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CURRENT OPINION

Getting personal in metastatic melanoma: neoantigen-based vaccines as a new therapeutic strategy

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Purpose of review
Cancer vaccines are facing renewed interest, thanks to the progress recently achieved in the immunotherapy field, including the success of immune checkpoint inhibitors (CPIs). The advances in understanding the CPI mode of action revealed a central role of neoantigens for the outcome of such treatments. Neoantigens became the preferred antigens for cancer vaccines and have been evaluated in several clinical trials. Here, we review the recent results from neoantigen-based vaccines in melanoma patients and discuss avenues for improvement.

Recent findings
The importance of neoantigens for tumor control comes from the positive correlation between tumor mutational burden (TMB) and response to CPI. Preclinical studies have proved the effectiveness of neoantigen vaccines in models, expediting their clinical testing. Tumor mutations are not shared in most tumor types including melanoma, mandating the need of a personalized approach. Several clinical studies have shown the safety, feasibility, immunogenicity and preliminary evidence of antitumor activity of personalized vaccination. Currently, new trials have been started aiming to both confirm clinical activity and combining vaccines with other immunotherapies for improved efficacy.

Summary
Personalized vaccines hold the promise for highly mutated and immunogenic cancers, including melanoma. Continuous efforts are underway to increase their likelihood of success.

Keywords
immune checkpoint, melanoma, mutational burden, neoantigen, T cells

INTRODUCTION

The goal of cancer vaccine is to induce an effective immune response against tumor antigens. However, while the use of vaccine for prevention of infectious diseases can certainly be considered a success, to date, several therapeutic vaccines have been tested in the clinic, including large phase III cancer vaccine studies, with unsatisfying results [1]. Induction of cell-mediated immunity rather than humoral immunity is considered important for the clearance of established infections or cancer. Nevertheless, two approved cancer vaccines exist: Bacillus Calmette–Guerin (BCG) for local treatment of early-stage bladder cancer, and Sipuleucel-T (Provenge), an autologous dendritic cell-based vaccine, for treatment of castration-resistant prostate cancer [2,3]. There are two major challenges in developing effective therapeutic cancer vaccines: the highly immunosuppressive microenvironment established by cancer cells and the selection of the cancer antigens. Recent advances in the field allowed to address these two main hurdles and significantly boosted interest in cancer vaccines.

It is now well known that cancer cells adopt a series of strategies to curtail antitumor immunity and finally achieve immune escape. One of these strategies relies on the expression of inhibitory ligands binding the so-called 'immune checkpoints'

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KEY POINTS

- Tumor-specific mutations can generate immunogenic neoantigens.
- Vaccination targeting tumor neoantigens has been proven to synergize with anti-PD-1 in tumor models with a high number of mutations.
- Mutations are unique to each individual tumor in the vast majority of tumor types, including melanoma, mandating a personalized intervention.
- Several neoantigen-based personalized vaccination approaches have been evaluated in melanoma phase-I trials.
- Neoantigen vaccination in melanoma patients has been shown to be feasible, immunogenic and with a favorable impact on clinical outcome.

expressed on immune cells to deliver inhibitory signals interfering with T-cell activation and function. As the discovery of immune checkpoints, much efforts were spent to develop strategies overcoming the inhibitory effects of tumor cells on T cells, leading to the development of CPIs [4]. The first Food and Drug Administration (FDA)-approved CPI was anti-cytotoxic T lymphocyte-associated protein 4 (CTLA-4) in 2011, for treatment of patients with metastatic melanoma [5]. In 2014, pembrolizumab and nivolumab, two antibodies that target programmed cell death protein-1 (PD-1), were also FDA-approved for unresectable metastatic melanoma, given the clinical benefits and improved overall survival achieved upon treatment [6]. Despite the clinical efficacy of CPIs, many melanoma patients still do not respond, and only a fraction of patients achieves durable remission, highlighting the need of improved therapeutic options [7]. The activity of anti-PD-1 relies on the presence of antigen-specific T cells by relieving immune suppression at the tumor site and rescuing T cells against tumor antigens spontaneously induced by the tumor. Therefore, there is a strong rationale for combining anti-PD-1 with cancer vaccine to boost already present antitumor T cells and trigger 'de novo' T cells for improved response rate.

