Haematology Oncology Research Journal
ISSN: 2997-6677|
Volume 13 Issue 1, January-March, 2025
Journal Homepage: https://ethanpublication.com/articles/index.php/E25
Official Journal of Ethan Publication

UNPACKING THE ROLE OF NEOANTIGENS AND TUMOR MUTATIONAL BURDEN IN CANCER IMMUNOTHERAPY

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DOI: https://doi.org/10.5281/zenodo.17405031

Abstract
Cancer immunotherapy has revolutionized oncology by leveraging the immune system to combat tumors. Among various biomarkers, neoantigens and tumor mutational burden (TMB) have emerged as critical factors in tailoring personalized treatments. Neoantigens are tumor-specific peptides displayed on cancer cell surfaces, derived from somatic mutations. Recognized as "non-self" by the immune system, they trigger T-cell responses and enable therapies like personalized vaccines and adoptive T-cell transfer. Critically, neoantigen potential correlates with TMB, which quantifies the total somatic mutations within a tumor genome. A higher TMB generally correlates with a greater likelihood of generating immunogenic neoantigens, making it a predictive biomarker for the efficacy of immune checkpoint inhibitors (ICI). Progress in high-throughput sequencing, bioinformatics, and immuno-peptidomics has significantly enhanced the accuracy of neoantigen prediction, including assessments of major histocompatibility complex (MHC) binding affinity and T-cell receptor recognition. Clinically, neoantigen-based therapies have shown efficacy in early trials, with strategies such as mRNA vaccines demonstrating synergy with ICI by boosting T-cell activation and overcoming immune suppression. Combining neoantigen-based therapies with chemotherapy and radiotherapy harnesses synergistic mechanisms to enhance efficacy, overcome resistance, and emerge as a pivotal oncology research focus. The integration of TMB into clinical practice has received regulatory approval as a biomarker for stratifying patients for ICI therapies. Furthermore, advanced methodologies like liquid biopsy and single-cell technologies have streamlined TMB measurement, improving its predictive value for personalized immunotherapy. Collectively, neoantigens and TMB have optimized the evolution of precision immuno-oncology by providing frameworks that maximize therapeutic efficacy, overcome resistance mechanisms, and advance durable cancer remission.

Keywords: Tumor neoantigens, Tumor mutational burden, Immunotherapy, Liquid biopsy, Clinical trials

Introduction

Cancer immunotherapy represents a transformative approach in cancer treatment, leveraging the host immune system to target and eliminate malignant cells [1]. The conceptual foundation of this approach dates back to the late nineteenth century, when William B. Coley, often regarded as the father of immunotherapy, utilized bacterial infections to induce an immune response against tumors [2, 3]. Over the past century, immunotherapy has evolved to encompass a variety of strategies, including immune checkpoint inhibitors, oncolytic viruses (OV), cytokines, and cancer vaccines [4]. These modalities operate

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through distinct mechanisms, targeting the complex interactions between immune cells and neoplastic cells within the tumor microenvironment (TME) [5, 6]. The effectiveness of immunotherapy hinges on the ability of T lymphocytes to identify tumor-specific antigens. This principle has guided the development of immune checkpoint blockade (ICB) therapies, including programmed cell death-1 (PD-1)/programmed cell death ligand-1 (PD-L1) inhibitor and cytotoxic T-lymphocyteassociated protein 4 (CTLA-4) inhibitor, both of which have achieved remarkable success in treating diverse cancers [7, 8]. However, the response rates to immunotherapy remain limited, with only a subset of patients deriving benefit due to tumor-intrinsic and extrinsic resistance mechanisms [7]. Consequently, the exploration of robust biomarkers in cancer immunotherapy is of paramount importance. Biomarkers can predict patient responsiveness to specific immunotherapeutic agents, thereby significantly enhancing the precision and efficacy of treatment [9]. They are also critical in identifying individuals at risk for severe adverse effects, thus improving the safety profile of these therapies [10]. Moreover, novel biomarkers can elucidate the mechanisms of resistance and toxicity, facilitating the development of innovative therapeutic strategies [9, 11]. The identification and validation of superior biomarkers are essential for broadening the therapeutic impact of immunotherapy to a wider patient population.

Fig. 1 Milestone events of neoantigen, TMB and cancer immunotherapy. The breakthrough in neoantigen or TMB, the major achievement of cancer immunotherapy and their combination events were reviewed retrospectively. CTLA-4: cytotoxic T-lymphocyte-associated protein 4, FDA: Food and Drug Administration, NCCN: National Comprehensive Cancer Network, NSCLC: non-small cell lung cancer, PD-1: programmed cell death-1, PFS: progression-free survival, TMB: tumor mutational burden, WES: whole-exome sequencing, WGS: whole-genome sequencing. Created with BioRender.com

The emergence of neoantigens and tumor mutational burden (TMB) as biomarkers in cancer immunotherapy signifies a substantial advancement in the field. The key development events about neoantigen, TMB and cancer immunotherapy are shown in Fig. 1. Neoantigens, arising from somatic mutations unique to individual tumors, have become crucial targets for personalized immunotherapies [8, 12]. Their identification is based on the premise that these tumor-specific antigens can be recognized by the host immune system, facilitating the development of tailored treatments to enhance antitumor immunity [13]. This progress has led to innovative therapeutic strategies, including personalized vaccines and adoptive T-cell therapies, which leverage neoantigens to provoke a targeted immune response [14, 15]. On the other hand, TMB quantifies the total number of somatic non-synonymous mutations within a tumor's genome, serving as an indicator of the potential neoantigen landscape [16]. The concept of TMB gained attention with the recognition that a higher mutational load might correlate with an increased likelihood of response to immune checkpoint inhibitors (ICI) [17]. This correlation has been validated by both retrospective and prospective studies, establishing TMB as a predictive biomarker for ICI response across various cancer types [18–20]. The incorporation of TMB into clinical practice represents a significant milestone in the application of biomarkers in immunotherapy. However, the journey of neoantigens and TMB has encountered several challenges. The heterogeneity of tumor mutations, the complexity of the TME, and the inherent variability in immunogenicity have posed significant obstacles to accurately predicting treatment response [16, 21]. Despite these obstacles, the role of neoantigens and TMB in immunotherapy is increasingly recognized, with ongoing research focused on refining their predictive capabilities and expanding their clinical applications [22]. As the field advances, the integration of neoantigen and TMB data with other biomarkers, such as PD-L1 expression and microsatellite instability [23, 24], is expected to further enhance the precision of immunotherapy, ultimately aiming to fulfill the full potential of this transformative treatment approach.

In the present review, we elucidate the definitions and interrelationship between neoantigens and TMB. The advancements in neoantigen-based treatments and the application of TMB in predicting immunotherapy responses are also explored. Additionally, we discuss the detailed mechanisms by which neoantigens and TMB contribute to cancer immunotherapy. This review also summarizes future challenges and potential strategies involving neoantigens and TMB, aiming to enhance neoantigen prediction and treatment efficacy in cancer immunotherapy.

Neoantigen and TMB: definition, interrelationship and assessment

Neoantigen and TMB are two pivotal concepts in cancer immunotherapy. Understanding their definitions and interrelationship is fundamental for grasping the mechanisms of immune recognition and therapeutic response **(**Fig. 2**)**. Neoantigens are derived from non-synonymous somatic mutations that are unique to individual tumors and absent in the normal germline genome [25, 26]. These neoantigens arise from alterations in the DNA sequence, leading to the production of novel or altered proteins that can be recognized as foreign by the immune system, particularly by T cells [27, 28]. The generation of neoantigens is a direct result of the genetic instability inherent in cancer cells, which accumulate a high number of mutations over time [29]. The immune system has the potential to recognize these neoantigens as non-self, triggering an anti-tumor immune response [30]. Conversely, TMB quantifies the total number of somatic nonsynonymous mutations within a tumor's genome [16]. It serves as a proxy for the potential neoantigen landscape of a tumor, as a higher mutational load suggests a greater likelihood of presenting a broader array of neoantigens to the immune system [31]. The relationship between neoantigens and TMB is intricate and complementary. While TMB provides a broad estimation of the neoantigen potential within a tumor, specific neoantigens offer a more targeted approach to understanding an individual tumor's immunogenicity [32, 33]. The interplay between these factors is critical in developing personalized cancer vaccines and selecting patients most likely to benefit from ICI. TMB-high tumors are often associated with a better response to ICI [32]; however, the presence of specific neoantigens can further refine patient stratification, potentially leading to more precise and effective immunotherapeutic strategies [34]. Thus, neoantigens and TMB are closely linked in cancer immunotherapy. Neoantigens represent personalized antigenic targets that can be exploited for targeted immunotherapy, while TMB serves as a predictive biomarker gauging the overall immunogenic potential of a tumor [35, 36]. The combination of these parameters holds promise for advancing precision medicine in oncology, allowing for the development of more tailored and effective treatment approaches.

