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Reinvigorating therapeutic cancer vaccines

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Lessons learned from the rapid deployment of vaccines during the COVID-19 pandemic are reinvigorating the cancer vaccine field. Using delivery platforms including mRNA and synthetic long peptides, recent clinical trials have demonstrated that cancer vaccines are safe, feasible, and can be associated with the generation of antigen-specific memory T cells and, in some cases, durable clinical responses. Despite these advances, fundamental questions remain regarding the optimal delivery platforms and antigen targets to use in cancer vaccines. Ongoing and future studies that harness advances in the identification of novel sources of antigens, the prediction of immunogenic antigens, and the use of single-cell technologies to profile antigen-specific T cells will hopefully reveal correlates with clinical outcomes and provide a mechanistic basis for future progress.

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Introduction

The COVID-19 pandemic has stimulated innovation in vaccine development and, as a result, witnessed impressively rapid deployment of vaccines using a diversity of delivery approaches. Among the most widely used are vaccines delivered by mRNA or by viral vectors. Longstanding and ongoing efforts in the cancer vaccine field, including the incorporation of genomic data analysis and the development of mRNA-based delivery technology which were only previously tested at small

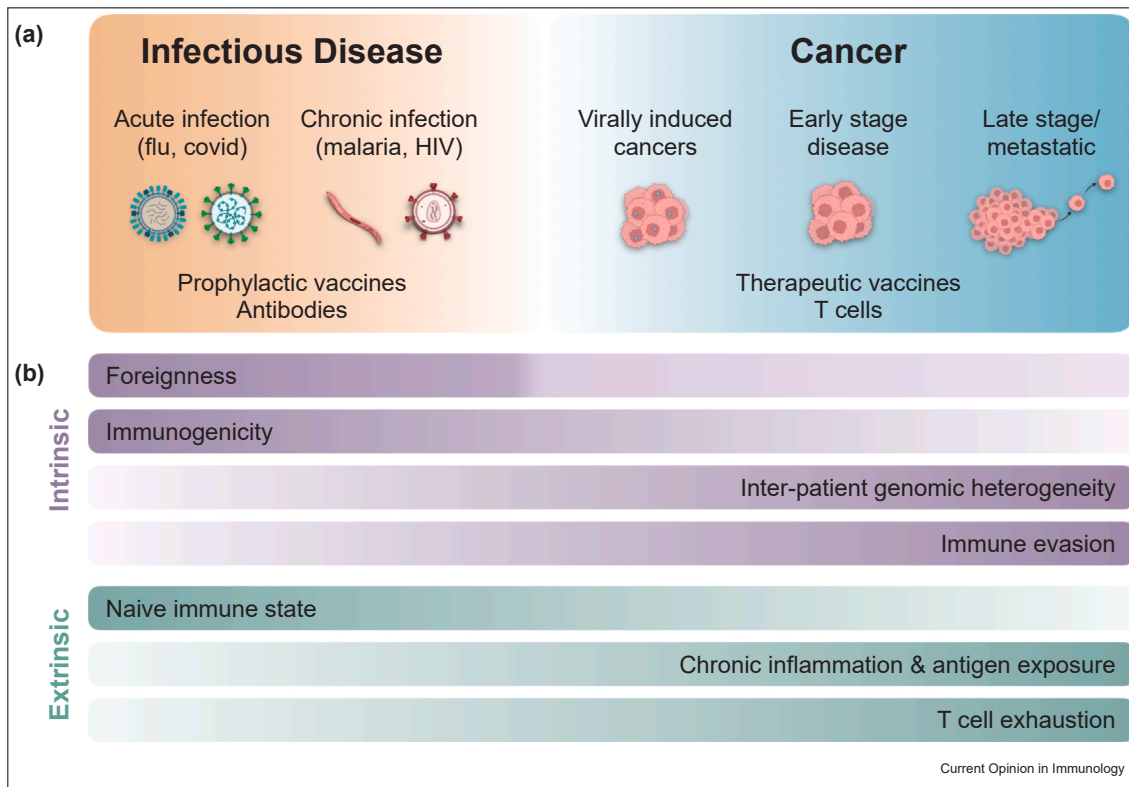
scale, certainly contributed a solid foundation from which to launch these urgent large-scale efforts [1,2]. With the Food and Drug Administration (FDA) approvals of these novel format COVID vaccines over the past two years, the time is ripe for renewing our focus on cancer and even incorporating the lessons learned from this recent time to our efforts.