An important challenge of cancer vaccine development is the selection of the tumor antigens. For effective vaccination, tumor antigens need to be sufficiently distinct from self-antigens in order to break the immunological tolerance that prevents undesired immune response against normal cells. In the last decades, the most common approach to cancer vaccination, including melanoma vaccines, employed shared tumor-associated antigens (TAAs) that are self-antigens abnormally expressed or overexpressed in tumors. Therapeutic vaccines based on TAAs have been largely unsuccessful likely because of the presence of immune tolerance [8▪]. In addition, TAAs are also expressed by non malignant cells, increasing the risk of vaccine-induced autoimmune toxicities.

The advent of next generation sequencing (NGS) has provided the opportunity to identify a new category of tumor-specific antigens, named neoantigens. Differently from TAA, neoantigens are mutated self-antigens, specific to each tumor and expressed in tumor cells only. Different reports have demonstrated that neoantigen-specific T cells are key for clinical response mediated by immune checkpoint inhibitors [9]. Tumors with high TMB, such as melanoma and non small cell lung cancer, which are likely to have a correspondently high number of neoantigens and to be more easily recognized by the immune system, respond better to CPI [10,11]. Because of their selective expression in tumors, neoantigens are recognized as non-self by the immune system, therefore, are highly immunogenic and well tolerated with minimal risk of inducing autoimmunity. The potential advantage of using neoepitopes as target of vaccination is to reduce the chance of off-target effects on normal healthy tissues. This becomes of high relevance, especially when considering the combination of CPI with cancer vaccine. Despite important clinical benefits, CPI therapy is often associated with a series of immune-related adverse events (iAEs). In this scenario, it is important to consider that the combination of personalized vaccines and CPI could improve the antitumor efficacy without additional toxicities differently from combination therapies such as anti-PD-1 and anti-CTLA-4 known to have an improved efficacy with concomitant increase of iAEs.

Thus, neoantigens represent the ideal target for therapeutic cancer vaccine, and their discovery has opened new avenues for the development of effective cancer vaccines.

PRECLINICAL EVIDENCES ON EFFECTIVENESS OF TUMOR NEOANTIGEN VACCINES

Compelling evidences in murine models have demonstrated that neoantigens represent a key target for therapeutic vaccination. The first demonstration of antitumor efficacy of a neoantigen-based vaccine comes from the study of Castle *et al.* [12] in the B16F10 melanoma model. By using NGS, they identified 563 expressed neoantigens. The immunogenicity of 50 identified mutations was demonstrated by immunizing mice with long peptides encoding

the mutated epitopes. The immunization conferred *in-vivo* tumor control both in protective and therapeutic settings. Similarly, in an independent study, Yadav *et al.* demonstrated immunogenicity and tumor control of peptide-based vaccination against selected neo-antigens identified in the MC38 tumor model [13]. Vaccination with synthetic mRNA vaccine encoding five selected neoantigens has also been shown to have antitumor activity in both in B16F10 melanoma and CT26 colon cancer models [14]. The work by D'Alise *et al.* on viral vector-based vaccines encoding multiple tumor neoantigens showed that immunization with a non-human great ape-derived adenoviruses (GAd) encoding 31 neoantigens selected from the CT26 cells elicited robust CD8+ and CD4+ T-cell responses and synergized with anti-PD-1 to eradicate large established murine tumors [15]. The antitumor efficacy correlated with increased potency and breadth of the neoantigen-specific T cells supporting combined treatment of neoantigen vaccine and anti-PD-1. In summary, preclinical studies have shown in models with a high number of mutations the effectiveness of therapeutic vaccines targeting neoantigens and its synergy with CPI treatment, prompting clinical evaluation in patients with high TMB tumors, such as melanoma.