Neoantigen identification and prediction methods have become increasingly sophisticated, facilitating the precise targeting of tumor-specific antigens in cancer immunotherapy [25]. The latest advances in the technologies used for defining or characterizing neoantigens are listed in Table 1 [22, 37–49].The process typically involves highthroughput sequencing to detect somatic mutations within the tumor genome, which are then correlated with the potential to generate neoantigens [50, 51]. Nextgeneration sequencing (NGS) serves as a cornerstone technology for neoantigen discovery by enabling comprehensive profiling of the tumor exome and identification of non-synonymous mutations that could lead to neoantigen production [52–54]. Besides, bioinformatics tools are essential for predicting mutations that can generate immunogenic peptides. Algorithms such as NetMHCpan and NetMHCIIpan predict the binding affinity of potential neoantigens to major histocompatibility complex

Fig. 2 The interrelationship between neoantigen, TMB and cancer immunotherapy. Neoantigens are derived from non-synonymous somatic mutations, leading to the production of novel or altered proteins that can be recognized as foreign by the immune system. When recognizing these neoantigens, the immune system triggers an anti-tumor immune response which represent personalized antigenic targets that can be exploited for targeted immunotherapy. On another hand, TMB quantifies the total number of somatic non-synonymous mutations within a tumor's genome. Higher mutational load suggests a greater likelihood of presenting a broader array of neoantigens, thereby providing a broad estimation of the neoantigen potential within a tumor. As a result, specific neoantigens offer a more targeted approach, while TMB serves as a predictive biomarker gauging the overall immunogenic potential of a tumor. Created with BioRender.com

(MHC) molecules [40, 55], a critical step in presenting antigens to T cells. These tools integrate peptide-MHC binding motifs and experimental binding data to predict the likelihood of a peptide binding to a specific MHC allele. Mass spectrometry (MS)-based approaches complement sequencing methods by directly analyzing peptides presented on the tumor cell surface, allowing for the identification of actual neoantigens [56]. This immunopeptidomic approach offers a more accurate assessment of the tumor's immunopeptidome, which comprises the peptides loaded onto MHC molecules and presented to the immune system [57]. Integrating NGS with MS data has also significantly improved neoantigen prediction accuracy [45, 58]. By comparing the tumor's mutational landscape with the peptides identified by MS, researchers

can pinpoint which mutations are likely to be presented as neoantigens [59]. This integrated approach helps narrow down potential neoantigens to those both expressed and presented on the tumor cell surface, making them more likely to be recognized by the immune system [60]. In summary, the identification and prediction of neoantigens involve combining NGS to detect tumor-specific mutations, bioinformatics tools to predict peptide-MHC binding, and MS to confirm neoantigen presence in the tumor's immunopeptidome. These methods are essential for developing personalized immunotherapies that can effectively target the unique neoantigens expressed by an individual's tumor.

The measurement of TMB is typically conducted using NGS technologies, with whole-exome sequencing (WES)

considered the gold standard [16, 61]. WES provides a comprehensive landscape of the coding mutations within the tumor genome [62]. However, due to its high cost and lengthy turnaround time, targeted panel sequencing is often used as a more practical alternative in clinical settings [61]. These panels sequence

specific genes or regions of interest, offering a more cost-effective and expedited approach compared to WES [63, 64]. Clinically, TMB has emerged as a predictive biomarker for response to ICI. Therapies such as pembrolizumab have received FDA approval for use in patients with TMB-high tumors, regardless of tumor type, signifying a pivotal step towards personalized medicine and the utilization of biomarkers to direct treatment decisions [20, 65]. Nevertheless, the implementation of TMB measurement in routine clinical practice faces challenges, including the standardization of methods, the determination of optimal cut-off, and the need for rigorous analytical and clinical validation [16]. Current research aims to refine TMB measurement and combine it with other biomarkers to enhance the precision of immunotherapy. The combination is expected to further improve patient selection for immunotherapies, potentially leading to more effective and personalized treatment strategies.

Advances of neoantigen in cancer immunotherapy Neoantigens, arising from tumor-specific somatic mutations, represent promising targets in cancer immunotherapy due to their potential for personalized tumor targeting [66]. Advances in genomic and computational technologies have deepened insights into how neoantigens enhance antitumor immunity. Mechanistically, neoantigens enhance T cell activation and cytotoxicity while potentiating ICI efficacy [67]. The concept of tumor neoantigen burden (TNB) has further emerged as a biomarker, correlating strongly with improved immunotherapy outcomes [68]. Clinically, neoantigen-based therapies, such as personalized vaccines and adoptive T cell therapies, have demonstrated efficacy in early-phase trials [30, 69, 70]. These advances highlight the transformative potential of neoantigens in precision oncology, leveraging the immune system's specificity to eradicate malignant cells. In this section, we elucidate the mechanisms by which neoantigens amplify T cell responses, synergize with immune checkpoint blockade, and guide the development of combination therapies to circumvent treatment resistance.

Mechanisms underlying neoantigen-based immunotherapy

The efficacy of cancer immunotherapy hinges on the immune system's ability to discriminate tumor cells from healthy tissues, primarily mediated by tumor-specific antigens recognition [71]. Unlike shared tumor-associated antigens, neoantigens are exclusively expressed by malignant cells, minimizing off-target toxicity and serving as ideal targets for T cell-based antitumor immunity [72]. The mechanisms underlying neoantigen-based immunotherapy are explained as follows **(**Fig. 3**)**. Recognition of neoantigen-MHC complexes by T cells triggers immune cascades that drive therapeutic responses [25, 73]. CD8 + cytotoxic T lymphocytes (CTL) are pivotal in this process, inducing tumor cell apoptosis via perforin and granzymes release upon binding neoantigen peptides presented on MHC class I molecules [74]. $CD4^+$ helper T cells augment this response by secreting cytokines which enhance CTL activation and prolong effector functions within the TME [75, 76]. Neoantigen immunogenicity is determined by their capacity to bind MHC molecules and engage T cell receptors (TCR) [77, 78]. High-affinity neoantigen-MHC binding is essential for robust T cell activation, with clinical studies demonstrating that tumors harboring neoantigens with strong MHC affinity exhibit improved responses to ICI [79, 80]. Additionally, neoantigens structurally

analogous to pathogen-derived epitopes may leverage pre-existing T cell memory, facilitating cross-reactive immune responses that bolster tumor elimination [78, 81].

Neoantigen-based therapies critically depend on functional T cell responses, yet are frequently compromised by T cell exhaustion—a dysfunctional state driven by persistent antigen exposure and immunosuppressive microenvironmental signals [82, 83]. This exhaustion manifests as progressive functional impairment, initiated by sustained overexpression of co-inhibitory receptors (e.g., PD-1, CTLA-4), which deliver intracellular inhibitory signals to paralyze T cell activation [84]. These defects are reinforced through profound epigenetic reprogramming, where stable chromatin modifications silence key transcription factors, locking in exhaustion-associated transcriptional programs [85]. Concurrently, metabolic dysregulation exacerbates dysfunction via mitochondrial impairment, compromised nutrient utilization, and accumulation of inhibitory metabolites, collectively impairing bioenergetic capacity [85]. Ultimately, these mechanisms induce hierarchical effector failure: beginning with loss of cytokine secretion, progressing to diminished proliferative potential, and eventually leading to irreversible decrease of cytotoxic granule exocytosis and target cell elimination. Notably in acute myeloid leukemia (AML), the bone marrow microenvironment actively drives T cell exhaustion through abundant inhibitory cytokines, immunosuppressive cells and metabolites [82, 86]. Solid tumors similarly foster exhaustion via hypoxia, nutrient depletion, and acidic conditions [87].

