

Neoantigen-Based Cancer Vaccines: Recent Advances and Clinical Applications

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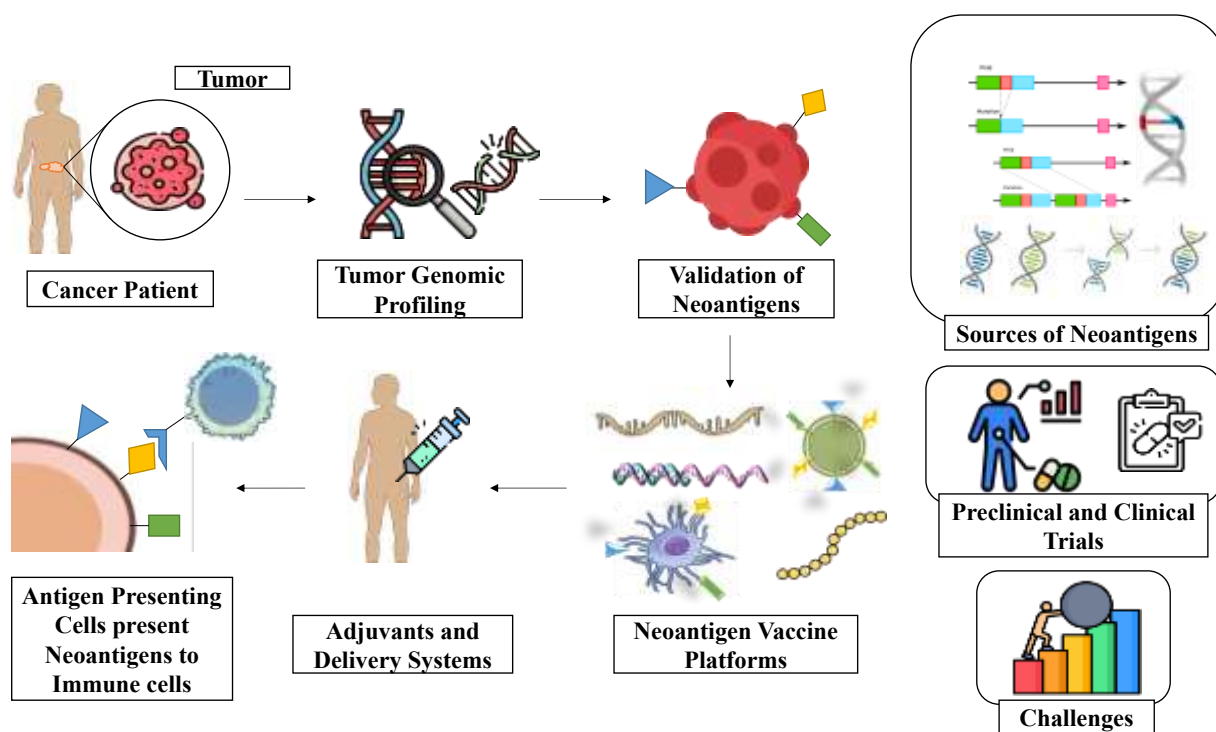
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Abstract

Cancer immunotherapy has emerged as one of the most promising modalities in the fight against malignancies. Among various therapeutic approaches, neoantigen-based vaccines have received considerable interest for their ability to deliver highly personalized treatments. Neoantigens are tumor-specific, mutated peptides resulting from somatic mutations in cancerous cells. They are identified by the immune system, positioning them as promising targets for immunotherapy. In this review, we explore the recent advances in the development of neoantigen-based vaccines, focusing on the strategies employed to identify, design, and deliver these vaccines. We also discuss the challenges faced in clinical application, including tumor heterogeneity, immune evasion, and vaccine delivery mechanisms. Lastly, we provide an overview of the clinical trials conducted to date and future directions on neoantigen-based cancer vaccines.

Graphical Abstract



Key Words

Neoantigens, Cancer vaccines, Personalized vaccines, T-cell receptors, Tumor-specific antigens, major histocompatibility complex

Background

Cancer remains one of the leading causes of both incidence and mortality worldwide. In 2020, there were 18.1 million new cancer cases and approximately 9.5 million cancer-related deaths [1]. Early-stage cancers are typically treated with surgery, radiation, and chemotherapy, while advanced stages often rely on palliative care [2]. Although these conventional treatments have shown some success, they are not ideal for cancers that have metastasized to areas where surgery is not feasible or when chemotherapy and radiation are not effective.

Cancer immunotherapies have emerged as a promising approach to overcome the limitations of traditional treatments [3]. One particularly promising strategy is the development of cancer vaccines that leverage the immune system's ability to target and treat tumors [4, 5]. Cancer cells can undergo various types of mutations, including point mutations, insertions/deletions, gene amplification, and translocations [5]. Some of these mutations result in non-synonymous somatic changes that alter the amino acid coding sequences, leading to the production of abnormal proteins that promote uncontrolled cell proliferation. The immune system might identify these altered proteins as foreign substances. Tumor-specific antigens, or neoantigens, result from genomic modifications like codon changes, editing, and differences in antigen processing and presentation [6, 7]. Neoantigens are presented on the cell surface by the major histocompatibility complex (MHC), also referred to as human leukocyte antigen (HLA) in humans, where they can be detected by T lymphocytes [5, 8]. Since neoantigens are specific to tumors and not found on normal cells, they represent ideal therapeutic targets [9]. Recent progress in neoantigen prediction methods, along with the integration of high-throughput omics technologies, has accelerated the creation of neoantigen-based vaccines [10].