CLINICAL EXPERIENCE WITH PERSONALIZED VACCINES IN MELANOMA PATIENTS

Neoantigens arise from somatic mutations in coding regions of the genome. Mutated proteins are cleaved into short peptides and displayed in the context of MHC molecules on the surface of the malignant cells, eliciting tumor-specific immune response. Tumor neoantigens can be distinguished into two types: shared neoantigens, conserved among patients, and personalized neoantigens, unique for each individual patient. The availability of rapid and cost-effective NGS techniques today allows the identification of tumor mutations, making feasible the individualized treatments [16]. As most neoantigens in melanoma result from random mutations, the majority of neoantigens are patient-specific and, as such, must be identified based on each patient's own tumor for a rational design of a personalized cancer vaccine. Neoantigen vaccine candidates are identified by NGS of the patient tumor DNA and comparing tumor DNA to the patient's normal DNA. As tumors can accumulate hundreds of mutations, bioinformatic algorithms have been developed to select the 'best' candidates and prioritize those with the highest probability of inducing T-cell responses [17]. The allelic frequency of the mutation, the abundance of the RNA transcript coding for the mutation and the theoretical MHC-binding affinity of the mutated peptide are the most commonly used parameters for the selection [18]. Single nucleotide variant mutations (SNVs) represent the major source of neoantigens, followed by nucleotide insertions or deletions (indels). A schematic representation of the procedures for personalized neoantigen-based vaccines preparation is depicted in Fig. 1. Vaccine preparation requires time to accomplish all the steps, and the manufacturing time is dependent on the choice of vaccine platform and may typically vary from 8 to 16 weeks [19]. Some tumors are more mutated than others thereby generating a difference in the likelihood of immunogenic neoantigen production among them [20–22]. Melanoma has a very high TMB similar to the one of the murine cell lines used in preclinical studies. Therefore, it represents the preferred tumor type to confirm in humans the results obtained with neoantigen vaccines in the models. Moreover, treatment with CPI exerts some tumor control also in patients who may not respond to the CPI treatment, allowing meanwhile vaccine preparation and injection also for this category of patients.

CLINICAL TRIAL LANDSCAPE OF NEOANTIGEN-BASED VACCINES IN MELANOMA

To date, personalized neoantigen vaccines based on different platforms, including dendritic cells, viral vectors, RNA and peptide-based vaccines, are in clinical evaluation in melanoma patients (Table 1). The first study of personalized vaccine in melanoma was the one from Carreno *et al.* [23], using an autologous dendritic cell-based vaccine. In this study, three stage III melanoma patients, previously treated with ipilimumab, were vaccinated with HLA- A∗02:01- specific tumor peptides loaded on dendritic cells. Vaccine was well tolerated, with no autoimmune adverse events observed. The analysis of the immune response on peripheral blood mononuclear cells (PBMCs) prevaccination and postvaccination demonstrated induction of CD8+ T cells. Both boosting of preexisting neoantigen responses and generation of de novo responses was demonstrated [23]. Thus, this was the first study showing that neoantigen vaccine can increase the breadth and diversity of neoantigen-specific T cells. Few years later, two studies were published, one using peptides and the second RNA vaccination [24▪▪,25,26]. Ott *et al.* [25] employed synthetic long 15–30-mer peptides with poly-ICLC (NeoVax) in six melanoma patients, four of whom with high-risk stage III and two with stage IV melanoma after surgical resection with curative intent. Vaccination with up 20 predicted MHC-I restricted

