological revolution that allows investigation into the tumor-immune interplay with unprecedented resolution. The most immediate evolution is in refining our genomic and transcriptomic insights. While NGS/WES provides the foundation for broad biomarkers like TMB, their application has matured to support a more specific assessment of tumor immunogenicity (192). This includes identifying specific resistance mutations, such as *STK11*, that predict ICI failure even in tumors with TMB-H, or sensitizing mutations like those in *POLE* that define a distinct “ultramutator” phenotype (124). This focus also extends to non-mutational drivers of immunogenicity; in virus-associated malignancies, for instance, the expression of viral antigens provides a source of highly potent T-cell targets. This was shown in the CheckMate 358 and CAN-2409 oncolytic virus studies, which exemplify how viral antigens can serve both as therapeutic targets and predictive tools (193, 194). To capture the functional state of this interplay, the field is moving beyond DNA alterations and toward transcriptomics (195). Pre-defined signatures offer a more nuanced predictor of a pre-existing anti-tumor immune response. For example, the 12-gene VIGex score includes 12 genes involved in immune activation and T-cell exhaustion (196). VIGex categorization has been associated with immunotherapy outcomes in patients enrolled in early phase clinical trials. Also, the 18-gene T-cell inflamed Gene Expression Profile (GEP) integrates the expression of multiple genes related to IFN- γ signaling and cytotoxic activity and has been associated with outcomes of patients treated with ICIs (197).

While deep genomic and transcriptomic profiling offers critical insights, tissue biopsies remain limited by their static, single-timepoint nature, failing to capture both spatial and temporal heterogeneity. Liquid biopsy platforms are addressing these limitations by enabling longitudinal assessment of therapeutic responses and clonal evolution. Notably, ctDNA may include data from all tumor sites, offering a systemic snapshot that can overcome

spatial heterogeneity inherent in single-site tissue biopsy. This enables the non-invasive assessment of established biomarkers, such as bTMB or MSI status, and can extend beyond ctDNA to other circulating components (198). Measuring circulating exosomal PD-L1, for instance, may provide a more representative readout of the total immunosuppressive landscape than a single tissue sample (199, 200). Another challenge of single-tissue biopsy is addressing the lack of spatial context. To resolve this limitation, technologies such as multiple immunohistochemistry and immunofluorescence (mIHC/IF) and spatial transcriptomics are finally elucidating the critical architecture of the TME, distinguishing among inflamed, immune-excluded, and desert phenotypes, and revealing interactions between nearby cells (201–203). Additionally, integrating diverse biological data with advanced computational tools may enable a comprehensive vision of clinical trials, moving beyond traditional endpoints and embracing quantitative, reproducible biomarker assessment (204). Furthermore, radiomics and computational tools may also provide a non-invasive method to explore this heterogeneity on a macroscopic scale by extracting quantitative features from standard medical imaging to create a complete digital portrait of the tumor burden (205).

The biomarker landscape is further expanding beyond the tumor to encompass systemic host factors that can profoundly modulate treatment efficacy. A prominent example is the gut

microbiota, where growing evidence suggests that the composition of intestinal flora can influence systemic immunity and patient response to ICIs (206, 207). For instance, taxa such as *Faecalibacterium* and *Ruminococcus* have been associated with improved outcomes. This systemic view is further enriched by metabolomics, which analyzes the metabolic products of both tumor cells and the host immune system, providing a real-time functional snapshot of the ongoing host-tumor interplay. Moreover, these discoveries not only provide information regarding potential biomarkers but may also support novel therapeutic strategies. For example, patient-derived fecal microbiota transplantation has shown promise in phase I melanoma trials (208). Artificial intelligence (AI) is emerging as necessary to integrate high-dimensional data and generate composite immune response scores. AI-driven approaches aim to overcome limitations of traditional statistical models in handling high-dimensional multi-omic data to identify predictive, not just prognostic, signatures (209, 210). For example, the proposed Predictive Biomarker Modeling Framework (PBMF) has demonstrated its capability by accurately identifying known IO biomarkers in phase 2 and 3 trials (209). Recently, SCORPIO, a machine learning model based on routine blood tests and clinical data, outperformed TMB and PD-L1 in predicting ICI benefit across multiple tumor types and settings (211). The promise of AI tools lies in their potential to uncover novel and non-linear interactions that could define responsive

