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# Personalized neoantigen vaccines: A new approach to cancer immunotherapy

Amanda R. Aldous, Jesse Z. Dong\*

Neon Therapeutics, Cambridge, MA 02139, USA

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## ABSTRACT

Neoantigens arise from somatic mutations that differ from wild-type antigens and are specific to each individual patient, which provide tumor specific targets for developing personalized cancer vaccines. Decades of work has increasingly shown the potential of targeting neoantigens to generate effective clinical responses. Current clinical trials using neoantigen targeting cancer vaccines, including in combination with checkpoint blockade monoclonal antibodies, have demonstrated potent T-cell responses against those neoantigens accompanied by antitumor effects in patients. Personalized neoantigen vaccines represent a potential new class of cancer immunotherapy.

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## 1. Background

Studies concerning the immune system and how to fight malignancies like cancer date back several decades, and our grasp on its mechanism is constantly evolving. In the late 1800s, Coley realized that cancer could be affected by stimulating the immune system through inoculation with inactivated toxins, which are still used today to treat bladder cancer.<sup>1–3</sup> In the early 1900s, Erlich proposed that the immune system is able to eliminate cancer cells via antibodies delivering toxins to those cells. Burnet and Thomas later expanded on Erlich's idea of immune surveillance in the 1950s to say that lymphocytes were the species that recognized malignant cells.<sup>4</sup> Then in the 1960s, tumor associated antigens that led to the rejection of tumors were discovered, but it would take several more years and sets of data until these ideas implicating immune cells in the fight against cancer were accepted by the greater community.<sup>5,6</sup>

Vaccines are immune-active methods being explored in the cancer field to either prevent (prophylactic) or therapeutically treat and eradicate disease. Vaccines are credited to Jenner when, in 1796, he vaccinated against smallpox using cowpox lesion from a local milkmaid.<sup>7</sup> Live attenuated vaccines later came into play with Pasteur's rabies vaccine in the 1880s, and more recently, with implementation of the influenza vaccines, whereas some vaccines over the years were developed against dead organisms or toxins.<sup>8</sup> After 1950, cell culture based vaccines were developed for vaccina-

tion against MMR, Polio, and versions of influenza.<sup>7,8</sup> Recently in the field of cancer immunotherapy, two prophylactic cancer vaccines have been approved by the U.S. Food and Drug Administration (FDA); one for human papillomavirus involved in many cervical cancers and another against the hepatitis B virus associated with hepatic cancer.<sup>3</sup> The first FDA approved therapeutic cancer vaccine, in 2010, was the *ex-vivo* generated dendritic cell (DC)-based Provenge (Sipuleucel-T) for the treatment of prostate cancer.<sup>3,9</sup>

There is a wide array of strategies being explored for personalized cancer vaccines that would induce the immune system to attack the unique traits of an individual tumor. These approaches include whole tumor cells, DCs, T-cells, DNA, RNA, viral vectors, protein and peptides.<sup>3,10–13</sup> Each method has distinct advantages and disadvantages, which have been thoroughly reviewed elsewhere.<sup>3,13–15</sup> In brief, whole tumor cell therapies are extremely limited to the availability of patient material. DC's, although have low toxicity and are the only existing FDA approved therapeutic modality for cancer, are not very potent and require additional steps to have antigens loaded and presented. Viral methods can accommodate large gene inserts and readily yield MHC Class I and II antigen presentation, but may have a high risk of infection or preexisting immunity. DNA plasmids offer the advantage of delivering multiple antigens and genes to express both short and long peptides, but mouse studies have been difficult to translate to human trials. mRNA has similar advantages to DNA, with less side effects and lower autoimmunity, but can undergo rapid degradation and clearance which may lead to lower potency. This review will focus on peptide-based cancer vaccines since peptides are easily produced, cost effective, multiple sequences can be incorpo-

\* Corresponding author.

E-mail address: [jdong@neontherapeutics.com](mailto:jdong@neontherapeutics.com) (J.Z. Dong).

rated into a formulation, and immune-monitoring of the vaccine efficacy is straightforward using just the vaccine components.<sup>3,15,16</sup>

