

#### EXPERT INSIGHT

## Neoantigen vaccines for cancer: ready for primetime

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Tumor neoantigens are somatic mutations specific to the tumor that can elicit immune responses. Genetic mutations are one of the hallmarks of cancer and as a result almost all tumors harbor neoantigens and are susceptible to elimination by the immune system. However, tumor cells often organize an immunosuppressive tumor microenvironment, which hampers the recruitment of immune cells into the tumors and the development of immune responses against these neoantigens. The use of vaccines can facilitate the development of an immune response against tumor neoantigens and increase the immune pressure on cancer, ideally driving tumor elimination. Several challenges step in the way of neoantigen vaccines becoming effective immunotherapies today. Here, we discuss these challenges and provide a picture of the current state of the art in neoantigen vaccines.

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#### TUMOR NEOANTIGENS

Tumor neoantigens are somatic mutations specific to the tumor that can elicit immune responses. The specific immune responses are

generated by engagement of the adaptive immune system (T cells and B cells) against the mutated peptides presented by Major Histocompatibility Complex (MHC) molecules.

Neoantigen presentation can activate anti-tumor activity, which has the potential of eliminating the tumor cells.

Unfortunately, tumors develop mechanisms to elude immune recognition and elimination. Some of these mechanisms for immune evasion involve developing an immunosuppressive microenvironment that prevents antigen presentation (such as through Human Leukocyte Antigen (HLA) loss) and/or T cell recognition, priming, infiltration or activation against the tumor cells. These issues prevent the expansion and infiltration of T cells that are able to recognize tumor neoantigens and target the tumor cells.

Vaccines allow presentation of relevant antigens to the immune system in conditions that favor the development of effective immune responses. Vaccination typically results in activation and expansion of B cells and T cells that can respond to the corresponding antigens via the humoral and cellular immune responses. In the context of cancer, vaccination can circumvent the difficulties imposed by the tumor immunosuppressive microenvironment facilitating the expansion of T cells specifically directed against the tumor neoantigens [1]. Targeting tumor neoantigens provides important advantages that can be exploited for cancer treatment (Table 1):

- ▶ Tumor neoantigens result from somatic mutations and are not subject to central tolerance. In contrast to tumor associated-antigens, the immune system has never encountered these neoantigens prior to their emergence in the tumor and thus, has not been trained to ignore them. This results in neoantigens having the potential to elicit strong immune responses, similar to those elicited against microbial or other foreign agents [2].
- ▶ Tumor neoantigens are specifically expressed in the tumor, consequently minimizing off-tumor adverse effects since the tumor driven somatic changes are not present in the germline. These observations

increase the safety profile of neoantigen therapies compared to targeting tumor associated antigens or other common pathways which have the potential to lead to adverse effects by targeting similar processes present in normal cells [3] onships.

- ▶ Somatic mutations are a hallmark of cancer development, which makes targeting neoantigens a therapeutic possibility in all cancer types [4,5]. While dysregulation of certain pathways or availability of tumor associated antigens to target is usually specific to certain tumor types, the presence of tumor somatic mutations occurs in all tumors, opening the possibility to use neoantigen-based treatments for any type of cancer.

Thus, neoantigen directed personalized vaccines check off all the boxes for an ideal cancer targeted therapy based on their high degree of tumor specificity and their ability to activate the immune system. Indeed, emerging data from checkpoint immunotherapy studies as well as a re-evaluation of historical anti-cancer immunotherapy data suggests that the observed clinical effects correlate well with the presence of cancer neoantigens and neoantigen directed T cell responses in the different tumor targets [6]. Not surprisingly, many academic and industry groups have developed methods for targeting neoantigens using vaccines based on multiple vaccine platforms (Table 2). Although most clinical studies are in Phase I or II, the early data bodes well for the safety profile of neoantigen targeting as a class of intervention. Indeed, even simply based upon the targeted enrollment of the reported ongoing studies, the cumulative exposure to neoantigen vaccines exceeds 3000 patients over more than 70 clinical trials across the different vaccine platforms. These studies have clearly demonstrated the induction of broad neoantigen directed T cell immunity. Additionally, clinical responses offered by neoantigen vaccines have demonstrated

► **TABLE 1**

**Current challenges and opportunities for neoantigen vaccines.**

Opportunities
<ul style="list-style-type: none"> <li>► Strong responses, not subject to central tolerance</li> <li>► Tumor-specific, minimizing off-target effects</li> <li>► Potentially present in all cancers</li> </ul>
Current challenges
<ul style="list-style-type: none"> <li>► Difficulty in generating CD8<sup>+</sup> responses</li> <li>► Ability to target a limited number of neoantigens</li> <li>► Long manufacturing timelines</li> </ul>

encouraging signs of viability of approach and early clinical proof-of concept. Next, we discuss the main hurdles that neoantigen vaccines are facing in their growth towards a game-changing therapy (Table 1):

