



PERSPECTIVE

Immunological and metabolic optimization of tumor neoantigen vaccines

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Tumor initiation and progression are highly intricate biological processes, and mutation-driven tumorigenesis is a primary underlying cause. Personalized cancer vaccines have been developed to exploit these specific mutations, particularly in the form of tumor neoantigens, to induce immune responses, particularly the activation of CD8⁺ T cells, which can attack malignant cells. Since tumor mutations result in protein sequence alterations distinct from those in normal tissues, therapies that precisely target these alterations could, in principle, confer effective tumor control while minimizing off-target effects.

Neoantigen-based therapies have garnered considerable scientific and clinical attention in recent years, as evidenced by more than 100 active or completed clinical trials. To date, a diverse array of neoantigen-targeting vaccine platforms have advanced to clinical trials, including peptide, DNA, RNA, and viral vector-based vaccines, each with their own advantages and limitations (Table 1). Notably, more than 50% of these trials have used synthetic long peptides (SLPs) in combination with adjuvants, typically poly-ICLC. Although these platforms can induce CD4⁺ T cell immune responses, their inherently low immunogenicity for CD8⁺ T cells remains a major challenge. Typically, neoantigen-specific CD8⁺ T cells are either undetectable or present at very low frequencies, accounting for 0.1% of the total CD8⁺ T cells in the peripheral blood. This proportion is far below the 10%–20% rate achievable

with highly immunogenic vaccines such as the yellow fever and vaccinia vaccine¹. Although the magnitude of CD8⁺ T cells is not the sole factor determining clinical outcomes, it is foundational to therapeutic efficacy. Sufficient CD8⁺ T cells are necessary for the effectiveness of other therapies aimed at reversing exhaustion and the immunosuppressive tumor microenvironment (TME).

This Perspective reviews current advances and ongoing challenges (Table 2) in the development of tumor neoantigen vaccines. Specifically, the efficacy of tumor vaccines is influenced by factors within the TME, such as metabolism, whose crucial role has been progressively elucidated in recent years; this aspect is also summarized and discussed. Finally, strategies for enhancing tumor neoantigen vaccines are described (Figure 1).

Sources of neoantigens

Tumor neoantigens originate primarily from mutation-dependent tumor antigens. Because of tumors' intrinsic heterogeneity, substantial variability exists in neoantigen expression, thus leading to inconsistent neoantigen presentation. Therefore, the identification of so-called "trunk mutations" that prominently control tumor proliferation has become critical. However, a major challenge arises from the high similarity between these trunk mutations and their corresponding wild-type sequences. During T cell development, neoantigen-specific T cell receptors (TCRs) often exhibit inherently low affinity for such neoantigens with minimally altered peptide sequences due to negative selection. Even when high-affinity TCRs do exist, the absolute abundance of such T cells remains extremely low². This mechanistic insight helps explain why neoantigen-specific CD8⁺ T cell responses are often undetected or are observed at only very low levels in patients, although the possible induction of immune responses against such antigens may occur.

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Table 1 Comparison of neoantigen vaccine platforms

Vaccine platform	Advantages	Limitations
Peptide	Simple and convenient production. Easy to modify and functionalize. High safety and low cost.	Poor immunogenicity, highly dependent on the adjuvant and delivery system.
DNA	Rapid production and low cost. Potential for cellular immune response induction.	Risk of genomic integration. Less effective in nonhuman primates and clinical studies than in mice.
RNA	Rapid development and scalable production. Elicits both humoral and cellular immune responses.	Poor stability, requiring delivery systems.
Viral vector-based	Strong immunogenicity, particularly for CD8 ⁺ T cell immune responses.	Vaccine efficacy potentially limited by pre-existing immunity to the vector. Potential risk of insertional mutagenesis, depending on the vector type.