## 2. Cancer immunology

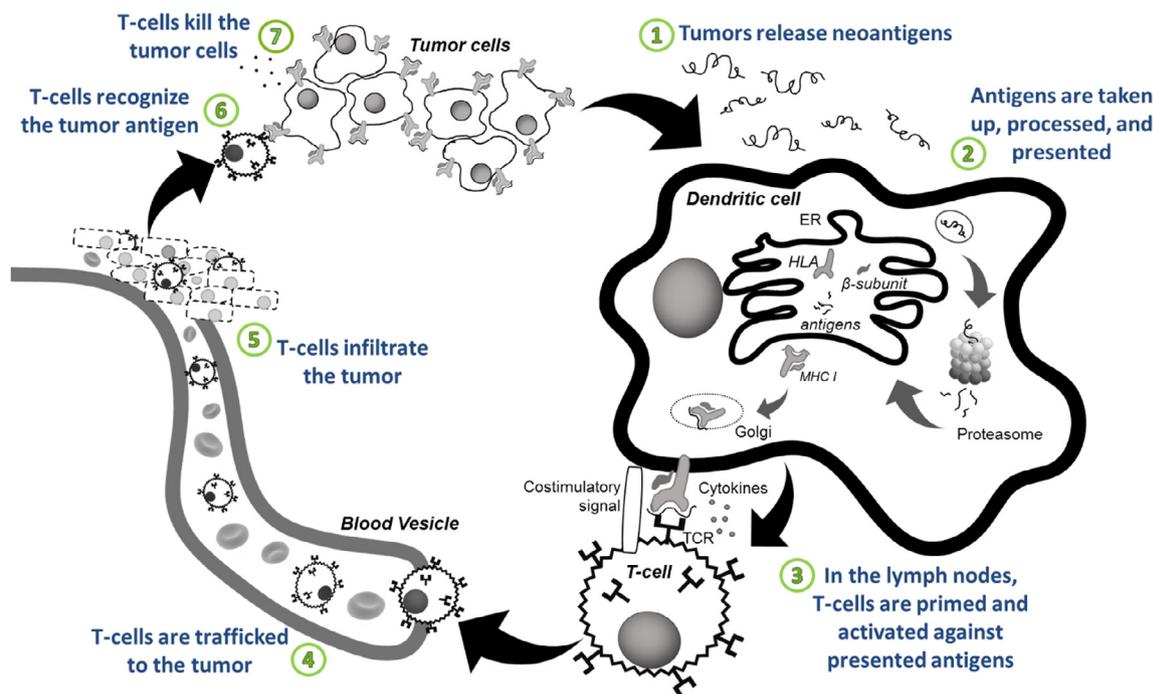
The immunology behind the success of peptide antigen targets is particularly complex. Fig. 1 shows a simple schematic of this process for CD8<sup>+</sup> T-cells (cytotoxic T-lymphocytes).<sup>15,17–22</sup> First, tumors will release the antigens, which are taken up by antigen presenting cells (APCs), such as DCs. The antigens are non-trivially processed within the DC, being broken down by the proteasome or cytosolic peptidases into shorter peptide segments. These peptides are trafficked to the endosomal reticulum (ER) within the cell by the transporter protein associated with antigen processing (TAP). While in the ER, the peptides must locate and bind the major histocompatibility complex (MHC), which for Class I is the assembly of a polymorphic alpha chain that recognizes the peptide and the conserved  $\beta$ -subunit required for proper structure and binding. The human population has a wide diversity of inherited alpha chain allele types that fall into three main subtypes for Class I MHC; human leukocyte antigen (HLA)-A, HLA-B, and HLA-C, each with hundreds of variations as well as unique peptide binding motifs. Once formed, the peptide-MHC is transported to the cell surface via the Golgi apparatus.<sup>15,17–22</sup> The now antigen loaded DC's migrate to the lymph nodes where they prime and activate T-cells against the antigen presented on the DC surface. For successful activation of CD8<sup>+</sup> T-cells, the T-cell receptor (TCR) must recognize the peptide (8–11mer) bound to Class I MHC on the DC. A costimulatory signal must also be exchanged between the T-cell and APC, which constitutes an interaction between CD80/86 and CD28 proteins on the cell surfaces, respectively. The third signal required for recognition is that of cytokine release from the DC. After activation against the peptide antigen, T-cell popula-

tions expand, traffic to the site of the tumor, and infiltrate the tumor. T-cells can then kill the cancer cells if they recognize the presented antigens, which are processed and presented in cancer cells via the same mechanism as previously described for DCs.<sup>15,17–23</sup>

Many possible complications could prevent sufficient immune responses. Firstly, antigens may not be taken up, processed, or presented properly by DCs. Secondly, cells can sometimes be limited in their expression of MHC complexes essential for peptide processing.<sup>13</sup> Signals required to prime and activate the T-cell may be missing or insufficient, or there are not T-cells with TCR's that are able to recognize the antigen.<sup>15,17–22</sup> There are many inhibitory signals present in the tumor environment that prevent T-cells from being activated, expanded, or infiltrating the tumor.<sup>13,15,17–22</sup> Overall, T-cell recognition of antigens is an important step of clinical efficacy, and vaccination provides the opportunity to overcome problems associated with the above steps in inducing T-cell response.<sup>24</sup>

## 3. History of peptide-based cancer vaccines

Peptides are an attractive choice as vaccines due to their potential to directly function as pivotal T-cell epitopes.<sup>10,13</sup> Other advantages of peptides include their low toxicity profiles, specificity for their target, low comparative cost, and straightforward chemical synthesis.<sup>10,12,13,25</sup> Additionally, a single vaccine can incorporate multiple peptide epitopes, increasing the chance of activating multiple T-cells and overcoming loss or changes of epitopes during tumor progression, which in result avoids tumor escape.<sup>10,13,26</sup> In fact, using multiple peptides within vaccines has correlated with positive clinical response in several clinical trials.<sup>27–29</sup> Though an analysis via ClinicalTrials.gov performed in 2014 found that no peptide-based cancer vaccines had reached the market, several