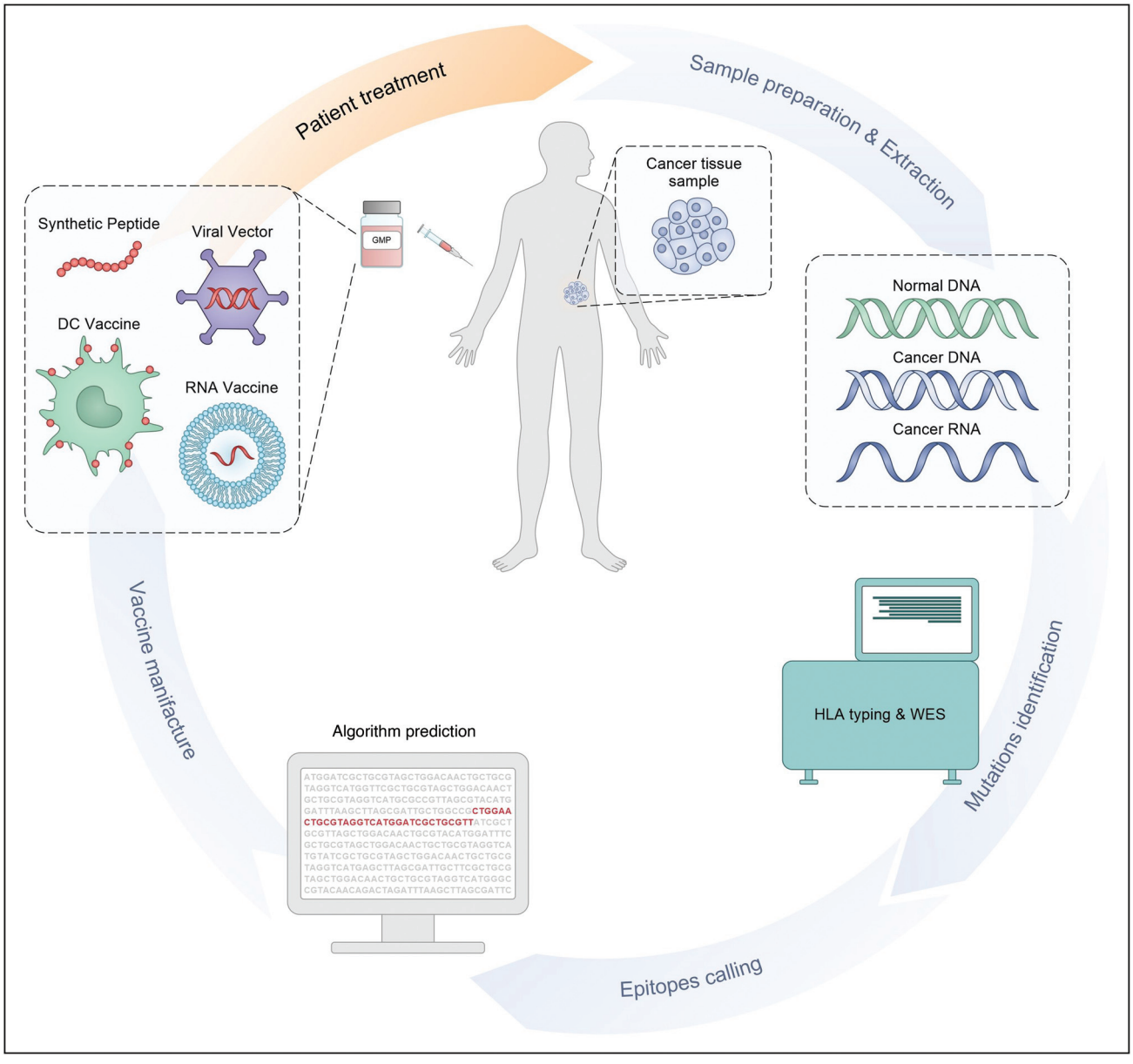

FIGURE 1. Overview of the process for generation of personalized neoantigen-based cancer vaccine. The first step for developing neoantigen cancer vaccine is the identification of mutated tumor-specific antigens from the patient tumor biopsy by whole exome/transcriptome sequencing and prediction of immunogenic MHC epitopes. Next, vaccines (e.g. viral vectors, mRNA, peptides) are manufactured and aministered in patients.