Table 2 Obstacles to therapeutic neoantigen vaccine development

Considerations	Obstacles
Antigens	Because of tumor heterogeneity, screening for key trunk mutations is difficult. Furthermore, tumor-mutated antigens are highly similar to self-antigens, thus resulting in minimal immunogenicity. Some cryptic antigens can serve as sources of neoantigens, but they exhibit substantial expression variability and insufficient immunogenicity, and consequently are prone to immune escape.
Prediction methods	Bioinformatics-based prediction methods can quickly obtain epitope information, but due to the influence of complex processes, such as the formation of defective ribosomal products and antigen presentation, current predictions have limited accuracy. Experimental methods such as transcriptomics and immunopeptidomics can improve accuracy, but they remain affected by issues such as complex detection procedures and low detection abundance.
Adjuvants	Inducing CD8 ⁺ T cell immune responses is difficult. Conventional adjuvants have limited effects on enhancing antigen cross-presentation by DCs.
Delivery systems	Some delivery systems cannot effectively reach immune-inducing sites, such as lymph nodes and spleen, thus resulting in limited T cell activation and expansion. Furthermore, accumulation of delivery materials in the liver raises safety concerns.
Metabolic factors	Tumor metabolic reprogramming decreases antigen presentation and causes problems such as an immunosuppressive TME and T cell exhaustion.

Beyond mutation-driven antigens, an alternative source of tumor neoantigens comes from mutation-independent mechanisms involving antigens derived from non-coding genomic regions. Their generation can occur through various pathways, such as the translation of circular RNAs, initiation from selective non-AUG start codons, and ribosomal frameshift errors³. Because some of these antigens exhibit substantial tumor specificity, they might potentially provide a rich source for novel immunotherapeutic targets. Notably, a recent study has revealed that such cryptic neoantigens can elicit effective cytotoxic responses against pancreatic cancer organoids⁴. However, notable challenges lie in the heterogeneous expression of these non-canonical antigen epitopes across tumor clones, coupled with their dispensability for tumor cell survival. These characteristics render them inherently susceptible

to immune editing and facilitate vaccine escape in the absence of robust epitope spreading. Consequently, although targeting this “dark proteome” offers compelling opportunities, its overall clinical utility remains to be rigorously validated.

Neoantigen identification and design

The advent of next-generation sequencing technologies has made large-scale genetic analysis of individual tumors and patients feasible. Coupled with advances in whole-exome sequencing and RNA sequencing, a wide range of bioinformatics pipelines and MHC-peptide binding prediction algorithms have been developed for the prospective identification of tumor neoantigens. Some predictive tools integrate multiple considerations, such as antigen cleavage, antigen transport,