**Fig. 1.** A simplistic diagram of the cancer immunity cycle, as proposed by Chen and Mellman.<sup>15,17–22</sup> The cancer releases antigens (1), which are taken up and processed by DCs (2). In the lymph nodes, T-cells are primed and activated against the presented epitopes (3). Once T-cells are trafficked to (4) and infiltrate (5) the tumor, they must recognize these same antigens on the cancer cells (6) to induce an immune response against the tumor (7). Also shown is the generally accepted processing and presentation of Class I MHC epitopes on the surface of DC's (2) and their recognition by CD8<sup>+</sup> T-cells (3), though Class I MHC epitopes can also be recognized at the surface without internal processing. CD4<sup>+</sup> helper T-cells also follow this cycle, except for two key points: the mode of tumor cell attack (which is still convoluted) and that CD4<sup>+</sup> cells recognize peptides that are much longer (greater than 15 amino acids).<sup>19–22</sup> Note these longer peptides bind to Class II MHC, which has a different structure from Class I MHC protein.

studies were in anti-cancer phase III studies and hundreds were being explored in phase I/II for several disease targets.<sup>19</sup> Similarly, several preliminary studies have demonstrated the potential success of peptide vaccines in overcoming challenges of cancer immunotherapy (Tables 1 and 2).<sup>19</sup> A list of characterized antigens can be found in sources such as the National Cancer Institute website.<sup>30,31</sup>

### 3.1. Self-antigen based vaccines

Most anti-cancer peptide vaccines, both past and present, immunize using non-mutated self-antigens. These tumor associated antigens (TAAs) are present in the normal genome, and often expressed in low levels in healthy cells, but are overexpressed in cancerous tissue.<sup>15,22,32,33</sup> Cancer testis antigens (CTAs) are a subgroup of TAAs, as they are present in the normal genome and in cancer cells, but their expression in normal cells is typically limited to germline cells.<sup>15,22</sup> MAGE, the first family of genes to encode for tumor antigens in humans, was discovered in 1991.<sup>32,34,35</sup> An extensive list of self-antigens, including MAGE-A3, Melan-A/Mart1, gp100, Her2/Neu, and NY-ESO-1, as well as their identified peptide epitope sequences, can be found in the literature.<sup>15,36,37</sup> The primary concern with TAAs is their presence in normal or germline tissues, which can prevent strong immune response due to central tolerance or result in autoimmunity if a strong response is generated against these antigens. These factors may contribute to the poor efficacy and safety issues observed with self-antigen vaccines in clinical trials.<sup>20,22,32,38–40</sup> Selecting mutation-based peptide antigens, on the other hand, is a simple solution to mitigate the central tolerance and autoimmunity concerns associated with TAAs.

### 3.2. Neoantigen based vaccines

Neoantigens, unlike self-antigens, are mutation-based peptide sequences found only in the tumor cells, so they are not subject to central tolerance and have lower risk of generating autoimmunity.<sup>15,22,32,33,41</sup> These tumor specific antigens (TSAs) can arise from viral proteins, post-translational modifications, or somatic muta-

tions such as point mutations, insertion-deletions, or changes in the open reading frame.<sup>22,24,33,42</sup> There are increasing numbers of studies showing T-cell reactivity to neoantigen sequences, in both CD4<sup>+</sup> and CD8<sup>+</sup> T-cells, which are essential to antitumor activity.<sup>43–48</sup> Amongst neoantigens are two subgroups; shared and personalized neoantigens. Shared, as the name implies, are mutation-based antigens common amongst certain tumor types or patients but are not found within the normal genome.<sup>10,42</sup> Shared peptide antigens have been identified which can be used to broadly vaccinate patients of the same cancer type when those patients commonly express that antigen (Table 1).<sup>49–54</sup>

On the other hand, neoantigens imply a further unique antigen difference from patient to patient, and tumor to tumor. Vaccines incorporating personalized neoantigens cater specifically to that individual patient or tumor, and so are promising targets to activate antitumor immunity (Table 2).<sup>32,41</sup> Since tumors of the same cancer type can be widely variable, a purely personalized approach is the best method to ensure response for every individual cancer. Recent animal studies by Sahin and Schreiber have shown personalized neoantigen peptide vaccines are effective against melanoma in murine models.<sup>55,56</sup> Sahin's group identified 50 peptides as being potentially immunogenic. After testing synthesized 27-mers of these antigens in combination treatment with the dsRNA poly I:C adjuvant, 11 peptides were validated to induce a response against the mutated epitope.<sup>55</sup> Schreiber's lab made 21-mers of two peptides (Lama4 and Alg8) predicted to be immunogenic in their methylcholanthrene (MCA) induced sarcoma mouse model and saw robust T-cell responses against both neoantigens in combination with poly I:C.<sup>56</sup>

The use of personalized neoantigen vaccines in clinical trials was pioneered nearly a decade ago by Wu, Hacohen, and other colleagues.<sup>20,57–59</sup> The Dana Farber Cancer Institute (DFCI) continues to be steadily involved in such clinical trials using synthetic long peptides and recently published promising clinical findings.<sup>15,29,33,35,60</sup> In the DFCI NeoVax trial, 4 of 6 patients treated with up to 20 long synthetic personalized neoantigen peptides plus poly IC:LC adjuvant had no recurrence at 25 months following treatment, with two other patients experiencing complete regres-

**Table 1**

Examples of shared neoantigen peptide vaccine studies.