neoantigens induced CD4+ and CD8+ T cells, with a greater fraction of the response consisting of CD4+ T cells. Of six vaccinated patients, four patients had no tumor recurrence at 25 months postvaccination, while two patients with recurrence experienced complete tumor regression when treated with Pembrolizumab (anti-PD-1). In a subsequent follow-up study (median of ~4 years after treatment), NeoVax induced-immune responses were shown to be durable, and an interesting phenomenon called epitope-spreading was described as the presence of de novo T-cell responses recognizing additional melanoma antigens not included in the vaccine [24▪▪]. Similarly, Sahin *et al.*, employed an RNA-based vaccine approach to immunize 13 patients at high risk of relapse of melanoma by intra-nodal administration. They demonstrated immunogenicity to 60% of encoded neoantigens [26]. Consistent with their preclinical data in murine models, the majority of mRNA vaccine-elicited T-cell responses were CD4+, as assessed by ex-vivo IFN-γ ELISpot assay, while CD8 + T cells could be rarely detected and only after in-vitro re-stimulation. Interestingly, in the Sahin *et al.* study, eight patients with no detectable disease

Table 1. Key clinical trials of neoantigen-based vaccines in melanoma

Trial No	Phase	Vaccine format/route	Combination therapy
NCT02035956	Phase 1, completed	RNA vaccine (naked)/intranodal	TAA RNA vaccine
NCT03815058	Phase 2, recruiting	RNA-Lipoplex/i.v.	Pembrolizumab
NCT03289962	Phase 1a/1b, Recruiting	RNA-Lipoplex/i.v.	Atezolizumab
NCT00683670	Phase 1 completed	Dendritic cell/i.v.	gp100 melanoma peptides
NCT01970358 (NeoVax)	Phase 1, completed	Peptide + poly-ICLC/subcutaneous	NA
NCT03929029 (NeoVax)	Phase 1b, recruiting	Peptide + poly-ICLC + Montanide	Nivolumab Ipilimumab
NCT03715985 (NeoPepVac)	Phase 1b, recruiting	Peptide + CAF09b/I.P. + i.m.	NA
NCT02897765 (Neo-PV-01)	Phase 1, completed	Peptide + poly-ICLC/subcutaneous	Nivolumab
NCT04990479 (Nous-PEV)	Phase 1b, recruiting	Gorilla adenovirus vector (prime)/modified vaccine Ankara (MVA)/i.m. injection	Pembrolizumab

i.m., intramuscular; i.v., intravenous; TAA, tumor associated antigen; ICLC, polyinosinic polycytidylic acid.

at the time of vaccination remained recurrence free and two of the five patients with metastatic disease experienced objective clinical responses. RNA neoantigen vaccine (RNA-lipoplex) was also tested by intravenous delivery (RO7198457). Recently, the administration of RNA-lipoplex encoding 20 individualized neoantigens is being investigated in combination with pembrolizumab in metastatic melanoma patients in a phase II study (Table 1; NCT03289962) [27]. These studies, either by using peptides or RNA vaccines, showed a preferential induction of CD4+ versus CD8+ T cells. The induction of CD8+ T-cell responses has been notoriously difficult to achieve in cancer patients. Data with preventive vaccines suggest that the choice of the vaccine platform is key to ensure the induction of CD8+ T-cell response in humans. In this context, viral vectored vaccine and in particular the heterologous prime/boost with Adenoviruses (Ad), followed by Modified Vaccinia Ankara (MVA), has demonstrated induction of powerful and durable CD8+ T-cell responses in humans [28]. Moreover, these viral vectors are particularly suitable to encode for very large antigens [29]. In the context of a cancer vaccine, the capability of these vectors to target multiple neoantigens simultaneously offers the advantage to face and potentially curtail tumor immune escape. At the same time, this approach of polyepitope-neoantigen vaccine would possibly allow to overcome the limits of the current prediction algorithms increasing the likelihood to include in the vaccine at least a subset of neoantigen eliciting effective tumor-specific T-cell responses. Not all mutations generate immunogenic neoantigens, and only few of them are indeed capable to spontaneously induce T cells. The proportion of neoantigens that spontaneously induce T cells has been roughly calculated to be 1–2% [30]. In this view, our group has developed a personalized vaccine based on heterologous prime/boost with Ad followed by MVA vector encoding 60 unique patient-specific neoantigens (Nous-PEV), currently in clinical trial in patients with metastatic melanoma and NSCLC in combination with Pembrolizumab (NCT04990479, Table 1). A similar approach is also currently employed to target shared neoantigens in metastatic patients with a deficiency in mismatch repair/microsatellite instability (dMMR/MSI) [31]. Recently published data from the latter trial showed robust and broad induction of neoantigen-specific CD8 T-cell response in vaccinated cancer patient [32▪,33]. Moreover, expansion and diversification of vaccine-induced T-cell recognizing neoantigens were observed post-treatment in the tumor infiltrate in patients with a long-term clinical response [32▪].

