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REVIEW

Cancer Focus

Advances in the development of personalized neoantigen therapies

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The ability to specifically engage tumor-reactive T cells for therapeutic benefit is the ultimate goal of cancer immunotherapy. Whereas currently approved immunotherapies leverage and modulate existing endogenous T cells in an antigen non-specific manner, cancer vaccines and neoantigen therapeutics promise the ability to selectively amplify T cells specific for targeted antigens. Advances in the identification of tumor-specific antigens coupled with a greater understanding of T cell biology and immunization platforms have culminated in recent trials where signs of clinical efficacy have been observed, particularly in randomized adjuvant clinical settings. In this review, we discuss the identification of tumor-specific antigens for cancer therapy, the benefits of including antigens recognized by CD4⁺ T cells, clinical data investigating novel immunization platforms, and emerging clinical settings where promotion of tumor-specific immunity may be optimal.

Introduction

Elimination of cancer by the adaptive immune system is dependent on the recognition of immunogenic tumor antigens by T cells. Antitumor T cells are primed by antigen-presenting cells (APCs) following the uptake, processing, and presentation of tumor antigens. In the clinic, pre-existing tumor-specific T cells may be activated in vivo by the administration of immune checkpoint blockade (ICB) therapies or ex vivo by the expansion and subsequent reinfusion of tumor-infiltrating lymphocytes (TIL) harvested from tumor tissue. Both of these therapeutic approaches have demonstrated efficacy and won Food and Drug Administration (FDA) approvals. If the patients most likely to derive benefit from these therapies are those with a robust pre-existing immune response to their tumors, where does this leave the majority of patients who do not respond?

Cancer vaccines and neoantigen (NeoAg) therapeutics continue to represent a promising strategy to generate a specific antitumor immune response in patients who are unable to mount a sufficient spontaneous T cell response to their tumors. Cancer vaccines may be able to engage tumor-specific T cells without the activation of self-reactive T cells, such as those that have been implicated in the development of immune-related adverse events following treatment with ICB (Axelrod et al., 2022; Damo et al., 2023; Blum et al., 2024). New reasons for excitement in the field are manifold. (1) We may have better antigens in the tumor to target. The technical ability to target personalized, mutated NeoAgs in days is now upon us. These truly tumor-specific antigen targets may be activating T cells that are not exhausted or dysfunctional from years of antigen exposure by the tissue of origin. (2) We may have superior immune activation platforms. DNA, mRNA, and other platforms have been refined over years of preclinical and clinical testing, with recent mRNA vaccine trials demonstrating signs of clinical efficacy. (3) We are testing vaccines in earlier disease settings, including both adjuvant and neoadjuvant (Table 1). While it is true that T cell–activating therapeutics have been shown to be "safe and immunogenic" for decades, they can now be tested before a mature tumor microenvironment is in place, harboring potentially multiple resistance mechanisms and increased tumor antigen heterogeneity.

In this review we will address new insights on antigen targets including personalized and public mutated antigen classes and how they are being identified. We will also discuss the role of CD4⁺ T cells in cancer vaccines, antigen delivery platforms being harnessed to promote optimal antitumor immunity, emerging clinical data from academic and biopharma efforts, optimal disease settings for intervention, and methods of monitoring and predicting patient responses (Fig. 1).

Selecting tumor-specific antigens for immunization

Ideal target antigens for cancer vaccines should be both immunogenic and tumor-specific. NeoAg, derived from the protein

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Table 1. **Hurdles for activation of tumor-specific immunity in vivo and approaches to address them**[a]

Hurdle	Approaches	Recent results
Quality of therapy-induced T cells	-Optimize priming signals by including costimulation and cytokines	Optimal pathway to trigger and molecule use TBD
	-Evaluate NeoAg targets and "dark" antigens	Single cell analysis and TCR-based tracking is allowing identification of antigen-specific T cells; broader antigen classes being tested
T cell polyfunctionality and exhaustion	-Evaluate truncal NeoAgs and more recently expressed "branch" antigen targets	Single cell analysis and TCR-based tracking is allowing identification and functional profiling of antigen-specific T cells
	-Optimize priming and/or boosting signals by including costimulation and cytokines	Optimal pathway and molecule use TBD, IL-12 continues to be evaluated
Vaccine trafficking to tumor and tumor tissue penetration	-Inject the tumor or tumor bed with an activating signal (chemokine, oncolytic virus, and/or stimulatory tumor killing agent) to optimize tumor targeting	Oncolytic viruses continue to be developed, cocktails of costimulation with checkpoint blockade have shown distant tumor regressions
Heterogeneity of antigen expression	-Include multiple antigens in the T cell activating drugs	Multiple antigens increasingly included in vaccines and NeoAg therapeutics (up to >200)
	-Promote epitope spreading	More commonly evaluated in trials
Antigen loss or MHC loss	-Include multiple antigens presented by multiple human lymphocyte antigen molecules	Multiple antigens increasingly included in vaccines
	-Promote epitope spreading	More commonly evaluated in trials
	-Provide intratumoral IFN-γ signal to up-regulate MHC class I	Optimal pathway and molecule use TBD

TBD, to be determined.
[a]Updated from Adamik and Butterfield (2022).

products of genes mutated as a result of tumorigenesis, meets both of these requirements. Tumor-specific genomic alterations, including single-nucleotide variants (SNVs), frameshifts resulting from nucleotide insertions or deletions, and fusions resulting from chromosomal rearrangements, give rise to a range of potential NeoAg that can be identified by next-generation sequencing (NGS) approaches.

Although somatic NeoAg can be effectively targeted by the immune system, the same mutational processes that generate NeoAg in tumors may also promote immune evasion. Tumors evolve over time from more homogeneous "truncal" mutation-containing tissues to heterogenous tissues consisting of "branches" deriving from distinct clonal progenitors following the acquisition of novel mutations. As such, an effective immune response may be thwarted by the emergence of tumor clones with low or no expression of identified target NeoAg, loss of human leukocyte antigen (HLA) molecules or other genes responsible for antigen presentation, and/or loss of interferon (IFN) response genes (Anagnostou et al., 2017; McGranahan et al., 2017). To overcome these resistance mechanisms, it is proposed that "truncal" NeoAg with homogenous expression across tumor cell clones should be prioritized in cancer vaccines (McGranahan et al., 2016). These are often activating mutations in oncogenes which promote tumor cell growth and division, such as KRAS, PIK3CA, and BRAF. Such "driver" mutations therefore confer a fitness advantage to tumor cell clones and their loss is unfavorable to the tumor. While not all driver mutations are capable of generating an immunogenic target in the context of the diverse range of HLA alleles, many specific peptide-HLA pairs have been described (Bear et al., 2021; Chandran et al., 2022; Conn et al., 2025; Kim et al., 2022). Nonetheless, HLA loss has been reported as a mechanism of immune evasion in cases where immunogenic oncogenes have been targeted with adoptive cell therapies (Tran et al., 2016; Nagarsheth et al., 2021)—therefore, it is also likely preferred to include as many immunogenic NeoAg as is feasible in a single vaccine to limit the effects of loss of any single mutation or HLA gene.