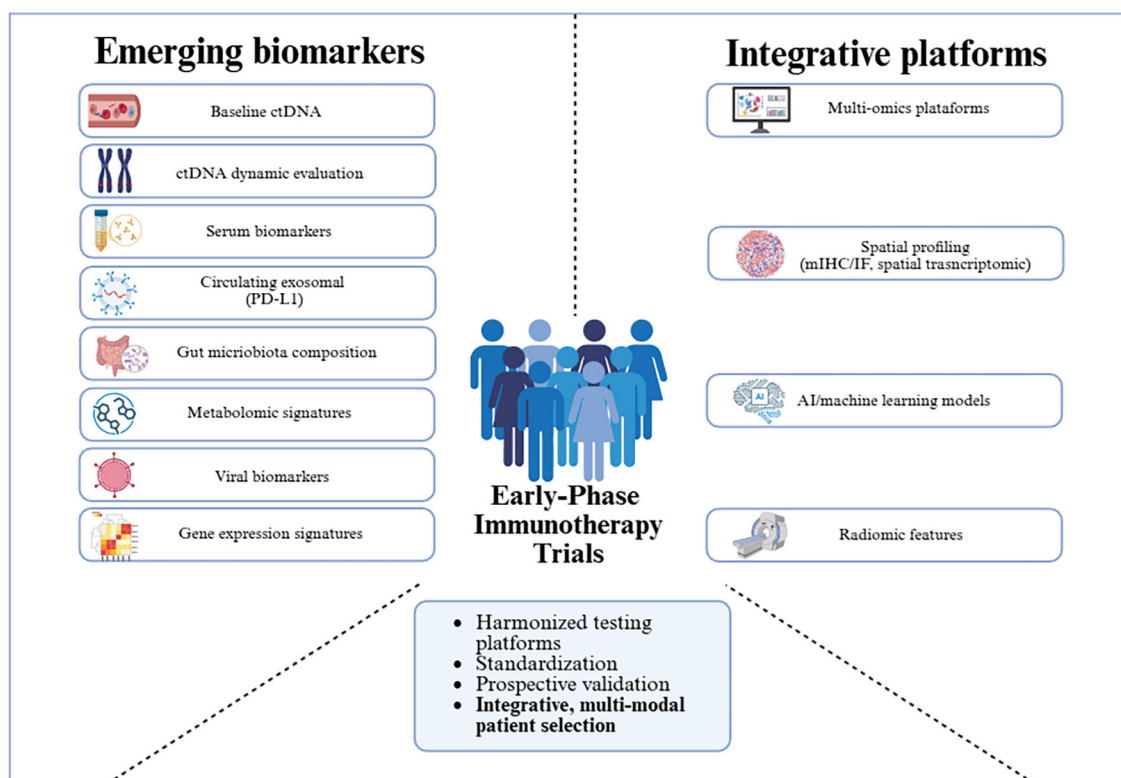


FIGURE 1

Summary of emerging biomarkers and integrative platforms under investigation in immunotherapy. To overcome the challenges associated with the implementation of novel biomarkers and multi-modal integration in early phase immunotherapy trials, efforts focused on harmonization, standardization, and prospective validation will be essential.

patient subsets for early-phase IO trials. However, the “black-box” nature of some AI algorithms, limited training datasets in early-phase settings, and the need for prospective validation limit their adoption in regulatory decision-making for early trials (212). These emerging integrative platforms, along with novel biomarkers, are summarized in Figure 1.

5.3 Regulatory challenges

Overcoming operational and regulatory hurdles is paramount to translating these multi-modal strategies from concept to clinic. The clinical maturation of less invasive biomarkers such as ctDNA, combined with the power of computational tools, already offers a pathway to faster and more efficient data generation. However, technology alone is insufficient without a concerted effort toward harmonization. Establishing unified frameworks through international collaboration is therefore essential. Landmark initiatives, such as the guidelines developed for ctDNA by Friends of Cancer Research and the FDA, provide a blueprint for standardizing data acquisition and reporting (213, 214). Such standardization is a prerequisite for streamlining regulatory processes, reducing clinical trial activation times, and building the large, high-quality datasets needed to robustly validate emerging biomarkers. In Europe, the *In Vitro* Diagnostic Regulation (IVDR) framework exemplifies this duality: while it enhances diagnostic assays’ reliability and patient safety through harmonized standards, its stringent requirements have also introduced practical delays in the activation of early-phase trials (215). Specifically, the regulation’s rigorous validation demands for laboratory-developed tests, which are essential for novel biomarkers, create significant cost and logistical hurdles that affect academic-led research. Ultimately, these collaborative frameworks between academia, industry and regulatory authorities will be key to accelerating the cycle of discovery, validation and clinical implementation. However, open questions remain on whether exploratory biomarkers for hypothesis generation require undergoing the complete clinical validation framework. In this regard, stringent criteria at early stages may hinder innovation, whereas inadequate rigor can promote non-reproducible findings. It may become essential to adopt a balanced approach that fosters innovation while maintaining methodological integrity.