In summary, the current clinical results show the safety and feasibility of personalized vaccination in human melanoma, as well their capability to induce neoantigen-specific immune response.

CONCLUSION

Nowadays, several clinical studies with different vaccine platforms and combination therapies are underway in melanoma patients, including randomized trials with vaccination approaches proven already to be well tolerated and immunogenic in the phase-I studies. Promising preliminary results reported on clinical activity encourage to believe that the therapeutic effectiveness will be finally demonstrated according to clinical standards. Meanwhile, the field is moving forward to tackle with critical aspects of this novel personalized intervention. Major directions of research activity are towards improvements on the overall vaccine production process to make it faster and cheaper. Moreover, a complementary area of great interest for the field is the validation

of biomarkers for selecting patients who would benefit most from neoantigen vaccination, including the ones that are less likely to respond the CPI monotherapy.

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Conflicts of interest

E.S. is a founder and employee of Nouscom. A.M.D is employee of Nouscom.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:
- ■ of special interest
- ■■ of outstanding interest

1. Vansteenkiste JF, Cho BC, Vanakesa T, *et al.* Efficacy of the MAGE-A3 cancer immunotherapeutic as adjuvant therapy in patients with resected MAGE-A3-positive nonsmall-cell lung cancer (MAGRIT): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2016; 17:822–835.
2. Guallar-Garrido S, Julian E. Bacillus Calmette-Guerin (BCG) therapy for bladder cancer: an update. Immunotargets Ther 2020; 9:1–11.
3. Handy CE, Antonarakis ES. Sipuleucel-T for the treatment of prostate cancer: novel insights and future directions. Future Oncol 2018; 14:907–917.
4. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012; 12:252–264.
5. Hodi FS, O'Day SJ, McDermott DF, *et al.* Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010; 363:711–723.
6. Robert C, Ribas A, Schachter J, *et al.* Pembrolizumab versus ipilimumab in advanced melanoma (KEYNOTE-006): posthoc 5-year results from an open-label, multicentre, randomised, controlled, phase 3 study. Lancet Oncol 2019; 20:1239–1251.
7. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell 2017; 168:707–723.
8. ■ Morse MA, Gwin WR, Mitchell DA. Vaccine therapies for cancer: then and now. Target Oncol 2021; 16:121–152.

Interesting review providing a historical overview of cancer vaccines.