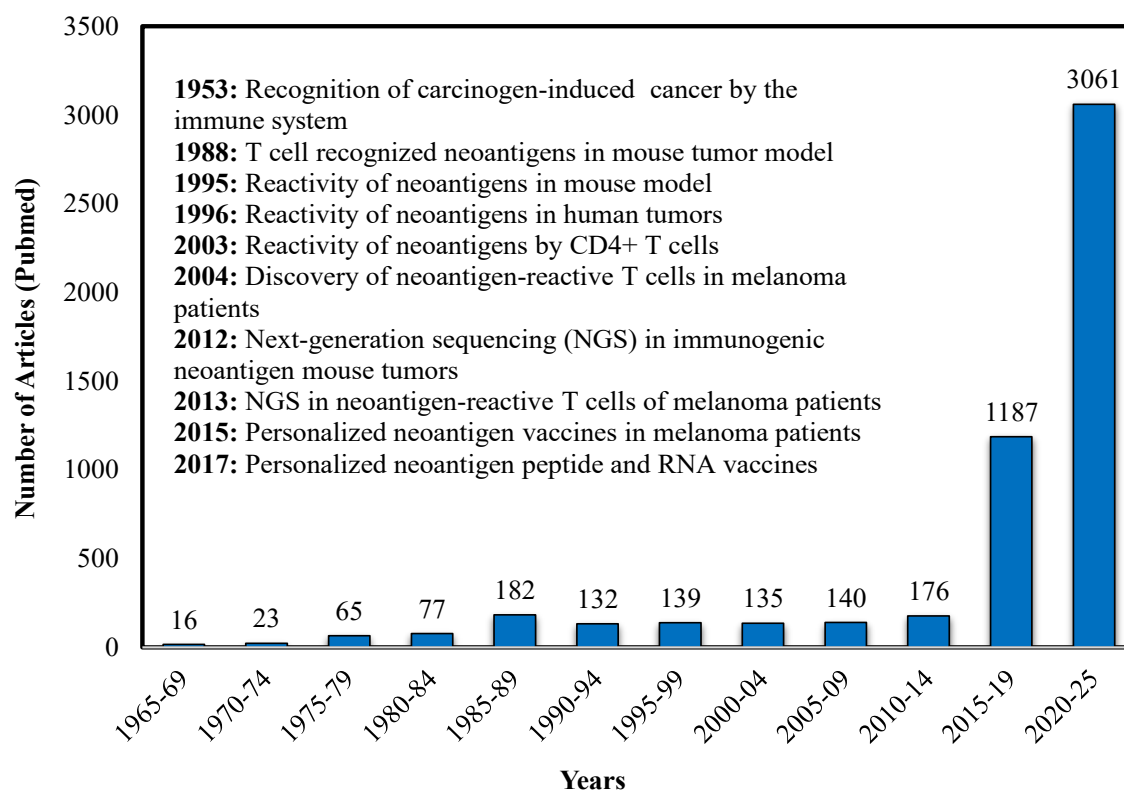


Fig. 1 Graph showing number of articles related to neoantigens published in Pubmed from the year 1965-2025. A historical analysis of tumor-specific neoantigens was performed by searching the PubMed database using the keyword "neoantigen." The publication count from 1965 to 2025 is displayed in a column chart.

Neoantigen-based cancer vaccines are designed to provoke a strong, targeted immune response against tumors by presenting these distinct, tumor-specific antigens to the immune system. Unlike traditional cancer vaccines that target shared tumor antigens, neoantigen vaccines are personalized, potentially reducing off-target effects and improving specificity [4, 11]. Recent advancements in next-generation sequencing (NGS) and bioinformatics have made it possible to identify and prioritize neoantigen candidates from a patient's tumor genome, facilitating the creation of customized cancer vaccines [12]. However, challenges remain in designing effective vaccines, optimizing delivery systems, and overcoming immune suppression within tumors. This review will explore these advances and challenges, as well as the current progress of clinical trials involving neoantigen-based vaccines.

Neoantigens: Identification and Sources

Neoantigens and Tumor Mutations

Cancer vaccine candidates should target tumor antigens that are highly expressed in tumor tissues. These antigens are classified as shared or personalized based on how frequently they appear [13, 14]. Shared antigens, or "public" antigens, contain common mutations found in patients with certain HLA alleles [15]. They target tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs). TAAs are autoantigens normally found in healthy tissues but are overexpressed in various cancers. These include cancer-testis antigens, tissue-differentiation neoantigens, and overexpressed antigens, as demonstrated in **Table 1**. In contrast, TSAs arise from somatic mutations, which can improve MHC presentation of antigen epitopes or alter T-cell receptor (TCR) recognition [5, 14, 16].

Table 1 Tumor-Associated Antigens (TAAs) versus Tumor-Specific Antigens (TSAs). [1]

Property	Tumor-Associated Antigens (TAAs)	Tumor-Specific Antigens (TSAs)
Present in Normal Cells	Yes	No
Present in Tumor Cells	Yes	Yes
Risk of Vaccine	High	Minimal
Examples	p53, Ras	HPV E6, E7, Her2/neu

Neoantigens are tumor-specific peptides present on proliferating tumor cells but absent in normal tissues [4, 17]. These peptides arise from various genetic alterations, with the type and frequency of mutations differing across cancer types. These alterations include point mutations, insertions or deletions (INDELS), and gene fusions or translocations, all of which alter protein sequences and generate novel epitopes that are absent in normal cells [18, 19, 20]. These neoantigens are displayed on the surface of tumor cells by MHC molecules, where they are detected by the TCRs of cytotoxic T lymphocytes (CTLs). This interaction triggers an immune response that specifically targets and destroys tumor cells expressing the neoantigen [21].