Indication	Epitope	Adjuvant	Safety/Response	Author
Colorectal, pancreatic, bile duct, and lung cancer	13-mers of the KRas mutations G12D, G12V, and G12C	Detox <sup>TM</sup> PC	Immune response to mutant Ras peptides was seen in 20/37 patients with overall survival of 16.9 months	Rahma et al. <sup>54</sup>
Hepatitis C virus (HCV)	Four HLA-A*24 9mers	IFA	No severe toxicity or liver damage observed, with dose dependent immune responses seen in >50% of patients	Yutani et al. <sup>53</sup>
Cervical cancer	25- to 35-mers: Nine HPV-16 E6 and four HPV-16 E7 epitopes	IFA	15/19 patients showed clinical responses, and 9 patients had complete response	Kenter et al. <sup>49</sup>

\* IFA: Incomplete Freund's adjuvant, also referred to as Montanide or Montanide ISA 51.

**Table 2**

Personalized neoantigen peptide vaccine clinical trials (with ClinicalTrail.gov trial identifications).

Institution	Trial ID	Details
DFCI	NCT01970358	<b>NeoVax:</b> Phase I trial for melanoma using SLP <sup>*</sup> neoantigens with poly IC:LC adjuvant <sup>15,22,29,33,35,60</sup>
DFCI	NCT02287428	<b>NeoVax:</b> Phase I trial for MGMT-unmethylated glioblastoma and glioblastoma multiforme using SLP neoantigens with poly IC:LC adjuvant <sup>15,22</sup>
Neon Therapeutics	NCT02897765	<b>NEOPV-01:</b> Phase I clinical trial for melanoma, non-small cell lung, and bladder cancers using personalized peptide vaccine with nivolumab <sup>10,33</sup>
Agenus	NCT02992977	<b>AutoSynVax:</b> Phase I trial for advanced cancer using synthetic peptides complexed to heat shock proteins with QS-21 Stimulon adjuvant <sup>33,63</sup>
WUSM <sup>**</sup>	NCT02427581	Phase I trial for triple negative breast cancer using SLP neoantigens with poly IC:LC adjuvant <sup>22</sup>
WUSM	NCT02510950	Phase 0 trial for glioblastoma multiforme astrocytoma using SLP neoantigens with poly IC:LC adjuvant <sup>22</sup>
MD Anderson Cancer Center	NCT02600949	Phase I trial for pancreatic and colorectal cancers using peptide vaccine plus IFA <sup>22</sup>

\* SLP: synthetic long peptides.

\*\* WUSM: Washington University School of Medicine.

sion after additional treatment with anti-PD-1 checkpoint inhibitor therapy.<sup>29,60</sup> This work demonstrates the efficacy of a personalized peptide based approach towards potent neoantigen vaccines. Based on the DFCI platform, Neon Therapeutics began phase I clinical trials in 2016 using synthetic peptides in combination with checkpoint inhibitor nivolumab for treatment against melanoma, smoking related non-small cell lung cancer (NSCLC), and bladder cancer.<sup>10,61</sup> Agenus also entered a clinical trial in early 2017 for advanced stage cancer using synthetic short peptides complexed to heat shock proteins (HSPs) in combination with QS-21 Stimulon adjuvant (a saponin extract).<sup>33,62,63</sup> These and other current personalized neoantigen peptide vaccine trials are detailed in Table 2.