To apply these lessons to cancer vaccines, we must acknowledge the critical differences between the biological contexts of cancer and infection. First, at the broadest level, infectious disease vaccines are used *prophylactically* and generally seek to activate B cells to produce antibodies, although the role of cell-mediated immunity is increasingly acknowledged [3]. By contrast, cancer vaccines — with the notable exception of vaccines against human papillomavirus (HPV) and hepatitis B virus (HPV) to prevent cervical and liver cancers [4] — are *therapeutic* vaccines that primarily aim to activate tumor-antigen specific T cells to induce long-term tumor control, although the role of B cells cannot be ignored [5] (Figure 1a). Second, pathogens are foreign to the host immune system and thus have high immunogenic potential, whereas cancer cells are self-derived and have variable immunogenicity (Figure 1b). This variability stems in part from broad inter-tumor and intra-tumor genomic heterogeneity, which results in variable expression of tumor antigens. Third, acute infections such as influenza are externally induced, stochastic events that occur in the context of a naïve immune state and generally resolve spontaneously. In contrast, both chronic infections and cancers are characterized by chronic inflammation and antigen exposure, which can lead to progressive immune dysfunction including T cell exhaustion. Tumors can also acquire cancer cell-intrinsic immune evasive features as they progress to more advanced disease states. Collectively, these features underscore the unique challenges for the development of cancer vaccines. Here, we highlight some of the recent clinical advances in cancer vaccine development and discuss how these unique challenges guide the path for future work.

Clinical advances in tumor-associated antigen vaccines: breaking immune tolerance

Most vaccine studies to date have used tumor-associated antigens (TAAs), which are derived from non-mutated self-proteins that are either (1) overexpressed in cancer

Figure 1



Infectious disease and cancer (a) are distinct biological contexts with (b) features intrinsic to pathogens or cancer cells as well as extrinsic features of the immune response.

compared with healthy tissues; (2) expressed during lineage differentiation and aberrantly expressed by cancer cells; or (3) restricted in expression to germ cells and aberrantly expressed by cancer cells [6]. Historically, vaccines targeting TAAs induced clinically ineffective antigen-specific T cell responses, likely due to a combination of insufficient T cell numbers, low antigen affinity, and suboptimal activation [4]. The TAA-specific T cells that are identified in patients with cancer are of relatively lower affinity because high-affinity T cells that recognize TAAs can be subject to central and peripheral immunological tolerance [6]. However, TAA vaccines remain under active investigation due to several potential advantages. For example, since TAAs are not unique to individual tumors but instead shared across many tumor types, they allow for more rapid and cost-effective 'off-the-shelf' vaccines that do not require personalization.

In one recent encouraging study, Sahin et al. reported an interim analysis of a phase I clinical trial (NCT02410733) of patients with melanoma treated with an mRNA vaccine encoding four melanoma TAAs, either alone or in combination with immune checkpoint inhibitors (ICIs) [7]. Seventy-five percent of evaluated

patients induced either CD8+ or, more commonly, CD4+ T cell responses specific for one or more vaccine TAAs. In the vaccine monotherapy arm, 11 of 25 patients derived clinical benefits (metabolic complete response, partial response, or stable disease). Additional features of vaccine-induced T cell activation, such as T cell trafficking to the tumor site and epitope spreading, remain to be investigated in this ongoing trial. However, these results demonstrate that the use of vaccine platforms with strong adjuvant activity and a dosing regimen with priming and repeated boosting can induce T cell activation despite central tolerance towards TAAs. Additional trials using this platform are ongoing in patients with triple-negative breast cancer, prostate cancer, and ovarian cancer [1]. Vaccines targeting melanoma TAAs have also recently been investigated using full-length protein [8] or synthetic long peptides (SLPs) [9].

Clinical advances in neoantigen vaccines

The overall limited efficacy of vaccines targeting TAAs, coupled with advances in next-generation sequencing technologies that enabled the identification of tumor somatic mutations, has led to a focus on neoantigens derived from these mutations as targets for cancer vaccine development. Because neoantigens are derived

from genetic alterations, such as non-synonymous single-nucleotide variants (SNVs) or frameshifts, that are unique to tumor cells, neoantigen-specific T cells are not deleted by central tolerance mechanisms and can be of relatively high affinity. These features make neoantigens attractive targets for cancer vaccines. Indeed, neoantigens are central to anti-tumor T cell responses in the context of several immunotherapies including adoptive T cell therapy, ICIs, and vaccines [10]. To identify tumor neoantigens, tumor-specific somatic alterations are detected by comparing whole-exome sequencing of patient tumor and healthy tissue, and RNA sequencing serves to confirm the expression of mutated genes. Patient human leukocyte antigen (HLA) typing is then performed, and HLA-binding peptides are predicted *in silico* [11]. Cancer vaccines targeting such personal neoantigens have been delivered using epitope-length peptides loaded on dendritic cells [12], intranodally as RNA [13], or subcutaneously as SLPs adjuvanted with poly-ICLC (polyinosinic and polycytidylic acid, stabilized with poly-l-lysine and carboxymethylcellulose) [14] and shown to be safe, feasible, and associated with the establishment of antigen-specific memory T cells. The latter two studies identified remarkably similar rates of CD8+ and (predominantly) CD4+ T cell responses against vaccine neoantigens [13,14].