Neoantigen Identification: From Discovery to Validation

Tumor Genomic Profiling

Identifying neoantigens requires thorough tumor profiling to detect somatic mutations that produce tumor-specific peptides [22, 23]. The widespread use of high-throughput techniques, particularly NGS, along with significant cost reductions, has made it easier to identify somatic mutation profiles and predict neoantigens. The most widely employed NGS-based approaches for this purpose are whole-genome sequencing (WGS) and whole-exome sequencing (WES) in combination with RNA sequencing (RNA-seq) [8, 22]. While WGS provides a comprehensive view, WES offers a more cost-effective approach by focusing on the 2-3% of the genome related to protein-coding genes, which are the primary sources of somatic mutations leading to mutated proteins [24]. When combined with RNA-seq, which provides gene expression data, WES can help prioritize genes more likely to produce functional proteins and, in some cases, serve as a starting point for identifying somatic mutations [25].

We can identify possible neoantigens by analyzing somatic mutations found in tumor biopsies or circulating tumor DNA from blood samples. These mutations are prioritized according to their capacity to bind with MHC molecules, essential for antigen presentation [26].

Bioinformatics Tools for Neoantigen Prediction

Bioinformatic algorithms play a crucial role in identifying neoantigens. Several software tools have been developed to predict which mutated peptides are likely to bind to MHC molecules and be presented to T-cells [4] as given in **Table 2**.

Table 2 Different Bioinformatics Tools for Neoantigen Prediction

Tool	Description
NetMHC	This tool forecasts how peptides will bind to MHC class I molecules, applicable to both known and new peptide sequences. The binding of peptides to MHC molecules plays a crucial role in antigen presentation, and NetMHC assists in identifying which peptides are likely to bind effectively [27].
MHCflurry	MHCflurry is an open-source software tool developed to predict MHC class I binding affinities. Its versatile command-line and programmatic interfaces allow easy integration into high-throughput bioinformatics workflows [28].
MultiRTA	This tool accounts for all possible peptide binding core configurations. It computes the MHC-peptide binding affinity as a weighted average over all potential configurations, providing a more comprehensive prediction of binding potential [29].
nextNEOpi	nextNEOpi predicts class I and II neoantigens from Single nucleotide variations (SNVs), INDELs, and gene fusions by analyzing raw sequencing data, and identifies a set of features linked to tumor immunogenicity and responses to immunotherapy [30].
NeoPredPipe	NeoPredPipe efficiently offers summary information to both researchers and clinicians on predicted neoantigen burdens, while also providing valuable insights into tumor heterogeneity based on somatic mutation calls and, optionally, patient HLA haplotypes [31].

These tools are essential for predicting the likelihood of a mutated peptide binding to specific MHC molecules, a critical factor in determining its immunogenicity. Peptides that bind strongly to MHC class I and II molecules are considered prime candidates for cancer vaccine development.

Validation of Neoantigens

Once neoantigen candidates are identified, they must undergo experimental validation through various techniques:

Mass Spectrometry

Mass spectrometry (MS) is an essential tool in proteomics, widely used in clinical settings to profile patient samples and support treatment decisions. Recent advancements in MS technology have enhanced the targeting and identification of cancer-specific antigens, which are critical for developing antigen-specific therapies [26, 32].

T-cell Assays

Identifying immunogenic neoantigen-reactive T-cells involves determining which neoantigens can effectively trigger CD8⁺ T-cell responses. For a neoantigen to be immunogenic, it must bind to MHC-I complexes and engage with TCRs on T-cells, which leads to T-cell activation [33, 34]. This activation leads to the production of cytokines like Tumor necrosis factor- α (TNF- α) and Interferon- γ (IFN- γ), secretion of Interleukin-2 (IL-2), T-cell proliferation, and enhanced cytotoxic activity in CD8⁺ T-cells. To identify these reactive T-cells, assays like cytokine detection (e.g., ELISA or intracellular staining) or tetramer-based assays are employed, where peptide-MHC complexes help detect T-cell binding and response [4, 35].

Tetramer Binding Assays

Tetramers are clusters of MHC molecules attached to specific peptides (neoantigens) [36]. They are utilized to detect and isolate T-cell populations that specifically recognize a given neoantigen. By linking MHC molecules to target peptides, tetramers can interact with TCRs on T-cells that are specific to the neoantigen. This method enables the detection and quantification of neoantigen-specific T-cells in patient samples, offering valuable information about the immune response to tumors [9].

