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Personal Neoantigen Vaccines for the Treatment of Cancer

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Abstract

Cancer vaccines can generate and amplify tumor-specific T cell responses with the promise to provide long-term control of cancer. All cancer cells harbor genetic alterations encoding neoantigens that are specific to the tumor and not present in normal tissue. Similar to foreign antigens targeted by T cells in infectious disease settings, neoantigens represent the long elusive immunogens for cancer vaccination. Since the vast majority of mutations are unique to individual tumors, neoantigen vaccines require custom design for each patient. The availability of rapid and cost-effective genome sequencing, along with advanced bioinformatics tools, now allows neoantigen-target discovery and vaccine manufacturing in sufficient time for the treatment of cancer patients. Clinical trials in melanoma and glioblastoma have demonstrated the feasibility, immunogenicity, and signals of efficacy of this personalized immunotherapy approach. Key unresolved areas include identification of the most effective vaccine delivery platforms, validation and consensus of neoantigen target selection, and optimal strategies for partnering immunotherapies. Given the universal presence of mutations in cancer and the patient-tailored paradigm, personalized neoantigen vaccines have potential applicability for all cancer patients.

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Effective and long-lasting anticancer immunity relies on robust, tumor-specific T cell responses. Forays into generating such responses in cancer patients by delivering inflammatory stimuli in the context of tumor antigens predate the advent of effective vaccines against infectious organisms; however, therapeutic cancer vaccines have been largely elusive to date. With the exception of sipileucel-c, an autologous dendritic cell vaccine that has demonstrated improvement in overall survival for patients with advanced prostate cancer (Kantoff et al. 2010), vaccines are not yet available for the standard treatment of patients with early stage or advanced malignancies.

GENERATING ANTITUMOR IMMUNITY WITH CANCER VACCINES VERSUS ADOPTIVE CELL THERAPY

Conceptually, a cancer vaccine is intended to generate de novo and amplify pre-existing tumor-specific T cell responses against tumors by providing tumor antigens along with an inflammatory signal (Hu et al. 2018). Similar to a vaccine against a virus or other infectious agent, vaccinating against cancer is attractive because: (*a*) the approach is highly specific—i.e., it should target only the relevant (tumor-specific) T cell populations; and (*b*) effector memory T cell populations are stimulated in vivo, which can lead to the generation of T cell responses that last for many years. At least in theory, cancer vaccines may therefore be able to provide durable antitumor immunity. We note that adoptive cell therapy (ACT) approaches, such as adoptive transfer of tumor-infiltrating lymphocytes (TILs), transgenic T cells, or chimeric antigen receptor T cells, are also highly specific to the tumor and have shown clinical efficacy, which can also last for many years. In contrast to the in vivo stimulation of T cell responses mediated by vaccines (active immunotherapy), ACT is termed passive immunotherapy based on the generation of high numbers of tumor-specific T cells in vitro with subsequent infusion to the patient.

VACCINE TARGETS: FROM NATIVE ANTIGENS TO NEOANTIGENS

Historically, a major impediment to the development of effective cancer vaccines has been the lack of truly tumor-specific antigens. The vast majority of clinical trials testing cancer vaccines to date have utilized tumor-associated antigens (TAAs), which include antigens that are selectively expressed in tumor cells, overexpressed, or involved in tissue differentiation such as NY-ESO-1, human epidermal growth factor receptor 2, and melanoma-associated antigen (Buonaguro et al. 2011, Chen et al. 1997, Disis et al. 2009). Because many of these antigens are also expressed in normal tissues, there is a concern of inducing autoreactive immune responses, leading to toxicities affecting healthy organs. In addition to autoimmunity, expression of vaccine antigens on healthy cells leads to negative selection of high-affinity T cells in the thymus during ontogeny, likely compromising the effectiveness of TAA-based vaccines due to low affinity of vaccine-induced T cell responses.

Because neoantigens are not expressed by healthy tissues and are not exposed to central tolerance mechanisms in the thymus, they should not cause autoimmunity and are potentially highly immunogenic. While neoantigens have been long recognized as valuable tumor targets (Lennerz et al. 2005; Mandelboim et al. 1994, 1995; Sensi & Anichini 2006), only the recent availability and affordability of next-generation sequencing have led to large-scale identification of these antigens. These efforts have established neoantigens as key targets of effective immune responses: (*a*) Tumor mutational burden and neoantigen load have been linked to more robust antitumor T cell responses and better outcomes for cancer patients both prognostically and in the context of different immunotherapies (Brown et al. 2014, Rizvi et al. 2015, Snyder et al. 2014, Van Allen et al.

2015); (*b*) frequencies of neoantigen-specific T cells were found increased in cancer patients who responded to immune checkpoint inhibition and other immunotherapies (Rizvi et al. 2015, van Rooij et al. 2013); and (*c*) direct in vivo cytotoxicity by neoantigen-specific T cells was demonstrated in various mouse models including sarcoma, melanoma, and colon cancer and in patients with treatment-resistant advanced cholangiocarcinoma or breast cancer who experienced tumor responses upon adoptive transfer of neoantigen-specific T cells (Tran et al. 2014, Zacharakis et al. 2018).

Another class of tumor-specific antigens includes those that are encoded by oncogenic viruses, including human papillomavirus found in head and neck and anogenital cancers; human herpes virus 8, identified in Kaposi sarcoma; and Epstein Barr virus (EBV), associated with B cell lymphoma, Hodgkin's disease, and nasopharyngeal carcinoma (Melief et al. 2015, Ott & Hodi 2013). Vaccines against human papillomavirus have demonstrated efficacy in the prevention and treatment of anogenital intraepithelial neoplasias (Joura et al. 2015, Kenter et al. 2009).

VACCINES TARGETING NEOANTIGENS: OFF THE SHELF VERSUS TRULY PERSONAL

Off-the-Shelf Neoantigen Vaccines

Targeting neoantigens encoded by oncogenic driver mutations could be attractive for biological reasons (the clonal nature of the mutations) and for practical purposes (driver mutations are shared by subsets of patient populations, and vaccines could therefore be produced in batches and be available for use as needed). However, only relatively small subsets of tumors harbor specific oncogenic drivers. Furthermore, the number of potentially immunogenic driver mutations is further reduced due to HLA diversity since the majority of neoantigens encoded by oncogenic clonal mutations harbored by an individual tumor will not bind to that individual's HLA molecules. Therefore, an off-the-shelf approach requires not only the incorporation of many irrelevant epitopes (potentially compromising efficacy) but also the selection of patients (see **Figure 1**). Therefore, many patients considered for such an approach may not be eligible for the treatment. Moreover, while this strategy may be feasible for subsets of patients with tumors generally harboring a relatively large number of driver mutations such as non-small-cell lung cancer (NSCLC), it is likely not a solution for the many tumor types with low numbers of mutations (including driver mutations).

Personal Neoantigen Vaccines

The vast majority of neoantigens are encoded by passenger mutations; these are for the most part irrelevant for the growth of tumor cells, but may be highly immunogenic. Consequently, a truly personal approach, in which target neoantigens are selected based on the mutational profile of an individual's tumor, takes advantage of a larger spectrum of neoantigens and can be tailored to targets with the highest potential for immunogenicity in that individual. We note that the complexity of the process is an intrinsic challenge of this approach. The time delay is particularly problematic for patients with advanced cancers. In the initially reported studies reviewed below, needle-to-needle lead time (i.e., the time from the tumor biopsy to the first vaccination) was between 6 and 18 weeks. However, while the vaccine generation workflows consist of a substantial number of individual steps (e.g., tumor tissue acquisition, pathologic review, DNA/RNA isolation, sequencing, mutational calling and prioritization, epitope selection, vaccine synthesis; see **Figure 2**), there are significant opportunities to accelerate and automatize; moreover, there are no inherent biological delays such as, for example, the necessity to culture T cells in vitro in order