While certain tumors may harbor as many as 10 somatic mutations per megabase, not all of these, when expressed as protein, will generate a peptide capable of binding to the patient's HLA proteins. Therefore, a host of bioinformatic tools have been developed to predict the immunogenicity of tumor antigens by leveraging HLA binding data inferred from immunopeptidomic datasets to identify conserved binding motifs corresponding to distinct HLA alleles (Jurtz et al., 2017; O'Donnell et al., 2018; Shao et al., 2020). Despite much interest in these tools, their capacity to accurately predict NeoAg capable of stimulating a T cell response in vivo remains limited. In early trials evaluating the immunogenicity of NeoAg vaccines in melanoma patients, peptides selected for their ability to bind to class I HLA molecules generated primarily $CD4^+$ T cell responses—this trend remains a feature of NeoAg vaccine trials with synthetic long peptides (SLPs) (Ott et al., 2017). This may reflect a relative bias for the generation of peptide complexes with HLA class II given the open ends of the peptide binding groove and the formation of such peptide complexes directly within the endocytic compartment, whereas class I peptides must be transferred from the cytosol to the endoplasmic reticulum via the formation of protein complexes in a less energetically favorable process (Roche and Furuta, 2015). In 2020, a global consortium assembled by the Parker Institute for Cancer Immunotherapy predicted 608 immunogenic T cell epitopes based on state of the art bioinformatic tools—of these, 37 (~6%) were found to be the targets of T cells (Wells et al., 2020). In a recent NeoAg vaccine trial in hepatocellular carcinoma (HCC), a similar proportion of

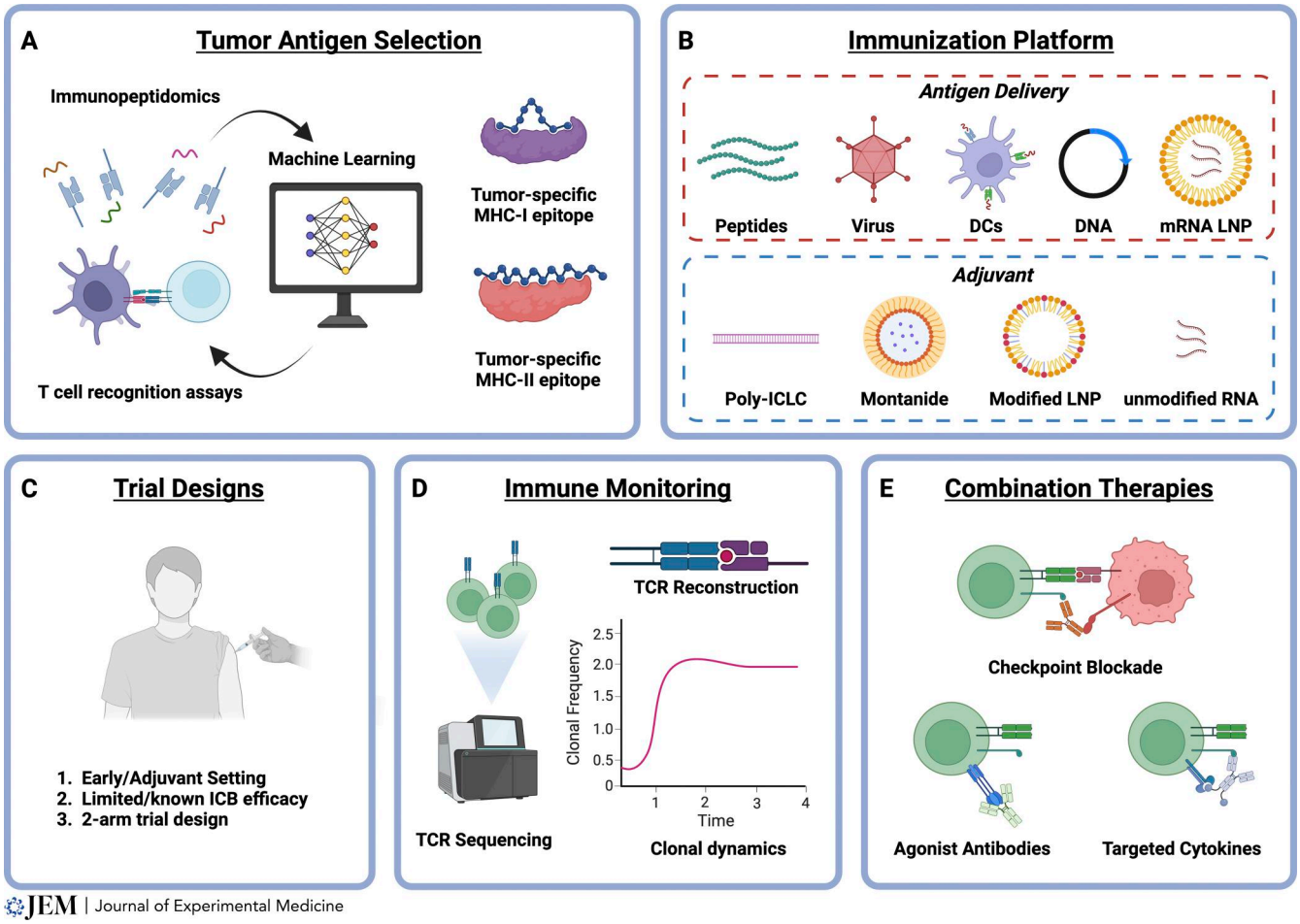

Figure 1. **Considerations for effective cancer vaccines. (A)** Optimal cancer vaccines require identification of tumor-specific, immunogenic antigens. Next-generation approaches are incorporating immunopeptidomic and functional T cell recognition datasets to train machine learning algorithms capable of predicting immunogenic CD4⁺ and CD8⁺ T cell epitopes from tumors. **(B)** Immunization platforms comprising antigen delivery and adjuvant components have been tested in numerous clinical trials, with newer mRNA platforms demonstrating enhanced signs of immunogenicity and efficacy. **(C)** Emerging clinical data suggests that the adjuvant setting may be optimal for cancer vaccine efficacy, with single-arm trials in tumors with low checkpoint blockade response rates and two-arm trials vs. standard of care alone as preferred designs. **(D)** New studies of mRNA LNP vaccines suggest a correlation between the frequency of circulating tumor-specific T cell clones and efficacy readouts, highlighting the importance of T cell receptor clonotype-based immune monitoring. **(E)** Combining cancer vaccines with additional immunotherapies, such as antibodies blocking immune checkpoints or agonizing costimulatory pathways and targeted cytokines, may improve efficacy. Figure created with BioRender.com.

T cell responses were generated in response to immunizing epitopes with predicted HLA binding affinities of above or below 500 nM, a common cutoff to stratify "low" versus "high" binding (Yarchoan et al., 2024). In another recently published trial from BioNTech, patients were immunized with mRNA vaccines encoding a median of 20 NeoAg predicted to bind HLA I, but for those patients where single epitope responses were evaluated, a median of 2 NeoAg were found to be immunogenic—although this may partially reflect immunodominance among vaccine target antigens as well as the complexity of identifying the breadth of responding T cells in a sensitive manner (Lopez et al., 2025). These results suggest that while we are now capable of rapidly identifying tumor-specific mutations with NGS and bioinformatics, prioritizing which of these mutations is most likely to generate a T cell response remains challenging.