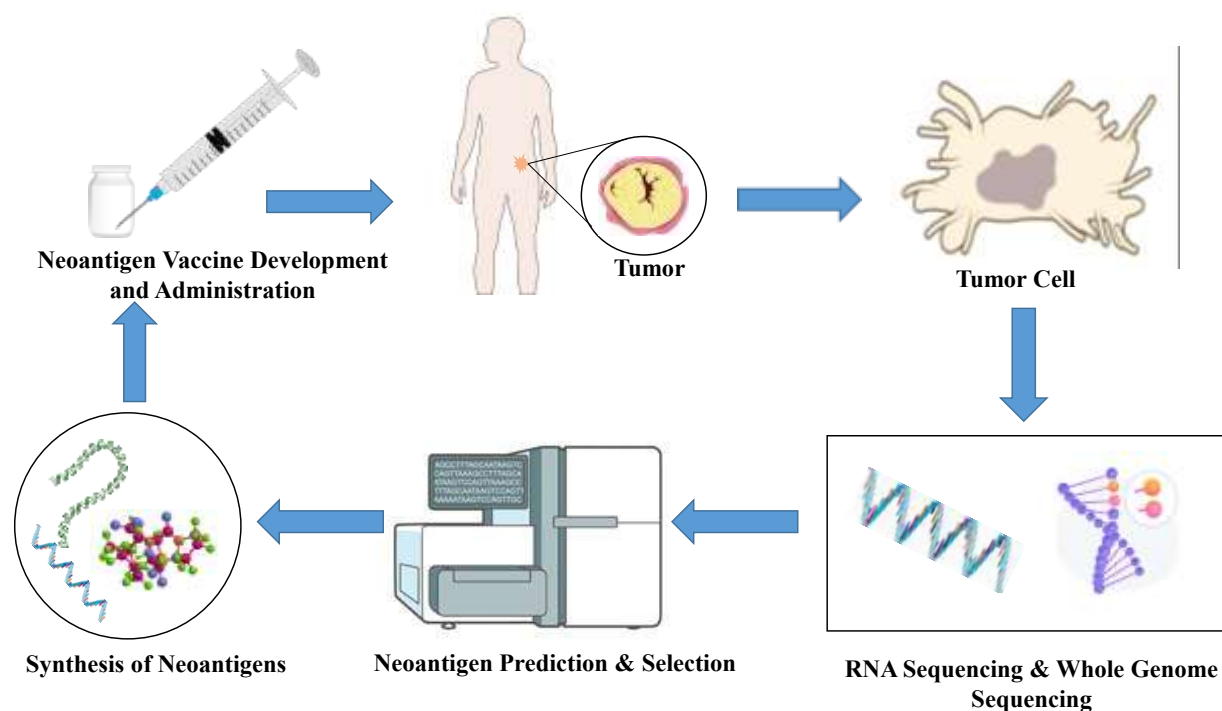


Fig. 2 Schematic diagram of the development of a neoantigen-based vaccine. This schematic diagram illustrates the process of neoantigen prediction, identification, and selection for neoantigen-based vaccine development through T-cell reorganization. Initially, malignant tumor cells are obtained via biopsy. Next, sequencing is carried out using NGS or other techniques. Following this, neoantigens are predicted, identified, and selected. These neoantigens are then synthesized. Ultimately, a vaccine construct is created and utilized for cancer immunization using the synthetic neoantigens.

Sources of Neoantigens

Neoantigens are perceived as foreign proteins not found in normal tissues, but they can arise in tumors utilizing various mechanisms. These include genetic mutations, abnormal transcriptomic variants, post-translational modifications (PTMs), and viral open reading frames (ORFs) [19, 37].

Single-Nucleotide Variants

Single nucleotide variations (SNVs) are the most prevalent type of mutation found in cancerous cells at the genomic level [8, 38]. These mutations can generate variant peptides that differ from the wild-type proteins, which are subsequently presented by MHC-I as distinct antigens. Research has demonstrated that cancer patients may possess hundreds of non-synonymous somatic mutations, resulting in an average of 150 potential neoantigenic peptides per person [8]. In melanoma, the primary source of neoantigens is SNVs, mainly caused by ultraviolet light-induced

DNA damage, leading to C > T mutations. These mutations can result in non-synonymous mutations that change amino acids [39, 40]. TCRs can often distinguish between the mutated and wild-type epitopes, reacting only to the mutated form. However, in rare cases, TCRs may recognize both the wild-type and mutated epitopes, in which case the mutation should not be considered a neoantigen [41].

Insertion, Deletion and Fusion

In addition to non-synonymous SNVs, other genomic alterations, including insertions, deletions, and gene fusions, can also result in changes to protein sequences, potentially making them recognizable by T-cells [41, 42]. INDEL mutations usually involve the addition or removal of base pairs in the genome, often causing the creation of non-synonymous novel ORFs, which are referred to as frameshift mutations [43, 44]. Frameshift INDELs have the potential to generate a wider variety of neoantigens with stronger MHC-I binding affinity, indicating that they may be more immunogenic than SNVs [8, 42]. Gene fusions, another critical mutation type in tumors, can also produce multiple neoantigens. These fusions can result from mesenchymal deletions, chromosomal translocations, or inversions [8, 37].

Post-Transcriptional and Post-Translational Alternations

Post-transcriptional events like alternative splicing, polyadenylation, RNA editing, and modifications to non-coding regions significantly contribute to the creation of diverse tumor-specific neoantigens, thereby expanding the variety of potential neoantigens [45, 46, 47]. Furthermore, dysregulated translation, a hallmark of cancer development, introduces additional tumor-specific neoantigens. Proteomic variants can also emerge from dysfunction in the transporter associated with antigen processing (TAP), altered proteasome processing, and abnormal PTMs, all of which can affect protein stability, folding, and degradation, potentially creating neoantigens that are recognized by the immune system [48, 49, 50].