Long-term outcomes were evaluated in patients with melanoma who received neoantigen SLP vaccines in the adjuvant setting [14] with a median follow-up of almost four years [15]. Of six patients reported in the initial study, all were alive although three had disease recurrence after vaccination. Single-cell transcriptional profiling revealed that neoantigen-specific T cells primarily exhibited memory phenotypes after vaccination, and neoantigen-specific T cells were identified up to 2.5–4 years after treatment, demonstrating long-term persistence of these cells (Figure 2a and b). TCR clonotypes analyzed from circulating neoantigen-specific T cells in three patients revealed that new clonotypes emerged throughout the 24-week course of vaccination, revealing diversification of T cell responses (Figure 2b). In one patient with disease recurrence after vaccination, TCR sequencing of the recurring tumor confirmed that neoantigen-specific T cells infiltrated into the tumor tissue (Figure 2c). In three patients, T cell reactivity against neoantigens and TAAs that were not included in the vaccines was assessed, and T cell responses against these non-vaccine antigens were identified after vaccination that were not detected in pretreatment samples. These results demonstrate that epitope spreading, an indirect indicator of tumor cell killing, had occurred (Figure 2c) [15].

Expanding the proof-of-principle studies beyond melanoma, in a phase I/Ib trial patients with glioblastoma

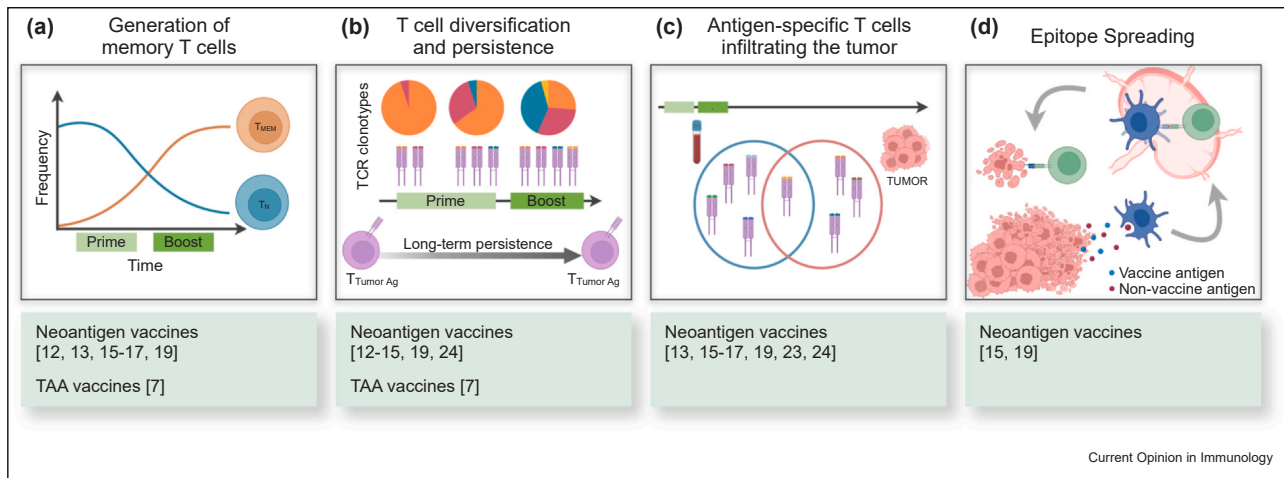
received personalized neoantigen vaccines consisting of SLPs adjuvanted with poly-ICLC in the adjuvant setting. In the subset of patients who did not also receive the immune-suppressing corticosteroid dexamethasone, vaccination induced neoantigen-specific polyfunctional CD8+ and CD4+ T cell responses [16]. Another phase I trial for patients with glioblastoma investigated peptide vaccines targeting both unmutated TAAs and patient-specific neoantigens and adjuvanted with granulocyte macrophage colony-stimulating factor (GM-CSF) and poly-ICLC [17]. Both glioblastoma trials identified vaccine-induced antigen-specific T cells within tumor tissue [16,17], demonstrating that neoantigen vaccination can induce antigen-specific T cell responses in the context of tumors that generally harbor lower mutational burdens and immunologically 'cold' tumor microenvironments [18]. Further, in a phase Ib study with a single-arm design, patients with melanoma, non-small cell lung cancer or bladder cancer received personalized neoantigen vaccines consisting of SLPs adjuvanted with poly-ICLC, in combination with anti-PD1. Vaccination induced newly primed neoantigen-specific T cells that migrated to the tumor site and exhibited markers of cytotoxic activity and a memory phenotype; multiple patients showed evidence of epitope spread [19]. However, due to the single-arm design, the encouraging radiographic and pathologic responses could not be definitively attributed to the administration of the neoantigen vaccine [19]. Randomized trials comparing ICI monotherapy versus ICIs plus neoantigen vaccination using a range of vaccine platforms are ongoing [1,20].