Newer NeoAg prediction approaches are utilizing machine learning models trained on datasets of known immunogenic epitopes. Additional high quality NeoAg immunogenicity data allows for improved modeling (Zeng et al., 2025). Many of these newer models more accurately identify immunogenic peptides compared to HLA binding affinity predictions alone (Bulik-Sullivan et al., 2019; Gartner et al., 2021; Müller et al., 2023). HLA binding affinity predictions may nominate peptides that, despite binding to HLA, are either not generated from their parent proteins efficiently or not recognized by the available T cell repertoire, "blind spots" that algorithms trained on T cell recognition data may account for. However, these models may have their own blind spots given that they are largely trained on pre-existing T cell responses present within TIL, which may not be representative of all possible NeoAg available for targeting by T cells.

While many cancer vaccine trials have now been conducted targeting tumor NeoAg derived from somatic mutations, advances in mass spectrometry and RNA sequencing approaches

are unveiling new classes of NeoAg, including circularized RNAs, endogenous retroviral elements (ERVs), and cryptic "dark matter" antigens. A recent report identified an evolutionarily conserved circular RNA, circFAM53B, as overexpressed in breast cancer tissues relative to healthy breast tissue (Huang et al., 2024). The junction of this circular RNA gives rise to antigenic peptides that may be recognized by both $CD4^+$ and $CD8^+$ T cells, suggesting this may be a public NeoAg for breast cancer patients.

ERVs have been hypothesized to be the target antigens for renal cell carcinoma (RCC) tumors responsive to IL-2 and checkpoint blockade (Panda et al., 2018; Wolf et al., 2023). Recent data on their regulation by HIF1a, known to be commonly over-expressed in RCC, further supports this hypothesis (Jiang et al., 2025). The potential for immunogenicity of the ERVs that are expressed and translated into protein is further substantiated in lung cancer in recent work correlating antibody responses to human endogenous retrovirus (human ERV) (HERV) and positive clinical outcomes (Ng et al., 2023). These studies support careful consideration of these targets for their potential in antitumor immunity. Their expression in normal adjacent tissue must also be examined (Kassiotis, 2014).

Short-lived, aberrantly translated peptides derived from DNA sequences outside of canonical protein-coding regions have also been termed dark matter antigens and can be eluted from HLA molecules on tumor cells. Advances in proteogenomic analyses have enabled the recent identification of tumor-specific dark matter antigens in melanoma, non-small cell lung cancer (NSCLC), and pancreatic cancer (Apavaloaei et al., 2025; Ely et al., 2025). While these peptides are too transient and unstable to be effectively cross-presented and therefore apparently unable to prime endogenous T cell responses, recent reports have demonstrated their immunogenicity following in vitro priming of donor T cells (Apavaloaei et al., 2025; Ely et al., 2025; Lozano-Rabella et al., 2023). These T cells specifically recognize tumor cells in vitro, highlighting the promise of such antigens. The biotechnology company UbiVac is investigating the efficacy of a cancer vaccine containing dark matter antigens, DPV-001, which they report can effectively prime T cells in vivo by recognizing these antigens (Moudgil, 2025). However, clinical efficacy data supporting the vaccine have not yet been reported.

Validation of tumor-specific immune responses to guide NeoAg selection

Given the low percentage of NeoAg predicted in silico that generate a T cell response in vivo, alternative approaches to experimentally validate NeoAg immunogenicity are being explored. One approach is to characterize the pre-existing immune response to tumor antigens in patient peripheral blood. While pre-existing immune responses to NeoAg may not be a requirement for the efficacy of cancer vaccines, the presence of circulating NeoAg-specific T cells primed under natural conditions provides evidence that (1) a given epitope is immunogenic and (2) the epitope is naturally presented by tumor cells and/or APC. In a recent study of patients with metastatic cancers, circulating NeoAg-specific $CD8^+$ T cells were identified by tetramer staining in 6/6 patients assessed at low frequencies of ~0.002% (Yossef et al., 2023). This study also described the surface phenotype of these cells as $CD45RO^+HLA^-DR^+CD39^+CD103^+$ and demonstrated that sorting on these markers can enrich for NeoAg-specific T cells >2,100-fold, potentially obviating the need to generate tetramers on a per-patient basis. While not described in that study, circulating $CD4^+$ T cells recognizing NeoAg have also been identified in the peripheral blood of patients with cancer (Veatch et al., 2019). Alternatively to identifying circulating tumor-specific T cells by surface phenotype, ex vivo expansion of antigen-specific T cells with NeoAg peptides or minigenes allows for the identification of functional antigen-specific T cells in patients with cancer (Danilova et al., 2018; Khateb et al., 2025, *Preprint*; Miller et al., 2024).

In two recently published trials of NeoAg vaccines from BioNTech and Geneos, pre-existing T cell responses to immunogenic vaccine epitopes were identified functionally by ELISPOT or at the single-cell level by TCR clonotype analysis in pre-treatment blood samples (Lopez et al., 2025; Yarchoan et al., 2024). In the BioNTech trial, the frequency of pre-existing NeoAg-specific clones in the peripheral blood often increased post-vaccine along with the emergence of new T cell clones recognizing the same antigen, highlighting two potential mechanisms whereby cancer vaccines may enhance the activity of pre-existing responses. In many cases, tumor-specific T cell responses emerging following vaccination are deemed "de novo" based on the absence of response in an ex vivo ELISPOT assay or the absence of a given TCR clonotype in a sequencing library derived from <1 × 10e6 peripheral T cells pre-treatment. Given the low frequency of tumor-specific T cells in peripheral blood, both of these assays may underestimate the presence of rare pre-existing responses.

The feasibility of targeting experimentally validated NeoAg has been demonstrated in the clinic. Genocea Biosciences developed an approach to functionally assess T cell responses by expressing patient-specific NeoAg in *Escherichia coli* which were then fed to autologous dendritic cells (DCs) (Lam et al., 2021). Patient T cells were then screened in an overnight assay for recognition of antigens presented by these DCs. Vaccines administered with identified NeoAg to patients with advanced cancers were immunogenic; however, there were limited signs of efficacy in combination with checkpoint blockade therapy. An academic trial in low mutational burden cancers targeting experimentally validated NeoAg was recently completed, further supporting the feasibility of functionally testing NeoAg-specific T cell responses to inform vaccine design (NCT03568058) (Miller et al., 2024). While screening patients upfront for pre-existing T cell responses may not be commercially scalable, efforts to generate larger datasets of verified immunogenic NeoAg may also provide more comprehensive datasets for training machine learning models as described above.