### Generation of CD8<sup>+</sup> T cell responses

While anti-tumor activity may be mediated by multiple mechanisms, CD8<sup>+</sup> T cells are recognized as the major contributors to cytolytic activity *in vivo* and therefore tumor elimination via direct killing of cells displaying cognate MHCI-peptide complexes on the tumor cell surface. Thus, the basis of most algorithms for generating neoantigen vaccines is the identification of MCHI epitopes that will drive expansion of neoantigen-targeted CD8<sup>+</sup> T cells. These expanded anti-neoantigen CD8<sup>+</sup> T cells will circulate, infiltrate the tumor, and promote immune elimination of the neoantigen-expressing tumor cells.

Early neoantigen vaccine clinical trials have used synthetic long peptides (SLPs) in combination with poly(I:C), dendritic cells loaded with HLA class I restricted peptides, or RNA vaccines encoding neo-epitopes. These clinical trials have demonstrated the induction of immune responses directed against an important fraction of the neoantigens delivered [7–11]. However, the vast majority of the immune responses elicited by RNA or SLPs have been CD4<sup>+</sup> T cells, both in these early clinical studies as well as in pre-clinical mouse studies preceding the clinical trials [7–10, 12,13]. This strong induction of CD4<sup>+</sup>

T cell responses occurs despite the fact that the epitopes were putatively selected *in silico* for high MHCI binding affinity in an effort to drive CD8<sup>+</sup> T cell responses. A potential explanation for the CD4<sup>+</sup> responses are the presence of overlapping epitopes that activate both CD4<sup>+</sup> and CD8<sup>+</sup> T cells [14,15]. Overlapping MCHI and MHCII binding epitopes can be commonly found around neoantigens using bioinformatic prediction tools. However, the unexpectedness of a predominant CD4<sup>+</sup> response was initially linked to the selection of epitopes used and much focus was directed towards improvement of the prediction methodology.

After more studies and additional clinical data across multiple different vaccine platforms and neoantigen selection approaches, the epitope selection hypothesis seems unlikely to entirely explain the preponderance of CD4<sup>+</sup> T cell responses. An alternate hypothesis is that the immune phenotype is linked to the method of immunization. Dendritic cell vaccination methodology loaded with HLA class I restricted neo-epitopes (9mers) was able to generate frequent CD8<sup>+</sup> T cell responses, as opposed to SLP and RNA [11]. Also, preclinical work with long neoantigen epitopes (33mers) encoded as DNA yielded primarily CD8<sup>+</sup> responses, especially in those epitopes with highest predicted MHC Class I binding affinity [16]. This preclinical work is in line with the frequency of CD8<sup>+</sup> responses seen in clinical trials using DNA encoded full tumor associated antigens or viral proteins [17–19]. The understanding of why the RNA and peptide

**TABLE 2**
**Representative clinical trials using neoantigen vaccines.**

Clinical trial number	Vaccine platform	Phase	Tumor types	Estimated enrollment
NCT01970358	Peptide	I	Melanoma	10
NCT03597282	Peptide	I	Melanoma	40
NCT03568058	Peptide	I	Solid tumors	30
NCT03380871	Peptide	I	NSCLC	15
NCT02897765	Peptide	I	UC, Melanoma, NSCLC	55
NCT03633110	Peptide	I/II	Melanoma, NSCLC, HNSCC, UC, RCC	99
NCT04161755	mRNA	I	PDAC	20
NCT03897881	mRNA	II	Melanoma	150
NCT03313778	mRNA	I	Solid tumors	90
NCT03815058	mRNA	II	Melanoma	132
NCT04267237	mRNA	II	NSCLC	80
NCT04486378	mRNA	II	CRC	201
NCT03289962	mRNA	I	NSCLC, CRC, UC, melanoma, TNBC, RCC, HNSCC, other solid	770
NCT04251117	DNA	I/II	HCC	12
NCT03548467	DNA	I/II	Solid tumors	65
NCT04015700	DNA	I	GBM	6
NCT03598816	DNA	II	RCC	48
NCT03092453	Dendritic cell	I	Melanoma	12
NCT03300843	Dendritic cell	II	Melanoma, GI, breast, ovarian, pancreatic	1
NCT01132014	Dendritic cell	I	Ovarian	67
NCT03639714	Viral vector + RNA	I/II	NSCLC, CRC, GEA, UC	214
NCT03953235	Viral vector + RNA	I/II	NSCLC, CRC, PDAC, other solid tumors	144
NCT03265080	Bacterial vector	I	NSCLC, CRC, HNSCC, UC, melanoma	5