#### 4. Personalized neoantigen vaccine selection, production, and delivery

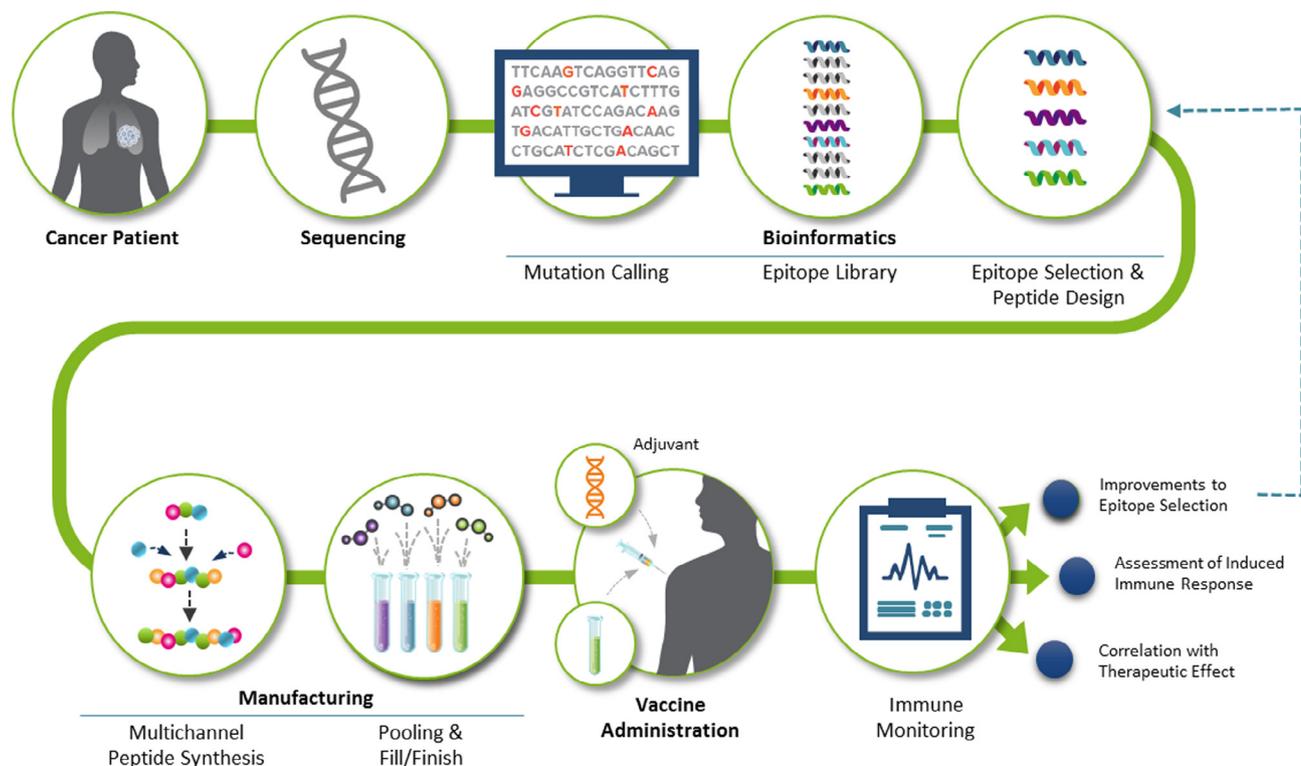
##### 4.1. Tumor biopsy, sequencing, and predictive algorithms

Due to the high polymorphism of human alleles and highly selective peptide-MHC binding, proper epitope prediction and selection is vital to future success of a personalized cancer vaccine.<sup>64</sup> After taking a tumor biopsy from the patient, DNA/RNA whole exome sequencing and computational processing of the data are the first steps toward identifying the mutanome (Fig. 2). Next generation sequencing data from tumor and healthy tissues are evaluated to look for point mutations, insertion-deletions, new open reading frames, structure changes, and copy number. This is compared in parallel to gene expression level data retrieved from cDNA microarray or RNAseq of the tumor to identify mutations within the sequences.<sup>17,32,35</sup>

The non-germline mutations elucidated from sequencing experiments are then screened using predictive algorithms to determine the most immunogenic antigens for incorporation into a personalized vaccine. The first widely used prediction tool, SYF-

PEITHI, was published in 1999 with over 2000 entries on experimentally derived MHC class I and II peptide motifs from humans and other species within its database.<sup>65</sup> This information includes the suspected role of amino acids in the reported ligands and T-cell epitopes, whether it be anchoring or auxiliary in binding with MHC, and their determined binding affinities to specific alleles.<sup>65,66</sup> In 2004, the immune epitope database (IEDB) was established as an official public source of this information, with data for over 120,000 epitopes recorded by 2015, including 3-D structure and binding data.<sup>67</sup> These tools have allowed researchers to query epitopes based on peptide lengths, sequence alignment, particular anchoring residues, and specific alleles of interest.<sup>65–67</sup> The prediction tool NetMHC, which was published in 2003, was trained on the experimentally derived information from the SYFPEITHI database and the IEDB, as well as proprietary affinity data.<sup>68,69</sup> NetMHC was modeled after the neural networking and learning capabilities of the brain and continues to be the most commonly used predictor today.<sup>68,69</sup> NetMHCpan was later developed as a means to extrapolate information from more common alleles to train and validate predictions for rare alleles that have little experimental data available.<sup>64,70</sup> Nevertheless, strong binders of MHC Class I have been routinely predicted using these algorithms.