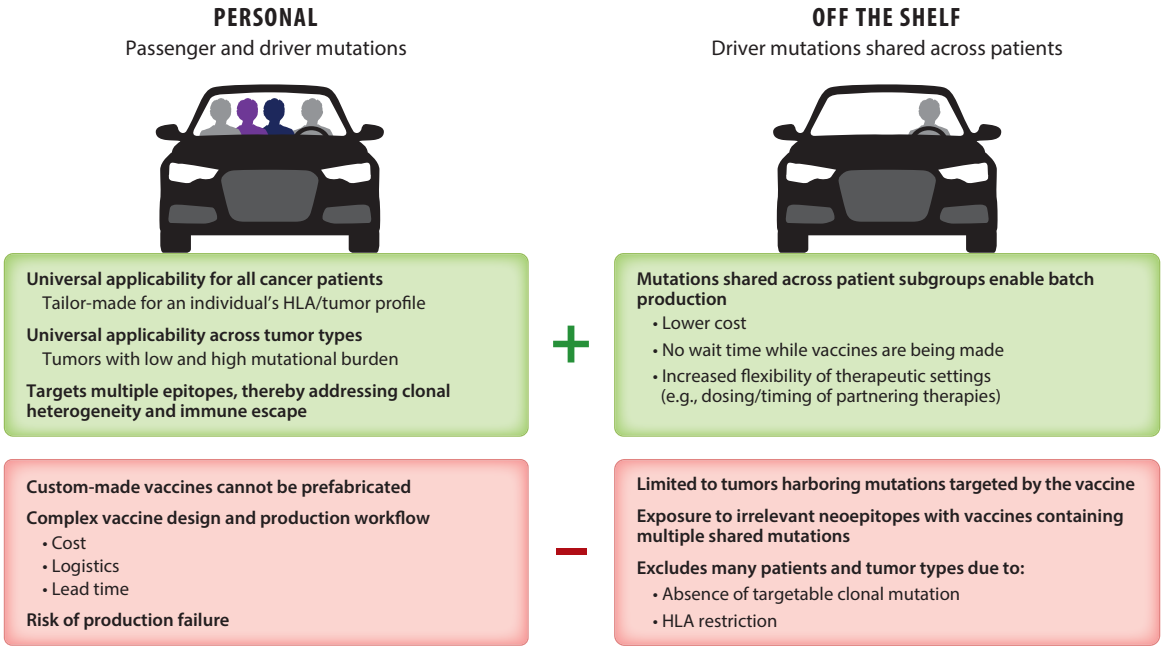

Figure 1

Personal versus off-the-shelf neoantigen-targeting vaccines: advantages (*green*) and disadvantages (*red*) of each. The ability of personal vaccines to target multiple diverse mutations may benefit more patients than off-the-shelf vaccines. However, reducing the manufacturing complexities of personal vaccines is essential for this therapeutic to be rapidly available in a clinical setting.

to obtain sufficient cell numbers, which is required for ACT. Optimizing parameters within each step of the workflow is imperative for scaling and sustaining the development of this treatment.

PRECLINICAL STUDIES HAVE DEMONSTRATED IMMUNOGENICITY AND ANTITUMOR ACTIVITY OF NEOANTIGEN-DIRECTED VACCINES

Early evidence pointing at neoantigens as targets of effective antitumor immune responses in mouse studies and from case reports in humans (Lennerz et al. 2005, Mandelboim et al. 1995, Sensi & Anichini 2006) led to deeper assessments of neoantigen vaccines in syngeneic mouse models. The first preclinical study testing neoantigen vaccines was conducted in the B16 mouse melanoma model (Castle et al. 2012). One-third of 50 validated mutations were found to be immunogenic, as tested by vaccinating mice with long peptides encoding mutated epitopes in combination with the Toll-like receptor agonist poly-ICLC; notably, a subset of the neoantigens mediated regression of established B16F10 melanoma.

In a subsequent study conducted in the MC38 murine colon cancer model, 6 potentially immunogenic neoepitopes were selected from 4,000 coding variations called in the tumor exome by applying filters including RNA expression, HLA binding [by NetMHC-3.4 (Lundegaard et al. 2008)], and mass spectrometry (Yadav et al. 2014). C57BL/6 mice were vaccinated with pools of long peptides containing these six neoepitopes administered in combination with anti-CD40 antibodies and poly-ICLC. Three of the six neoepitopes induced $CD8^+$ T cell responses in the periphery. $CD8^+$ T cells specific for these immunogenic epitopes were also enriched in

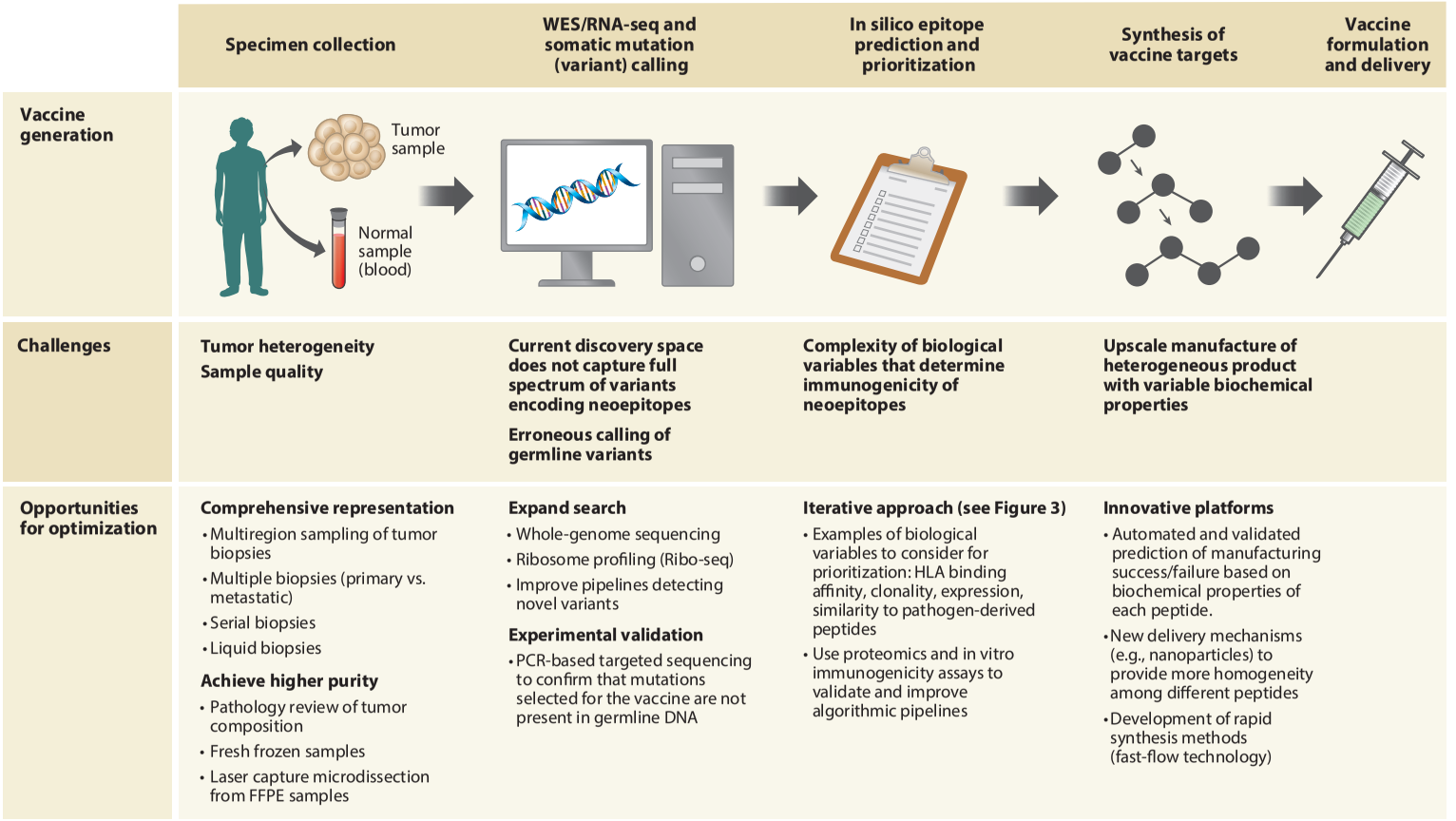

Figure 2

Generating personalized peptide-based neoantigen vaccines for cancer patients: challenges and opportunities. The process of designing and manufacturing a cancer vaccine tailored to an individual cancer patient requires additional precision for effective use. Here, we highlight selected opportunities to address the challenge for each step. Abbreviations: FFPE, formalin-fixed paraffin-embedded; PCR, polymerase chain reaction; Ribo-seq, ribosome sequencing; RNA-seq, RNA sequencing; WES, whole-exome sequencing.

nonvaccinated C57BL/6 mice bearing MC38 tumors. Vaccination of C57BL/6 mice bearing MC38 tumors with Reps1, Adpgk, and Dpagt1 epitopes led to increased frequencies of Adpgk-specific $CD8^+$ T cells, but not Reps1 or Dpagt1, indicating that Adpgk is an immunodominant epitope. Importantly, the neoantigen peptide vaccines mediated regression of established tumors in the majority of vaccinated mice.