NSCLC: Non-small cell lung cancer; UC: Urothelial carcinoma; HNSCC: Head & neck squamous cell carcinoma; RCC: Renal cell carcinoma; PDAC: pancreatic adenocarcinoma; CRC: Colorectal carcinoma; TNBC: Triple negative breast cancer; HCC: Hepatocellular carcinoma; GI: Gastrointestinal; GEA: Gastro esophageal adenocarcinoma.

based vaccines induce infrequent CD8<sup>+</sup> T cell responses remains unclear, although other mRNA and peptide/protein vaccines targeting viral antigens have also generated predominantly CD4<sup>+</sup> T cell responses, suggesting that this phenomenon could be platform dependent [20–22].

### Number of epitopes targeted

Adoptive cell transfer experience has shown that targeting CD8<sup>+</sup> T cells against a single epitope can be sufficient for tumor elimination [23,24]. These studies typically target proteins encoded by driver genes or proteins

required for cell growth or function such as KRAS, TP53 or CD19. Targeting proteins that are required for cell function helps avoid immune evasion, in which the tumor cell ‘gets rid’ of the therapeutic target by loss or mutation, avoiding recognition by the immune system [25].

Tumor neoantigens occur frequently as passenger mutations, not driver mutations. The fact that these mutations are not required for tumor survival makes it easier for the tumor to eliminate the target to avoid immune detection without consequences for its growth or survival. Targeting a low number of neoantigens could easily result in antigen loss and tumor escape from the immune pressure. Initial clinical trials using neoantigen vaccines started targeting up to 20 neoantigens [7, 8]. Currently, there are clinical trials targeting up to 40 neoantigens per patient, which in some tumor types translate into targeting 100% of their neoantigens for a high number of patients.

Subclonality in cancer also makes targeting a higher number of neoantigens beneficial. Cancer starts with the accumulation of driver mutations (oncogenes and tumor suppressor genes) in a single cell. This results in a transformed cell growing in a dysregulated manner. Dysregulated growth results in different mutations accumulating in different cells, establishing subclones, which will outgrow by competitive advantage. Outgrowth of different subclones becomes more dramatic following metastasis, chemotherapy or targeted therapies. The different subclones have a set of common and divergent mutations from each other. Additionally, the tumor neoantigen landscape does not present a static, invariant picture. Indeed, large scale genomic analysis of cancer patient tumor samples have provided evidence of the clonal heterogeneity within (i) different sections of the same resected tumor; and (ii) between tumors sampled at different metastatic sites within the same patient [26]. There is also the potential for an altered neoantigen landscape between tumors sampled longitudinally within a patient between primary and recurrent tumors or in response to various treatment regimens

[27,28]. Significantly, at least one analysis by O’Donnell et al concludes that even when clonal heterogeneity is observed longitudinally, these are likely resulting from mutational processes that are already underway in the earliest tumors [29]. Selection of a limited number of mutations for a vaccine can result in targeting only certain subclones, while not capturing neoantigens that are common to all subclones. A higher number of neoantigens or ideally targeting all discovered neoantigens will facilitate the targeting of all subclones present in that tumor, limiting also the possibility of tumor escape [30].

Finally, the ability to include more neoantigens in the vaccine reduces the need for perfection in the neoantigen selection strategy. Many groups select neoantigens based on bioinformatic prediction tools trained on binding affinity and mass spectrometry-eluted ligands. Prediction algorithms can also be trained using immune responses to the neoantigens used in clinical trials. The ability of these tools to predict CD8<sup>+</sup> T cell responses have been criticized due to the inability of early clinical trials to drive CD8<sup>+</sup> T cells, however, as noted above, this inability may be vaccine platform dependent. The Parker Institute for Cancer Immunotherapy is leading a bioinformatics effort coordinating scientists from over 40 industry, non-profit and academic groups to find the best way to predict neoantigens for treating patients [31]. However, the use of weakly or inconsistently immunogenic platforms, the different vaccine design and delivery methods, patient variability including tumor type and immunosuppressive elements, and immunologic readouts are limitations that can confound prospective evaluation and validation of prediction algorithms. Some groups adopt the approach of pre-validating the neoantigens by testing *in vitro* the patients’ T cells against predicted neoantigens identified by sequencing patient tumors and selecting only those neoantigens for inclusion in the vaccine for which preexisting T cell responses are detected. Inclusion of a higher number of neoantigens to the vaccine will be helpful in both