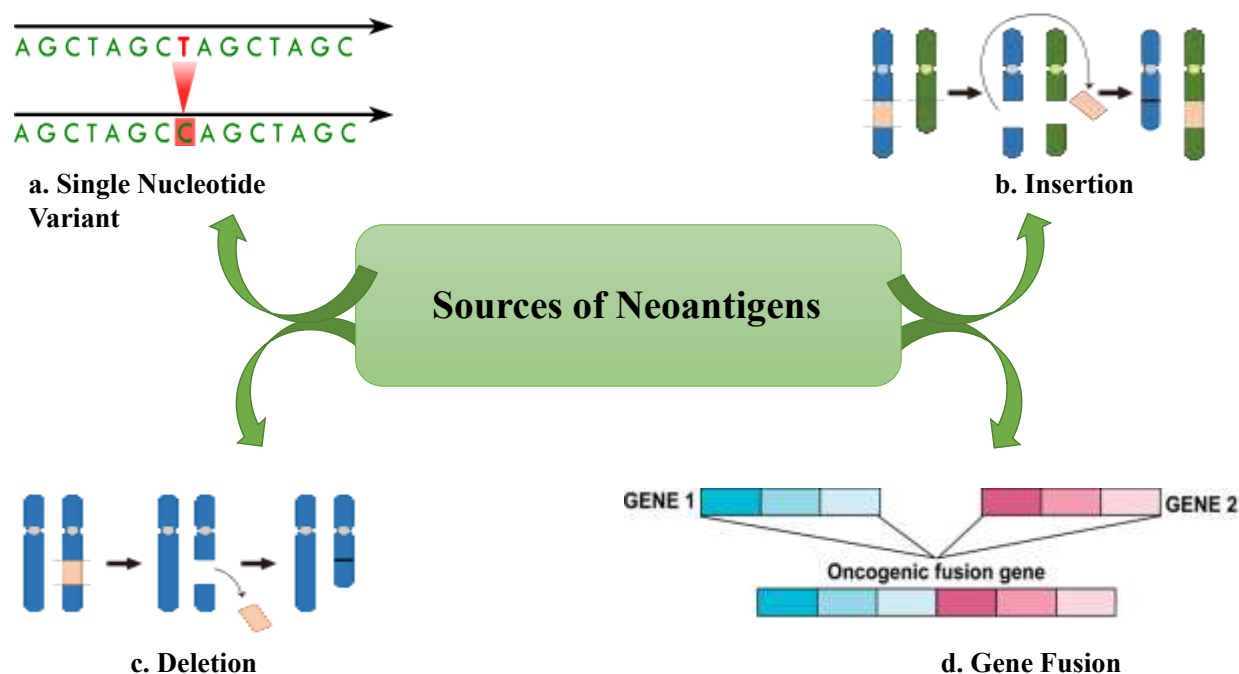


Fig. 3 Schematic presentation of Different Sources of Neoantigens. a) SNVs (Single Nucleotide Variants): Single base changes in the DNA sequence. b) Insertions: Addition of one or more nucleotides into the DNA sequence. c) Deletions: Loss of one or more nucleotides from the DNA sequence. d) Gene Fusions: Joining of two previously separate genes, often leading to abnormal gene function.

Strategies for Neoantigen Vaccine Development

The development of neoantigen-based vaccines involves several key strategies aimed at optimizing the immune response and overcoming challenges related to tumor evasion mechanisms.

Personalized Vaccine Approaches

Given that neoantigens are tumor-specific, the most promising approach to neoantigen vaccine development is the personalized or patient-specific vaccine [4, 51]. The process involves sequencing the patient's tumor, identifying neoantigens, and developing a vaccine tailored to those antigens. Personalized neoantigen vaccines have been shown to induce strong immune responses and hold the potential to improve clinical outcomes [23, 52]. For example, studies have demonstrated that personalized vaccines can lead to a robust T-cell response and shrinkage of metastatic tumors [33, 51].

Vaccine Platforms for Neoantigen Delivery

Several vaccine platforms can be used to deliver neoantigens, each with its advantages and challenges:

Peptide-Based Vaccines

Synthetic peptides typically consist of 20 to 30 amino acids, designed to correspond to the specific epitopes of tumor neoantigens and form the basis of peptide-based vaccines [5]. These synthetic peptides mimic the altered proteins present on the surface of tumor cells and serve as targets for the immune system [53]. However, one challenge with using these peptides is their short half-life, which means that they are quickly degraded or cleared from the body before they can effectively stimulate a sustained immune response [54]. This limitation can reduce the overall effectiveness of the vaccine. To overcome this, synthetic peptides are often combined with adjuvants (substances that enhance the body's immune response to the vaccine). Adjuvants help to activate the immune system more effectively, promoting a stronger and more long-lasting response against the tumor [55, 56].

DNA-Based Vaccines

Neoantigen DNA vaccines consist of synthetic DNA fragments that encode specific neoantigens. Once delivered into cells, these fragments are translated into proteins, which are presented to the immune system, prompting an immune response that targets and eliminates cancer cells expressing the relevant neoantigens [8, 57]. One advantage of DNA vaccines is their ability to deliver multiple neoepitopes simultaneously, using vectors designed to carry genes encoding tumor neoantigens along with integrated adjuvants. DNA-based platforms offer a stable and cost-effective way to deliver neoantigens. However, they face challenges in achieving high transfection efficiency. The use of electroporation techniques has enhanced vaccine uptake and improved immunogenicity [53].

RNA-Based Vaccines

Neoantigen RNA vaccines are an emerging cancer vaccine strategy that uses mRNA to instruct cells to produce tumor-specific neoantigens, triggering an immune response against the patient's cancer cells. The rapid progress of mRNA vaccine technology during the COVID-19 pandemic has highlighted the potential of this approach [58, 59, 60]. Recent advancements in technology

have enhanced the stability, backbone structure, delivery techniques, and cost-effectiveness of mRNA-based vaccines. RNA vaccines can be delivered through several methods, including viral vectors, micro-vectors, gene guns, microneedles, or in situ electro-transformation. As a result, numerous clinical trials exploring mRNA vaccine therapies for various cancers are currently recruiting patients [23, 53, 56].

Dendritic Cell-Based Vaccines

Antigen-presenting cells (APCs), such as dendritic cells (DCs), play a key role in continuously presenting antigens to the immune system, making them an ideal platform for neoantigen delivery. Autologous DCs can be harvested from patients, loaded with neoantigens, and then reintroduced into the patient to elicit specific immune responses targeting the neoantigens [8, 57]. Strategies to enhance DC migration to lymph nodes have also been developed, including the use of chemokines and adjuvants [53, 56, 61].

Neoantigens can be delivered to DCs using several approaches, such as exposing them to whole mRNA from autologous tumors, synthetic peptides, autologous whole tumor lysate (WTL), or through fusion with tumor cells [62]. mRNA transfection is the most direct method for generating intracellular neoantigens in DCs. Besides delivering neoantigens, mRNA electroporation can also introduce functional proteins into DCs, providing extra activation and maturation signals [8, 63].

Viral Vector-Based Vaccines

Viral vector-based vaccines utilize recombinant viral vectors to deliver tumor-associated neoantigens, stimulating a strong immune response [64, 65]. These vectors offer the advantage of robust antigen expression and the ability to trigger potent immune responses, as they can infect host cells and efficiently deliver genetic material [66, 67].