However, it is crucial to consider that most programs do not take into account all the various other factors impacting immunogenicity, which can include peptide processing by the proteasome, stability of HLA binding, and differences in amino acid properties and positions which affect recognition of the peptide by the TCR.<sup>26,42</sup> Predictors also fail to consider genetic insertion-deletions and fusions that may cause even greater immunogenicity than point mutations due to larger variation compared to wildtype. In addition there is far less data available for making Class II MHC predictions.<sup>22,43,55,71</sup> In an attempt to improve upon previous algorithms, which are mostly trained on biochemical affinity data, a scalable mono-allelic strategy was recently developed which gives



**Fig. 2.** Diagram of the personalized neoantigen peptide vaccine workflow. After diagnosis, a patient's tumor is biopsied, and both cancerous and normal tissues are sequenced. Bioinformatics processing, including mutation calling and application of predictive algorithms, is performed to find the most immunogenic antigen sequences. These peptides are then manufactured. After final formulation and quality control, the peptide vaccine is administered to the patient and immune responses are monitored.

largely unbiased insight to cleavage, expression, and antigen presentation while requiring less sample material.<sup>21</sup> This work aims to deconvolute antigen presentation by using LC–MS/MS to more effectively predict peptide–HLA binding. The large LC–MS/MS data set collected was deconvoluted in combination with bioinformatic analysis and then utilized to train a predictive algorithm in the context of learning more about endogenous processing.<sup>21</sup> There are also many other algorithms in existence, as different institutions are developing their own algorithms to increase the accuracy of the predictions, and automated benchmarking tools are being established to help determine which predictors are most effective for a given query.<sup>72</sup>

One main approach to validate neoantigens elucidated from algorithm predictions is to then test the synthetic version of those peptides and assess binding affinities to the corresponding HLA alleles. (Fig. 2).<sup>19,73</sup> Brown et al. looked at over 500 patients spanning six different tumor types and evaluated the number of mutational epitopes found in each tumor.<sup>74</sup> Hacohen and colleagues expanded such analysis by looking at thousands of data sets from tumor biopsies in the TCGA (The Cancer Genome Atlas). The result was a cytolytic index matrix that correlates to neoantigen load in over 18 tumor types.<sup>22,75</sup> This matrix identified tumors sensitive to spontaneous cytolytic activity and immune elimination, which revealed information about tumor–immune cell interactions and the presence of indications to predict immunogenicity. In another example of validating the accuracy of predictive methods, Castle et al. performed whole exome sequencing finding 563 non-synonymous somatic point mutations that were expressed in genes in B16F10 murine melanoma cells.<sup>55,71</sup> Fifty mutations were selected based on MHC class I binding predictions and validated using a prophylactic personalized neoantigen peptide vaccine of 27-mer sequences. Out of the fifty peptides, 11 were found to induce T-cell responses against the mutated epitopes; three of which were endogenously processed and two having resulted in inhibited tumor growth.<sup>17,22,55</sup> Studies such as these are able to experimentally demonstrate the effectiveness of prediction algorithms to select immunogenic neoantigens.

#### 4.2. Short vs. long synthetic peptides

Since peptides involved in immune responses vary greatly in length, immunological considerations are made in conjunction with prediction results to determine what lengths and sequences will be ultimately incorporated into the vaccine. Short length peptides are comprised of purely the epitope sequence bound to the HLA, which is approximately 8–11 amino acids. These epitope length peptides bind exogenously to Class I MHC on the cell surface, including Class I MHC on non-professional DCs, which can result in tolerance. This non-specific binding can also lead to a lack of T-cell responses against the neoantigen. Thus, short peptides must be administered in combination with a potent adjuvant for efficacy. In one example, use of Toll-like receptor 9 or 3 (TLR9 or TLR3) agonists induced favorable CD8<sup>+</sup> responses for short peptides by activating DC's and, therein, the immune system.<sup>32,76</sup> Short peptides are important for CD8<sup>+</sup> recognition, but are not as effective as long peptides in both binding class II MHC and provoking immune response via a CD4<sup>+</sup> T-cell pathway.

One way around this is to pursue synthetic long peptides (SLPs) which tend to be 15 amino acids or greater in length. The recognition epitope of these sequences is typically flagged at either terminus with additional adjacent amino acids from the parent protein sequence. The intracellular processing mechanism required for long peptides ensures antigen presentation by professional cells, which can lead to higher T-cell responses. In addition, SLPs are recognized by both CD4<sup>+</sup> and CD8<sup>+</sup> T-cells, allowing for broader T-cell responses.<sup>15</sup> This is particularly advantageous over short peptides

since CD4<sup>+</sup> T-cells are essential to fully activate CD8<sup>+</sup> T-cells. Moreover, increasing evidence supports CD4<sup>+</sup> T-cells themselves having cytotoxic activity.<sup>77</sup> These longer peptide sequences can also be designed to contain multiple epitopes for multiple HLA types within a single peptide chain, broadening the potential efficacy of long peptides as vaccines.