cases. In the case of bioinformatic predictions, it is likely that some predictions will not result in T cell responses in that patient. Targeting a higher number will therefore result in an increased probability of driving more neoantigen responses. *Ex vivo* experimental selection is closer to *in vivo* reactivity in principle, as there is confirmation that the selected epitope is presented by MHC molecules, recognized by the relevant T cell receptors (TCR) and shown to activate the T cells. However, the methodology is limited by the sensitivity of the detection assays and susceptible to *ex vivo* assay artifacts. The disadvantage of experimental selection is that it identifies only the epitopes with the strongest preexisting responses, regardless of their functional relevance to the anti-tumor response. Additionally, targeting T cells already present in the blood in significant numbers can also be detrimental as they could have a more exhausted phenotype, as opposed to priming *de novo* responses. Here again, targeting higher number of epitopes will increase the likelihood that the vaccine can effectively expand the desired T cells *in vivo* and that those T cells will be relevant to targeting the tumor.

A potential limitation for the immunization with large antigenic numbers is the possibility of antigen competition. There is preclinical and clinical data suggesting that simultaneous immunization with large quantities of antigen can result in lower T cell responses to the desired antigens [32,33]. However, this varies depending on the number of antigens to be delivered, the vaccine platform, adjuvants or the immune fitness of the individual receiving the vaccine. There is preclinical data using DNA neoantigen vaccines which show no impairment to antitumor responses of with up to 60 neoepitopes in which a single neoepitope was found to drive the antitumor response [16].

### Manufacturing time

Tumor neoantigens are largely unique [34]. This makes neoantigen vaccines a truly

personalized therapy. To manufacture these personalized vaccines, the minimal elements required are exome and RNA sequencing, neoantigen identification and GMP manufacturing of the patient-specific vaccine (Figure 1).

Most cancer therapies are off-the-shelf and can be dosed immediately once indicated. Neoantigen vaccines, however, require patient specific manufacturing time which is typically weeks or months. The longer the time required for vaccine manufacturing, the higher is the risk that the tumor can progress and make the patient ineligible for receiving the vaccine. Time is a critical factor in cancer treatment, and the speed in which a personalized vaccine can be manufactured is of high importance.

Published clinical trials report manufacturing timelines of over 12 weeks [7-10], which can result in the trials losing patients due to disease progression. Additionally, the extended manufacturing timelines make clinical studies to elucidate the efficacy of neoantigen vaccines challenging both as single agents (risk of disease progression) or as combinations with other agents. For example, with PD1/PDL1 checkpoint immunotherapy becoming standard of care in many cancer settings, the neoantigen vaccine clinical trials are often designed for the vaccine to be administered in combination with the checkpoint inhibitor (CPI). The combination approach is well supported by pre-clinical model studies as well as sound scientific rationale of combining a T cell inducer (vaccine) with a T cell activity facilitator (CPI). However, the long manufacturing lead times for current neoantigen modalities have meant that patients begin CPI therapy prior to vaccine availability and often have already received 3+ cycles of CPI therapy prior to the first vaccination. This delay in vaccine administration makes it difficult to determine if the effects observed in uncontrolled vaccine-CPI combination clinical trials are attributable to the vaccine or the CPI alone.

Recently, groups manufacturing DNA and RNA vaccines are claiming timelines for



vaccine production down to 6 weeks per patient. While 6 weeks is a significant advantage over previous standards, the treatment of cancer requires more aggressive manufacturing timelines that allow patient therapy to become available. Here, the fledgling neoantigen field need only look to their colleagues in the autologous/allogeneic CART therapy space. The initial therapies started with turnaround times of several months. After multiple years of process innovation, the most advanced CAR-T therapies are now being turned around in 4 weeks from apheresis to administration. This manufacturing change was certainly driven by the first clinical successes in the treatments impacting patient outcomes. In the meantime, the favorable safety profile, together with the longer time to treat available, makes adjuvant cancer therapy an especially well-suited space for neoantigen vaccines.