Fig. 3 Mechanisms underlying neoantigen-based immunotherapy. Recognition of neoantigen-MHC complexes by T cells triggers immune cascades that drive therapeutic responses. CTL are pivotal in this process, inducing tumor cell apoptosis via perforin and granzymes release. CD4+ helper T cells augment this response by secreting cytokines which enhance CTL activation and prolong effector functions within the TME. Combination therapies further exploit neoantigens to amplify T cell activity. Radiotherapy releases neoantigens by inducing immunogenic cell death. OV similarly enhance neoantigen exposure by lysing tumor cells and activating innate immune pathways. Neoantigen-based mRNA vaccines directly expand tumor-reactive T cell populations. When combined with ICI, these vaccines counteract immunosuppressive mechanisms such as PD-L1 upregulation and sustain T cell effector functions. MHC I: major histocompatibility complex I, PD-L1: programmed cell death ligand-1, TCR:
T cell receptor. Created with BioRender.com

Immune checkpoint blockade amplifies neoantigenbased T cell responses by blocking inhibitory signals [8]. Anti-PD-1/PD-L1 antibodies reinvigorate exhausted T cells within the TME, thereby restoring their cytotoxic function and proliferative capacity [88]. CTLA-4 inhibitors similarly enhance T cell priming in lymphoid organs by disrupting co-inhibitory signals, broadening the diversity and magnitude of neoantigen-specific T cell clones [88]. Clinical evidence indicates that tumors with high TMB, a predictor of an expanded neoantigen repertoire, respond more favorably to ICI, highlighting the synergistic relationship between neoantigen abundance and checkpoint modulation [32, 65, 89]. Beyond ICI, complementary approaches to reverse exhaustion include cytokine-based interventions, such as interlukin

(IL)−7, IL-12, or IL-15 administration, which promote T cell survival, proliferation, and functional recovery, with IL-15 showing particular promise in AML for its effects on NK and T cells [90–92]. Epigenetic modifiers, like histone deacetylase (HDAC) inhibitors or DNA methyl transferase (DNMT) inhibitors, aim to reset the exhaustion-associated epigenetic landscape and restore T cell functionality in AML [93–95]. Additionally, targeting alternative inhibitory pathways, such as transforming growth factor-β (TGF-β) signaling or adenosine receptors, represents an active area of investigation applicable to both solid tumors and hematologic malignancies [96, 97]. Combination therapies further exploit neoantigens to amplify T cell activity. Radiotherapy induces immunogenic cell death, releasing neoantigens that act as in situ vaccines to prime naïve T cells and recruit effector T cells to distant tumor sites (abscopal effect) [98, 99]. OV similarly enhance neoantigen exposure by lysing tumor cells and activating innate immune pathways, thereby promoting a pro-inflammatory TME that facilitates T cell infiltration [100, 101]. Neoantigen-based vaccines, which deliver personalized mutant peptides or RNA-encoded epitopes, directly expand tumor-reactive T cell populations [101]. When combined with ICI, these vaccines counteract immunosuppressive mechanisms such as PD-L1 upregulation and sustain T cell effector functions [102, 103]. In summary, neoantigens serve as molecular beacons that direct T cell-mediated tumor destruction. Their integration of neoantigen-targeted strategies with checkpoint blockade and adjunct therapies represents a paradigm shift in oncology, leveraging the precision of adaptive immunity to achieve durable antitumor responses.

Tumors also evade immune recognition through two primary mechanisms: downregulation of human leukocyte antigen (HLA) molecules and disruption of antigen processing [104, 105]. In HLA downregulation, tumor cells employ genetic deletions, transcriptional repression, or epigenetic silencing to reduce surface HLA expression, which directly impairs $CD8^+$ T cell recognition of tumor neoantigens [106, 107]. Concurrently, tumors disrupt antigen processing by compromising multiple steps: impaired immunoproteasome function limits antigenic peptide generation; defects in peptide transporters hinder peptide translocation into the endoplasmic reticulum; and molecular chaperone deficiencies destabilize HLA-peptide complexes [106, 108, 109]. Together, these defects create an "immunological invisibility" state by preventing functional antigen-HLA complexes from reaching the cell surface. Therapeutically, restoring antigen presentation leverages complementary strategies targeting distinct layers of tumor-induced suppression. Interferon-γ (IFN-γ) priming acts as a master transcriptional activator: by engaging Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling, it directly induces the expression of HLA class I molecules, antigen-processing machinery components, and peptide-loading chaperones [110–113]. These mechanisms re-establish the functional capacity for antigen processing and surface presentation. Epigenetic modulators operate at a foundational level, reversing tumor-driven epigenetic silencing of HLA and antigen-processing genes [95]. By demethylating promoters or enhancing histone acetylation, they restore basal transcriptional accessibility, enabling cells to respond to immunomodulatory signals like IFN-γ. Critically, these approaches act in concert—epigenetic

reprogramming primes gene responsiveness, while IFN-γ drives robust expression—creating a synergistic restoration of antigen presentation that prevents tumor immune evasion.

Increasingly, TNB has emerged as a biomarker demonstrating potential to optimize precision oncology and therapeutic efficacy in cancer [68]. TNB quantifies immunogenic neoantigens arising from tumor-specific somatic mutations, including nonsynonymous singlenucleotide variants (SNV), insertions/deletions (indel), and gene fusions [27, 114]. These neoantigens are processed and presented by MHC molecules to trigger T cell-mediated antitumor immunity. Although TNB correlates with TMB, this correlation varies significantly across cancer types and even within individual tumors. TMB serves as a crude proxy for the potential neoantigen load, reflecting the raw number of mutations, while TNB aims to capture the actual immunogenic burden by predicting which mutations are likely to generate antigens presented by the patient's specific HLA alleles and capable of eliciting an immune response. Consequently, only a subset of mutations generates functional neoantigens, as their immunogenicity hinges on MHC binding affinity, TCR recognition, and the immunosuppressive TME [77, 78, 115]. Consequently, TNB is superior to TMB in predicting immunotherapy efficacy. For instance, in nonsmall cell lung cancer (NSCLC) and melanoma, patients exhibiting high TNB demonstrate enhanced responses to PD-1/CTLA-4 inhibitors and prolonged progression-free survival (PFS) [67, 116]. Tumors with DNA mismatch repair deficiency (dMMR) or high microsatellite instability (MSI-H) accumulate elevated TNB due to frameshiftderived neoantigens, which foster pro-inflammatory microenvironments accompanied by robust T cell infiltration [117].

TNB has demonstrated several clinical applications to date. For instance, TNB serves as a predictive biomarker for immunotherapy response by identifying patients most likely to benefit from ICI [79]. In metastatic melanoma, high TNB is associated with enhanced cytolytic activity and durable clinical responses [79]. Additionally, TNB-guided neoantigen selection facilitates the development of personalized RNA or peptide-based vaccines [118, 119]. When combined with ICI, these vaccines augment T cell clonality and overcome ICI resistance [118, 119]. TNB can also be integrated synergistically with conventional therapies. Radiotherapy and chemotherapy induce immunogenic cell death, releasing neoantigens that augment the efficacy of ICI [120, 121]. For example, neoadjuvant chemoradiotherapy in rectal cancer increases neoantigen diversity and stimulates antitumor immunity [122]. Furthermore, TNB can be longitudinally monitored through liquid biopsy. Liquid biopsy is a minimally invasive diagnostic approach that analyzes circulating biomarkers in bodily fluids (such as plasma, urine, or saliva) to detect cancer and other diseases [123]. Circulating tumor DNA (ctDNA) analysis enables tracking of TNB dynamics, permitting real-time assessment of treatment efficacy and early detection of immune escape mechanisms such as neoantigen loss or HLA defects [124]. Despite its potential, challenges remain in standardizing TNB quantification, addressing tumor heterogeneity, and identifying high-quality neoantigens. Future efforts must integrate multi-omics data to refine TNBbased precision immunotherapy strategies.

Advances of neoantigen-based immunotherapy in clinical trials

The exceptional specificity of neoantigens has established them as critical targets in the rapidly advancing field of cancer immunotherapy [25]. Building upon the foundational understanding of neoantigen biology and the synergistic interaction with TMB, recent clinical research has transitioned from theoretical exploration to practical application [16]. The clinical trials of neoantigen-based immunotherapy in recent 3 years are shown in Table 2. These advancements aim to leverage the immune system's capacity to recognize neoantigens as foreign, thereby triggering robust, tumor-specific responses. Clinical trials have increasingly concentrated on four primary therapeutic strategies: personalized neoantigen vaccines, adoptive cell therapies, monoclonal antibodies, and OV [125]. Each of these approaches harnesses distinct mechanisms to enhance neoantigen immunogenicity, overcome immunosuppressive barriers, and improve therapeutic precision. For example, personalized vaccines deliver patient-specific mutant peptides or RNA-encoded epitopes to prime and expand neoantigen-reactive T cell populations [126, 127]. Adoptive cell therapies, such as engineered TCR or chimeric antigen receptor (CAR)-T cells, directly infuse neoantigen-targeted lymphocytes to mediate tumor eradication [128]. Monoclonal antibodies, particularly when combined with ICI, enhance T cell activation by blocking co-inhibitory signals in the tumor microenvironment [129, 130]. OV further complement these strategies by inducing immunogenic cell death and releasing neoantigens to stimulate systemic immunity [131]. This section delves into the clinical progress of these neoantigen-based strategies, underscoring their transformative potential in precision oncology.