Another study was conducted in a highly immunogenic sarcoma mouse model (d42m1-T3) (Gubin et al. 2014). Using a rigorous selection process, two mutant epitopes (mAlg8 and mLama4) were identified from a list of almost 3,000 expressed nonsynonymous mutations as the most likely target antigens. Both epitopes were also detected by targeted selected reaction mass spectrometry in d42m1-T3 sarcomas. $CD8^+$ T cells isolated from TILs of tumors that regressed after treatment with anti-PD-1 and/or CTLA-4 antibodies were found to be reactive to tetramers loaded with mAlg8 and mLama4, but not to 64 other strong binding predicted neoepitopes. Similar to the observations in the B16 melanoma and MC26 models described above, vaccination with mAlg8 and mLama4 long peptides plus poly-ICLC resulted in tumor regression in the sarcoma model.

The immunogenicity of neoepitope RNA vaccines was studied in the murine CT26 colon carcinoma, the 4T1 breast cancer, and the B16F10 melanoma models (Kreiter et al. 2015). A substantial proportion of nonsynonymous mutations in all three models were found to be immunogenic; interestingly, the majority of mutations were found to be recognized by $CD4^+$ T cells. Liposomal RNA vaccines, containing either one or up to five neoepitopes (pentatopes), mediated effective tumor control. In B16F10 melanoma, T cells infiltrating tumor lesions that regressed upon vaccination with the dominant class II B16 melanoma neoantigen M30 (B16-M30) were specific for this epitope and were found in substantially higher frequencies compared to tumors from nonvaccinated control mice, indicating that the vaccines were able to mediate trafficking of neoantigen vaccine–specific T cells into tumors. Notably, frequencies of myeloid derived suppressor cells and T regulatory cells in the tumor were decreased, indicating that the vaccines can modulate the composition of immune cell populations in tumors in favor of tumor-specific T cell responses.

Collectively, these four independent studies, which were conducted in a variety of mouse tumor models and used different vaccine delivery approaches, demonstrated antitumor activity and robust immunogenicity of vaccination with rigorously selected mutated epitopes.

GENERATING PERSONALIZED NEOANTIGEN VACCINES FOR CANCER PATIENTS

Because of the documented immunogenicity and high mutational load, melanoma was chosen as the clinical setting for the three initial clinical studies testing personalized neoantigen vaccines in humans. Each of the three studies used a different vaccine format. In the first study, three patients with stage III melanoma who had been previously treated with ipilimumab received intravenous administrations of mature, autologous dendritic cells loaded with seven HLA-A*02:01-restricted short (9-mer) neoantigen peptides in addition to two gp100-derived control peptides (Carreno et al. 2015). De novo vaccine-induced $CD8^+$ T cell responses were detected against 6 of 21 peptides contained in the vaccines, while pre-existent $CD8^+$ T cell responses against an additional three peptides were augmented after vaccination. Vaccine-induced $CD8^+$ T cells were highly avid and exhibited cytotoxicity. Of note, proteomic analyses utilizing an HLA-A*02:01^+-restricted melanoma line transfected with vaccine neoepitopes demonstrated processing and presentation of two of seven neoantigens contained in the vaccines in one patient. In a second study, eight patients with surgically resected melanoma [no evidence of disease (NED)] who were at high risk for recurrence and five patients with advanced disease were treated with intranodally administered RNA vaccines encoding up to ten personal neoantigens (Sahin et al. 2017). The patients who were NED

at the time of vaccine initiation remained without recurrence during 12–23 months of observation, while two objective responses related to vaccination were observed in the five patients with metastatic disease in addition to a complete response in a third patient who was treated with vaccine in combination with PD-1-blocking antibody therapy. T cell responses against 60% of the 125 vaccinating neoepitopes with a preponderance of $CD4^+$ T cells over $CD8^+$ T cells were detected. Two-thirds of these responses were de novo and one-fifth were detected ex vivo. Of note, T cells reactive to a vaccinating neoepitope were seen in a lymph metastasis, indicating that the vaccines were able to mediate trafficking of T cells into metastatic tumors. In a third study, six patients with stage III or IV surgically resected melanoma were vaccinated with 15–30-mer peptides encompassing neoepitopes (up to 20 peptides per patient), formulated with poly-ICLC. Similar to the RNA vaccine study described above, T cell responses against the majority of vaccinating epitopes were detected, with a predominance of $CD4^+$ versus $CD8^+$ T cell responses. T cell responses were triggered by autologous tumor cell lines in three of the six patients. Notably, two patients with progressive disease during or after the vaccinations had complete responses after four doses of pembrolizumab; the repertoire of vaccine neoepitope–specific T cells persisted and expanded after one year of ongoing complete responses in these two patients.

These three studies, all conducted in melanoma patients, demonstrated that personalized neoantigen vaccines are feasible, safe, and immunogenic. They also provide initial evidence of antitumor activity based on the observations of patients experiencing tumor regression with vaccines alone or subsequent treatment with immune checkpoint inhibition.

Beyond melanoma, two recent studies demonstrated robust immunogenicity of personalized peptide vaccines in patients with glioblastoma multiforme (GBM). In one study, 10 GBM patients received one to two long peptides [ca. 19 amino acids (AAs)] targeting neoepitopes that were formulated with poly-ICLC and granulocyte-macrophage colony stimulating factor in addition to a set of shared, nonmutated peptides selected from a premanufactured library (Hilf et al. 2019). Neoepitopes were selected based on predicted immunogenicity and class MHC class I binding affinity. Of note, none of 643 nonsynonymous mutations identified across the 15 patients enrolled on the study was detected by high-sensitivity mass spectrometry in the HLA class I and II peptidomes. Eleven of the 13 immunizing neoantigen peptides administered across 10 patients induced T cell responses. These responses were predominantly T_H1 (T helper cell type 1) $CD4^+$ and were polyfunctional. In another study, GBM patients who had received standard-of-care surgical resection and radiation were treated using the same vaccination approach as described above for the melanoma trial (Keskin et al. 2019). Robust de novo T cell responses directed against multiple neoepitopes contained in the vaccines were observed in two of five patients who had received at least one booster vaccination and had not required dexamethasone for management of neurological symptoms. These responses were detected ex vivo by IFN-γ ELISPOT (enzyme-linked immunosorbent spot), exhibited an antigen-experienced phenotype, and were polyfunctional. Vaccine-specific T cells that had trafficked into an intracranial tumor were identified by single-cell T cell receptor (TCR) α and β sequencing.

PERSONALIZED NEOANTIGEN VACCINES FOR CANCER PATIENTS: KEY AREAS AND OPPORTUNITIES FOR FURTHER DEVELOPMENT

As described above, the early experience with neoantigen vaccines has demonstrated safety, robust immunogenicity, and preliminary evidence for antitumor activity. These studies are a first foray into the field, demonstrating that a truly personalized approach to cancer therapy is feasible in the clinic. Rapidly evolving new technologies in many areas relevant to the complex vaccine production workflows, including computational genomics, biochemistry, pharmacology, immune

monitoring, and immunogenomics, provide a rich set of opportunities for further improvement of personalized vaccines. Key areas including vaccine manufacturing and delivery, neoepitope target discovery, validation, prioritization, and assessment of vaccine-induced immune responses are discussed below.