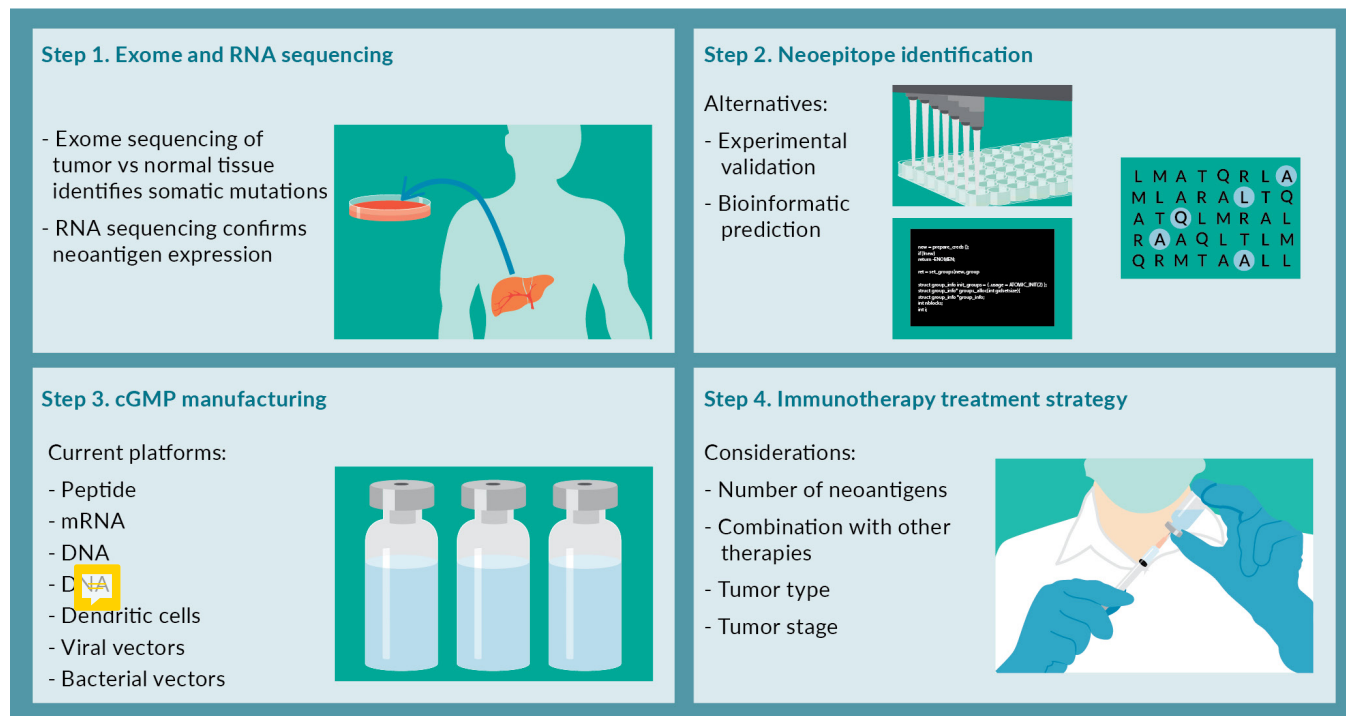
Neoantigen vaccines are patient friendly and benefit from requiring only a minimal starting sample from the patient - tumor biopsy and normal tissue as opposed to a large volume of cells collected via apheresis of the patient. We are confident that successful neoantigen vaccine results will drive research and development efforts and manufacturing optimization which will further refine the time and scale required for these new personalized therapies.

## CONCLUSION

Neoantigen vaccines are at the forefront of personalized medicine and present a very promising alternative to treat many different kinds of cancer. However, it is still a new therapy and it requires optimization. This will involve finding the best vaccination platforms to elicit

## ► FIGURE 1

Minimal elements required for manufacturing of neoantigen vaccines.



The minimal elements required for manufacturing neoantigen vaccines are: (1) exome and RNA sequencing, informs of the tumor-specific somatic changes and their expression; (2) neoantigen identification, can be done through experimental validation or bioinformatic prediction; (3) cGMP manufacturing: the selected neoantigens can be manufactured in different vaccine platforms; (4) the immunotherapy strategy defines how the vaccine will be given to the patient, considerations to make at this point are the number of neoantigens that will be used, potential combination with other therapies and the type and stage of tumor treated.

potent CD8<sup>+</sup> T cell responses, finding the best regimens and therapeutic combinations, increasing the number of neoantigens that can be targeted and finding the most appropriate time for vaccination, for which shortening the manufacturing time will be an important step. The field as a whole is learning from the past experiences and making better neoantigen

vaccines that will be able to change cancer treatment in the near future. What makes this possibility even more tantalizing is that because all cancers harbor somatic changes to some degree, neoantigen vaccines if successful, have the potential to be agnostic of tumor type, and could be used to treat all cancers based on their molecular (mutational) profile.

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## AUTHORSHIP & CONFLICT OF INTEREST

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