The role of $CD4^+$ T cells in cancer vaccines

In many cancer vaccine trials, NeoAg is selected on the basis of their predicted binding affinity to MHC-I, highlighting a focus on generating cytotoxic $CD8^+$ T cell responses capable of directly recognizing and eliminating tumor cells. However, there is

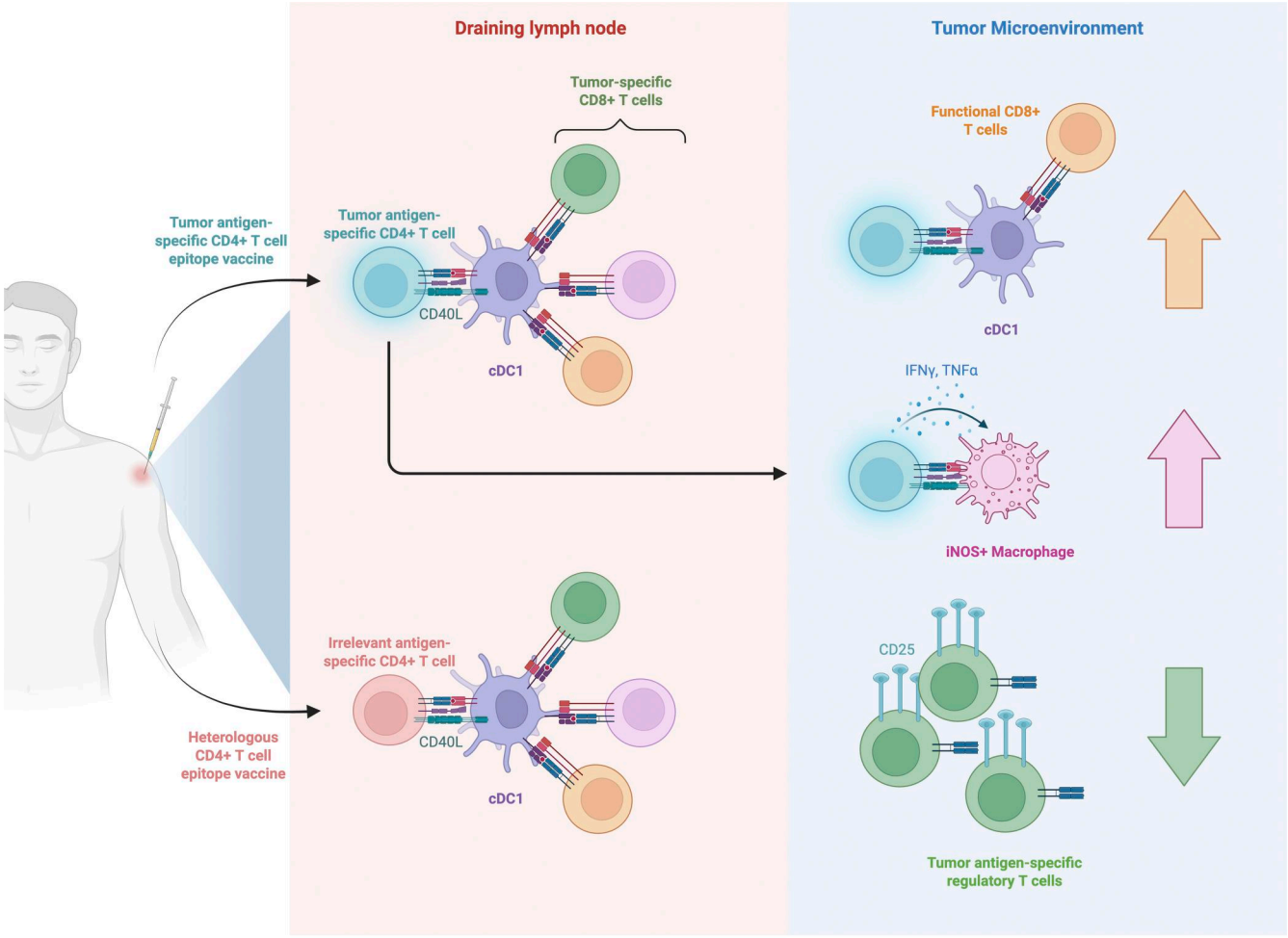

Figure 2. **Mechanisms of cancer vaccines including tumor-specific CD4⁺ T cell epitopes.** CD4⁺ T cells help the priming of tumor-specific CD8⁺ T cells in the draining lymph nodes can be achieved either by inclusion of "heterologous help" with a tumor-irrelevant MHC Class II epitope or by including tumor-specific antigens recognized by CD4⁺ T cells. Unlike heterologous help, inclusion of tumor-specific CD4⁺ T cell antigens may promote beneficial mechanisms within the tumor microenvironment that have been observed in preclinical and clinical studies, including (1) the formation of intratumoral clusters comprised of DCs, tumor-specific CD4⁺ T cells, and progenitor exhausted CD8⁺ T cells, which benefit from these interactions, (2) recruitment and activation of inducible nitric oxide synthase (iNOS+) effector macrophages, and (3) a reduction in the frequency of tumor antigen-specific CD4⁺ Treg. Figure created with BioRender.com.

substantial evidence that tumor antigen-specific CD4⁺ T cells are critical for effective antitumor immunity (Fig. 2). Indeed, adoptive transfer of NeoAg-specific CD4⁺ T cells alone can control solid tumors in humans and mouse models (Tran et al., 2014; Brightman et al., 2023; Lowery et al., 2025). It has long been appreciated in the context of vaccination that CD4⁺ T cells provide "help" during the priming phase of CD8⁺ T cells by licensing antigen-presenting DC via CD40L-CD40 interactions (Ferris et al., 2020; Schoenberger et al., 1998; Wu et al., 2022).

In cancer vaccines, CD4⁺ T cell help during priming has, in some trials, been provided by inclusion of an immunogenic tumor-irrelevant epitope, such as the pan-DR binding epitope (PADRE) (Snook et al., 2019), keyhole limpet hemocyanin (Aarntzen et al., 2012), or tetanus toxoid peptide (Saxena et al., 2025), alongside tumor-specific CD8⁺ T cell epitopes. Such heterologous help has been included in many shared antigen vaccine trials and T cell responses recognizing these xenoantigens can serve as biomarkers of vaccination (Aarntzen et al., 2012; Saxena et al., 2025). While heterologous help may replicate some of the cellular mechanisms required to effectively prime a CD8⁺ T cell response, the antitumor role of CD4⁺ T cells extends beyond that of help in the lymph nodes (Fig. 2). Studies in mouse models have demonstrated that tumor antigen-specific T cells accumulate at tumor-invasive margins where they interact with APCs and promote the recruitment and activation of inducible nitric oxide synthase (iNOS+) effector macrophages (Kruse et al., 2023). Tumor antigen-specific CD4⁺ T cell support for CD8⁺ T cells likely continues within the tumor microenvironment. A recent study demonstrated that adoptive cellular therapy (ACT) with in vitro primed, tumor-specific CD4⁺ and CD8⁺ T cells promotes tumor clearance in a mouse model where ACT with CD8⁺ T cells alone is ineffective (Espinosa-Carrasco et al., 2024). The authors propose a model where CD4⁺ T cell help locally within tumors promotes the functionality of previously primed CD8⁺ T cells, which distinguishes this phenotype from that of T cell help during priming in lymph nodes. Local CD4⁺

T cell help was dependent on the formation of cellular groups containing CD4+ T cells, CD8+ T cells, and CD11c+ APCs simultaneously presenting antigens recognized by both T cell subsets. CD8+ T cells in immune triads expressed lower levels of exhaustion-associated markers, including TOX and PD-1. In human cancer patients receiving ICB therapy, the presence of these "immune triads" within tumors positively correlated with response (not unlike tertiary lymphoid structures). A separate study demonstrated that in a cohort of patients with HCC, clinical response to ICB and the expansion of functional effector CD8+ T cells within tumors was associated with the presence of intratumoral CD4+ T cells interacting with DCs (Magen et al., 2023). Vaccine-expanded CD4+ T cells infiltrate human tumors, which suggests that including tumor-specific antigens for CD4+ T cells in cancer vaccines may promote these beneficial mechanisms (Awad et al., 2022; Ott et al., 2020). Recently published studies further support that cancer vaccines including tumor-specific CD4+ T cell epitopes may lead to better responses than those with tumor-irrelevant CD4+ T cell epitopes (De Graaf et al., 2024; Ninmer et al., 2024).