Target Selection and Validation

While the availability of relatively rapid and cost-effective next-generation sequencing has enabled the targeting of personal tumor mutations in cancer patients and the general framework of identifying suitable vaccine targets seems relatively straightforward (**Figure 2**), there are numerous challenges and opportunities to improve the pipelines and workflows that were employed for target selection in the initial clinical trials. The clinical cancer setting requires timely identification of vaccine targets, usually from limited amounts of tumor material. This reality necessitates a compromise between, on the one hand, steps that might be on the wish list for target validation—such as conducting mass spectrometry to physically detect computationally predicted epitopes or performing in vitro immune assays to validate the antigenicity of the selected epitopes in autologous peripheral blood mononuclear cells (PBMCs)—and, on the other hand, the clinical necessity of accelerating the process of manufacturing a personalized therapeutic for a cancer patient.

Identification of Nonsynonymous Mutations in Clonally Diverse Metastatic Tumors

Nonsynonymous tumor mutations (i.e., those affecting a change in the AA sequence of the encoded protein) are identified by comparing whole-exome sequencing (WES) data from a tumor sample with sequencing data from matched normal cells (for example, PBMCs). However, due to intra- and intertumoral heterogeneity, the relatively small tumor sample that is typically obtained from a metastatic site (selected based mainly on clinical accessibility) almost certainly does not reflect the entire spectrum of targetable mutations in a given patient (Dagogo-Jack & Shaw 2018, Hanahan & Weinberg 2011). Intratumoral clonal heterogeneity and tumor evolution that result in the outgrowth of subclones exhibiting resistance to selective pressure (such as the host's immune response) have been identified as key features of tumor progression (Greaves & Maley 2012, Landau et al. 2013). There is also evidence that immunotherapy itself can sculpt the spectrum of neoepitopes expressed by cancer cells, leading to downregulation of highly expressed neoantigens, whereby the tumors become less visible to the immune system and thereby evade attack by the immune response (George et al. 2017, Hu et al. 2018, Verdegaal et al. 2016). By targeting multiple neoepitopes rather than a single antigen, a personalized vaccine has the ability to address some of these challenges. In addition to clonal heterogeneity within a given tumor, there is also regional or geographic heterogeneity that results from divergent clonal evolution (Gerlinger et al. 2012).

Tumor Procurement

Multiregion core biopsies obtained from a given tumor metastasis or even several accessible metastatic sites could be utilized to address the issue of tumor heterogeneity and resulting limitations of a single core biopsy (Fennemann et al. 2019). Serial tumor biopsies, particularly of progressing (i.e., therapy-resistant) tumor lesions, may reveal newly emerging mutations encoding for neoantigens that could be targeted in an adapted, subsequently administered vaccine for an individual patient. While standard protocols are available to extract DNA and RNA from fresh frozen and formalin-fixed paraffin-embedded (FFPE) tumor tissue, variability of individual tumor samples including the amount and type of tumor content, downstream processing steps, and other variables can all affect the quality of the respective tumor DNA or RNA sample that is utilized for

sequencing (Oh et al. 2015). The method for procuring and storing tumor biopsies can therefore substantially impact the quality of the input needed for sequencing. DNA/RNA extracted from fresh frozen tumor samples is generally of higher quality compared to DNA/RNA isolated from FFPE tissues. Conversely, FFPE tumor samples are more practical for procurement, shipping, and storage and can be more readily evaluated by a pathologist to determine tumor content; an additional advantage is that they are amenable to laser microdissection in order to achieve higher purity of the tumor sample being utilized for DNA/RNA extraction (Datta et al. 2015).

Another potentially simpler and more elegant approach to obtain representative tumor material for the generation of a personalized vaccine is the collection of serial liquid biopsies. Sequencing of DNA and RNA from circulating cell-free tumor material collected (potentially across multiple time points) from the blood has the promise of providing a more comprehensive representation of the tumor genome in an individual patient, aside from being substantially more practical and cost effective compared to core biopsies, which typically require an interventional radiology-based approach and are not without risk for the patient (Adalsteinsson et al. 2017, Quandt et al. 2017).

Quality of Mutation Calls

While available algorithms to detect single-nucleotide variants (SNVs) (Carter et al. 2012, Saunders et al. 2012), insertions, or deletions (indels) provide reasonably accurate mutation calls for nonclinical use, there is currently no consensus with regards to the best validation tools that should be used to ascertain highest confidence and to prevent erroneous calling of germline variants as neoepitopes, which could potentially result in autoimmune toxicity. Manual review in Integrative Genomics Viewer should be performed (Barnell et al. 2019, Robinson et al. 2011), and additional experimental validation, for example, by PCR-based targeted sequencing, can be considered to confirm that the mutations selected for inclusion in the vaccines are present in the patient's tumor and not present in germline DNA.

Mutation Types and Mutation Discovery Space

Indels or fusion genes arising from deletions, translocations, or inversions can lead to frameshifts, thereby creating entire stretches of substantially altered AA sequences. Compared to SNVs, these variants are less common and more challenging to detect due to difficulties with standard read alignment methods (Jiang et al. 2012, Ratan et al. 2015). The resulting novel peptides/proteins have the potential to contain highly antigenic epitopes given the degree of foreignness compared to mere exchanges of single AAs resulting from SNVs (Maby et al. 2015, Turajlic et al. 2017). Beyond these established mutational types, new technologies have the potential to broaden substantially the neoantigen discovery space, which is currently largely limited to the exome (Chong et al. 2020, Laumont et al. 2018). For example, ribosomal profiling (Ribo-seq) allows the high-resolution measurement of translational events on a genome-wide scale (Calviello & Ohler 2017, Godet et al. 2008, Ingolia 2016). Epigenetic events such as DNA methylations and histone modifications, posttranscriptional modifications such as variants derived from alternative RNA splicing, and posttranslational modifications are currently untapped and could further increase the spectrum of neoantigens for targeting by personal vaccines (Frankiw et al. 2019, Jaganathan et al. 2019).

Prioritization of Mutant Epitopes for Inclusion in Personalized Vaccines

As discussed above, the clonal evolution and heterogeneity that are hallmarks of tumors imply that personalized cancer vaccines should incorporate many neoantigens rather than a few potentially dominant epitopes. Nevertheless, considerations such as manufacturing capability, cost, and the

possibility of competition for binding on HLA molecules on antigen-presenting cells (APCs) in the draining lymph node necessitate limiting the number of neoantigens contained in a personalized vaccine. Prioritizing the most immunogenic neoepitopes is therefore critical for vaccine design. Of note, this task may be different between highly mutated tumors with large numbers of potentially highly immunogenic mutations and tumors with limited mutations, where the challenge might be to select a minimum number of reasonable targets. There is currently no consensus on how to optimally prioritize neoantigen targets.

In Silico Neoepitope Prediction

A cornerstone of identifying the most immunogenic neoepitopes is the prediction of their binding affinity to HLA molecules. Because of the large numbers of potential peptides that can result from a genetic variant by way of proteosomal processing and the polymorphism of HLA alleles, the accurate prediction of peptides that will bind to a particular HLA molecule is challenging. Most pipelines for target epitope prioritization to date have employed in silico computational approaches involving machine learning algorithms (**Figure 3**). NetMHC and NetMHCpan, among