Several alternative approaches circumvent the need for *in silico* identification of personalized neoantigens, for example, by vaccinating with dendritic cells pulsed with whole tumor lysates, which encompass the full spectrum of a patient's tumor antigens [21]. Neoantigens that are shared across patients, including those derived from mutations in oncogenes [22–24], recurring alterations [25], or viral proteins [26], can also reduce the need for personalized neoantigen identification. Empirical neoantigen identification strategies that bypass *in silico* prediction algorithms are also being used to potentially ensure a higher proportion of vaccine neoantigens induce T cell responses in patients (NCT03633110) [27].

Ongoing challenges and next steps in cancer vaccine development

Despite recent advances in the identification of immunogenic neoantigens, this process is effort and time intensive, and defining characteristics of optimal neoantigen targets remains unclear [28,29]. Although initial major histocompatibility complex (MHC) class I (MHC-I) neoantigen prediction algorithms were based on peptide-MHC binding affinity data, they have been improved by incorporating mass spectrometry data from

Figure 2



Durable long-term responses to vaccination can include: **(a)** the generation of antigen-specific memory T cells; **(b)** diversification of T cell clonotypes and long-term T cell persistence; **(c)** infiltration of antigen-specific T cells into the tumor; and **(d)** epitope spreading. Examples of clinical studies in which each feature was reported are shown below.

peptides eluted from tumor samples or MHC mono-allelic cell lines, and by the use of machine learning approaches [30–33]. Additional peptide features, such as the mutation position within the peptide sequence, can also impact predicted binding affinity and immunogenicity [34]. The prediction of MHC-II restricted peptide binding is less established than predictions for MHC-I peptides, yet the crucial role of CD4+ T cells in anti-tumor immune responses [35,36] demonstrates the need for improved identification of MHC-II restricted peptides for neoantigen vaccine design. Much like for MHC-I peptide predictions, mass spectrometry data have improved prediction algorithms for MHC-II [37–39]. Ongoing and future preclinical and clinical studies will reveal if these advances improve the prioritization of immunogenic neoantigens.

A second area for improvement in vaccine design is to harness novel sources of neoantigens beyond those derived from SNVs or frameshift mutations [40–42]. Genetic alterations such as gene fusions [43]; transcriptional sources including alternative splicing [44]; RNA editing [45]; and translation from noncoding regions including endogenous retroelements [46] or unannotated open reading frames [47,48] have been identified as sources of neoantigens. Nonclassical MHC molecules [49] or alterations in peptide processing [50] also provide potential alternative antigenic sources.

Fundamental questions also remain regarding the optimal cancer vaccine delivery platform, which includes cells, viral vectors, proteins, peptides, mRNA, and DNA [4]. Importantly, each platform must be uniquely optimized: peptide production and purification is the rate-

limiting step in the development of peptide vaccines, whereas mRNA modification and encapsulation for increased adjuvanticity and stability were crucial steps forward in mRNA vaccines. Although no definitive frontrunner has been identified, the success of mRNA vaccines for SARS-CoV-2 provides a strong rationale for exploring this platform further in the context of cancer [1,7].

Recent advances in single-cell technologies [51] have enabled comprehensive phenotyping of T cells that can be specifically identified based on their TCR clonotypes [52]. By linking T cell phenotypes and TCR clonotypes with TCR antigen specificities, the features of tumor-antigen specific T cells within tumor compartments are being identified [53,54]. Employing these novel approaches in the context of cancer vaccines [15] can yield detailed information about the phenotypic and clonotypic features of vaccine-induced antigen-specific T cells, including the potential to correlate these features with clinical efficacy. Single-cell technologies can also be used to deeply examine cells within the tumor compartment in order to identify microenvironmental cell states and interaction networks that can either promote or inhibit the activity of vaccine-induced antigen-specific T cell responses, as well as cancer-cell intrinsic features that can promote immune evasion or recognition upon vaccination.