The clinical translation of personalized neoantigen vaccines has advanced rapidly due to breakthroughs in RNA technology and computational neoantigen prediction [132]. Building on the foundational understanding of neoantigen-T cell interactions, recent trials have demonstrated significant synergy between these vaccines and ICI [133]. A landmark phase 2b trial (KEYNOTE-942) showed that combining the personalized mRNA vaccine mRNA-4157 (V940) with pembrolizumab in resected stage III/IV melanoma reduced recurrence risk by 44% compared to pembrolizumab monotherapy, with 18-month recurrence-free survival rates of 78.6% versus 62.2% [134]. This trial underscored the synergy between neoantigen vaccines and ICI, where vaccines expand tumor-reactive T cell clones while ICI reverses T cell exhaustion [134, 135]. Notably, the vaccine induced $CD8^+$ T cell responses against multiple neoantigens, with clonal expansion correlating with prolonged survival [134, 135]. In pancreatic ductal adenocarcinoma (PDAC), a traditionally immunologically "cold" tumor, Rojas et al. reported that adjuvant mRNA neoantigen vaccines combined with PD-L1 blockade and chemotherapy elicited durable CD8 + T cell responses in 50% of patients, with vaccine-expanded T cell clones detectable for up to 1.9 years post-treatment [35]. Remarkably, patients with vaccine-induced T cells showed delayed recurrence, challenging the assumption that low-TMB tumors are resistant to vaccine [35]. This study also highlighted the importance of neoantigen quality—defined by MHC binding affinity and dissimilarity to wild-type peptides— in determining immunogenicity, with only 24% of predicted neoantigens eliciting detectable T cell responses

[35]. Advances in RNA vaccine technology have further improved efficacy. For instance, codon optimization, nucleoside modifications, and lipid nanoparticle (LNP) delivery systems enhance mRNA stability, translational efficiency, and lymph node-targeted antigen presentation [136–138]. Using unmodified RNA in cancer vaccines, as opposed to modified RNA in pathogen vaccines, retains intrinsic adjuvanticity by activating Toll-like receptors (TLR) and retinoic acid-inducible gene I (RIG-I) pathways, which promote robust type-I interferon responses critical for dendritic cell maturation and cross-priming [138]. Additionally, incorporating MHC-II neoantigens into vaccines has shown promise in preclinical models by broadening $CD4^+$ T cell help and sustaining CD8+ T cell memory [139]. Parallel advances in DNA vaccine platforms, exemplified by liposome-encapsulated multiepitope neoantigen constructs, have demonstrated potent tumor regression and reduced lung metastasis in preclinical melanoma models by enhancing intratumoral $CD8^+$ T cell infiltration and cytotoxicity [140]. In personalized T cell therapies, neoantigen-expanded autologous T cells induced polyclonal TCR repertoires and tumor regression in metastatic ovarian cancer, with durable TCR clonotypes persisting in circulation for over 15 months [141]. For DC-based vaccines, neoantigen-pulsed dendritic cells combined with immune adjuvants (e.g., TLR agonists) or checkpoint inhibitors elicited antigen-specific T cell responses in pancreatic cancer clinical trials, showing safety and potential synergy with chemotherapy [142]. In summary, personalized neoantigen vaccines represent a paradigm shift in precision oncology, with early clinical successes in both "hot" and "cold" tumors [138]. Future trials focused on minimally diseased hosts, combination therapies, and iterative vaccination strategies hold promise for overcoming resistance and achieving durable antitumor immunity.

Adoptive cell therapy, particularly CAR-T cell therapy, has emerged as a groundbreaking strategy for targeting neoantigens in solid tumors [143]. Unlike hematologic malignancies, solid tumors present distinct challenges, including antigen heterogeneity, immunosuppressive microenvironments, and the risk of off-target toxicities [144, 145]. Recent advancements in CAR-T engineering and neoantigen selection have begun to address these hurdles, demonstrating promising clinical outcomes [143, 146]. A key focus has been the targeting of clonal neoantigens derived from driver mutations, such as epidermal growth factor receptor variant III (EGFRvIII) in glioblastoma (GBM) [147]. This constitutively active variant of the epidermal growth factor receptor (EGFR), characterized by an extracellular domain deletion, functions as a tumor-specific antigen [148]. Early-phase trials of EGFRvIII-directed CAR-T cells demonstrated antigen reduction in post-treatment resections [149]. However, limited clinical efficacy highlighted issues such as adaptive immune resistance and regulatory T cell infiltration [143]. To enhance specificity, combinatorial antigen-sensing systems, such as synthetic Notch (SynNotch) receptors, have been engineered [150]. These logic-gated CAR require dual antigen recognition to activate cytotoxicity, reducing off-target effects while improving tumor discrimination [150]. Preclinical models demonstrated that SynNotch circuits enhance CAR-T cell persistence and mitigate tonic signaling, offering a blueprint for clinical translation [151]. Another breakthrough involves targeting public neoantigens, such as tumor-associated mucin 1 (Tan-

MUC1), which exhibits aberrant glycosylation in cancers [152]. Early trials of Tan-MUC1-directed CAR-T cells reported stable disease in patients with solid tumors without severe toxicity, underscoring its potential as a pan-cancer target [143]. Additionally, disialoganglioside (GD2), an oncofetal antigen re-expressed in neuroblastoma and diffuse midline glioma, has demonstrated remarkable efficacy [153]. In a phase I trial, third-generation GD2 CAR-T cells achieved a 63% overall response rate in neuroblastoma, with complete responses in 33% of patients [154]. As synthetic biology and multi-omics converge, CAR-T therapies targeting neoantigens are poised to redefine precision immunotherapy for solid tumors.

Monoclonal antibodies, particularly ICI, have shown significant potential in neoantigen-based immunotherapy by enhancing T cell responses against tumorspecific mutations [155]. Recent clinical trials have advanced the understanding and application of neoantigen-based immunotherapy in combination with ICI. For instance, a phase 1 trial of a shared neoantigen vaccine combined with immune checkpoint blockade in patients with advanced metastatic solid tumors demonstrated promising antitumor activity, with some patients achieving objective responses and a manageable safety profile [156]. In advanced hepatocellular carcinoma, a phase 1/2 trial of personalized neoantigen vaccine combined with pembrolizumab induced robust T cell responses and showed encouraging antitumor activity, with some patients experiencing partial responses [157]. Additionally, a phase 1b trial investigated the use of neoadjuvant nivolumab or nivolumab plus lymphocyte activation gene-3 (LAG-3) inhibitor relatlimab in resectable esophageal/gastroesophageal junction cancer [158]. This trial revealed promising pathological responses, with 2-year recurrence-free survival (RFS) and overall survival (OS) rates observed in 72.5% of patients receiving nivolumab monotherapy and 82.6% in the combination arm, as well as a high R0 resection rate of 100% [158]. Lastly, autogene cevumeran is a uridine messenger RNA lipoplex-based individualized neoantigen-specific immunotherapy designed from tumor-specific somatic mutation data [159]. A phase 1 trial of autogene cevumeran, with or without atezolizumab, in advanced solid tumors demonstrated the feasibility and immunogenicity of this approach, with some patients experiencing durable clinical benefits, including a patient with NSCLC who had a durable response (> 1 year on treatment) [159].

Collectively, these studies highlight the potential of combining neoantigen-based therapies with ICI to enhance antitumor immunity and improve patient outcomes across various cancer types.

Recent clinical trials have also demonstrated significant progress in OV-based therapies, particularly through strategic engineering and combination approaches [131]. T-VEC (talimogene laherparepvec), a herpes simplex virus (HSV)−1-derived OV expressing granulocyte–macrophage colony-stimulating factor (GM-CSF), remains a cornerstone, delivering durable responses in advanced melanoma [160]. In 2021, Japan approved DELYTACT (G47Δ-modified HSV-1) for malignant glioma, marking the first OV approval for brain tumors [161]. A phase 1/2 trials with rQNestin34.5v.2, another oncolytic herpes simplex virus (oHSV), revealed prolonged survival in glioma patients, with a median OS of 12.2 months compared to a historical median of 5.6 months. Combination therapies have further amplified efficacy [131]. A Phase 1b

trial combining T-VEC with anti-PD-1 antibody pembrolizumab achieved overall and complete response rates of 62% and 33%, respectively, in metastatic melanoma, highlighting synergistic immune activation [162]. Similarly, JX-594 (a vaccinia virus expressing GM-CSF) demonstrated survival benefits in colorectal cancer and hepatocellular carcinoma via intravenous delivery [163, 164]. Adenoviral vectors, such as DNX-2401, showed tumor reduction in pediatric diffuse intrinsic pontine glioma when paired with radiotherapy, with 9 out of 12 patients exhibiting immune activation [165]. Recently, emerging strategies have focused on arming OV with immunomodulators. For instance, oHSV-IL-12 enhanced TME infiltration of effector T cells and natural killer (NK) cells in preclinical models, while IL-15-armed OV improved CAR-NK cell persistence in glioblastoma [166, 167]. These advancements underscore the potential of OV to transform "cold" tumors into immunogenic hotspots, paving the way for next-generation combinatorial regimens with ICI, CAR-T/NK cells, and neoantigenbased therapies [168].

The applications of TMB in cancer immunotherapy

Mechanisms of TMB in Predicting Immunotherapy

Response

The predictive value of TMB in immunotherapy depends on its mechanistic association with neoantigen generation and subsequent immune activation [28]. As a surrogate marker for tumor immunogenicity, TMB reflects the probability of generating neoantigens capable of eliciting T cell-mediated antitumor responses [21]. This relationship can be delineated into four interrelated processes: neoantigen generation, antigen presentation, immune recognition, and host immune competency
(Fig. 4**)** [28, 30].