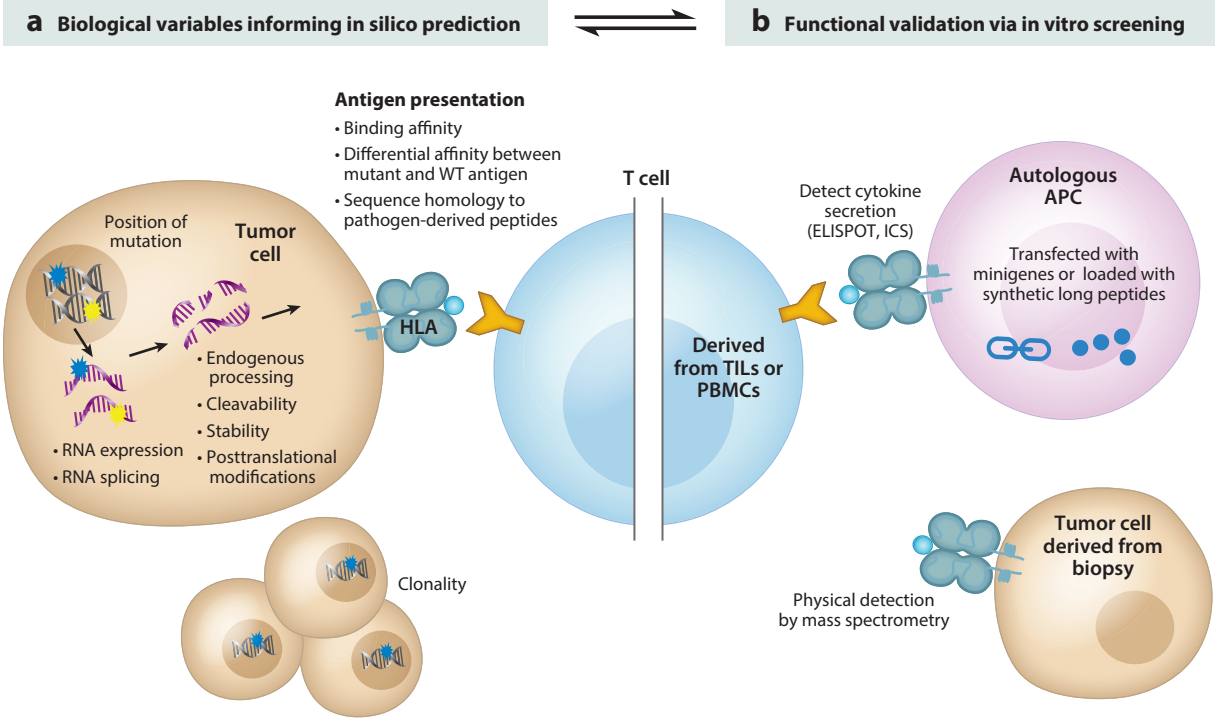

Figure 3

Iterative process between in silico prediction and in vitro screening to better inform the selection of immunogenic neoepitopes. Improvements to in silico approaches to select for immunogenic neoepitopes involve (*a*) expanding data sets to include biological variables ranging from the mutation's inherent position on a DNA strand to its clonality, RNA expression, processing, and likelihood of presenting itself on the tumor surface via an HLA molecule and (*b*) functional validation of both the neoantigen's physical presence using mass spectrometry and its ability to elicit a T cell response, as measured by cytokine detection assays on patient-derived T cells and APCs. Abbreviations: APC, antigen-presenting cell; ELISPOT, enzyme-linked immunosorbent spot; ICS, intracellular cytokine staining; PBMC, peripheral blood mononuclear cell; TIL, tumor-infiltrating lymphocyte; WT, wild-type.

the most widely used approaches, are neural network–based algorithms that rely on training by measurement of biochemical affinity of synthetic peptides and are incorporated in the Immune Epitope Database (Larsen et al. 2005, Nielsen & Andreatta 2016). One limitation of many previously used algorithms is their reliance on relatively small data sets (with limited numbers of trained peptides and a bias toward common HLA alleles). Neoepitope prediction tools are available that provide information on endogenous processing and intracellular transport prior to binding of the peptides to HLA (Larsen et al. 2005, Nielsen et al. 2005). A potentially critical parameter that many in silico epitope prediction algorithms have not taken into account in the past is the expression of the mutated gene in the tumor. Of note, only a small fraction of predicted binding peptides has been directly detected by mass spectrometry, and low correlation between protein abundance and peptide presentation was previously reported. However, recent studies using novel high-throughput mass spectrometry and improved immune purification approaches allowing for more stringent and accurate detection of large numbers of peptides have established a clear association between protein abundance and presentation of peptides on HLA (Bassani-Sternberg et al. 2015). Further studies employing RNA sequencing for gene expression analysis, thereby capturing exome-wide information at high sensitivity, confirmed this correlation and established an interrelationship between these two variables (high expression can compensate for low affinity and vice versa) (Abelin et al. 2017), allowing the information from both to jointly inform epitope selection. Of note, additional variables including subcellular location of the source proteins, as well as cleavability and predicted stability, influenced peptide presentation to variable degrees.

Additional limitations of target selection based on expression levels and in silico prediction of MHC affinity include the relatively low specificity of available prediction algorithms and the fact that naturally processed epitopes may be altered by posttranslational modifications (Dalet et al. 2011, Hanada et al. 2004). Incorporation into these algorithms of large mass spectrometry–derived HLA-ligandome data sets from monoallelic cell lines covering a much wider spectrum of HLA alleles, as well as of mass spectrometry data from tumor HLA–bound peptides, has already substantially improved the predictive accuracy of existing algorithms (Abelin et al. 2017, Bulik-Sullivan et al. 2018, Sarkizova et al. 2020). There is also emerging evidence that the quality of neoantigens as determined by the degree of differential HLA affinity between mutant and wild-type peptides, as well as sequence homology to pathogen-derived peptides, may supersede neoantigen quantity as an indicator of immunogenicity (Balachandran et al. 2017, Luksza et al. 2017). Computational prediction of MHC class II neoepitopes is more challenging than class I prediction, largely due to the more promiscuous binding characteristics of the class II molecule, but also because class II neopeptides can be processed and presented by various types of APCs in addition to tumor cells. Nevertheless, inroads into in silico selection of $CD4^+$ T cell neoepitopes have been made in mouse models by combining MHC binding prediction and RNA expression analysis, as well as more recently in humans by utilizing proteomics to develop algorithms that predict class II epitopes presented by professional APCs (Abelin et al. 2019).

Discovery and Prioritization of Target Neoepitopes by In Vitro Immune Screening

While the in silico target discovery and prioritization approach is relatively cost effective, fast, and has comparatively low input specimen requirements, it does not provide direct evidence that a given neoepitope is able to induce a T cell response, i.e., whether it is immunogenic. In vitro screening of T cell responses against libraries of candidate neoantigens may enable direct functional validation of neoantigen targets in individual patients. One approach pioneered at the National Cancer Institute surgical branch is the use of tandem minigenes encoding mutated gene

products identified by WES. These constructs are transfected into APCs, such as autologous B cells or immature dendritic cells expressing each of the individual HLA class I gene products expressed by the tumor of interest (Lu et al. 2014; Tran et al. 2014, 2015). Instead of tandem minigenes, pools of long (ca. 25 AAs) peptides loaded onto APCs can also be utilized for this unbiased in vitro screening method. Using this approach, a variety of neoepitopes targeted by TILs have been delineated (Prickett et al. 2016; Tran et al. 2014, 2015; Zacharakis et al. 2018). TILs represent the perhaps most preferred effector T cell population to identify and validate neoepitopes given their location in the tumor and presumed clonal expansion in the context of antigens presented in the tumor microenvironment. By some measure, unbiased screening of TILs could even represent a gold standard for neoantigen target discovery: This method has led to the identification of mutant antigens that were the targets of T cells that, when adoptively transferred to patients, mediated regression of solid tumors including melanoma, cholangiocarcinoma, and breast cancer, providing compelling evidence for their immunogenicity and validity as targets of effective immune responses (Prickett et al. 2016; Tran et al. 2014, 2015; Zacharakis et al. 2018). It should be noted, however, that this technology has been used predominantly in the adoptive T cell transfer setting, where harvesting TILs from surgical tumor samples is part of the treatment workflow.

Given the generally limited availability of TILs, PBMCs have been used as an alternative effector T cell population for in vitro screening, as demonstrated by Genocea's ATLAS™ platform. Here, every predicted neoantigen from the patient tumor is expressed in autologous APCs using *Escherichia coli*. After incubating these APCs with T cells derived from the patient's PBMCs, a cytokine readout authenticates the predicted antigens and determines whether they are immunogenic or inhibitory (Dadali et al. 2019).