Conclusions

Despite recent clinical advances in the development of cancer vaccines, clinical efficacy has been limited, and ongoing challenges include the time and cost of manufacturing cancer vaccines, particularly for personal

neoantigen vaccines. In addition, the use of cancer vaccines in combination with other cancer therapies will require careful optimization of therapeutic sequences and combinations. However, there are ample opportunities to refine vaccine development approaches. First, novel antigen sources will broaden the available pool of antigens, and encouraging advances in the *in silico* prediction of neoantigens will allow for improved identification of immunogenic neoantigens for use in cancer vaccines. Second, the success of mRNA vaccines for COVID-19 has galvanized the field of mRNA vaccines and hopefully accelerated a pathway for next-generation cancer vaccines. Finally, single-cell technologies will allow for detailed profiling of antigen-specific T cells, the immune microenvironment, and cancer cells following vaccination, which can be correlated with clinical outcomes to provide a mechanistic basis for future advancements.

Conflict of interest statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: CJW holds equity in BioNTech, Inc and receives research support from Pharmacyclics. The remaining author declares no competing interests.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest.

1. Beck JD, Reidenbach D, Salomon N, Sahin U, Tureci O, Vormehr M, Kranz LM: **mRNA therapeutics in cancer immunotherapy.** *Mol Cancer* 2021, **20**:69.
2. Kranz LM, Diken M, Haas H, Kreiter S, Loquai C, Reuter KC, Meng M, Fritz D, Vascotto F, Hefesha H, *et al.*: **Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy.** *Nature* 2016, **534**:396-401.
3. Panagioti E, Klenerman P, Lee LN, van der Burg SH, Arens R: **Features of effective T cell-inducing vaccines against chronic viral infections.** *Front Immunol* 2018, **9**:276.
4. Hollingsworth RE, Jansen K: **Turning the corner on therapeutic cancer vaccines.** *NPJ Vaccines* 2019, **4**:7.
5. Cui C, Wang J, Fagerberg E, Chen PM, Connolly KA, Damo M, Cheung JF, Mao T, Askari AS, Chen S, *et al.*: **Neoantigen-driven B cell and CD4 T follicular helper cell collaboration promotes anti-tumor CD8 T cell responses.** *Cell* 2021, **184**:6101-6118 e6113.
6. Coullie PG, Van den Eynde BJ, van der Bruggen P, Boon T: **Tumour antigens recognized by T lymphocytes: at the core of cancer immunotherapy.** *Nat Rev Cancer* 2014, **14**:135-146.
7. Sahin U, Oehm P, Derhovanessian E, Jabulowsky RA, Vormehr M, Gold M, Maurus D, Schwarck-Kokarakis D, Kuhn AN, Omokoko T, *et al.*: **An RNA vaccine drives immunity in checkpoint-inhibitor-treated melanoma.** *Nature* 2020, **585**:107-112.
- Interim analysis of a clinical trial of an mRNA vaccine encoding four TAAs, which demonstrated that optimized platforms with potent adjuvant activity can induce T cell activation despite central tolerance to TAAs.
8. Pavlick A, Blazquez AB, Meseck M, Lattanzi M, Ott PA, Marron TU, Holman RM, Mandeli J, Salazar AM, McClain CB, *et al.*: **Combined vaccination with NY-ESO-1 protein, Poly-ICLC, and montanide improves humoral and cellular immune responses in patients with high-risk melanoma.** *Cancer Immunol Res* 2020, **8**:70-80.
9. Patel SP, Petroni GR, Roszik J, Olson WC, Wages NA, Chianese-Bullock KA, Smolkin M, Varhegyi N, Gaughan E, Smith KT, *et al.*: **Phase I/II trial of a long peptide vaccine (LPV7) plus toll-like receptor (TLR) agonists with or without incomplete Freund's adjuvant (IFA) for resected high-risk melanoma.** *J Immunother Cancer* 2021, **9**:e003220.
10. Schumacher TN, Scheper W, Kvistborg P: **Cancer neoantigens.** *Ann Rev Immunol* 2019, **37**:173-200.
11. Peters B, Nielsen M, Sette A: **T cell epitope predictions.** *Ann Rev Immunol* 2020, **38**:123-145.
12. Carreno BM, Magrini V, Becker-Hapak M, Kaabinejadian S, Hundal J, Petti AA, Ly A, Lie WR, Hildebrand WH, Mardis ER, *et al.*: **Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells.** *Science* 2015, **348**:803-808.
- Clinical trial that demonstrated the safety, feasibility, and immunogenicity of neoantigen vaccines using epitope-length peptides loaded on dendritic cells.
13. Sahin U, Derhovanessian E, Miller M, Kloke BP, Simon P, Lower M, Bukur V, Tadmor AD, Luxemburger U, Schrors B, *et al.*: **Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer.** *Nature* 2017, **547**:222-226.
- Clinical trial that demonstrated the safety, feasibility, and immunogenicity of neoantigen vaccines using RNA delivered intranodally.
14. Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, Zhang W, Luoma A, Giobbie-Hurder A, Peter L, *et al.*: **An immunogenic personal neoantigen vaccine for patients with melanoma.** *Nature* 2017, **547**:217-221.
- Clinical trial that demonstrated the safety, feasibility, and immunogenicity of neoantigen vaccines using SLPs.
15. Hu Z, Leet DE, Allesoe RL, Oliveira G, Li S, Luoma AM, Liu J, Forman J, Huang T, Iorgulescu JB, *et al.*: **Personal neoantigen vaccines induce persistent memory T cell responses and epitope spreading in patients with melanoma.** *Nat Med* 2021, **27**:515-525.
- Study that evaluated long-term outcomes for patients treated with neoantigen SLP vaccines, which demonstrated that vaccination induced long-term persistence of neoantigen-specific memory T cells, diversification of TCR clonotypes, and epitope spreading.
16. Keskin DB, Anandappa AJ, Sun J, Tirosh I, Mathewson ND, Li S, Oliveira G, Giobbie-Hurder A, Felt K, Gjini E, *et al.*: **Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial.** *Nature* 2019, **565**:234-239.
17. Hilf N, Kuttruff-Coqui S, Frenzel K, Bukur V, Stevanovic S, Gouttefangeas C, Platten M, Tabatabai G, Dutoit V, van der Burg SH, *et al.*: **Actively personalized vaccination trial for newly diagnosed glioblastoma.** *Nature* 2019, **565**:240-245.
18. Lawrence MS, Stojanov P, Polak P, Kryukov GV, Cibulskis K, Sivachenko A, Carter SL, Stewart C, Mermel CH, Roberts SA, *et al.*: **Mutational heterogeneity in cancer and the search for new cancer-associated genes.** *Nature* 2013, **499**:214-218.
19. Ott PA, Hu-Lieskovan S, Chmielowski B, Govindan R, Naing A, Bhardwaj N, Margolin K, Awad MM, Hellmann MD, Lin JJ, *et al.*: **A phase Ib trial of personalized neoantigen therapy plus anti-PD-1 in patients with advanced melanoma, non-small cell lung cancer, or bladder cancer.** *Cell* 2020, **183**:347-362 e324.
20. Blass E, Ott PA: **Advances in the development of personalized neoantigen-based therapeutic cancer vaccines.** *Nat Rev Clin Oncol* 2021, **18**:215-229.