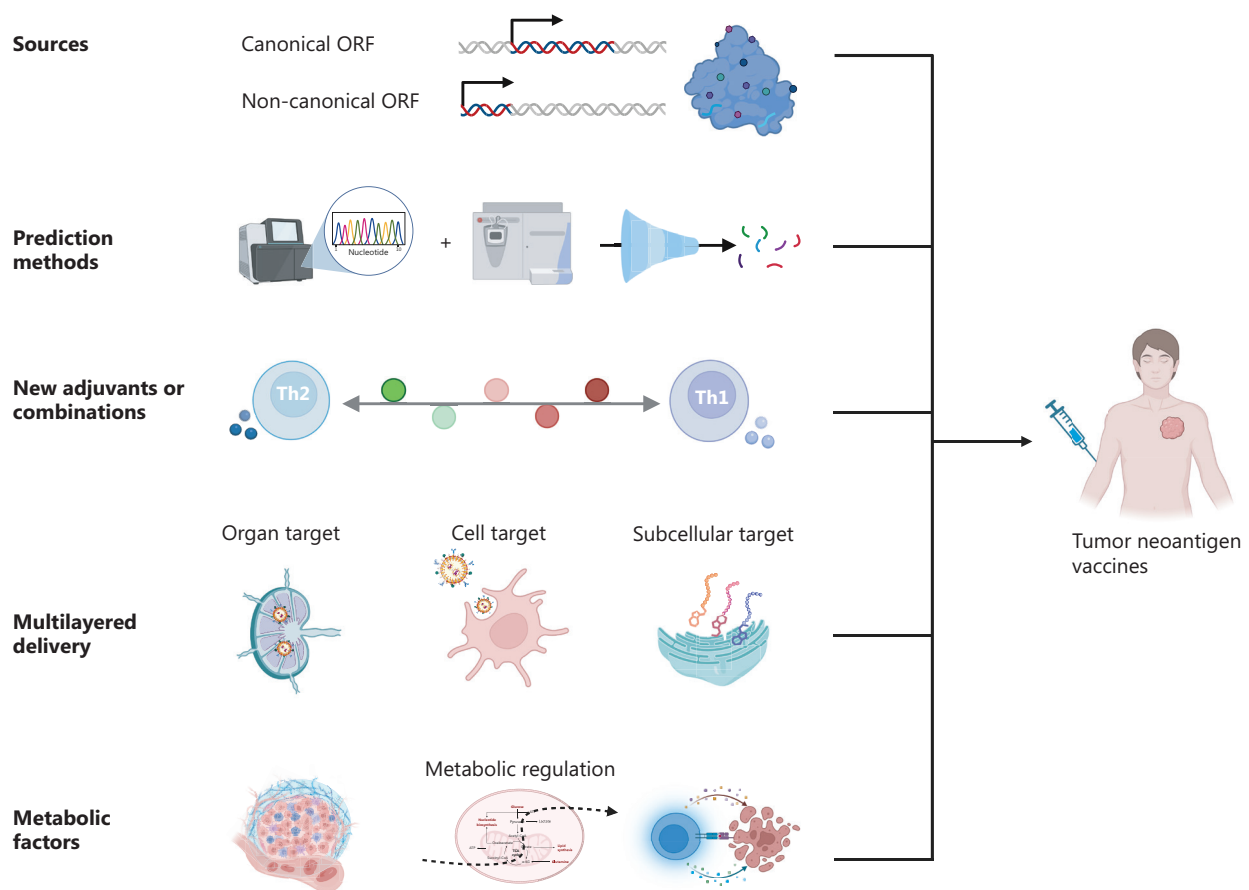


Figure 1 Strategies to overcome the low immunogenicity of neoantigen vaccines focus on 5 key areas: antigen design, prediction methods, adjuvant development, delivery system optimization, and metabolic regulation. 1) Target trunk mutations, or shared canonical or non-canonical antigens, optimize antigen length and screen neoantigens to induce epitope spreading. 2) Whole exome sequencing (WES), ribosome profiling (RiboSeq), immunopeptidomics, and mass spectrometry-based detection methods are integrated to screen for preferable neoantigens. 3) Novel multifunctional adjuvants or combinations of existing adjuvants are developed. 4) The composition of the delivery system is optimized, targeted molecules and multi-level targeting are explored, and new delivery materials with high safety are developed. 5) Metabolic regulation promotes antigen presentation, and reverses tumor suppressor environments and T cell exhaustion. (Created with BioRender.com).

cellular presentation, and TCR recognition. In recent years, new artificial intelligence technologies have also been applied to enhance accuracy.

Despite these technical advancements, the practical effectiveness of these bioinformatics approaches remains limited. In some cases, less than 2.7% of predicted neoantigens are ultimately recognized by patient-derived T cells⁵. Similarly, the Tumor Neoantigen Selection Alliance (TESLA) global consortium has reported that, even with a comprehensive pipeline—starting with MHC binding prediction and followed by validation using patient-matched T cells—only approximately 6% of the predicted neoantigens elicit T cell recognition⁶.

These findings illustrate the limitations of current methods in tumor neoantigen prediction and highlight the urgent need for improved algorithms and validation frameworks.

The accuracy of neoantigen prediction depends not only on the sophistication of the computational algorithms but also critically on intrinsic biological factors pertaining to the tumor cells, antigen-presenting cells, and antigen processing preferences. Therefore, complementary approaches enabling direct integration of mass spectrometry immunopeptidomics data might provide better results. For instance, the NeoDis computational framework integrates data from genomic, transcriptomic, and immunopeptidomic sources to accurately identify

neoantigens. Among the 66 HLA-I neoantigenic peptides for T cell screening assays, 11 were found to be immunogenic⁷. Nevertheless, these direct detection methods face their own technical challenges, including immunopeptidome dilution due to non-tumor cells infiltration, the requirement for large quantities of tumor tissue or cells, and frequently low MHC class I expression on tumor cells. These challenges might be potentially overcome by strategies such as cultivating tumor organoids to enrich for homologous peptides or using methods to enhance MHC class I expression⁸.

Adjuvants for tumor neoantigen vaccines

Because of the inherently low immunogenicity of tumor neoantigens, administration of the antigen alone is generally insufficient to provoke robust immune response, thereby necessitating a combination of adjuvants. Certain vaccine platforms, such as plasmid DNA and mRNA-based vaccines, can partially function as self-adjuvants and boost the immune response, whereas SLP neoantigen vaccines require the addition of adjuvants to achieve effective immunogenicity. An adjuvant is an additive to vaccines that enhances the immunogenicity of antigens, common adjuvants include ligands of pattern recognition receptors, cytokines, and immune checkpoint inhibitors.