TMB quantifies somatic non-synonymous mutations within a tumor genome [16]. Elevated TMB increases the probability of immunogenic neoantigen generation, as coding-region mutations may produce altered peptides perceived as"non-self"by the immune system [30]. These neoantigens arise from diverse mutational processes, such as SNV, indel, and gene fusions [30]. For instance, tumors with dMMR or polymerase epsilon/delta (POLE/ POLD1) mutations exhibit hypermutated genomes enriched in frameshift-derived neoantigens——structurally distinct from self-peptides and inherently immunogenic [169, 170]. Mechanistically, neoantigen abundance correlates with TMB, expanding the antigenic repertoire available for immune recognition [21]. However, only mutations exhibiting high binding affinity to MHC molecules and sufficient dissimilarity from self-antigen confer functional immunogenicity [77, 171].

Neoantigen presentation via MHC class I and II molecules is critical for T cell activation [172]. Elevated TMB increases the probability of neoantigen-MHC binding with sufficient affinity for surface presentation [173, 174]. Defects in antigen presentation machinery, such as loss of β2-microglobulin (B2M) or MHC downregulation, can disrupt this process even in TMB-high tumors [28, 175]. Additionally, tumor heterogeneity, marked by spatial and temporal variations in neoantigen expression, may limit presentation consistency [176, 177]. Clonal evolution under therapeutic pressure further modulates immunogenicity

through dynamic neoantigen presentation [178]. In TMB-high tumors, neoantigen diversity also increases the probability of TCR recognition [179]. Neoantigenspecific T cells infiltrate the TME and initiate cytotoxic responses. Pre-existing neoantigen-specific T cell clones in peripheral blood or tumor-infiltrating lymphocytes (TIL) correlate with improved ICI efficacy [180–182]. Conversely, tumors with low neoantigen clonality often evade immune detection due to suboptimal T cell activation [183]. Structural similarity between neoantigens and pathogen-derived epitopes may also engage crossreactive memory T cells, amplifying antitumor immunity [184]. For instance, viral peptide-like neoantigens can recruit pre-existing memory T cell populations, accelerating immune responses [185, 186].

Host immune competency, governed by systemic and local factors, determines the response efficacy of immunotherapy [187]. Elevated TMB alone is insufficient in immunosuppressive TME characterized by PD-L1 expression, regulatory T cell (Treg) infiltration, and myeloidderived suppressor cells (MDSC) or inhibitory cytokine [188–191]. ICI like anti-PD-1/PD-L1 agents restore exhausted T cell cytotoxicity by blocking inhibitory signals [192]. However, TMB-high tumors with low CD8+ T cell infiltration ("immune-excluded"phenotypes) frequently resist ICI, highlighting the necessity of a permissive TME [193]. Germline polymorphisms in immune-related genes, including HLA alleles, further influence outcomes [194]. Broad peptide-binding HLA class I supertypes enhance neoantigen presentation, whereas HLA loss of heterozygosity (LOH) compromises immunity [194, 195].

Immunotherapy efficacy hinges on the interplay between TMB and these four pillars. TMB-high tumors with intact antigen presentation, robust T cell infiltration, and favorable immune contexts are more responsive to ICI. Discordances arise when TMB overlooks neoantigen quality or immune evasion mechanisms. For instance, hypermutated tumors with dMMR may resist therapy due to impaired antigen presentation or dominant immunosuppressive [196, 197]. Thus, TMB requires integration with complementary biomarkers—such as PD-L1 expression, immune gene signatures, and HLA status—to enhance predictive accuracy and predicts immunotherapy response by approximating neoantigendriven immunogenicity, contingent on host immune competence. Future research should prioritize multiomics integration to elucidate tumor-immune dynamics and optimize patient stratification.

Detection of TMB in cancer immunotherapy

Recent advancements in TMB measurement have prioritized enhancing accuracy, scalability, and clinical applicability, especially in immunotherapy contexts. Although WES remains the gold standard for comprehensive TMB assessment, emerging technologies overcome its limitations, such as high costs and lengthy processing times, while improving compatibility with low-input or lowpurity clinical samples [16, 61]. The primary detection methods of TMB are shown in Fig. 5.

Liquid biopsy has emerged as a non-invasive alternative to tissue-based TMB analysis, particularly for inaccessible tumors or those with spatially heterogeneous mutational profiles [198]. Blood TMB (bTMB) measures somatic mutations in ctDNA, capturing the cumulative mutational load across primary and metastatic sites [199]. Recent studies have validated assays such as GuardantOMNI and PredicineATLAS

for bTMB estimation, showing correlation with tissue TMB in different cancers [200, 201]. For example, synthetic reference standards generated by spiking tumor cell line DNA into donor-matched lymphoblastoid DNA at low-tumor-fraction thresholds (0.5%–2%) have enabled bTMB assay calibration, mitigating challenges like ctDNA fragmentation and low variant allele frequencies (VAF) [202]. Advanced bioinformatics pipelines now employ noise-reduction algorithms to filter artifacts from clonal hematopoiesis or sequencing errors, improving specificity for true tumorderived mutations [202]. Next-generation targeted panels have also advanced to enhance sensitivity and broaden genomic coverage. Hybrid capture-based panels paired with unique molecular identifiers (UMI) improve detection of low-VAF mutations (< 1%) in low-tumor-content samples [202, 203]. Furthermore, multiplex PCR-based approaches, including the Oncomine Tumor Mutation Load Assay, facilitate rapid and cost-effective TMB estimation by targeting mutation hotspots and immune-relevant genomic regions [204]. These panels are increasingly integrated with machine learning algorithms to predict neoantigen load and immunogenicity, associating TMB quantification with functional immune response metrics. Single-cell sequencing, a newly developed highthroughput technology, allows investigation of genomics, transcriptomics, and epigenetics on a single-cell level [205]. Single-cell DNA sequencing (scDNA-seq) and single-cell RNA sequencing (scRNA-seq) are transforming TMB analysis by dissecting intratumoral heterogeneity and distinguishing clonal neoantigens [206, 207]. Spatial transcriptomics, such as Visium Spatial Gene Expression, provides spatial context for TMB within the TME, correlating mutational hotspots with immune-excluded regions or PD-L1 expression [208]. These methods elucidate interactions between TMB and local immune activity, refining its role as a dynamic biomarker.

Standardization efforts have resulted in synthetic reference materials, incorporating predefined mutations at specified VAF for cross-platform validation and reduced interlaboratory variability. Furthermore, the Friends of Cancer Research TMB Harmonization Consortium has developed guidelines for panel design, bioinformatics pipelines, and clinical reporting, standardizing criteria [209]. In summary, contemporary TMB measurement methods prioritize precision, scalability, and integration with complementary biomarkers. Liquid biopsy, ultrasensitive targeted panels, and single-cell technologies are broadening TMB's clinical utility, while standardization initiatives mitigate reproducibility challenges. Future directions will likely emphasize dynamic TMB assessment through ctDNA analysis and multi-omics integration to optimize immunotherapy stratification.

Clinical applications of TMB in predicting immunotherapy response

The clinical utility of TMB as a predictive biomarker for ICI response has been validated across diverse cancer types, with growing evidence supporting its role in patient stratification. Clinical trials of cancer immunotherapy closely related to TMB are shown in Table 3 [210–212]. The FDA's 2020 tumor-agnostic approval of pembrolizumab for advanced solid tumors with TMB ≥ 10 mutations per megabase (mut/Mb), based on the phase II KEYNOTE-158 trial, constituted a pivotal milestone [20]. This approval underscores

TMB's potential as a pan-cancer biomarker, though its application remains context-dependent and requires histology-specific integration.

In NSCLC, TMB has demonstrated robust predictive value. Retrospective analyses of the CheckMate-026 and CheckMate-227 trials revealed that patients with TMBhigh tumors (≥ 10 mut/Mb) treated with nivolumab or ipilimumab demonstrated significantly improved PFS and objective response rates (ORR) compared to chemotherapy [213, 214]. Similarly, in melanoma, TMB correlates with durable responses to anti-PD-1/CTLA-4 therapies, with high TMB associated with enhanced cytolytic activity and prolonged survival [215]. Notably, tumors harboring dMMR or POLE/POLD1 mutations, such as colorectal and endometrial cancers, exhibit exceptionally high TMB and marked sensitivity to ICI [216, 217]. However, TMB's predictive power varies by cancer type. For example, in microsatellite-stable (MSS) colorectal and gastric cancers, tumors with TMB ≥ 10 mut/Mb often exhibit limited response to immune checkpoint inhibitors unless accompanied by specific molecular features, reflecting the dominant immunosuppressive microenvironment and lack of immunogenic neoantigens in MSS tumors [218]. This observation aligns with the broader recognition that TMB's predictive utility is contingent on both mutational load and the functional immunogenicity of the resulting neoantigens, which may be compromised in non-hypermutated or immune-excluded tumors. These discrepancies underscore the need for histologyspecific TMB thresholds and validation.