21. Tanyi JL, Chiang CL, Chiffelle J, Thierry AC, Baumgartener P, Huber F, Goepfert C, Tarussio D, Tissot S, Torigian DA, *et al.*: **Personalized cancer vaccine strategy elicits polyfunctional T cells and demonstrates clinical benefits in ovarian cancer.** *NPJ Vaccines* 2021, **6**:36.
22. Cafri G, Gartner JJ, Zaks T, Hopson K, Levin N, Paria BC, Parkhurst MR, Yossef R, Lowery FJ, Jafferji MS, *et al.*: **mRNA vaccine-induced neoantigen-specific T cell immunity in patients with gastrointestinal cancer.** *J Clin Invest* 2020, **130**:5976-5988.
23. Platten M, Bunse L, Wick A, Bunse T, Le Cornet L, Harting I, Sahn F, Sanghvi K, Tan CL, Poschke I, *et al.*: **A vaccine targeting mutant IDH1 in newly diagnosed glioma.** *Nature* 2021, **592**:463-468.
24. Li F, Deng L, Jackson KR, Talukder AH, Katailaha AS, Bradley SD, Zou Q, Chen C, Huo C, Chiu Y, *et al.*: **Neoantigen vaccination induces clinical and immunologic responses in non-small cell lung cancer patients harboring EGFR mutations.** *J Immunother Cancer* 2021, **9**:e002531.
25. Kloor M, Reuschenbach M, Pauligk C, Karbach J, Rafiyan MR, Al-Batran SE, Tariverdian M, Jager E, von Knebel Doeberitz M: **A frameshift peptide neoantigen-based vaccine for mismatch repair-deficient cancers: a phase I/IIa clinical trial.** *Clin Cancer Res* 2020, **26**:4503-4510.
26. Youn JW, Hur S-Y, Woo JW, Kim Y-M, Lim MC, Park SY, Seo SS, No JH, Kim B-G, Lee J-K, *et al.*: **Pembrolizumab plus GX-188E therapeutic DNA vaccine in patients with HPV-16-positive or HPV-18-positive advanced cervical cancer: interim results of a single-arm, phase 2 trial.** *Lancet Oncol* 2020, **21**:1653-1660.
- Interim analysis of a clinical trial of a DNA vaccine targeting HPV E6 and E7, which reported that vaccination combined with pembrolizumab had a 42% overall response rate in patients with recurrent or advanced cervical cancer.
27. Lam H, McNeil LK, Starobinets H, DeVault VL, Cohen RB, Twardowski P, Johnson ML, Gillison ML, Stein MN, Vaishampayan UN, *et al.*: **An empirical antigen selection method identifies neoantigens that either elicit broad antitumor T-cell responses or drive tumor growth.** *Cancer Discov* 2021, **11**:696-713.
28. Roudko V, Greenbaum B, Bhardwaj N: **Computational prediction and validation of tumor-associated neoantigens.** *Front Immunol* 2020, **11**:27.
29. Lang F, Schrors B, Lower M, Tureci O, Sahin U: **Identification of neoantigens for individualized therapeutic cancer vaccines.** *Nat Rev Drug Discov* 2022, **261**:292.
30. Abelin JG, Keskin DB, Sarkizova S, Hartigan CR, Zhang W, Sidney J, Stevens J, Lane W, Zhang GL, Eisenhaure TM, *et al.*: **Mass spectrometry profiling of HLA-associated peptidomes in mono-allelic cells enables more accurate epitope prediction.** *Immunity* 2017, **46**:315-326.
31. Bulik-Sullivan B, Busby J, Palmer CD, Davis MJ, Murphy T, Clark A, Busby M, Duke F, Yang A, Young L, *et al.*: **Deep learning using tumor HLA peptide mass spectrometry datasets improves neoantigen identification.** *Nat Biotechnol* 2018, **37**:55-63.
32. Sarkizova S, Klaeger S, Le PM, Li LW, Oliveira G, Keshishian H, Hartigan CR, Zhang W, Braun DA, Ligon KL, *et al.*: **A large peptidome dataset improves HLA class I epitope prediction across most of the human population.** *Nat Biotechnol* 2020, **38**:199-209.
- Study which profiled peptides presented by MHC monoallelic cell lines using mass spectrometry and developed HLATHENA, an improved prediction algorithm for identification of MHC-I bound peptides.
33. Gartner JJ, Parkhurst MR, Gros A, Tran E, Jafferji MS, Copeland A, Hanada KI, Zacharakis N, Lalani A, Krishna S, *et al.*: **A machine learning model for ranking candidate HLA class I neoantigens based on known neoepitopes from multiple human tumor types.** *Nat Cancer* 2021, **2**:563-574.
34. Capietto AH, Jhunjunwala S, Pollock SB, Lupardus P, Wong J, Hansch L, Cevallos J, Chestnut Y, Fernandez A, Lounsbury N, *et al.*: **Mutation position is an important determinant for predicting cancer neoantigens.** *J Exp Med* 2020, **217**:e20190179.