Such functional validation can feed into in silico predictions. The Parker Institute for Cancer Immunotherapy is focused on improving and benchmarking these algorithms to better predict neoantigens and formed a consortium of over 40 academic, nonprofit, and industry research groups called TESLA (Tumor Neoantigen Selection Alliance) to functionally validate the predicted peptides and provide more accurate predictions. Predicted neoantigens from all collaborators are being evaluated through iterative, empirical testing, eventually selecting effective neoantigens.

VACCINE DELIVERY

Delivery platforms for personalized cancer vaccines include synthetic peptides, nucleic acids (DNA and mRNA), viral vectors, and dendritic cells, each with distinct characteristics (Guo et al. 2018, Hu et al. 2018, Sahin & Tureci 2018).

Synthetic long peptides (SLPs) are approximately 15–30 AAs in length and have a proven track record of safety and immunogenicity in human trials including personalized neoantigen vaccines (Keskin et al. 2019, Ott et al. 2017, Schumacher et al. 2014). As opposed to short peptides, which are 8–10 AAs in length in order to allow binding to HLA class I molecules and thus exclusively engage $CD8^+$ T cells, SLPs are approximately 15–30 AAs long. SLPs contain $CD4^+$ epitopes and require processing and presentation by professional APCs that provide optimal T cell stimulation; SLP vaccines have demonstrated effective stimulation of $CD4^+$ and $CD8^+$ T cell responses (Kenter et al. 2008, 2009). Peptides are a versatile vaccine format, as they can be combined with a range of immune adjuvants (Obeid et al. 2015, Slingluff 2011). Synthesis is often challenging, and its feasibility depends on the length and AA sequence of a given peptide, for example, the presence of cysteine residues or a hydrophobic sequence. However, innovative approaches can, at least in part, overcome manufacturability issues. It was recently shown that automatic flow synthesis enables faster production of peptides at higher purity and with a higher success rate compared to traditional batch synthesis (Truex et al. 2020).

Messenger RNA vaccines, previously hampered by challenges related to instability and inefficient in vivo delivery, have emerged as an attractive format for personalized cancer vaccines (Pardi et al. 2018). Improved formulations, for example, through liposomal delivery, have enabled intravenous administration, which directs the vaccine-immunogen to APCs in all lymphoid compartments. This systemic delivery has demonstrated robust immunogenicity and is superior to local administration, for example, into lymph nodes or tumor lesions (Kranz et al. 2016). RNA vaccines have built-in immune adjuvants, as they contain pathogen-associated molecular patterns that engage Toll-like receptors leading to the production of type I interferons and an innate immune response. An additional advantage is the relative ease and speed of mRNA production, allowing effective scaling and thus timely and cost-effective manufacturing. Personalized RNA vaccines targeting neoantigens are currently being tested in multiple tumor types, including NSCLC and melanoma (NCT03289962, NCT03815058, NCT04267237, NCT03313778, NCT03897881).

DNA plasmids are a versatile vaccine platform that can incorporate any neoantigen sequence without manufacturability concerns (Rice et al. 2008, Tondini et al. 2019). Production of DNA plasmids is relatively simple and cost effective. Given that DNA needs to enter the nucleus of a cell in order for successful transfection to occur, effective targeting of dendritic cells is more challenging compared to RNA and peptide vaccines (Rice et al. 2008, Sahin & Tureci 2018). Technologies to improve transfection efficiency of DNA plasmids include electroporation, gene guns, and tattooing (Luxembourg et al. 2006, Porgador et al. 1998). Incorporation of DNA into a viral vector, for example, a recombinant vaccinia virus or adenovirus vector, enables transfection and expression of the neoantigen DNA in APCs, thereby increasing immunogenicity (Larocca & Schlom 2011). However, production, quality control, and administration of viral vectors are challenging; furthermore, immune responses against the viral vector itself can compromise the specificity and effectiveness of the vaccine. DNA-based personalized neoantigen vaccines are being tested in clinical trials in patients with solid tumors including GBM, breast cancer, pancreatic cancer, and prostate cancer (NCT03199040, NCT03122106, NCT03532217, NCT04015700).

ASSESSMENT OF VACCINE-INDUCED IMMUNE RESPONSES

The personalized neoantigen vaccine setting provides a unique opportunity to dissect T cell responses that result from tumor-specific immune intervention. ELISPOT, intracellular cytokine staining, and MHC multimers have been used widely to characterize immune responses elicited by personalized neoantigen vaccines. These technologies can provide useful information on the magnitude, phenotype, and some aspects of functionality of vaccine-induced polyclonal T cell responses. However, as these assays are performed on bulk T cell populations, they do not provide clonal-level information. Single-cell immungenomics tools are now available to assess the clonality of neoepitope-specific T cell populations without the selection bias caused by different in vitro growth kinetics inherent to the traditional in vitro cloning approach. TCRs of vaccine-induced neoantigen-specific T cells can be identified by determining the TCRα and β CDR3 sequences of flow cytometric–sorted or tetramer single neoepitope–reactive T cells through alignment of sequenced reads to the International Immunogenetics database. In single-cell TCR repertoire analyses of vaccine-induced neoepitope-specific $CD4^+$ T cell responses isolated by tetramers in patients with high-risk melanoma who had received personal neoantigen long-peptide vaccines, dozens of distinct clones in T cell populations reactive to a single vaccine neoepitope were observed (Hu et al. 2019). The TCR repertoires evolved throughout the vaccinations with the persistence of clones throughout the priming and boosting phases, but also with the emergence of multiple novel clones many months after initial vaccination.

Single-cell RNA sequencing (scRNA-seq) also allows for the analysis of transcriptional changes in T cells and other immune cells in relation to vaccination in the peripheral blood and tumors.

The assessment of tetramer isolated neoantigen-specific $CD4^+$ T cells from high-risk melanoma patients who had received personalized neoantigen vaccines by scRNA-seq allowed for the identification of broad shifts in the transcriptional profiles between pre- and postvaccination time points (Ott et al. 2017). Reconstructed TCRs can also be expressed in TCR-deficient Jurkat cell lines, which can be functionally tested for their specificity to antigen. This technology therefore also allows for the linkage of an antigen-specific immunologic response to specific TCR sequences. As described above, this technology was recently used to identify vaccine-induced, neoantigen-specific T cells in a posttreatment intracranial tumor of a patient with GBM (Keskin et al. 2019).

CONCLUDING REMARKS

After successful feasibility testing in an initial set of small trials, personalized neoantigen vaccines have proceeded from the concept stage into wider clinical investigation and now are being pursued in a broad range of tumor types. Key questions and challenges that need to be tackled in the coming years include further optimizing neoantigen target discovery, accelerating and streamlining vaccine manufacturing, clinical testing of novel vaccine adjuvants and delivery platforms, and identifying the most suitable partnering therapies. Lessons learned from preclinical studies (for example, recent studies in mice demonstrating detrimental effects of PD-1 inhibition on the functionality of vaccine-induced CD8 memory responses given prior to vaccination) provide important information for the most effective combination strategies. Assessment of immune responses using state-of-the-art immunogenomics tools providing single-cell resolution and T cell clonal-level information will help researchers gain deep insights into the diversity, dynamics, and functionality of the specific vaccine approaches used. Choosing the most appropriate treatment setting for a given tumor type, such as earlier disease stages, and identifying appropriate endpoints for the assessment of antitumor activity will be critical to maximizing success for cancer patients in the clinic.