The KEYNOTE-158 trial established TMB's role as a companion diagnostic biomarker. In this study, pembrolizumab achieved an ORR of 29% in TMB-high (≥ 10 mut/ Mb) patients across 10 cancer types, including rare malignancies such as small cell neuroendocrine carcinoma and sarcoma [20]. Response rates increased to 37% in tumors with TMB > 13 mut/Mb, suggesting a dose–response relationship between TMB and ICI efficacy [16, 20]. Despite these advances, the 10 mut/Mb threshold remains debated, as some cancers (e.g., melanoma) may benefit from higher cutoffs, while others (e.g., MSI-H tumors) respond robustly even at lower TMB levels [219, 220].

Integrating TMB with PD-L1 expression enhances predictive accuracy, as these biomarkers capture complementary aspects of tumor immunogenicity: TMB quantifies neoantigen load (reflecting genomic instability and potential T-cell recognition), while PD-L1 measures adaptive immune resistance within the tumor microenvironment. In NSCLC, the CheckMate-227 trial demonstrated that patients with TMB-high (≥ 10 mut/ Mb) and PD-L1-positive tumors derived the greatest benefit from nivolumab plus ipilimumab, with a median PFS of 7.2 months compared to 3.2 months in TMB-low counterparts [221]. This synergy arises because high TMB increases antigenicity, while PD-L1 expression indicates pre-existing immune engagement. Conversely, in PD-L1-negative tumors, TMB retains predictive value as a stand-alone biomarker, highlighting its independence from PD-L1—particularly valuable in cancers like small cell lung cancer where PD-L1 expression is often absent or heterogeneous. Combined Positive Score (CPS) and Tumor Proportion Score (TPS), which assess PD-L1 expression differently, further refine TMB-based stratification: TPS quantifies only tumor-cell membrane staining (percentage of viable tumor cells), while

CPS includes immune cells (number of PD-L1-positive tumor and immune cells per 100 tumor cells), making it more sensitive in cancers with prominent stromal immune infiltration. For instance, in gastric cancer, the phase III KEYNOTE-062 trial showed that TMB-high (≥ 10 mut/ Mb) patients with CPS ≥ 1 (a broader cutoff than TPS, validated for gastrointestinal malignancies) exhibited superior survival with pembrolizumab versus chemotherapy [222]. These findings suggest that dual biomarker approaches mitigate the limitations of single-marker strategies—such as spatial heterogeneity in PD-L1 expression or TMB's inability to reflect immune evasion mechanisms—particularly in cancers with heterogeneous PD-L1 expression. However, drawbacks persist: technical variability in TMB quantification and scoring discordance between CPS/TPS assays can lead to inconsistent classifications, while overlapping predictive value sometimes reduces the incremental benefit of combined testing. In conclusion, TMB has emerged as a critical biomarker for ICI response prediction, particularly in TMBhigh malignancies. Its integration with PD-L1, CPS, and TPS refines patient selection, though histology-specific validation and technical standardization are imperative. As precision immuno-oncology advances, TMB is poised to serve as a cornerstone in multi-omics frameworks, guiding personalized therapeutic strategies across diverse cancer types.

Challenges and perspectives

Potential advantages and challenges of neoantigens and TMB in immunotherapy

Neoantigens represent a cornerstone of precision immunotherapy due to their capacity to trigger tumor-specific immune responses while sparing healthy tissues [71]. This tumor-exclusive expression pattern fundamentally distinguishes them from tumor-associated antigens that

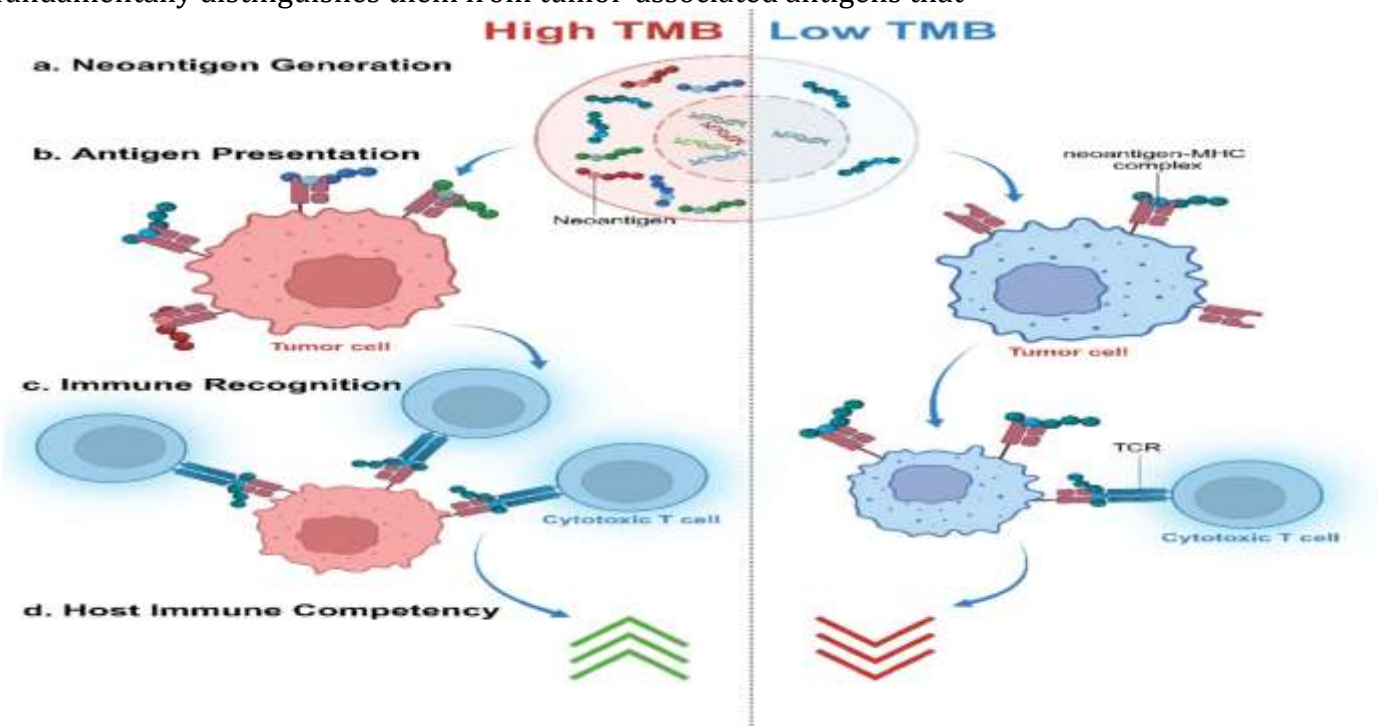
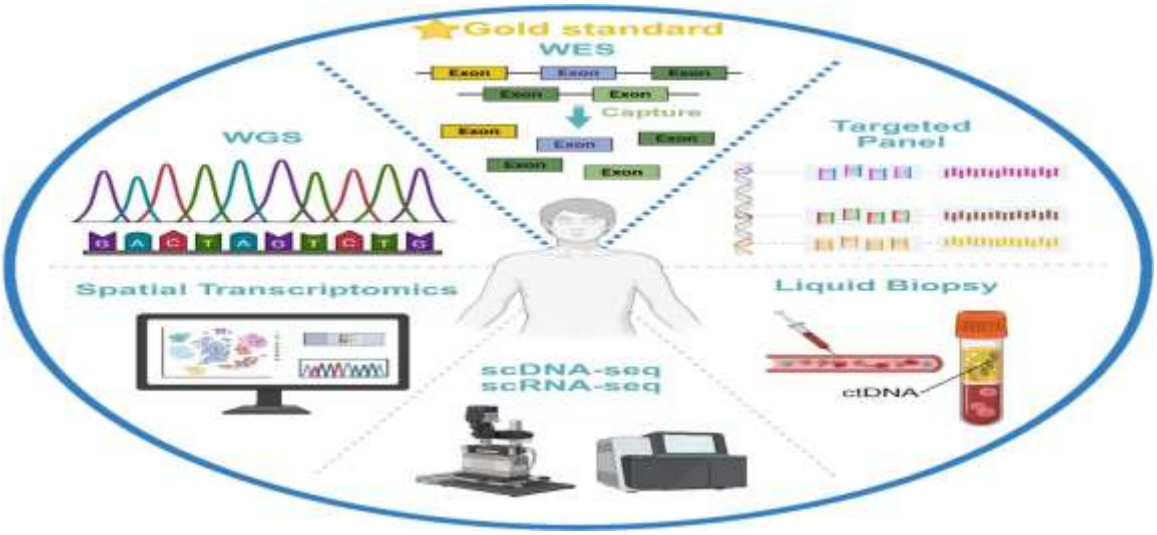