35. Kreiter S, Vormehr M, van de Roemer N, Diken M, Lower M, Diekmann J, Boegel S, Schrors B, Vascotto F, Castle JC, *et al.*: **Mutant MHC class II epitopes drive therapeutic immune responses to cancer.** *Nature* 2015, **520**:692-696.
36. Alspach E, Lussier DM, Miceli AP, Kizhvatov I, DuPage M, Luoma AM, Meng W, Lichti CF, Esaulova E, Vomund AN, *et al.*: **MHC-II neoantigens shape tumour immunity and response to immunotherapy.** *Nature* 2019, **574**:696-701.
37. Reynisson B, Barra C, Kaabinejadian S, Hildebrand WH, Peters B, Nielsen M: **Improved prediction of MHC II antigen presentation through integration and motif deconvolution of mass spectrometry MHC eluted ligand data.** *J Proteome Res* 2020, **19**:2304-2315.
38. Abelin JG, Harjanto D, Malloy M, Suri P, Colson T, Goulding SP, Creech AL, Serrano LR, Nasir G, Nasrullah Y, *et al.*: **Defining HLA-II ligand processing and binding rules with mass spectrometry enhances cancer epitope prediction.** *Immunity* 2019, **51**:766-779 e717.
39. Racle J, Michaux J, Rockinger GA, Arnaud M, Bobisse S, Chong C, Guillaume P, Coukos G, Harari A, Jandus C, *et al.*: **Robust prediction of HLA class II epitopes by deep motif deconvolution of immunopeptidomes.** *Nat Biotechnol* 2019, **37**:1283-1286.
40. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, Bignell GR, Bolli N, Borg A, Borresen-Dale AL, *et al.*: **Signatures of mutational processes in human cancer.** *Nature* 2013, **500**:415-421.
41. Roudko V, Bozkus CC, Orfanelli T, McClain CB, Carr C, O'Donnell T, Chakraborty L, Samstein R, Huang KL, Blank SV, *et al.*: **Shared immunogenic poly-epitope frameshift mutations in microsatellite unstable tumors.** *Cell* 2020, **183**:1634-1649 e1617.
- Study that identified recurring frameshift alterations as a source of immunogenic neoantigens in patients with microsatellite instability-high tumors.
42. Turajlic S, Litchfield K, Xu H, Rosenthal R, McGranahan N, Reading JL, Wong YNS, Rowan A, Kanu N, Al Bakir M, *et al.*: **Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: a pan-cancer analysis.** *Lancet Oncol* 2017, **18**:1009-1021.
43. Yang W, Lee KW, Srivastava RM, Kuo F, Krishna C, Chowell D, Makarov V, Hoen D, Dalin MG, Wexler L, *et al.*: **Immunogenic neoantigens derived from gene fusions stimulate T cell responses.** *Nat Med* 2019, **25**:767-775.
- Study that identified gene fusions as a source of neoantigens that are immunogenic in patients with head and neck cancers, including those with low mutation burden.
44. Kahles A, Lehmann KV, Toussaint NC, Huser M, Stark SG, Sachsenberg T, Stegle O, Kohlbacher O, Sander C, Cancer Genome Atlas Research N, *et al.*: **Comprehensive analysis of alternative splicing across tumors from 8,705 patients.** *Cancer Cell* 2018, **34**:211-224 e216.
- Study that identified alternative splicing as a source of putative neoantigens in patient tumors from The Cancer Genome Atlas.
45. Zhang M, Fritsche J, Roszik J, Williams LJ, Peng X, Chiu Y, Tsou CC, Hoffgaard F, Goldfinger V, Schoor O, *et al.*: **RNA editing derived epitopes function as cancer antigens to elicit immune responses.** *Nat Commun* 2018, **9**:3919.
46. Laumont CM, Vincent K, Hesnard L, Audemard É, Bonnel É, Laverdure J-P, Gendron P, Courcelles M, Hardy M-P, Côté C, *et al.*: **Noncoding regions are the main source of targetable tumor-specific antigens.** *Sci Transl Med* 2018, **10**:eaau5516.
- Study that identified noncoding regions of the genome as a source of tumor antigens and demonstrated that prophylactic vaccination targeting these antigens can prolong survival of tumor-bearing mice.
47. Chong C, Muller M, Pak H, Harnett D, Huber F, Grun D, Leleu M, Auger A, Arnaud M, Stevenson BJ, *et al.*: **Integrated proteogenomic deep sequencing and analytics accurately identify non-canonical peptides in tumor immunopeptidomes.** *Nat Commun* 2020, **11**:1293.
48. Ouspenskaia T, Law T, Clauser KR, Klaeger S, Sarkizova S, Aguet F, Li B, Christian E, Knisbacher BA, Le PM, *et al.*: **Unannotated proteins expand the MHC-I-restricted immunopeptidome in cancer.** *Nat Biotechnol* 2021, **40**:209-217.