In recent years, extensive researches have focused on the roles of pattern recognition receptors, such as Toll-like receptors, and the cGAS-STING pathway. Ligands targeting these receptors are typically small molecules with well-defined mechanisms of action and favorable drug potential, and therefore highly promising for clinical translation. SLP neoantigen vaccines critically require dendritic cell (DC)-mediated antigen cross-presentation to effectively elicit CD8⁺ T cell immune responses. However, in the absence of adequate adjuvant support, mobilizing DCs to achieve potent T cell activation and expansion remains difficult. Inadequate DC activation can even lead to undesirable immune tolerance, thereby diminishing CD8⁺ T cell responses.

To circumvent this limitation, neopeptides are frequently co-administered with adjuvants. Nonetheless, the enhanced immunogenicity achieved through adjuvant addition is often modest and falls short of the robust and durable immune responses required for effective tumor control. This shortcoming arises from a predominant focus on the adjuvant's

immunostimulatory function, without adequately addressing the inherent inefficiency of the cross-presentation process itself. Crucially, because the cross-presentation process of exogenous antigens by DCs is inherently intricate and often inefficient, effectively presenting neoantigen epitopes is difficult. Therefore, to stimulate high-level specific CD8⁺ T cell immune responses to an extent comparable level to those induced by viral vaccines, not only adjuvant activity but also antigen cross-presentation by DCs must be optimized⁹.

Our recent study has offered new insights to address this important issue. We have developed a new series of STING agonists, collectively termed STING Agonist-Based ER targeting molecules (SABER), to not only potently activate DCs but also critically target antigens directly to the endoplasmic reticulum (ER). These characteristics depend on SABER's high binding affinity toward the STING protein, which resides within the ER, a critical hub for antigen processing. Our findings have demonstrated that after neoantigens delivered to the ER, they can form a localized "micro-reactor" that effectively promotes antigen cleavage and transport. This mechanism subsequently augments the specific CD8⁺ T cell immune response. Compared with conventional adjuvanted SLPs, SABER-based neoantigen vaccines induce tenfold greater CD8⁺ T cell induction. Our findings strongly suggest that the "last mile" of antigen delivery, specifically from the cytoplasm to the ER, is crucial for optimizing antigen cross-presentation¹⁰.

Delivery system for tumor neoantigen vaccines

Beyond the essential role of adjuvants, delivery systems are equally important for enhancing the T-cell immune response to neoantigens. In recent years, mRNA has attracted considerable attention, because of its rapid production and broad therapeutic scope across a diverse array of diseases. Despite its advantages, mRNA's inherent instability and negative charge present major obstacles to efficient cellular uptake and membrane translocation. Consequently, encapsulation and transport *via* lipid nanoparticles (LNPs) have become imperative. LNPs not only protect mRNA against rapid degradation *in vivo* but also notably target antigen-presenting cells, which have a propensity for nanoparticle phagocytosis. Moreover, by precisely modulating the lipid composition and physicochemical properties of LNPs, organ and tissue-specific targeting can be optimized¹¹. For instance, our research group

has successfully used biomimetic nanoparticles to encapsulate 2',3'-cyclic guanosine monophosphate-adenosine monophosphate, the natural ligand for the STING protein, thereby eliciting effective antigen-specific CD8⁺ T cell immunity in the lungs¹². Additionally, the use of polymeric materials or inorganic nanoparticles has also been demonstrated to effectively enhance CD8⁺ T cell induction. However, a key impediment is the safety profiles of these delivery systems, particularly concerns regarding off-target organ accumulation and the potential induction of undesirable nanoparticle-triggered antibody or inflammatory responses. Therefore, we developed an *in vivo* electroporation system as an alternative delivery system^{13,14}.

Metabolic effects on tumor neoantigen vaccines

Despite substantial progress in tumor neoantigen vaccines, some post-injection challenges must be addressed to achieve meaningful clinical outcomes. These obstacles include tumor immune evasion, immunosuppressive TME, and T cell exhaustion. Recent evidence has highlighted the critical roles of metabolic changes in shaping the immune landscape of neoantigen-based vaccines. These metabolic alterations affect key processes, such as antigen presentation in tumor cells and DCs, as well as the overall state of T cells.

The immunopeptidome is highly plastic and is modulated by cellular metabolic activity. For example, altering cellular metabolism *via* inhibiting the mammalian target of rapamycin (mTOR) results in dynamic changes in the composition of the immunopeptidome and leads to high abundance of MHC class I-presented peptides¹⁵. Furthermore, autophagy, a key metabolic process in the degradation and recycling of various cytoplasmic components, enhances antigen processing in antigen-presenting cells. Notably, active autophagy facilitates the delivery of cytosolic antigens to MHC class II loading compartments, thereby promoting CD4⁺ T cell responses to intracellular antigens¹⁶.