Fig. 4 Mechanisms of TMB in predicting immunotherapy response. The mechanisms of TMB in predicting immunotherapy response can be delineated into four interrelated processes: neoantigen generation, antigen presentation, immune recognition, and host immune competency. **a.** Elevated TMB increases the probability of immunogenic neoantigen generation, as coding-region mutations may produce altered peptides perceived as"non-self"by the immune system. **b.** Elevated TMB increases the probability of neoantigen-MHC binding with sufficient affinity for surface presentation. **c.** In TMB-high tumors, neoantigen diversity also increases the probability of TCR recognition. **d.** Host immune competency, governed by systemic and local factors, also determines the response efficacy of immunotherapy. MHC: major histocompatibility complex, TCR: T cell receptor, TMB: tumor mutational burden. Created with BioRender.com

May exist in normal tissues, thereby minimizing autoimmune complications. The patient-specific nature inherently reduces off-target toxicity and circumvents central or peripheral immune tolerance mechanisms, positioning them as ideal targets for personalized therapies like vaccines and adoptive T-cell therapies [72, 223]. The avoidance of immune tolerance is particularly critical, as conventional tumor antigens often undergo negative selection during thymic education, whereas neoantigens emerge from somatic mutations after immune system maturation. Additionally, TNB strongly correlates with improved responses to ICI, especially in hypermutated cancers such as melanoma and NSCLC, where high TNB is associated with elevated cytolytic activity and durable clinical benefits [67, 79, 116]. This correlation stems from the increased probability of generating immunodominant epitopes when mutational load exceeds a critical threshold, effectively transforming "cold" tumors into "hot" immunogenic microenvironments.

Despite these advantages, neoantigen-based therapies face substantial clinical challenges. Tumor heterogeneity remains a major obstacle, as subclonal neoantigens— expressed only in specific tumor subpopulations—drive immune escape under therapeutic pressure [224]. This spatial and temporal heterogeneity creates evolutionary bottlenecks where therapy-resistant clones lacking targetable



neoantigens eventually dominate the tumor ecosystem. Deficiencies in antigen presentation, including B2M loss or MHC downregulation, further obscure neoantigen visibility to T cells, even in tumors with high TMB [28, 175]. Critically, the extreme patient-specificity of somatic mutations generating neoantigens creates a fundamental limitation: each individual's tumor possesses a unique mutanome, with only a little fraction yielding immunogenic epitopes. This intrinsic variability is compounded by the vast polymorphism of HLA alleles across human populations [225]. Specific HLA allotypes exhibit differential binding affinities for peptide

Fig. 5 Detection of TMB in cancer immunotherapy. The primary detection methods of TMB are shown in figure. WES remains the gold standard for comprehensive TMB assessment. Emerging technologies overcome the limitations of WES, such as high costs and lengthy processing times. ctDNA: circulating tumor DNA, scDNA-seq: single-cell DNA sequencing, scRNA-seq: single-cell RNA sequencing, WES: whole-exome sequencing, WGS: whole-genome sequencing. Created with BioRender.com

Sequences, meaning identical mutations may generate immunogenic neoantigens in some patients but remain immunologically inert in others due to HLA mismatch [226]. Consequently, the combinatorial complexity of patient-specific mutations interacting with diverse HLA haplotypes severely restricts the universal applicability of neoantigen-targeting therapies, rendering most neoantigens private to individual patients. To overcome these barriers, innovative strategies are emerging. One approach focuses on "shared neoantigens" derived from recurrent driver mutations commonly found across patients with specific cancer types [156, 227]. These public epitopes offer potential for off-the-shelf therapies targeting broader patient cohorts [228]. Complementarily, leveraging HLA supertypes—groups of HLA alleles with similar peptide-binding preferences—can enhance population coverage [229, 230]. For instance, designing vaccines targeting epitopes presented by HLA supertype alleles could theoretically benefit larger patient subsets despite individual HLA variations [229, 230]. Such strategies aim to transform neoantigen therapeutics from purely personalized paradigms toward population-level solutions.

In terms of the translational and implementation challenges, significant translational hurdles must be overcome to realize the full potential of neoantigen-based strategies. Personalized neoantigen therapies face complex manufacturing logistics, including rapid turnaround times for vaccine production and scalable infrastructure for adoptive cell therapies, which currently limit widespread accessibility. Assay standardization remains critical for both biomarkers, particularly for TMB quantification across sequencing platforms and TNB validation in clinical settings. For TNB to serve as a robust stratification or response marker, it must demonstrate consistent reproducibility across institutions, establish validated predictive thresholds linked to clinical outcomes, and prove cost-effectiveness relative to existing biomarkers. While TNB offers superior specificity over TMB by filtering immunogenic mutations, neither

biomarker alone suffices to capture the multifaceted nature of treatment response. Combining TNB/TMB with indicators of immune activation—such as T-cell infiltration, PD-L1 expression, or MSI-H/MSS status—will likely yield more reliable predictive models. Furthermore, TMB's utility as a standalone biomarker is constrained by tumor heterogeneity, temporal variations during therapy, and discordance between tissue and liquid biopsy measurements. These biological and technical gaps underscore the need for dynamic monitoring approaches and integrated frameworks that account for tumor-immune coevolution. Ultimately, the path forward necessitates not only refining the biomarkers themselves but also developing multifactorial frameworks that synthesize diverse data streams. Addressing these gaps through collaborative standardization initiatives and health economics research will be pivotal for equitable clinical implementation. Furthermore, prospective clinical trials explicitly designed to validate these integrated approaches and their impact on patient outcomes are essential to bridge the current translational divide.

The immunosuppressive TME, enriched with Treg, MDSC, and inhibitory cytokines, also suppresses neoantigen-specific T-cell activity [188–191]. Notably, this suppression operates through both direct cell–cell contact mechanisms and paracrine signaling networks that establish regional immune privilege zones within tumors. Moreover, current prediction pipelines frequently overlook post-translational modifications, alternative splicing, or non-canonical antigen presentation, resulting in overestimated immunogenic neoantigens [30, 231, 232]. These compounding individualization factors further exacerbate the computational oversimplification arises from the predominant focus on exome-derived mutations while neglecting the complex proteomic processing required for actual immunogenicity. Collectively, these biological and computational hurdles necessitate integrative strategies to refine neoantigen selection and address resistance.

Enhancing neoantigen prediction accuracy and immunogenicity

Improving neoantigen identification demands advances in multi-omics integration and computational modeling. The current paradigm shift recognizes that neoantigen immunogenicity is not merely a function of mutation presence but requires coordinated expression, processing, presentation, and T-cell recognition. Current approaches predominantly rely on WES and tools like NetMHCpan to predict MHC binding affinity [40, 62]. However, only a minority of predicted neoantigens are naturally processed and presented on MHC molecules, underscoring the gap between in silico predictions and in vivo immunogenicity [233]. This discrepancy highlights the crucial role of proteasomal cleavage patterns and peptide transport efficiency—biological filters largely absent in current algorithms. Emerging solutions combine genomics with immunopeptidomics—MS-based profiling of MHC-bound peptides—to directly identify presented neoantigens [57]. By anchoring predictions to empirically verified MHC ligands, this approach bypasses theoretical assumptions about antigen processing machinery. For instance, integrating MS data with RNA sequencing enhances detection of splice variant- or frameshift-derived neoantigens, which conventional pipelines often miss [234]. Such integration effectively bridges the genotype–phenotype divide by correlating transcriptional output with actual peptide presentation.

Machine learning models trained on immunopeptidomic datasets now prioritize neoantigens using features beyond MHC binding, such as TCR recognition probability, peptide stability, and dissimilarity to self-antigens [44, 46, 235]. These multidimensional models simulate the immunological "fitness" of neoantigens by quantifying their likelihood to complete the entire immune recognition cascade. These models can also incorporate tumor-specific variables, including HLA diversity, mutation clonality, and immune contexture, to better predict functional immunogenicity [236–238]. For example, clonality-adjusted prediction weights account for the therapeutic relevance of targeting truncal versus subclonal mutations. Experimental validation remains critical: high-throughput TCR screening and in vitro T-cell activation assays confirm neoantigen-induced immune responses [239, 240]. These functional assays serve as essential reality checks by quantifying the magnitude and specificity of T-cell responses against predicted epitopes. Meanwhile, scRNA-seq and spatial transcriptomics map neoantigen-specific T-cell clones within the TME, elucidating their spatial distribution and functional states [241, 242]. This spatial resolution reveals microenvironmental niches where neoantigen-specific T-cells become functionally impaired, informing combination therapy strategies.