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LITERATURE CITED

Abelin JG, Harjanto D, Malloy M, Suri P, Colson T, et al. 2019. Defining HLA-II ligand processing and binding rules with mass spectrometry enhances cancer epitope prediction. *Immunity* 51:766–79.e17

Abelin JG, Keskin DB, Sarkizova S, Hartigan CR, Zhang W, et al. 2017. Mass spectrometry profiling of HLA-associated peptidomes in mono-allelic cells enables more accurate epitope prediction. *Immunity* 46:315–26

Adalsteinsson VA, Ha G, Freeman SS, Choudhury AD, Stover DG, et al. 2017. Scalable whole-exome sequencing of cell-free DNA reveals high concordance with metastatic tumors. *Nat. Commun.* 8:1324

Balachandran VP, Luksza M, Zhao JN, Makarov V, Moral JA, et al. 2017. Identification of unique neoantigen qualities in long-term survivors of pancreatic cancer. *Nature* 551:512–16

Barnell EK, Ronning P, Campbell KM, Krysiak K, Ainscough BJ, et al. 2019. Standard operating procedure for somatic variant refinement of sequencing data with paired tumor and normal samples. *Genet. Med.* 21:972–81

Bassani-Sternberg M, Pletscher-Frankild S, Jensen LJ, Mann M. 2015. Mass spectrometry of human leukocyte antigen class I peptidomes reveals strong effects of protein abundance and turnover on antigen presentation. *Mol. Cell Proteom.* 14:658–73

Brown SD, Warren RL, Gibb EA, Martin SD, Spinelli JJ, et al. 2014. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. *Genome Res.* 24:743–50

Bulik-Sullivan B, Busby J, Palmer CD, Davis MJ, Murphy T, et al. 2018. Deep learning using tumor HLA peptide mass spectrometry datasets improves neoantigen identification. *Nat. Biotechnol.* 37:55–63

Buonaguro L, Petrizzo A, Tornesello ML, Buonaguro FM. 2011. Translating tumor antigens into cancer vaccines. *Clin. Vaccine Immunol.* 18:23–34

Calviello L, Ohler U. 2017. Beyond read-counts: Ribo-seq data analysis to understand the functions of the transcriptome. *Trends Genet.* 33:728–44

Carreno BM, Magrini V, Becker-Hapak M, Kaabinejadian S, Hundal J, et al. 2015. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. *Science* 348:803–8

Carter SL, Cibulskis K, Helman E, McKenna A, Shen H, et al. 2012. Absolute quantification of somatic DNA alterations in human cancer. *Nat. Biotechnol.* 30:413–21

Castle JC, Kreiter S, Diekmann J, Lower M, van de Roemer N, et al. 2012. Exploiting the mutanome for tumor vaccination. *Cancer Res.* 72:1081–91

Chen YT, Scanlan MJ, Sahin U, Tureci O, Gure AO, et al. 1997. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. *PNAS* 94:1914–18

Chong C, Muller M, Pak H, Harnett D, Huber F, et al. 2020. Integrated proteogenomic deep sequencing and analytics accurately identify non-canonical peptides in tumor immunopeptidomes. *Nat. Commun.* 11:1293

Dadali T, Tjon E, Agnihotri P, Croshier M, Cabral C, et al. 2019. *ATLAS™ identifies relevant neoantigens for therapeutic anti-tumor vaccination and may serve as a biomarker for efficacy of immunotherapy of solid tumors*. Poster presented at 34th Annual Meeting of the Society for Immunotherapy of Cancer (SITC 2019), Washington, DC

Dagogo-Jack I, Shaw AT. 2018. Tumour heterogeneity and resistance to cancer therapies. *Nat. Rev. Clin. Oncol.* 15:81–94

Dalet A, Robbins PF, Stroobant V, Vigneron N, Li YF, et al. 2011. An antigenic peptide produced by reverse splicing and double asparagine deamidation. *PNAS* 108:E323–31

Datta S, Malhotra L, Dickerson R, Chaffee S, Sen CK, Roy S. 2015. Laser capture microdissection: big data from small samples. *Histol. Histopathol.* 30:1255–69

Disis ML, Wallace DR, Gooley TA, Dang Y, Slota M, et al. 2009. Concurrent trastuzumab and HER2/*neu*-specific vaccination in patients with metastatic breast cancer. *J. Clin. Oncol.* 27:4685–92

Fennemann FL, de Vries IJM, Figdor CG, Verdoes M. 2019. Attacking tumors from all sides: personalized multiplex vaccines to tackle intratumor heterogeneity. *Front. Immunol.* 10:824

Frankiw L, Baltimore D, Li G. 2019. Alternative mRNA splicing in cancer immunotherapy. *Nat. Rev. Immunol.* 19:675–87

George S, Miao D, Demetri GD, Adeegbe D, Rodig SJ, et al. 2017. Loss of PTEN is associated with resistance to anti-PD-1 checkpoint blockade therapy in metastatic uterine leiomyosarcoma. *Immunity* 46:197–204

Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J, et al. 2012. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N. Engl. J. Med.* 366:883–92

Godet Y, Moreau-Aubry A, Guilloux Y, Vignard V, Khammari A, et al. 2008. MELOE-1 is a new antigen overexpressed in melanomas and involved in adoptive T cell transfer efficiency. *J. Exp. Med.* 205:2673–82

Greaves M, Maley CC. 2012. Clonal evolution in cancer. *Nature* 481:306–13

Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, et al. 2014. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature* 515:577–81

Guo Y, Lei K, Tang L. 2018. Neoantigen vaccine delivery for personalized anticancer immunotherapy. *Front. Immunol.* 9:1499

Hanada K, Yewdell JW, Yang JC. 2004. Immune recognition of a human renal cancer antigen through post-translational protein splicing. *Nature* 427:252–56

Hanahan D, Weinberg RA. 2011. Hallmarks of cancer: the next generation. *Cell* 144:646–74

Hilf N, Kuttruff-Coqui S, Frenzel K, Bukur V, Stevanovic S, et al. 2019. Actively personalized vaccination trial for newly diagnosed glioblastoma. *Nature* 565:240–45

Hu Z, Leet D, Sarkizova S, Holden R, Sun J, et al. 2019. *Personalized neoantigen-targeting vaccines for high-risk melanoma generate long-term memory T cell response and epitope spreading*. Paper presented at Fifth CRI-CIMT-EATI-AACR International Cancer Immunotherapy Conference, Paris, Sept. 25–28

Hu Z, Ott PA, Wu CJ. 2018. Towards personalized, tumour-specific, therapeutic vaccines for cancer. *Nat. Rev. Immunol.* 18:168–82

Ingolia NT. 2016. Ribosome footprint profiling of translation throughout the genome. *Cell* 165:22–33

Jaganathan K, Kyriazopoulou Panagiotopoulou S, McRae JF, Darbandi SF, Knowles D, et al. 2019. Predicting splicing from primary sequence with deep learning. *Cell* 176:535–48.e24

Jiang Y, Wang Y, Brudno M. 2012. PRISM: pair-read informed split-read mapping for base-pair level detection of insertion, deletion and structural variants. *Bioinformatics* 28:2576–83

Joura EA, Giuliano AR, Iversen OE, Bouchard C, Mao C, et al. 2015. A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. *N. Engl. J. Med.* 372:711–23

Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, et al. 2010. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N. Engl. J. Med.* 363:411–22

Kenter GG, Welters MJ, Valentijn AR, Lowik MJ, Berends-van der Meer DM, et al. 2008. Phase I immunotherapeutic trial with long peptides spanning the E6 and E7 sequences of high-risk human papillomavirus 16 in end-stage cervical cancer patients shows low toxicity and robust immunogenicity. *Clin. Cancer Res.* 14:169–77

Kenter GG, Welters MJ, Valentijn AR, Lowik MJ, Berends-van der Meer DM, et al. 2009. Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. *N. Engl. J. Med.* 361:1838–47

Keskin DB, Anandappa AJ, Sun J, Tirosh I, Mathewson ND, et al. 2019. Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. *Nature* 565:234–39

Kranz LM, Diken M, Haas H, Kreiter S, Loquai C, et al. 2016. Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. *Nature* 534:396–401

Kreiter S, Vormehr M, van de Roemer N, Diken M, Lower M, et al. 2015. Mutant MHC class II epitopes drive therapeutic immune responses to cancer. *Nature* 520:692–96

Landau DA, Carter SL, Stojanov P, McKenna A, Stevenson K, et al. 2013. Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. *Cell* 152:714–26

Larocca C, Schlom J. 2011. Viral vector-based therapeutic cancer vaccines. *Cancer J.* 17:359–71

Larsen MV, Lundegaard C, Lamberth K, Buus S, Brunak S, et al. 2005. An integrative approach to CTL epitope prediction: a combined algorithm integrating MHC class I binding, TAP transport efficiency, and proteasomal cleavage predictions. *Eur. J. Immunol.* 35:2295–303