Metabolic shifts in tumors frequently result in a highly immunosuppressive TME. Accumulated lactate, a byproduct of aerobic glycolysis, inhibits DC functions which are essential for effective T cell priming. Moreover, lactate-driven acidosis destabilizes peptide-MHC class I complexes and impairs antigen presentation, particularly *via* the cross-presentation pathway¹⁷. Cross-presentation is an energy-intensive process sensitive to the metabolic fitness of DCs. Recent studies have

demonstrated that optimal cross-presentation requires finely tuned metabolic programs, including glycolysis, oxidative phosphorylation, and fatty acid metabolism¹⁸. Nevertheless, aberrant lipid accumulation in tumor-infiltrating DCs impairs their ability to present antigens, whereas pharmacological inhibition of fatty acid synthesis has been shown to substantially enhance the efficacy of cancer vaccines¹⁹.

In parallel, T cell metabolism, which underpins the effector function of CD8⁺ T cells responding to neoantigen vaccines, is also governed by nutrient availability within the TME. Notably, recent findings have revealed that lactate, rather than being merely immunosuppressive, also serves as a bioenergetic and biosynthetic fuel that supports CD8⁺ T cell activity in certain metabolic contexts²⁰.

Conclusion and future prospects

The identification and subsequent utilization of tumor neoantigens have led to paradigm shift in the understanding of malignancies and are highly promising avenue for cancer immunotherapy. Nevertheless, despite these bright prospects, substantial challenges persist. A major obstacle is that the high-level similarity between neoantigens and their wild-type counterparts often renders neoantigens poor immunogenicity and makes them concealed from the immune system. Consequently, stimulating comparably robust immune responses to those elicited by potent foreign substances, such as viruses, is inherently difficult.

Although bioinformatics approaches based on whole-exome sequencing and RNA sequencing have rapidly advanced, their predictive accuracy requires further refinement. Future developments are expected to rely on the seamless integration of machine learning and big data analytics to discern broader spectrum of epitope features, alongside the application of immunopeptidomics to more precisely identify antigen sequences. Moreover, ongoing advancements in mass spectrometry and organoid culture technologies are expected to facilitate the discovery of novel neoantigen epitopes.

Crucially, antigen optimization alone is insufficient, because the inherently low immunogenicity of neoantigens limits the host's ability to mount robust, antigen-specific CD8⁺ T cell responses. Consequently, optimization of adjuvants and delivery systems is imperative. Although many adjuvants and delivery systems have been developed to date, their overall efficacy remains constrained. The immunostimulatory bias inherent to various adjuvants requires evaluation; moreover,

most delivery systems function primarily at the organ or tissue level, and therefore fall short of achieving precise subcellular antigen delivery. Therefore, future efforts must focus on refining adjuvant structures and identifying more efficacious adjuvant combinations, also improving delivery systems that significantly enhance cross-presentation efficiency.

Furthermore, to overcome the overarching challenges inherent in tumor treatment, post-vaccination bottlenecks must be addressed. Metabolic reprogramming in tumor cells and the TME establishes a hostile immunological niche that adversely affects nearly every step involved in neoantigen vaccine efficacy, from antigen generation to cross-presentation and T cell activation. To fully harness the therapeutic potential of neoantigen-based cancer vaccines, these metabolic barriers must be overcome through rational combinatorial strategies that modulate both tumor-intrinsic and microenvironmental metabolic constraints.

In summary, substantial progress has been made in the identification of tumor neoantigens and the development of novel technologies to induce effective neoantigen-specific CD8⁺ T cell responses. However, multiple impediments persist, particularly regarding vaccine delivery and anti-tumor efficacy. Addressing these limitations through innovative and integrated strategies will be essential to fully achieve the clinical potential of neoantigen vaccines and deliver meaningful therapeutic benefits to patients with cancer.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

Conceived and designed the analysis: Jianfeng Huang, Ji Wang.
Collected the data: Xiaofeng Wang, Zhangping Huang, Lin Peng, Shuoxi Xu.

Wrote the paper: Xiaofeng Wang.

Reviewed and revised the paper: Jianfeng Huang, Ji Wang.

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