However, one challenge is that pre-existing immunity to viral vectors, due to previous exposure or vaccination, may limit the effectiveness of these vaccines. This pre-existing immunity could lead to a reduced immune response or even rapid clearance of the vector before it can deliver its payload. To address this challenge, novel viral vectors are being explored, such as adenovirus serotype 26 (Ad26) and vesicular stomatitis virus (VSV). These vectors are being investigated for their ability to bypass pre-existing immunity while still delivering the neoantigens effectively [68].

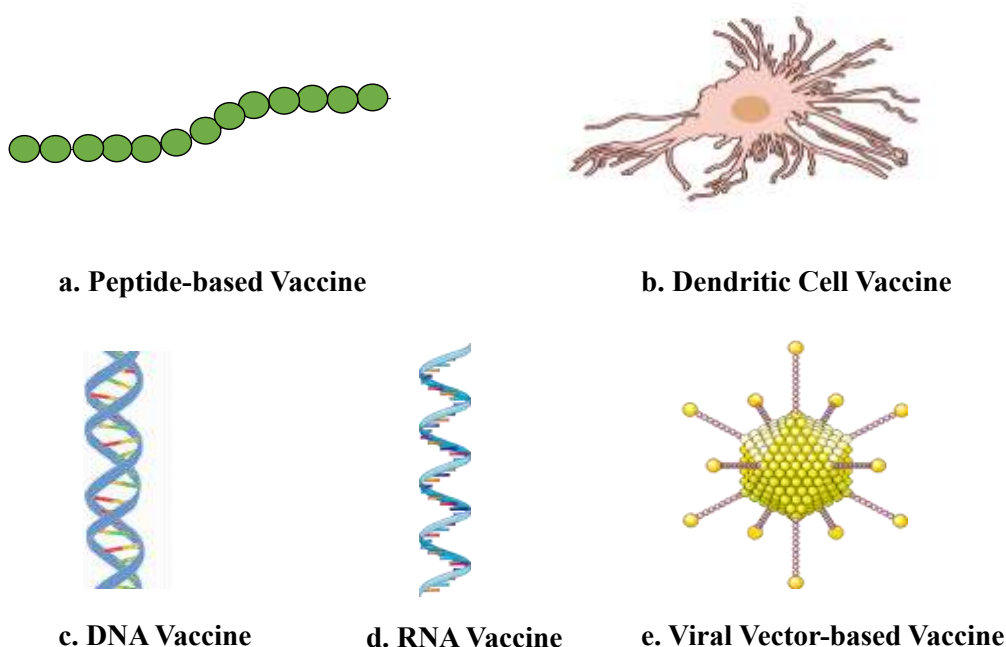


Fig. 4 Diagrammatic presentation of Different Platforms for Neoantigen-based Vaccine Delivery. **a)** Neoantigens of interest are delivered to the immune system as antigenic peptides. **b)** Dendritic cells are loaded with several tumor antigens in the dendritic cell vaccine. **c)** And **d)** DNA or RNA encoding tumor antigens are delivered to the immune system using several delivery platforms. **e)** Engineered viruses serve as vectors to introduce tumor antigens into the immune system.

Adjuvants and Delivery Systems

Adjuvants are crucial elements of neoantigen-based cancer vaccines, as they boost the immune response and promote effective tumor targeting. The role of adjuvants in boosting both innate and adaptive immunity is critical for improving vaccine efficacy. Commonly used adjuvants include:

TLR (Toll-like receptor) agonists

TLR ligands or agonists are potent immunomodulators that activate innate immune pathways, triggering responses such as the activation of cytotoxic Natural killer cells (NK cells), T-cells, and the maturation of dendritic cells [69, 70]. These agonists have shown significant therapeutic efficacy against various diseases, including cancer, and have been found to enhance the effectiveness of immunotherapies, such as cell-based therapies. Additionally, TLR agonists can sensitize cancer cells to conventional treatments like radiation and chemotherapy [71]. Recently, synthetic TLR agonists have shown significant improvement as adjuvants in cancer vaccines, particularly for poorly immunogenic tumors, by boosting TH1 (Helper T-cells 1) or TH2 (Helper T-cells 2)-mediated immune responses [72].

Cytokines

Cytokines, crucial regulators of immune responses, can also serve as adjuvants to enhance cancer vaccine effectiveness. By stimulating both innate and adaptive immunity, cytokines promote communication between immune cells and strengthen the overall immune response [41, 73, 74]. IFN α has been used as an adjuvant in treating high-risk melanoma and other cancers, while high-dose interleukin-2 (HDIL-2) has shown efficacy in metastatic renal cell carcinoma and melanoma. Other cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN- γ , IL-7, IL-12, and IL-21, have also been evaluated in clinical trials as potential adjuvants to improve the immune response in cancer immunotherapy, although newer therapies are now being explored [75, 76].

Lipid Nanoparticles

Lipid nanoparticles (LNPs) are a crucial delivery system for mRNA-based vaccines, particularly in cancer immunotherapy, as they efficiently encapsulate and protect the mRNA from degradation while ensuring its safe delivery into cells [77]. LNPs enhance the stability of the mRNA, which is essential for effective translation into tumor-specific neoantigens. Once inside the cell, LNPs assist in mRNA translation, leading to the expression of neoantigens and triggering the activation of the immune response [78, 79].

These adjuvants and delivery systems are crucial for enhancing the immune response in neoantigen-based vaccines, improving tumor targeting, and fostering robust immune activation for more effective cancer therapy.