#### 4.3. Peptide manufacturing

With modern genetic sequencing and bioinformatic processing, potential epitope peptide sequences are readily identified and selected for incorporation into a vaccine. Due to solid-phase peptide synthesis (SPPS) technology, it is straightforward and relatively inexpensive to generate peptide vaccines compared to others that are comprised of recombinant proteins, cells, or that are based on adoptive cell transfer therapies.<sup>12,13,19,25</sup> Peptides themselves are also stable, particularly when stored at low temperatures, and have been shown to be safe in over 100 clinical trials.<sup>26</sup>

Peptides for personalized therapy have synthetic challenges because each patient requires a unique set of peptides for the vaccine that is completely different from those of other patients, with varying amino acid composition and physicochemical properties. Thus, there is no “bulk” manufacturing of any peptide that is stored until the next patient demand. As a result, manufacturing is a constant process which is not only fast and robust but also suitable for different sequences.<sup>20,78,79</sup> After synthesis, the peptides must be purified, which is no trivial task when dealing with different sequences of varying hydrophobicity. Many manufacturers provide a selection of instruments to choose from which range from traditional RP–HPLC to flash-like systems, with a wide array of software and detector capabilities to fit any given need. Depending on the peptide properties, some purification methods may be more advantageous than others in obtaining high yields and purities. Replacing the number of manual operations involved with purification with automated processes will further improve lead time, which has already been observed with liquid handling systems, UPLC, and auto-sampling systems.<sup>80,81</sup> After purification to the required quality, the peptide is lyophilized to yield solid powder before proceeding to release testing and formulation.

Formulation of personalized drug substance is another challenging step in production due to varying physicochemical properties for each peptide, especially degradation or aggregation.<sup>25,79</sup> Excipients in the formulations may include buffering agents, surfactants, and preservatives, to name a few, in order to improve solubility and stability.<sup>25</sup> The formulation may also be derived from delivery methods like poly(lactic-co-glycolic) acid (PLGA), liposomes, virosomes, or nanoparticles.<sup>15,19,82,83</sup> After aseptic fill-finish of final drug product, the vaccine is shipped to clinical sites for administration to patients. During administration, the peptide vaccine is usually combined with an adjuvant that stimulates the immune system, such as Montanide or MF59 emulsions, dsRNA poly I:C or poly IC:LC, or Toll-like receptor targeting agents, such as CpG oligonucleotides.<sup>84–88</sup> Many of the current clinical trials are also investigating a combination approach, particularly personalized vaccine with checkpoint inhibitors.

### 5. Combination therapy of personalized neoantigen vaccines and checkpoint inhibitors

Checkpoints are stages in the cancer immunity cycle that can lead to lack of immune response, for a number of reasons. Recall (in Fig. 1) that one stage requires activated T-cells to recognize the tumor antigen on the cell surface. However, cancer commonly sends inhibitory signals and disguises itself from the T-cells, which has been a main cause of tumor immunosuppression.<sup>15,17–22</sup> The

first target identified to enhance T-cell immunity was CTLA-4 (cytotoxic T lymphocyte antigen 4) in the 1990s, when Allison and colleagues showed CTLA-4s role in suppressing T-cell activity against recognizing tumor antigens.<sup>22,89</sup> Specifically, the checkpoint CTLA-4 functions to inhibit T-cell priming by interacting with CD 80/86, a signal that puts the breaks on T-cell activation, therein preventing any immune response.<sup>22,89</sup> Based on this rationale, monoclonal antibodies were developed to block checkpoints as new approaches in developing cancer therapeutics. In 2011, the FDA approved ipilimumab, a monoclonal antibody against CTLA-4, for use against melanoma.<sup>22,33</sup> This checkpoint inhibitor is responsible for unmasking tumor cell signals that are dampening the immune response to that cancer, and is also able to generate specific T-cell responses to antigens present.<sup>33</sup> Since then, several checkpoints have been discovered.<sup>90</sup> Other common checkpoints and their inhibitors are detailed in Table 3.

In studies where checkpoint blockade has exhibited T-cell responses, the tumors typically carried high mutational burden, creating many neoantigens.<sup>10,22,92</sup> For example, non-small cell lung cancer and melanoma, which often have high neoantigen load, have a more positive response to the checkpoint blockade and greater overall survival rate.<sup>23,24,33,93,94</sup> Comparably, tumors with lower numbers of mutations, like thyroid cancer or acute myeloid leukemia, tend to have poor response rates.<sup>94</sup> Even though response rates for high mutational burden tumors have exhibited high clinical efficacy, the response rate for those “sensitive” tumors is still low, ranging from 20 to 40%.<sup>95–97</sup> The idea of supplementing checkpoint blockade with an additional personalized neoantigen vaccine should not only improve response rates against “sensitive” tumor types, but also promote responses by tumors previously “insensitive” to checkpoint inhibition.<sup>20,44,46,56,96,98,99</sup>

### 5.1. Identifying the role of neoantigens in checkpoint blockade

Seminal work led by Schumacher and researchers supports the use of checkpoint blockade to amplify neoantigen T-cell response, as well as the use of whole exome sequencing to successfully predict those immunogenic neoantigens. An initial 1657 somatic mutations were identified from whole exome sequencing of a stage IV melanoma tumor. Using RNAseq data, as well as NetChop and NetMHC predictions, neoantigen sequences were identified, synthesized, and tested against the patient’s bulk tumor infiltrating lymphocytes. T-cell responses against two specific neoantigens were observed, with the dominant response arising from a serine to leucine mutation in a serine/threonine protein kinase in the tumor. These responses demonstrated that there exists T-cell reactivity that is neoantigen-specific, and these are the first results to reveal these responses via cancer exome sequencing. Additionally, just weeks into combination treatment with the checkpoint inhibitor ipilimumab, Schumacher was able to see an added 5-fold increase in response to the neoantigen as well as a 25% reduction