Laumont CM, Vincent K, Hesnard L, Audemard E, Bonneil E, et al. 2018. Noncoding regions are the main source of targetable tumor-specific antigens. *Sci. Transl. Med.* 10:eaau5516

Lennerz V, Fatho M, Gentilini C, Frye RA, Lifke A, et al. 2005. The response of autologous T cells to a human melanoma is dominated by mutated neoantigens. *PNAS* 102:16013–18

Lu YC, Yao X, Crystal JS, Li YF, El-Gamil M, et al. 2014. Efficient identification of mutated cancer antigens recognized by T cells associated with durable tumor regressions. *Clin. Cancer Res.* 20:3401–10

Luksza M, Riaz N, Makarov V, Balachandran VP, Hellmann MD, et al. 2017. A neoantigen fitness model predicts tumour response to checkpoint blockade immunotherapy. *Nature* 551:517–20

Lundegaard C, Lamberth K, Harndahl M, Buus S, Lund O, Nielsen M. 2008. NetMHC-3.0: accurate web accessible predictions of human, mouse and monkey MHC class I affinities for peptides of length 8–11. *Nucleic Acids Res.* 36:W509–12

Luxembourg A, Hannaman D, Ellefsen B, Nakamura G, Bernard R. 2006. Enhancement of immune responses to an HBV DNA vaccine by electroporation. *Vaccine* 24:4490–93

Maby P, Tougeron D, Hamieh M, Mlecnik B, Kora H, et al. 2015. Correlation between density of $CD8^+$ T-cell infiltrate in microsatellite unstable colorectal cancers and frameshift mutations: a rationale for personalized immunotherapy. *Cancer Res.* 75:3446–55

Mandelboim O, Berke G, Fridkin M, Feldman M, Eisenstein M, Eisenbach L. 1994. CTL induction by a tumour-associated antigen octapeptide derived from a murine lung carcinoma. *Nature* 369:67–71

Mandelboim O, Vadai E, Fridkin M, Katz-Hillel A, Feldman M, et al. 1995. Regression of established murine carcinoma metastases following vaccination with tumour-associated antigen peptides. *Nat. Med.* 1:1179–83

Melief CJ, van Hall T, Arens R, Ossendorp F, van der Burg SH. 2015. Therapeutic cancer vaccines. *J. Clin. Investig.* 125:3401–12

Nielsen M, Andreatta M. 2016. NetMHCpan-3.0; improved prediction of binding to MHC class I molecules integrating information from multiple receptor and peptide length datasets. *Genome Med.* 8:33

Nielsen M, Lundegaard C, Lund O, Kesmir C. 2005. The role of the proteasome in generating cytotoxic T-cell epitopes: insights obtained from improved predictions of proteasomal cleavage. *Immunogenetics* 57:33–41

Obeid J, Hu Y, Slingluff CL Jr. 2015. Vaccines, adjuvants, and dendritic cell activators—current status and future challenges. *Semin. Oncol.* 42:549–61

Oh E, Choi YL, Kwon MJ, Kim RN, Kim YJ, et al. 2015. Comparison of accuracy of whole-exome sequencing with formalin-fixed paraffin-embedded and fresh frozen tissue samples. *PLOS ONE* 10:e0144162

Ott PA, Hodi FS. 2013. The B7-H1/PD-1 pathway in cancers associated with infections and inflammation: opportunities for therapeutic intervention. *Chin. Clin. Oncol.* 2:7

Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, et al. 2017. An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature* 547:217–21

Pardi N, Hogan MJ, Porter FW, Weissman D. 2018. mRNA vaccines—a new era in vaccinology. *Nat. Rev. Drug Discov.* 17:261–79

Porgador A, Irvine KR, Iwasaki A, Barber BH, Restifo NP, Germain RN. 1998. Predominant role for directly transfected dendritic cells in antigen presentation to $CD8^+$ T cells after gene gun immunization. *J. Exp. Med.* 188:1075–82

Prickett TD, Crystal JS, Cohen CJ, Pasetto A, Parkhurst MR, et al. 2016. Durable complete response from metastatic melanoma after transfer of autologous T cells recognizing 10 mutated tumor antigens. *Cancer Immunol. Res.* 4:669–78

Quandt D, Dieter Zucht H, Amann A, Wulf-Goldenberg A, Borrebaeck C, et al. 2017. Implementing liquid biopsies into clinical decision making for cancer immunotherapy. *Oncotarget* 8:48507–20

Ratan A, Olson TL, Loughran TP Jr., Miller W. 2015. Identification of indels in next-generation sequencing data. *BMC Bioinform.* 16:42

Rice J, Ottensmeier CH, Stevenson FK. 2008. DNA vaccines: precision tools for activating effective immunity against cancer. *Nat. Rev. Cancer* 8:108–20

Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, et al. 2015. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 348:124–28

Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, et al. 2011. Integrative genomics viewer. *Nat. Biotechnol.* 29:24–26

Sahin U, Derhovanessian E, Miller M, Kloke BP, Simon P, et al. 2017. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature* 547:222–26

Sahin U, Tureci O. 2018. Personalized vaccines for cancer immunotherapy. *Science* 359:1355–60

Sarkizova S, Klaeger S, Le PM, Li LW, Oliveira G, et al. 2020. A large peptidome dataset improves HLA class I epitope prediction across most of the human population. *Nat. Biotechnol.* 38:199–209

Saunders CT, Wong WS, Swamy S, Becq J, Murray LJ, Cheetham RK. 2012. Strelka: accurate somatic small-variant calling from sequenced tumor-normal sample pairs. *Bioinformatics* 28:1811–17

Schumacher T, Bunse L, Pusch S, Sahm F, Wiestler B, et al. 2014. A vaccine targeting mutant IDH1 induces antitumour immunity. *Nature* 512:324–27

Sensi M, Anichini A. 2006. Unique tumor antigens: evidence for immune control of genome integrity and immunogenic targets for T cell-mediated patient-specific immunotherapy. *Clin. Cancer Res.* 12:5023–32

Slingluff CL Jr. 2011. The present and future of peptide vaccines for cancer: single or multiple, long or short, alone or in combination? *Cancer J.* 17:343–50

Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, et al. 2014. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N. Engl. J. Med.* 371:2189–99

Tondini E, Arakelian T, Oosterhuis K, Camps M, van Duikeren S, et al. 2019. A poly-neoantigen DNA vaccine synergizes with PD-1 blockade to induce T cell-mediated tumor control. *OncoImmunology* 8:1652539

Tran E, Ahmadzadeh M, Lu YC, Gros A, Turcotte S, et al. 2015. Immunogenicity of somatic mutations in human gastrointestinal cancers. *Science* 350:1387–90

Tran E, Turcotte S, Gros A, Robbins PF, Lu YC, et al. 2014. Cancer immunotherapy based on mutation-specific $CD4^+$ T cells in a patient with epithelial cancer. *Science* 344:641–45

Truex NL, Holden RL, Wang BY, Chen PG, Hanna S, et al. 2020. Automated flow synthesis of tumor neoantigen peptides for personalized immunotherapy. *Sci. Rep.* 10:723

Turajlic S, Litchfield K, Xu H, Rosenthal R, McGranahan N, et al. 2017. Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: a pan-cancer analysis. *Lancet Oncol.* 18:1009–21

Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, et al. 2015. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* 350:207–11

van Rooij N, van Buuren MM, Philips D, Velds A, Toebes M, et al. 2013. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. *J. Clin. Oncol.* 31:e439–42

Verdegaal EM, de Miranda NF, Visser M, Harryvan T, van Buuren MM, et al. 2016. Neoantigen landscape dynamics during human melanoma–T cell interactions. *Nature* 536:91–95

Yadav M, Jhunjhunwala S, Phung QT, Lupardus P, Tanguay J, et al. 2014. Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. *Nature* 515:572–76

Zacharakis N, Chinnasamy H, Black M, Xu H, Lu YC, et al. 2018. Immune recognition of somatic mutations leading to complete durable regression in metastatic breast cancer. *Nat. Med.* 24:724–30