CD4+ T cells adopt a range of phenotypes tailored to specific contexts, including FOXP3+ regulatory T cells (Treg). Treg maintains peripheral tolerance by suppressing local effector T cells through a variety of mechanisms and their abundance in tumors limits effective antitumor immunity. While there is evidence that Treg primarily recognize self-peptide antigens, recent studies have demonstrated that NeoAg-specific Treg exist in both human and murine tumors (Griswold et al., 2025; Oliveira et al., 2022; Sultan et al., 2024). Therefore, might cancer vaccines including NeoAg recognized by CD4+ T cells inadvertently promote the expansion of pre-existing NeoAg-specific Treg? This does not seem to be the case based on current evidence—in murine models where spontaneous NeoAg-specific CD4+ T cells are found, therapeutic vaccination promotes a reduction in their relative frequency compared to CD4+ T cells with effector-associated phenotypes recognizing the same antigen (Griswold et al., 2025; Sultan et al., 2024). Similarly, in human melanoma patients, circulating NeoAg-specific CD4+ T cells were profiled by single-cell RNA sequencing following vaccination and no clusters with Treg-associated genes were identified (Awad et al., 2022; Hu et al., 2021).

Vaccine platforms and their features

While many cancer vaccine trials in humans to date have utilized SLPs to deliver tumor-specific antigens, novel vaccine platforms are under investigation in the clinic that may improve our ability to selectively expand tumor-specific T cells. These include the use of modified peptides, viral vectors, DC, DNA plasmids, and lipid nanoparticles (LNPs) encapsulating mRNA as vaccine platforms. Controlled trials comparing platforms have rarely been performed, and it is difficult to compare vaccine platforms across multiple single-arm clinical trials with distinct NeoAg prediction methodologies and disease settings. Here, we will summarize trends apparent in the early clinical data.

Peptides

SLPs remain the most commonly utilized cancer vaccine platform given their known safety profile and relative ease of manufacturing. While SLP vaccines are capable of eliciting T cell responses (Bijker et al., 2008) according to numerous trials (Kenter et al., 2008), the magnitude of resulting T cell responses detected in peripheral blood is often low and skewed towards the generation of CD4+ T cell responses. However, NeoAg-specific T cells generated in melanoma patients immunized with SLPs were detectable when tested ex vivo at a median follow-up time of 4 years, suggesting T cell responses generated with SLP vaccines may be long-lived (Hu et al., 2021). Furthermore, in several single-arm trials of SLP vaccines in the adjuvant setting, patients have experienced long periods of recurrence-free survival (RFS), including in melanoma (Hu et al., 2021), renal cancer (Braun et al., 2025), and other advanced cancers (Keskin et al., 2019; Saxena et al., 2025). However, given the small size of these trials and the single-arm design, it is impossible to determine whether these encouraging clinical signs are truly a result of the vaccine.

Peptide modifications may improve their immunogenicity profile. Post-translational modifications can be processed and presented and recognized by T cells, including tumor-specific phosphorylated peptides (Zarling et al., 2000). Phosphorylated peptides have demonstrated immunogenicity in humans (Engelhard et al., 2020). The addition of an amphiphilic tail to immunizing peptides enables binding to endogenous albumin, which allows for greater trafficking to the lymph nodes (Liu et al., 2014). Elicio Therapeutics demonstrates that this approach is feasible and safe in the context of an "off the shelf" vaccine targeting mutant KRAS epitopes (Pant et al., 2024). Alternatively, the addition of hydrophobic amino acid sequences to the flanks of peptide antigens results in formation of "self-assembling nanoparticles" (Lynn et al., 2020). Preclinical studies suggest that these modifications may have a dramatic effect on the magnitude of T cell responses generated in vivo due to the size and structure of the resulting nanoparticles, enabling efficient uptake by APCs (Baharom et al., 2021).

Viral vectors

Viral vectors continue to be explored for cancer vaccines. PROSTVAC, which leverages a modified poxvirus, failed in a phase III trial in metastatic castration-resistant prostate cancer. However, its safety, immunogenicity, and epitope spreading track record provide a rationale for the development of similar viral vector vaccines encoding multiple shared and/or NeoAg (Sater et al., 2020). Adenoviruses like Ad5 also have a strong safety, immunogenicity and stability record (Butterfield et al., 2008; Butterfield et al., 2014; Butterfield et al., 2019). Great ape adenoviral vectors are under evaluation in the clinic and are of interest given their large DNA cargo capacity, allowing for the inclusion of many NeoAg in the same vaccine. Nouscom and Gritstone are investigating adenoviral vector vaccines with heterologous boosting strategies employing Modified Vaccinia Ankara (MVA) and self-amplifying RNA, respectively. Nouscom recently published results from their personalized NeoAg vaccine trial in combination with anti-PD-1 in metastatic NSCLC and melanoma. Among 5 patients receiving both MVA and adenovirus encoding up to 60 NeoAg, both CD8+ and CD4+ T cell responses were generated with mean pooled ex vivo ELISPOT of ~700 spot forming units per million cells (SFU) (D'Alise et al.,

2024). Similar results were observed with Gritstone's trial treating microsatellite stable colorectal cancer in combination with anti-PD-1, with patients achieving moderate to high magnitude ex vivo ELISPOT responses (Palmer et al., 2022). Despite immune responses, the GRANITE trial failed to achieve their primary endpoint of circulating tumor DNA (ctDNA) reduction compared to a chemotherapy control arm.

DCs

As the most potent APC, DCs have been used to both stimulate and shape antitumor T cell responses since 1995 (Mukherji et al., 1995; Hsu et al., 1996). The early studies were necessarily in late stage cancer patients and targeted shared, non-mutated antigens as well as uncharacterized tumor lysates (Banchereau et al., 2001; Butterfield et al., 2003). A meta-analysis of vaccine platforms found that DCs were superior to other forms of antigen delivery used at the time (Rosenberg et al., 2004). The field has struggled to improve on a 7% response rate in late stage disease, and substantial effort has been spent on isolation of specific circulating DC subsets, modification of the DCs through maturation cocktails, culture conditions and genetic engineering (Arthur et al., 1997; Butterfield et al., 2019). As an autologous personalized cellular product, the cells are highly variable. Cells from late stage patients may appear phenotypically acceptable, yet be metabolically skewed and have functional defects in key antigen presentation capabilities (Arthur et al., 1997). Nonetheless, clinical trials testing DC vaccines have shown some successes, as a recent randomized trial in glioblastoma indicated (Liau et al., 2023).