6 Conclusion

Optimizing early-phase IO trials requires a paradigm shift, integrating genomic, transcriptomic, spatial, and systemic host data into a cohesive patient portrait, where adaptive trial designs and collaborative, transparent validation efforts converge. While this multi-dimensional approach offers exciting prospects for precision IO, translating these into validated, actionable tools for selecting patient populations in early-phase trials remains a significant challenge. Overcoming hurdles in analytical standardization, achieving robust prospective validation and

ensuring their practical and economic feasibility are key to their successful clinical integration. Ultimately, focusing on these aspects is essential to move the path forward from exploratory observations into strategies that accelerate the development of next-generation immunotherapies.

Author contributions

GC: Conceptualization, Investigation, Methodology, Visualization, Writing – original draft, Resources. DM: Conceptualization, Investigation, Methodology, Visualization, Writing – original draft, Resources. OM: Supervision, Writing – review & editing. VS: Supervision, Writing – review & editing. PN: Supervision, Writing – review & editing. GV: Supervision, Writing – review & editing. RD: Supervision, Writing – review & editing. AV: Supervision, Writing – review & editing. EG: Supervision, Writing – review & editing. AH: Conceptualization, Investigation, Methodology, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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Supplementary material

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Glossary

ICIs	Immune checkpoint inhibitors	WGS/WES	Whole-genome/exome sequencing
mAb	Monoclonal antibody	tTMB	Tissue-based TMB
anti-PD-1	anti-programmed cell death 1	bTMB	Blood TMB
anti-PD-L1	anti-programmed cell death ligand 1	ctDNA	Circulating tumor DNA
irAEs	Immune-related adverse events	EDMs	Exonuclease domains
IO	Immuno-oncology	POLE	DNA polymerases epsilon
MSI-H	Microsatellite instability-high	POLD1	DNA polymerases delta 1
dMMR	Mismatch repair-deficient	TME	Tumor microenvironment
FDA	U.S. Food and Drug Administration	STK11/LKB1	Serine/Threonine kinase 11
TMB	Tumor mutational burden	MRD	Molecular residual disease
TMB-H	Tumor mutational burden high	ctMoniTR	ctDNA for Monitoring Treatment Response
Mb	Megabase	VAF	Variant allele frequency
ORR	Overall response rate	ALC	absolute lymphocyte count
PD-L1	Programmed death-ligand 1	NLR	Neutrophil-to-lymphocyte ratio
IHQ	Immunohistochemistry	LMR	Lymphocyte-to-monocyte ratio
NSCLC	Non-small cell lung cancer	PLR	Platelet-to-lymphocyte ratio
TNBC	Triple-negative breast cancer	dNLR	Derived NLR
HNSCC	Head and neck squamous cell carcinoma	SII	Systemic immune-inflammation index
TCs	Tumor cells	CRP	C-reactive protein
ICs	Immune cells	LDH	Lactate dehydrogenase
TPS	Tumor Proportion Score	Tregs	Regulatory T cells
CPS	Combined Positive Score	MDSCs	Myeloid-derived suppressor cells
RCC	Renal cell carcinoma	CNS	Central nervous system
OS	Overall survival	IVD	<i>In-vitro</i> diagnostic device
MMR	Mismatch repair	CPTAC	Clinical Proteomic Tumor Analysis Consortium
EC	Endometrial cancer	GEP	Gene Expression Profile
CRC	Colorectal cancer	mIHC/IF	Immunohistochemistry and immunofluorescence
PCR	Polymerase chain reaction	AI	Artificial intelligence
NGS	Next-generation sequencing	PBMF	Predictive Biomarker Modeling Framework
PFS	Progression-free survival		