**Table 3**  
Examples of common checkpoint inhibitors.

Target	Commercial antibodies	Function of the checkpoint inhibitor
CTLA-4	Ipilimumab (yervoy), tremelimumab	Block CTLA-4 and CD80/86 interactions which turn off T-cell activation <sup>22,89</sup>
PD1	Nivolumab (Opdivo), Pembrolizumab (Keytruda)	Block the PD1 receptor on T-cells from binding PDL1 ligands, which are expressed by the tumor to inactive T-cells <sup>22,33</sup>
PD-L1	Atezolizumab (TCENTRIQ)	Block the PD-L1 ligands expressed by tumors that can inactivate T-cells by binding the T-cell PD1 receptor <sup>91</sup>

in tumor load.<sup>100</sup> These results further show the effectiveness of checkpoint therapy at diminishing immunosuppression and permitting strong T-cell recognition of the neoantigen epitopes.<sup>44</sup>

Another case study by Schreiber and colleagues suggests that administered neoantigen peptide vaccines are similarly effective compared to checkpoint inhibitor treatment alone.<sup>44,56,96,99</sup> Using a MCA sarcoma murine model, tumor cells were subjected to sequencing, mutation calling, and filtering of peptide sequences based on affinity and processing predictions. This resulted in identification of two neoantigens, Alg8 and Lama4, which were validated by screening TILs from checkpoint inhibitor anti-PD-1 treated MCA mice. T-cell responses against these antigens were then tested for therapeutic efficacy in naïve mice with a synthetic long peptide vaccine consisting of the two neoantigen epitope peptides and poly I:C adjuvant. Vaccinated mice had 85% survival compared to a control group treated with poly I:C alone (15% survival) or with poly I:C plus a shared peptide based on HPV viral mutations (10% survival). The combination of personalized neoantigen peptides and poly I:C was also more effective than peptides alone, and worked comparably to only anti-PD-1 treated mice. This work also demonstrated that using a combination of multiple checkpoint inhibitors (anti-PD-1 with anti-CTLA-4) expanded T-cell responses.<sup>56</sup>

These and other studies beg the question of whether a combination of checkpoint inhibitor and neoantigen peptide vaccine would provide synergistic effects compared to either treatment alone.<sup>101</sup> To probe this question, one study looked at administering a four-component combination which included a peptide and DNA conjugate vaccine, anti-PD-1 checkpoint inhibitor, a tumor antigen specific antibody, and an interleukin molecule.<sup>102</sup> Each component alone showed only modest effects, but in combination regressed tumors and had prolonged responses (cures) in 75% of B16F10 melanoma mice. They found that for these difficult to treat, resistant tumors, all four components were required to elicit response, that only two or three components were necessary for other tumors, and that the importance of each component varied with tumor type. Since a four-component system may be challenging in the clinic, Moynihan and coworkers imply that modifying their protocol and incorporating sequencing and neoantigen prediction may be ways to discover alternate therapies.<sup>102</sup> The trial being conducted by Neon Therapeutics is directly testing the possibility of the synergistic effects of a neoantigen peptide vaccine with checkpoint inhibitors by dosing with ipilimumab prior to vaccination comprised of multiple neoantigen peptides and poly LC:IC adjuvant. Results from this work will expand the field of information and shed light on this combination approach for cancer immunotherapy.

## 6. Conclusion

In targeting neoantigens, we are approaching a new chapter of cancer vaccine development. Aiming at neoantigens has the potential to overcome central tolerance and risk of autoimmunity, which have hampered cancer vaccine research and development and significantly contributed to the failures of previous cancer vaccines. Several studies discussed have shown that neoantigen specific T-cell responses exist and contribute to decreases in tumor growth, portraying them as obvious targets for cancer immunotherapy. However, there remain hurdles to overcome to optimally utilize personalized vaccines. For example, we need more data regarding the processing and presentation of neoantigens to improve prediction algorithms to select the most ideal immunogenic epitopes. Faster and less expensive vaccine production is equally vital to servicing patients effectively, and in a timely manner. Developing better synthesis, purification, and automation technologies will allow

for rapid manufacturing of personalized vaccines to meet required short production turnaround times. Furthermore, based on culminating evidence, the strategy of combining neoantigen vaccines with checkpoint blockade is anticipated to produce synergistic effects. In fact, this will be directly tested in the Neon Therapeutics trial combining neoantigen peptide vaccine and checkpoint blockade. Addressing these questions should aid in reaching broader populations and malignancies with greater efficacy than current immunotherapies.

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## A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.bmc.2017.10.021>.

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