DNA plasmids

DNA plasmids encoding NeoAg can be delivered via injection and subsequent in vivo electroporation. Trials have shown that shared antigens encoded in plasmid DNA can be immunogenic and a specific $CD8^+$ phenotype increased by the vaccination was significantly correlated with survival in patients with pancreatic cancer (Vonderheide et al., 2021). Geneos recently published results in HCC demonstrating that the combination of pembrolizumab with up to 40 DNA-encoded NeoAg and IL-12 resulted in an objective response (OR) rate of 30.6%, greater than the historical OR rate for pembrolizumab monotherapy (12–18%) (Yarchoan et al., 2024). A greater number of distinct NeoAg targets were recognized by T cells following vaccination with pooled ex vivo peripheral blood ELISPOT responses ranging from ∼-0–600 SFU per patient, highlighting the immunogenicity of this platform. However, another recently published study of DNA plasmid vaccines in triple-negative breast cancer only generated low magnitude T cell responses observable after in vitro stimulation, but not ex vivo (Zhang et al., 2024). Unlike the Geneos trial, these vaccines were not given in combination with either plasmid-encoded IL-12 or anti-PD-1 antibodies, suggesting that combination therapy may be critical for plasmid DNA vaccine immunogenicity.

mRNA LNPs

After decades of research leading to the highly effective mRNA vaccines during the SARS-CoV-2 pandemic, there has been great interest in applying this platform to NeoAg therapeutics. Data from trials from BioNTech and a collaboration between Merck and Moderna demonstrate the immunogenicity of mRNA NeoAg vaccines in various tumor types. In contrast to the low frequencies of vaccine-induced $CD8^+$ T cells generally detectable in blood following immunization with peptides, mRNA vaccines such as BioNTech's autogene cevumeran have generated NeoAg-specific $CD8^+$ T cell responses comprising 1–5% of the circulating repertoire in patients with pancreatic cancer, gastric cancer, and triple-negative breast cancer (Lopez et al., 2025).

In addition to impressive immunogenicity data, signs of clinical efficacy are emergent in mRNA vaccine trials. In a cohort of patients with pancreatic cancer treated in the adjuvant setting, 8/16 patients mounted a measurable immune response to mRNA vaccination (Rojas et al., 2023). After a median follow-up time of 3.2 years, the median RFS for the 8 patients without detectable immune responses was 13.4 months, while only 2/8 patients with detectable immune responses recurred at the same time (median RFS not reached) (Sethna et al., 2025). In Merck and Moderna's phase 2b study in resected melanoma, in which patients were randomized 2:1 to receive the individualized neoantigen therapy (INT) mRNA LNP with pembrolizumab vs. standard of care pembrolizumab, the combination with the INT significantly prolonged RFS compared to pembrolizumab alone (Weber et al., 2024). The treatment expanded T cells to immunizing epitopes with evidence of de novo immune responses (Gainor et al., 2024).

Given these recent examples of apparent clinical benefit in mRNA cancer vaccine trials, it is possible that mRNA vaccines may provide advantages over previously tested vaccine platforms. To promote a $CD8^+$ T cell response, SLPs must be taken up by mature DCs and processed into short peptides presented on MHC-I (Bijker et al., 2008). Given their low molecular weight, injected SLPs are likely rapidly cleared from the injection site, making this process inefficient. Conversely, mRNA LNPs injected intramuscularly in preclinical models promote a local inflammatory response and antigen translation for up to 10 days, as well as efficient antigen expression in draining lymph nodes (Lutz et al., 2017; Pardi et al., 2015; Blizard et al., 2025). This combination of local inflammation and prolonged antigen expression may better mimic natural infection and therefore promote an optimal downstream immune response. The generation of a high magnitude NeoAg-specific $CD8^+$ T cell response in the recent BioNTech trials seems to correlate with readouts of efficacy—in a recently published follow-up study to the pancreatic cancer trial, the first 2 patients to recur among 8 with measurable T cell responses were those with the lowest cumulative frequency of NeoAg-specific T cell clones over the follow-up window (Sethna et al., 2025). Among the 6 patients without relapse, frequencies of NeoAg-specific $CD8^+$ T cells remained elevated in the periphery for years following initial treatment and retain effector functionality, suggesting mRNA vaccination may lead to durable immune surveillance. Both of these promising trials were also conducted in the adjuvant setting and in combination with ICB therapy, highlighting the importance of disease setting selection and combination therapies, both topics which we will cover further in this review.

Selection of optimal vaccine adjuvants

Delivery of immunogenic peptide antigens alone is not sufficient to initiate a productive T cell response. Activation of the innate immune system via pattern recognition receptors (PRRs) promotes the maturation of APCs by modulating their capacity for antigen presentation, costimulation, and migration to secondary lymphoid organs. Vaccine adjuvants include both synthetic PRR agonists and formulations that promote the delivery of vaccine antigens to APCs such as by forming a local depot of antigen at the injection site.

FDA-approved adjuvants for infectious diseases include aluminum salts, cytosine phosphoguanine (CpG 1018), saponins (Matrix-M), oil-in-water emulsions (MF59), and formulations with monophosphoryl lipid A (MPLA), a synthetic analog of lipopolysaccharide (LPS). However, these adjuvants have been primarily developed and tested in the context of vaccines for infectious diseases where eliciting a strong neutralizing antibody response may be sufficient to promote protection from pathogens. Therefore, alternative adjuvant strategies may be better suited for the primary goal of cancer vaccines, which is to elicit a sustained and polyfunctional T cell response. Indeed, different PRR agonists can have distinct effects on induced T cell responses. For example, a preclinical study in mice found that animals immunized with a DC vaccine adjuvanted with MPLA had reduced memory $CD8^+$ T cell formation relative to those immunized with LPS as an adjuvant, despite both adjuvants triggering the toll-like receptor 4 (TLR4) pathway and promoting a similar expansion of effector-like cells following vaccination (Cui et al., 2014).

To date, the majority of SLP cancer vaccine trials in the clinic have employed poly inositic-polycytidylic acid/poly L-lysine (Poly-ICLC), a synthetic double stranded RNA adjuvant that stimulates toll-like receptor 3 (TLR3) and MDA5. In an effort to enhance the potency of SLP vaccines, a recently published trial administered vaccines with Poly-ICLC and montanide, an oil-in-water emulsion adjuvant, to provide both PRR stimulation and antigen depot effects (Blass et al., 2025). Compared to historical data, the authors reported an increase in the magnitude of ex vivo T cell responses measured following vaccination as well as a relative increase in the frequency of NeoAg-specific $CD8^+$ T cells. However, in addition to the administration of two adjuvants, patients in this trial were also treated with local ipilimumab and systemic nivolumab—therefore, any differences in observed T cell responses cannot be attributed to this adjuvant combination alone.

mRNA vaccines contain immunostimulatory components inherent in their formulation that act as adjuvants. In the case of mRNA LNP vaccines, the lipids encapsulating the mRNA payload have distinct adjuvant effects. Studies in mice demonstrate that empty LNPs can enhance the activity of protein subunit vaccines by promoting T follicular helper cell and B cell responses in an IL-6 dependent manner (Alameh et al., 2021). Another study in mice demonstrated that adjuvant activity of mRNA LNP vaccines was dependent on MDA5, a viral RNA sensor, suggesting that byproduct RNA species encapsulated within LNPs may also contribute (Li et al., 2022). A recent study screened distinct LNP formulations to identify those capable of enhancing the activity of therapeutic effects of cancer vaccines (Zhu et al., 2024). Interestingly, the authors demonstrated that LNPs capable of triggering both cellular and humoral Th2 responses, in addition to Th1 responses, were superior in their ability to limit tumor progression in preclinical models. In addition to tuning the chemistry of LNPs, synthetic small molecule adjuvants may be chemically conjugated to LNPs to promote additional adjuvant mechanisms (Han et al., 2023). Efforts to supplement the native adjuvant activities of mRNA vaccines may result in further enhanced antitumor efficacy.