9. Darvin P, Toor SM, Sasidharan Nair V, Elkord E. Immune checkpoint inhibitors: recent progress and potential biomarkers. Exp Mol Med 2018; 50:1–11.
10. Rizvi NA, Hellmann MD, Snyder A, *et al.* Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in nonsmall cell lung cancer. Science 2015; 348:124–128.
11. Van Allen EM, Miao D, Schilling B, *et al.* Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science 2015; 350:207–211.
12. Castle JC, Kreiter S, Diekmann J, *et al.* Exploiting the mutanome for tumor vaccination. Cancer Res 2012; 72:1081–1091.
13. Yadav M, Jhunjhunwala S, Phung QT, *et al.* Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. Nature 2014; 515:572–576.
14. Kreiter S, Vormehr M, van de Roemer N, *et al.* Mutant MHC class II epitopes drive therapeutic immune responses to cancer. Nature 2015; 520:692–696.
15. D'Alise AM, Leoni G, Cotugno G, *et al.* Adenoviral vaccine targeting multiple neoantigens as strategy to eradicate large tumors combined with checkpoint blockade. Nat Commun 2019; 10:2688.
16. Morganti S, Tarantino P, Ferraro E, *et al.* Next Generation Sequencing (NGS): a revolutionary technology in pharmacogenomics and personalized medicine in cancer. Adv Exp Med Biol 2019; 1168:9–30.
17. Richters MM, Xia H, Campbell KM, *et al.* Best practices for bioinformatic characterization of neoantigens for clinical utility. Genome Med 2019; 11:56.
18. Leoni G, D'Alise AM, Tucci FG, *et al.* VENUS, a novel selection approach to improve the accuracy of neoantigens' prediction. Vaccines (Basel) 2021; 9.
19. Richard G, Princiotta MF, Bridon D, *et al.* Neoantigen-based personalized cancer vaccines: the emergence of precision cancer immunotherapy. Expert Rev Vaccines 2022; 21:173–184.
20. Lawrence MS, Stojanov P, Polak P, *et al.* Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 2013; 499:214–218.
21. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science 2015; 348:69–74.
22. Vogelstein B, Papadopoulos N, Velculescu VE, *et al.* Cancer genome landscapes. Science 2013; 339:1546–1558.
23. Carreno BM, Magrini V, Becker-Hapak M, *et al.* Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. Science 2015; 348:803–808.
24. ■■ Hu Z, Leet DE, Allesoe RL, *et al.* Personal neoantigen vaccines induce persistent memory T cell responses and epitope spreading in patients with melanoma. Nat Med 2021; 27:515–525.

This work demonstrates the important finding that a personal neoantigen vaccine can stimulate a durable immune T-cell response in patients with melanoma.

25. Ott PA, Hu Z, Keskin DB, *et al.* An immunogenic personal neoantigen vaccine for patients with melanoma. Nature 2017; 547:217–221.
26. Sahin U, Derhovanessian E, Miller M, *et al.* Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature 2017; 547:222–226.
27. Lopez JS, Camidge R, Iafolla M, *et al.* Abstract CT301: a phase Ib study to evaluate RO7198457, an individualized Neoantigen Specific immunoTherapy (iNeST), in combination with atezolizumab in patients with locally advanced or metastatic solid tumors. Cancer Res 2020; 80(16_Suppl); CT301-CT.
28. Sasso E, D'Alise AM, Zambrano N, *et al.* New viral vectors for infectious diseases and cancer. Semin Immunol 2020; 50:101430.
29. Hartnell F, Brown A, Capone S, *et al.* A novel vaccine strategy employing serologically different chimpanzee adenoviral vectors for the prevention of HIV-1 and HCV coinfection. Front Immunol 2018; 9:3175.
30. Karpanen T, Olweus J. The potential of donor T-cell repertoires in neoantigen-targeted cancer immunotherapy. Front Immunol 2017; 8:1718.
31. Leoni G, D'Alise AM, Cotugno G, *et al.* A genetic vaccine encoding shared cancer neoantigens to treat tumors with microsatellite instability. Cancer Res 2020; 80:3972–3982.
32. ■ D'Alise AM, Brasu N, De Intinis C, *et al.* Adenoviral-based vaccine promotes neoantigen-specific CD8+ T cell stemness and tumor rejection. Sci Transl Med 2022; 14:eabo7604.

Important work providing new insights into how adenoviral vector-based neoantigen cancer vaccine can drive efficacy beyond that seen with checkpoint inhibition alone, promoting expansion and diversification of neoantigen-specific CD8+ T cells with a stem-like phenotype.

33. Overman M, Fakih M, Le D, *et al.* 410 phase I interim study results of Nous-209, an off-the-shelf immunotherapy, with pembrolizumab, for the treatment of tumors with a deficiency in mismatch repair/microsatellite instability (dMMR/MSI). J Immunother Cancer 2021; 9:A441.