Combining neoantigen-based therapies with conventional treatments

The integration of neoantigen-based therapies with conventional treatments—such as chemotherapy, radiotherapy, and ICI—may exploit synergistic mechanisms to improve therapeutic efficacy and overcome resistance, positioning this strategy as a pivotal focus in contemporary oncology research. This combinatorial approach leverages the complementary strengths of each modality: conventional therapies debulk tumors and modulate microenvironments, while neoantigen-targeted therapies provide immunological specificity. Chemotherapy and radiotherapy induce immunogenic cell death, releasing tumor-derived antigens and activating antigen-presenting cells (e.g., dendritic cells), thereby priming adaptive immunity for neoantigen-specific responses [120, 243]. The resulting "antigen storm" enhances cross-presentation of therapy-exposed neoantigens, effectively converting tumor debris into endogenous vaccines. For instance, the abscopal effect of radiotherapy, which triggers systemic antitumor immunity, could synergize with neoantigen vaccines when administered sequentially to enhance localized treatment outcomes [244]. Timing optimization is crucial here, as radiation-induced inflammation may create temporal windows of enhanced immune receptivity. Concurrently, ICI mitigate T-cell exhaustion, sustaining the cytotoxic activity of neoantigen-reactive T lymphocytes. This PD-1/PD-L1 axis blockade essentially "releases the brakes" on neoantigen-specific clones that have infiltrated tumors but become functionally anergic. Preclinical evidence indicates that such combination regimens augment effector T-cell infiltration within the TME, thereby prolonging immune-mediated tumor control [245].

Within biomarker-guided precision oncology, TMB serves as a predictive biomarker to stratify patients for combination therapies. This stratification acknowledges the biological continuum of tumor immunogenicity, where therapeutic strategies must adapt to each patient's neoantigen landscape. TMB-high tumors, characterized by greater neoantigen diversity, demonstrate increased susceptibility to

immunotherapies, whereas TMB-low malignancies may benefit from chemotherapy- or radiotherapy-induced antigen exposure to counteract neoantigen paucity [24, 246, 247]. In TMB-low scenarios, conventional therapies essentially function as neoantigen amplifiers by inducing DNA damage and subsequent mutation generation. Furthermore, optimizing the temporal sequencing and dosing of personalized regimens is critical; for instance, post-chemotherapy immune microenvironment remodeling may enhance the therapeutic window for neoantigen vaccine efficacy [248]. his phased approach allows chemotherapy to first eliminate immunosuppressive elements like MDSC, creating a more permissive environment for vaccine-primed T cells.

Despite these advances, challenges persist, including immunosuppressive off-target effects of conventional therapies, tumor clonal evolution-driven antigen escape, and the logistical complexity of personalized therapeutic platforms. The dynamic nature of tumorimmune coevolution demands real-time monitoring approaches to adjust combination regimens as resistance mechanisms emerge. Future research must prioritize elucidating mechanistic synergies between treatment modalities, standardizing biomarker validation, and deploying computational tools to refine combination strategies. Advanced systems biology approaches could decode the nonlinear interactions between chemotherapy-induced stress responses and neoantigen presentation dynamics. Innovations in neoantigen prediction algorithms, multi-omics integration, and scalable manufacturing technologies are poised to advance this paradigm toward more durable and precise cancer therapies, ultimately improving clinical outcomes. The ultimate goal resides in creating adaptive treatment ecosystems where conventional and immunotherapeutic approaches mutually reinforce each other through precisely orchestrated molecular and cellular interactions.

Conclusions

The exploration of neoantigens and TMB has transformed the landscape of cancer immunotherapy, offering novel opportunities for precision oncology. Neoantigens, tumor-specific antigens arising from somatic mutations, represent ideal targets for personalized therapies due to their absence in normal tissues, thereby minimizing offtarget toxicity. Concurrently, TMB serves as a robust biomarker for predicting immunotherapy response by estimating tumor immunogenicity. Together, these parameters have reshaped our understanding of tumorimmune interactions and propelled the development of innovative therapeutic strategies.

Neoantigen-based therapies exhibit significant clinical potential. Early-phase trials demonstrate synergistic effects between neoantigen vaccines and ICI, with vaccines expanding tumor-reactive T-cell clones and ICI reversing T-cell exhaustion. Advances in CAR-T engineering have similarly enhanced specificity and persistence in solid tumors, overcoming challenges such as antigen heterogeneity and immunosuppressive microenvironments. These breakthroughs highlight the necessity of tailoring therapies to individual tumor mutational profiles. TMB has emerged as a critical biomarker for identifying patients likely to benefit from ICI, particularly in hypermutated cancers such as melanoma, NSCLC, and dMMR tumors. The FDA's tumor-agnostic approval of pembrolizumab for TMB-high solid tumors

represented a milestone in biomarker-driven oncology. However, TMB's predictive utility remains context-dependent, requiring integration with complementary biomarkers such as PD-L1 expression, immune gene signatures, and HLA status. Liquid biopsy and ultra-sensitive sequencing panels have improved TMB measurement scalability, enabling dynamic monitoring of mutational load and resistance mechanisms. Despite progress, challenges persist in standardizing TMB thresholds, addressing tumor heterogeneity, and differentiating immunogenic from non-functional mutations.

The interplay between neoantigens and TMB underscores the complexity of tumor immunogenicity. While TMB quantifies mutation abundance, only a subset generates immunogenic neoantigens capable of eliciting T-cell responses. This discrepancy emphasizes the need for multi-omics approaches combining genomics, immunopeptidomics, and single-cell technologies to refine neoantigen prediction. Machine learning models trained on MHC-eluted ligand datasets and TCR recognition patterns have improved prediction accuracy, bridging in silico algorithms and in vivo immunogenicity. Furthermore, spatial transcriptomics and single-cell sequencing elucidate tumor-immune dynamics, revealing niches where neoantigen-specific T cells are functionally suppressed. Nonetheless, clinical translation faces hurdles: tumor heterogeneity and clonal evolution drive antigen escape, while defects in antigen presentation render neoantigens undetectable to immune surveillance. Future strategies should prioritize combinatorial approaches, such as combining neoantigen vaccines with chemotherapy or radiotherapy to induce immunogenic cell death and amplify antigen exposure. Additionally, realtime monitoring via ctDNA may enable adaptive therapy adjustments to counteract resistance.

Advancing neoantigen-based immunotherapy will require integrating artificial intelligence, synthetic biology, and multi-omics platforms. Standardizing biomarker validation, optimizing manufacturing scalability, and fostering interdisciplinary collaboration are essential to make these therapies widely accessible. Ultimately, the goal is to establish adaptive treatment ecosystems where conventional and immunotherapeutic modalities synergize to achieve durable remission. As the field evolves, neoantigens and TMB will remain cornerstones of precision immuno-oncology, guiding the development of therapies that harness the immune system's full potential to combat cancer.

Abbreviations

AE	Adverse event
AML	Acute myeloid leukemia
AUC	Area under the plasma concentration–time curve
BAM	Binary Alignment/Map format
BED	Biologically effective dose
B2M	β2-Microglobulin
bTMB	Blood tumor mutational burden
CAR	Chimeric antigen receptor

CBR	Clinical benefit rate
CCA	Cholangiocarcinoma
CDR3	Complementarity determining region 3
cfDNA	Cell free DNA
CGP	Comprehensive genomic profiling
Cmax	Maximum concentration
CNV	Copy number variant
CPS	Combined Positive Score
CR	Complete response
CRC	Colorectal cancer
cRDE	Recommended dose for expansion for combination
ctDNA	Circulating tumor DNA
CTL	Cytotoxic T lymphocyte
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
CUP	Cancer of unknown primary
DC	Dendritic cell
DCR	Disease control rate
DFS	Disease-free survival
DFS2	Disease-free survival 2
DIPG	Diffuse intrinsic pontine glioma
DLT	Dose-limiting toxicity
DMFS	Distant metastasis-free survival
DMG	Diffuse midline glioma
dMMR	DNA mismatch repair deficiency
DNMT	DNA methyl transferase
SAE	Severe adverse event
ScDNA-seq	Single-cell DNA sequencing scRNA-seq Single-cell RNA sequencing
SD	Stable disease
SHERPA	Systematic HLA Epitope Ranking Pan Algorithm
SLP	Synthetic long peptide
SNV	Single-nucleotide variant
SoC	Standard of care
SynNotch	Synthetic Notch
TACE	Transhepatic arterial chemotherapy and embolization
Tan-MUC1	Tumor-associated mucin 1
TCR	T cell receptor

TEAE Treatment emergent adverse event
TEIM-Res TCR–Epitope Interaction Modelling at Residue Level
TESAE Treatment-emergent serious adverse event
TGF-β Transforming growth factor-β
TIL Tumor-infiltrating lymphocyte
TIminer Tumor Immunology miner
TLR Toll-like receptor
Tmax Time to peak drug concentration
TMB Tumor mutational burden TME Tumor microenvironment
TNB Tumor neoantigen burden
TPS Tumor Proportion Score
TRAE Treatment-related adverse event
Treg Regulatory T cell
TSA Tumor-specific antigen
TSNAD Tumor-specific neoantigen detector
TT Targeted therapy
TTF Time to treatment failure
TTNT Time to next treatment
TTP Time to progression
TTR Time to remission
UMI Unique molecular identifier
VAF Variant allele frequency
VCF Variant call format
WES Whole-exome sequencing
WGS Whole-genome sequencing

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