Table 3 Pros and Cons of Various Neoantigen Delivery Vaccine Platforms

Vaccine types	Advantages	Disadvantages
Peptide-Based Vaccines	• Highly specific and safe • Low risk of autoimmunity	• Requires an appropriate adjuvant • Weak immune responses
DNA Vaccines	• Can deliver multiple antigens in one vaccine • A versatile platform that supports molecular engineering	• Risk of integration into the host genome • Low transfection efficiency
RNA Vaccines	• No risk of integration into the genome • High immunogenicity	• Rapid degradation • Potential for inducing an inflammatory response
Dendritic Cell Vaccines	• Dendritic cells are crucial for priming CD8 T-cells • Can be engineered to express both neoantigens and costimulatory molecules	• High expense • Labor intensive
Viral Vector-Based Vaccines	• Highly immunogenic • Durable immune response	• Potential risk of vector-induced immunogenicity • Need for specialized storage conditions

Preclinical and Clinical Advances

Preclinical Studies

Recent research has revealed that neoantigen vaccines are capable of generating powerful antitumor responses in mice [80]. In the B16F10 murine melanoma model, Castle et al. (2017) vaccinated C57BL/6 mice with synthetic peptides derived from 50 identified mutations. Of these, 16 peptides were found to be immunogenic, as demonstrated by an IFN- γ ELISPOT assay [81]. Notably, vaccination with two mutant antigens, MUT30 (Kif18b K739N) and MUT44 (Cpsf3l D314N), induced significant antitumor effects in both preventive and therapeutic contexts [82, 83].

Clinical Trials

Early-Phase Trials

The detection of neoantigen-specific CD8⁺ as well CD4⁺ T-cells in tumor-infiltrating lymphocytes (TILs) from melanoma patients who responded to checkpoint blockade therapy, along with promising preclinical findings, has fueled a growing interest in advancing the clinical development of neoantigen vaccines [83, 84]. Studies have reported the safety and immunogenicity of neoantigen vaccines in melanoma, lung cancer, and glioblastoma patients. For instance, a Phase I trial in melanoma patients demonstrated durable T-cell responses and improved survival outcomes [51, 85].

Combination Therapies

The effectiveness of immune checkpoint inhibitors in cancer treatment is revolutionizing patient care and driving advancements in cancer research. Neoantigen vaccines combined with immune checkpoint inhibitors have shown synergistic effects, enhancing anti-tumor immunity. Ongoing trials are investigating combinations with anti-PD-1/PD-L1 and anti-CTLA-4 antibodies to overcome immune evasion mechanisms [37, 86].

Long-Term Monitoring

Longitudinal studies are being conducted to evaluate the durability of immune responses elicited by neoantigen vaccines. Biomarkers such as T-cell persistence and cytokine profiles are under investigation to correlate immune activity with clinical outcomes [87].

Table 4 List of clinical trials using neoantigen-based cancer vaccine. This information can be accessed at <https://clinicaltrials.gov/> and <https://www.chictr.org.cn/indexEN.html> (data accessed on 4th March 2025).

ClinicalTrial.gov identifier	Phase	Cancer Type	Status	Vaccine Format
NCT01970358	I	Melanoma	completed	Synthetic peptide
NCT02348320	I	Triple-negative breast cancer (TNBC)	completed	DNA
NCT03908671	I	Esophageal cancer	Recruiting	mRNA
NCT00683670	I	Advanced-stage melanoma	completed	Dendritic cell
NCT03639714	I/II	Non-Small Cell Lung Cancer	completed	GRT-C901 adenovirus-based
NCT03380871	Ib	Non-Small Cell Lung Cancer	completed	Synthetic peptide
NCT02035956	I	Melanoma	completed	RNA

Challenges in Neoantigen-based Cancer Vaccines

Tumor Heterogeneity and Neoantigen Variability

It is widely recognized that tumor heterogeneity is primarily driven by irregularities in the tumor microenvironment, including a high mutational burden, hypoxic conditions, and abnormal blood vessel formation [4, 88]. A major challenge in identifying neoantigens is the high level of intra-tumoral heterogeneity. Tumors consist of genetically diverse subclones, each potentially carrying different mutations that result in the expression of unique neoantigens [89, 90]. This heterogeneity makes it difficult to design universal vaccines, as a neoantigen-based vaccine may only target specific subclones of the tumor [8].

Immune Evasion

A major hurdle in cancer treatment is immune evasion, which hampers the effectiveness of neoantigen-based immunotherapies. Tumors can evade immune surveillance through several mechanisms, including the loss of neoantigen expression, changes in antigen presentation via MHC molecules, and the immunosuppressive characteristics of the tumor microenvironment (TME) [91, 92]. The TME inhibits immune cell activity by promoting factors like regulatory T-cells, myeloid-derived suppressor cells, and immunosuppressive cytokines, thereby reducing the efficacy of neoantigen-targeting therapies. The development of combination therapies, where neoantigen vaccines are paired with immune checkpoint inhibitors or other immunotherapies, holds promise in overcoming these barriers [8].

Loss of Neoantigen Expression

Tumors can use the loss of tumor-specific neoantigens as a key strategy for immune evasion, particularly because many neoantigens are incidental by-products of tumorigenesis and are not essential for the survival of tumor cells [93]. Depleting neoantigens may act as a resistance mechanism to anti-tumor immunity, making it a significant challenge for the effectiveness of personalized neoantigen-based immunotherapies [8].

Impaired Presentation of Neoantigen Peptides

Tumors can acquire mutations that impact not only neoantigen expression but also HLA heterozygosity and MHC stability in response to immune pressure. These changes can interfere with neoantigen processing and presentation, preventing effective T-cell recognition and tumor

elimination [82, 94]. Furthermore, tumors may be able to avoid being detected by adoptively transferred T-cells due to changes in important antigen presentation genes like $\beta 2M$ or a loss of variety in HLA alleles [8].

The Immunosuppressive Tumor Microenvironment (TME)

Tumor cells use a variety of immune evasion techniques, such as loss of neoantigens and poor antigen presentation. Via several mechanisms within the immunosuppressive TME, tumor cells can also hinder T-cell activation and neoantigen identification [95, 96]. These include the inhibition of certain TME cells, the release of ions or proteins from cancer cells post-necrosis, and the overexpression of immunological checkpoint ligands, notably PD-L1 and CTLA-4, which hinder T-cell development and function [8].