Selecting tumor types and disease stages for success

It is apparent that FDA-approved immunotherapies such as ICB and TIL therapy have higher OR rates in certain types of cancer. In particular, highly mutated forms of cancer such as melanoma appear to respond best to these immunotherapies, owing to an abundance of antigens available to the immune system and the resultant endogenous priming of T cell responses (Cristescu et al., 2018). While cancer vaccines promise to promote effective T cell responses even in lower mutational burden tumors, whether certain tumor types may respond better than others to these therapies remains to be seen, as signs of clinical efficacy are limited at this time. To date, NeoAg vaccines have demonstrated the capacity to generate functional T cell responses across a range of cancers, including melanoma, RCC, HCC, CRC, NSCLC, pancreatic cancer, glioblastoma, and glioma. While NeoAg vaccines may conceptually promote effective immunity across multiple cancer types, the benefit may be more apparent in certain tumor types with implications for clinical trial design. For example, the efficacy signal in BioNTech's pancreatic cancer trial is suggestive despite its single-arm design, given the historical low response rate to ICB and other immunotherapies as well as the short RFS window following surgical resection (Rojas et al., 2023). Conversely, tumor types with longer RFS periods following standard of care treatment will pose a greater challenge for demonstrating efficacy without randomization.

It is equally critical to identify the optimal point of intervention for cancer vaccines. Given that tumor tissue is a prerequisite for the identification of patient-specific NeoAg, truly prophylactic cancer vaccines are limited to efforts targeting recurrent tumor alterations and other shared antigens. Furthermore, prophylactic cancer vaccines such as the human papillomavirus vaccine differ in that they prevent tumorigenesis via protection from an oncogenic virus, a mechanism dependent on the development of neutralizing antibodies as opposed to cytotoxic T cells. Most trials of therapeutic cancer vaccines to date have treated patients in late stage disease, typically in patients failed by standard of care and often in combination with ICB. Preclinical murine models suggest that treatment with cancer vaccines may improve the efficacy of ICB in the setting of established tumors (Liu et al., 2022; Dolina et al., 2023). Therefore, several trials have investigated the combination of ICB and cancer vaccines in the advanced disease setting in tumor types known to respond poorly to ICB alone. Unfortunately, there have been limited signs of improved efficacy in these trials relative to

historical response rates. This may be due to the fact that advanced stage cancers are more likely to exhibit many of the hallmark features associated with immunotherapy failure, including intratumoral heterogeneity and loss of HLA or NeoAg among fractions of tumor clones (McGranahan et al., 2016, 2017), mutations in IFN signaling genes (Shin et al., 2017), exhausted T cells (Chow et al., 2022), and a tumor microenvironment comprising immunosuppressive cell types (Kieffer et al., 2020; Tay et al., 2023; Lasser et al., 2024) and soluble factors (Mariathasan et al., 2018; Tauriello et al., 2018; Lacher et al., 2024; Morotti et al., 2024). Furthermore, advanced stage cancer patients may also exhibit systemic immunosuppression resulting from many first-line therapies such as radiation and chemotherapy.

Recent trials have investigated treatment in the adjuvant setting with the goal of prolonging RFS. In contrast with advanced disease stages, intervention in the adjuvant setting may harness an intact immune response and avoid established and immunosuppressive tumor microenvironments. Long periods of RFS and OS have been described in pancreatic cancer, melanoma, and RCC (as discussed above) (Hu et al., 2021; Braun et al., 2025; Sethna et al., 2025). These results suggest that cancer vaccines may be best positioned to prevent recurrence by eliminating residual disease following surgery as opposed to augmenting responses to ICB in late stage disease. Indeed, trials have been published investigating BioNTech's mRNA vaccine autogene cevumeran in the adjuvant setting and in patients with advanced cancers. Despite inducing similarly high frequencies of NeoAg-specific $CD8^+$ T cells in a subset of patients investigated with advanced cancers, few of these patients experienced durable clinical benefit.

Monitoring immunity to cancer vaccines

As cancer vaccines are evaluated in the clinic, it is critical to monitor resulting T cell responses, mechanisms of tumor recurrence, and additional biomarkers associated with clinical response. While assays such as longitudinal ex vivo ELISPOT and flow cytometric analysis of T cell activation remain standards in the field for rapid assessment of post-intervention T cell responses, single-cell peripheral blood mononuclear cells (PBMC) and TIL RNA sequencing approaches have enabled unprecedented tracking of the phenotype and clonal repertoire of T cells following immunotherapy intervention. The challenge is confirming the tumor antigen specificity of the TCR sequences, which can be accomplished by TCR reconstruction studies in the laboratory. Specific T cell clones that expand following vaccination can be tracked across time and tissues, permitting quantitative and qualitative assessments that may associate with clinical response. In the previously mentioned study of mRNA vaccines in patients with pancreatic cancer, the abundance of vaccine-expanded T cell clones over time appeared to correlate with recurrence; of 8 patients with verified $CD8^+$ T cell responses following vaccination, 2 patients with the lowest frequency of circulating vaccine-associated T cells recurred while the rest remain free of disease (Sethna et al., 2025). In another recent study of mRNA vaccines, progressive disease was associated with a loss of detectable circulating NeoAg-specific T cells in a patient with gastric cancer and another with triple negative breast cancer (Lopez et al., 2025).

As the cost of T cell repertoire sequencing continues to fall, TCR reconstruction and functional testing in the context of cancer vaccine trials has become increasingly common. A library of synthetic TCRs may be generated from a limited blood volume, enabling analysis beyond what is possible with standard methods. Synthetic TCRs can be expressed in primary donor T cells or engineered reporter T cell lines and used for medium-to high-throughput screening assays to determine their specificities (Cetin et al., 2024; Kuilman et al., 2025; Moravec et al., 2025a; Moravec et al., 2025b). TCR functional affinity and cross-reactivity, as well as the number of distinct clonotypes generated against a given antigen, each may provide useful biomarkers of patient response to cancer vaccines. TCR reconstruction studies in the BioNTech trial of NeoAg vaccines in pancreatic cancer identified a correlation between TCR cross-reactivity and clonal half-life, suggesting that clones with a higher relative affinity for the NeoAg compared to wildtype peptide may have relatively reduced persistence in the periphery following priming doses (Sethna et al., 2025). Another recent study suggests that TCRs with higher structural avidity, measured as the dissociation kinetic of a given TCR and its target peptide-MHC antigen complex, preferentially reside within tumors, providing another potential biomarker of vaccine-induced T cells (Schmidt et al., 2023).