49. Crowther MD, Dolton G, Legut M, Caillaud ME, Lloyd A, Attaf M, Galloway SAE, Rius C, Farrell CP, Szomolay B, *et al.*: **Genome-wide CRISPR-Cas9 screening reveals ubiquitous T cell cancer targeting via the monomorphic MHC class I-related protein MR1.** *Nat Immunol* 2020, **21**:178-185.
50. Garrido G, Schrand B, Levay A, Rabasa A, Ferrantella A, Da Silva DM, D'Eramo F, Marijt KA, Zhang Z, Kwon D, *et al.*: **Vaccination against nonmutated neoantigens induced in recurrent and future tumors.** *Cancer Immunol Res* 2020, **8**:856-868.
51. Stuart T, Satija R: **Integrative single-cell analysis.** *Nat Rev Genet* 2019, **20**:257-272.
52. Pai JA, Satpathy AT: **High-throughput and single-cell T cell receptor sequencing technologies.** *Nat Methods* 2021, **18**:881-892.
53. Oliveira G, Stromhaug K, Klaeger S, Kula T, Frederick DT, Le PM, Forman J, Huang T, Li S, Zhang W, *et al.*: **Phenotype, specificity and avidity of antitumour CD8(+) T cells in melanoma.** *Nature* 2021, **596**:119-125.
54. Caushi JX, Zhang J, Ji Z, Vaghasia A, Zhang B, Hsiue EH, Mog BJ, Hou W, Justesen S, Blosser R, *et al.*: **Transcriptional programs of neoantigen-specific TIL in anti-PD-1-treated lung cancers.** *Nature* 2021, **596**:126-132.