Discussion and Conclusion

Neoantigen-based cancer vaccines provide a promising and tailored approach to cancer immunotherapy. Advancements in genomic sequencing and bioinformatics have made it possible to identify neoantigens on an individual level, opening the door to the development of personalized vaccines. Despite promising preclinical and early-phase clinical results, challenges such as tumor heterogeneity, immune evasion, and vaccine delivery need to be addressed. Ongoing clinical trials and the development of combination therapies may further improve the efficacy of neoantigen vaccines and their role in the treatment of cancer. As the field progresses, neoantigen-based vaccines could become a cornerstone of cancer immunotherapy.

The future of neoantigen-based vaccines is increasingly centered on the scaling of personalized therapies. With advancements in technology that improve the identification of neoantigens, these vaccines have the potential to become a standard cancer treatment [86]. As NGS techniques, bioinformatics tools, and artificial intelligence (AI) continue to evolve, the process of identifying relevant neoantigens will become faster, more accurate, and more cost-effective [23]. These developments are particularly significant for detecting rare or complex mutations, which are often challenging to target with conventional treatments [4]. These innovations hold the potential to deliver highly targeted therapies, significantly enhancing patient outcomes by customizing treatment to the unique genetic profile of each tumor. Furthermore, the integration of AI-driven algorithms into the design and optimization of vaccines could lead to more efficient vaccine development pipelines, reducing both the time and costs associated with bringing these treatments

to clinical practice. The combination of neoantigen-based vaccines with immune checkpoint inhibitors (such as PD-1/PD-L1 inhibitors and CTLA-4 inhibitors) is an exciting and promising avenue for cancer treatment [37]. Immune checkpoint inhibitors work by disrupting the regulatory pathways tumors use to evade immune detection, thereby enhancing the immune system's ability to identify and target cancer cells [86]. When combined with neoantigen vaccines that target the unique mutations of a patient's tumor, this approach has the potential to synergistically enhance the body's immune response, greatly improving treatment effectiveness. Clinical trials are currently exploring these combinations in various cancers, including melanoma and non-small cell lung cancer, with preliminary results showing promise in improving both the durability and strength of the anti-tumor response [97, 98]. This combination therapy could offer a more comprehensive and tailored approach to cancer treatment by not only training the immune system to target specific tumor markers but also ensuring that the immune response remains active and effective against the cancer cells.

Researchers are focused on refining vaccine delivery strategies to more efficiently target dendritic cells, which are essential for processing and presenting neoantigens to T-cells [8, 57]. Enhancing the ability of neoantigen vaccines to engage dendritic cells is critical, as these cells play a central role in initiating and amplifying the immune response. To improve the delivery, stability, and overall immune response generated by these vaccines, innovative technologies such as lipid nanoparticles, nanoparticles, and viral vectors are being optimized. These delivery systems can help ensure that neoantigens are effectively transported to the right locations within the immune system, allowing for more potent and targeted immune activation. Additionally, the inclusion of adjuvants can enhance the immune system's capacity to recognize and target tumor cells more effectively [8]. This combined approach of optimizing delivery systems alongside immune stimulators holds significant potential to maximize the effectiveness of neoantigen vaccines. This progress in vaccine delivery and immune modulation is an exciting step toward improving the precision and efficacy of cancer therapies.

In the future, neoantigen-based vaccines will focus on identifying the most immunogenic neoantigens and designing vaccines capable of targeting a broad spectrum of tumor subclones. Tumors are often characterized by significant genetic and molecular heterogeneity, which can result in the emergence of subclones that escape immune detection. To overcome this challenge, advances in single-cell sequencing and multi-omic approaches, combining genomics, proteomics,

and transcriptomics, will allow for a more detailed understanding of tumor biology. These technologies will enable the identification of neoantigens that are highly expressed across different regions of a tumor, reducing the risk of tumor escape by ensuring that the immune system targets a comprehensive set of neoantigens. Additionally, T-cell repertoire analysis will play a crucial role in pinpointing the most effective neoantigen candidates by evaluating which neoantigens are most likely to provoke a robust T-cell response [4, 8]. This integrated approach will be critical in developing next-generation personalized cancer vaccines that can more effectively address the complex nature of tumor evolution and immune evasion. This emerging strategy holds great promise for transforming cancer treatment by providing a more comprehensive and adaptive immune response. As these technologies advance, the ability to identify and target a broader array of tumor variants, while ensuring that the immune system can recognize and eliminate even the most elusive cancer subclones, will significantly improve the success rates of neoantigen-based vaccines.

Strategies to overcome immune tolerance will be a critical area of focus for future research in neoantigen-based vaccines. One promising approach involves combining these vaccines with therapies that modulate the TME to reduce immune suppression and enhance the body's natural immune response [91, 92]. The TME is often a significant barrier to effective cancer immunotherapy, as it can suppress immune cell activity and create an environment where tumors can evade immune detection. By targeting these immune-suppressive mechanisms within the TME, researchers hope to create a more conducive environment for neoantigen vaccines to work effectively. In addition, scientists are investigating how to generate more robust T-cell responses through epitope spreading, a process in which the immune system not only targets the neoantigen initially presented by the vaccine but also expands its focus to include other tumor-associated antigens. This broadens the immune response and reduces the risk of tumor escape, as it prevents the tumor from evading immune detection by altering or hiding a single neoantigen [8]. These innovative strategies offer significant promise in overcoming the current limitations of cancer immunotherapy. By addressing immune tolerance and the immune-suppressive tumor microenvironment, neoantigen vaccines can potentially achieve more durable and effective anti-tumor responses.

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