Biomarkers of response to cancer vaccines

Biomarkers in PD-1 blockade have been established, and include tumor mutation burden (TMB) (Cristescu et al., 2018), circulating ctDNA, tumor cell PD-L1, tumor lymphocytic infiltrate, and interferon gene expression profile (NanoString). These related aspects of tumor biology (high TMB leading to greater opportunity for T cell activation and tumor infiltration leading to interferon-related gene activation and PD-L1 upregulation) signify tumors more likely to respond to multiple types of immune-based therapies. These tumor biomarkers have also been tested as correlates of response and patient stratification in recent vaccine and INT trials. Interestingly, patient subset translational analyses suggest INT + pembrolizumab may benefit a broad patient population irrespective of the status of PD-L1, TMB, ctDNA, and HLA heterozygosity (Weber et al., 2024).

More exploratory biomarkers are also under investigation, including circulating antibodies. While our understanding of the role of tumor-specific antibodies remains incomplete, a recent study of SLP cancer vaccines evaluated the emergence of antibodies specific to the synthetic NeoAg peptides used for immunization. While 12/13 patients evaluated demonstrated the emergence of NeoAg-specific circulating IgG, there was minimal correlation between the NeoAg bound by IgG and concurrently measured T cell responses (Saxena et al., 2025). While autoimmunity development is a known toxicity associated with many forms of immunotherapy, broad development of auto-antibodies (AutoAb) to self-proteins is an emerging prognostic biomarker. Melanoma patients can have thousands of AutoAb detectable in

the circulation, compared to ~200 in healthy donors (unpublished data). The ability to perform broad screens with both native protein shapes (REAP and others) and phage immunoprecipitation sequencing (PhIPseq) makes this area technically tractable for exploration of reactivities of AutoAb as a way to test for epitope spreading and other tumor-specific responses.

Combination approaches to improve outcomes

Multiple combination approaches to enhance the potency and clinical efficacy of cancer vaccines are under investigation. The goal in these cases is for the additional therapy to enhance the activation of T cells targeted by the vaccine and/or promote complementary mechanisms, such as broadening the T cell response to include additional antigens or modulating the activity of other relevant cell types. Many cancer vaccine trials in the last 10 years have incorporated antibodies that block either PD-1/L1 or CTLA-4 with the antigen delivery platform (Ott et al., 2020; Rojas et al., 2023; Weber et al., 2024; Braun et al., 2025; Blass et al., 2025). ICB with an anti-PD-1/L1 is meant to amplify endogenously primed and vaccine primed T cell responses. Recent data from BioNTech's pancreatic cancer trial suggests that T cell clones expanding following treatment with anti-PD-L1 are largely distinct from those expanding following vaccination and more likely consist of clones already found within the tumor, highlighting the logic behind this combination (Sethna et al., 2025). Anti-CTLA-4 has been utilized to diversify antigenic responses and promote epitope spreading, and is also a logical choice for a vaccine combination. The concerns come from single-arm combination trials where one of the agents (the anti-PD-1/L1) has known clinical activity, making discernment of the impact of the vaccine component challenging to impossible to identify. For smaller academic and biotech company trials, two-arm trials with patients randomized to one ± both can be financially untenable. Single-arm combinations in settings where the ICB therapy has minimal efficacy can circumvent some of this concern.

When delivering tumor antigens, increasing activation of APCs and T cells via costimulation is also a reasonable combination. Many costimulatory molecules have been well characterized for their ability to promote T cell activation and quality, including CD28, ICOS, OX40, and CD40. These important molecules trigger critical pathways in APCs and T cells in vitro and in preclinical models, but agonist antibodies targeting these pathways have yet to be efficacious in clinical trials (Lim et al., 2024). Optimal dose and schedule remain a critical challenge.

The addition of cytokine support to act as a growth factor for T cells (IL-2, IL-15) or as a trigger for skewing T cell responses towards type 1 (IL-12p70) are common strategies to augment T cell responses. Systemic delivery of these cytokines has significant toxicity, and targeting an optimal amount at the best time and location has been challenging. Efforts to harness these molecules and pathways is ongoing, including the development of targeted immunocytokines (Codarri Deak et al., 2022; Kaptein et al., 2024; Moynihan et al., 2024) and conditionally active-cytokines (Hsu et al., 2021; Mansurov et al., 2022), both of which are intended to limit systemic and off-target activity associated with toxicity while preserving antitumor function. While immunocytokines and conditionally-active cytokines may promote optimal cytokine signaling within the tumor, local administration of certain cytokines alongside vaccination may selectively benefit vaccine-induced T cells. For example, co-delivery of mRNA or DNA encoding IL-12 alongside tumor antigens may promote the priming and Th1 phenotype of vaccine-induced T cells (Aunins et al., 2025; Yarchoan et al., 2024).

Conclusions and future perspectives

Recent advances in our understanding of effective immunity against cancer, enabled by maturing preclinical and clinical trial data and novel translational science methodologies, provide critical support for the continued development of efficacious cancer vaccines and NeoAg therapeutics. Tumor NeoAg derived from somatic mutations can be readily identified and early signs of clinical efficacy in immunotherapy-resistant tumor types and standard of care controlled two-arm trials suggest targeting these antigens, particularly in the adjuvant setting, is a strategy showing improved outcomes (Rojas et al., 2023; Weber et al., 2024; Sethna et al., 2025). Approaches to improve the accuracy of in silico NeoAg prediction models are ongoing. In addition, cryptic tumor-specific antigens have been discovered which may outnumber traditional NeoAg and/or provide antigen targets more likely to be shared across patients (Apavaloaei et al., 2025; Ely et al., 2025). Trials investigating the efficacy of targeting such antigens are in development. A breadth of preclinical and clinical data suggests that it is likely beneficial to include tumor-specific epitopes recognized by $CD4^+$ T cells as opposed to heterologous helper epitopes, highlighting a need for complementary antigen identification platforms specifically for HLA class II.

Recent clinical results suggest that the efficacy of cancer vaccines may be most apparent in the adjuvant setting rather than in advanced disease cases. Therefore, future studies comparing immunization platforms and therapeutic combinations may be most likely to yield meaningful data when assessed in patients following surgery; in general, intervention with immunotherapies in earlier disease stages before a mature, immunosuppressive tumor microenvironment is established appears broadly beneficial. Advances in techniques used to monitor T cell immunity and tumor immune evasion may provide an opportunity to intervene with additional therapies in cases where relapse appears likely due to, for example, changes in T cell response characteristics or detection of increased ctDNA. Tracking tumor antigen-specific T cells molecularly, by TCR sequencing, may further enable understanding the dynamics of antitumor immunity. We remain optimistic that targeting the right tumor-specific antigens with the most effective immunization platforms will lead to further meaningful efficacy for patients.

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