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Authors
Adamik, Juraj
Butterfield, Lisa H

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Q18 : Expanded name of HLA correct? Or should this be defined as “human lymphocyte antigen“?

Response: ok

CANCER IMMUNOTHERAPY

What’s next for cancer vaccines?[Q1]

Cancer Vaccines

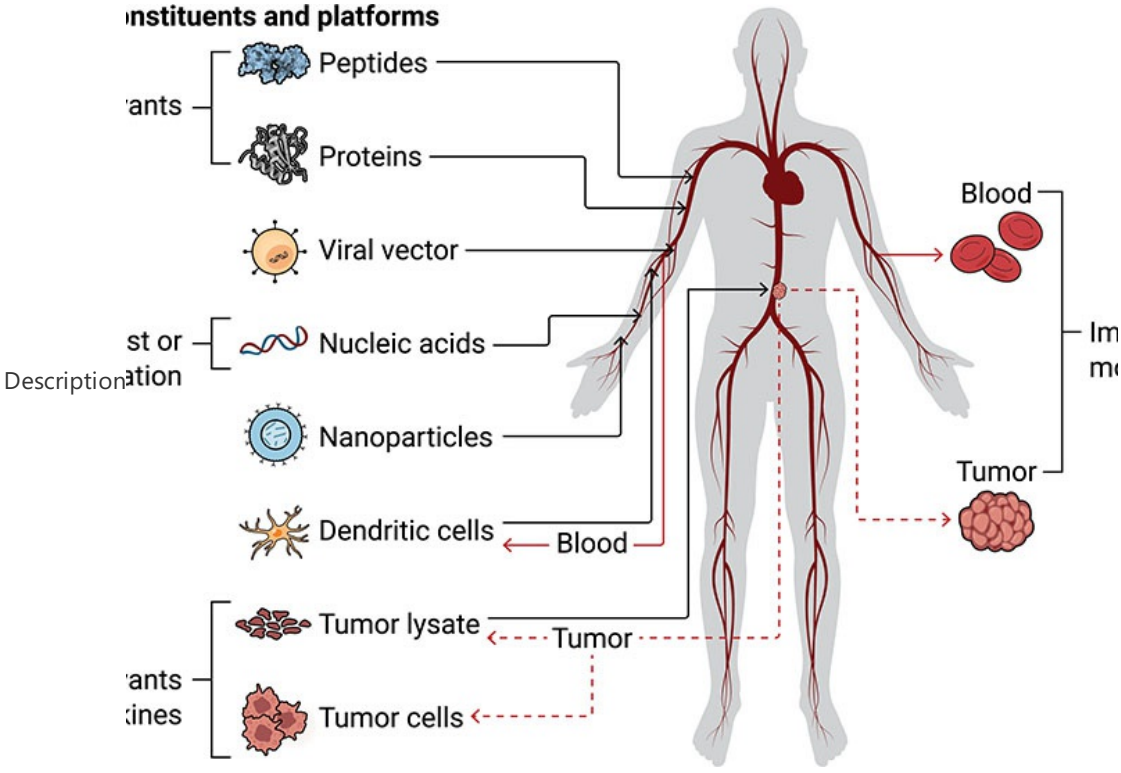
Juraj Adamik and [Q2] Lisa H. Butterfield*

Parker Institute for Cancer Immunotherapy, University of California, San Francisco, Suite D3500, 1 Letterman Drive, San Francisco, CA 94129, USA.

* Corresponding author. Email: lbutterfield@parkerici.org[Q3]

ABSTRACT

Cancer vaccines have been shown clinically to drive tumor-reactive cell activation, proliferation, and effector function. Unfortunately, tumor eradication by treatment with cancer vaccines has been unsuccessful in many patients. Critical steps are underway to improve vaccine efficacy and combine them with immunotherapy and standard-of-care treatments.



Cancer vaccination can promote antitumor immunity in patients, and optimization of components and new combinations are needed for clinical efficacy.

INTRODUCTION[Q4]

Antitumor immunity is initiated by the release of tumor antigen from dying tumor cells, followed by uptake, processing, and presentation by antigen-presenting cells (APCs[Q5]). Tumor antigen–specific T cells can be activated to proliferate and traffic to tumors for targeted killing under optimal conditions of costimulation and appropriate positive signals (*1*).

Although spontaneous antitumor immune responses can occur in some individuals, cancer vaccines are designed to promote these same effects and can be harnessed for broader clinical impacts. What is missing from cancer vaccines to date? In short, robust clinical efficacy. Many small trials in late-stage patients have concluded that vaccines are safe and immunogenic, are associated with interesting immune effects, and provide provocative case reports with clinical responses (*2*, *3*). Several studies have shown significant **[Q6]** improvements in progression-free survival and/or relapse-free survival in adjuvant and minimal residual disease settings (*4*). Such data suggest that cancer vaccination may be insufficiently effective alone in later stages and metastatic tumor settings but can have positive clinical impacts in lower tumor burden settings. Successful vaccination has remained an elusive goal despite robust and reproducible results across vaccine platforms and evidence of increased antitumor effector cells in the periphery.

What is limiting efficacy of stand-alone cancer vaccination? How can cancer vaccination be incorporated into our therapeutic armamentarium? The success of checkpoint blockade has highlighted a need for preexisting tumor-specific immunity for optimal responses. Therefore, vaccines may be a critical tool to promote antitumor immunity in those who do not develop these responses spontaneously. How can this field move **[Q7]** forward [Table 1]? In this viewpoint, we discuss antigen targets, vaccine platforms, rational combinations, and immune monitoring to set the stage for future progress.

Antigen targets

Ideally, cancer vaccines need to target tumor-specific antigens (TSAs) to avoid potential autoimmune responses and issues with central tolerance. Many vaccines fall short of this specificity by targeting overexpressed tumor-associated antigens (TAAs) (*5*, *6*). Indeed, in April 2010, the U.S. Food and Drug Administration (FDA) approved sipuleucel-T (*7*), an autologous cellular immunotherapy targeting the nonmutated TAA prostate acid phosphatase, for the treatment of patients with asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer. Such antigens have largely proven to be safe and immunogenic in clinical trials and underwent systematic evaluation by the National Cancer Institute Pilot Project for the Acceleration of Translational Research on the prioritization of cancer antigens **[Q8]** (*8*). Nonetheless, concerns remain for the potential autoimmune toxicity associated with targeting this class of antigens in the form of vaccines or adoptive T cell therapy (*9*).

What is antigenically unique about the tumor? It is well established that tumors can be detected as foreign tissues by the immune system. Kras and p53 are examples of commonly mutated oncogenic drivers. Vaccines targeting epitopes from these mutated proteins are inefficiently presented by major histocompatibility complex (MHC) molecules, can induce antigen-specific regulatory T cell (T_{reg}), and have had limited efficacy to date (*10*, *11*). For about 15% of the tumors that are virally driven, proteins derived from viruses, such as human papilloma virus (HPV) proteins E6 and E7, are TSAs that have been targeted successfully (*12*). NY-ESO-1 and MAGE family proteins are nonmutated “cancer-testes” antigens expressed in a variety of tumor cells. A deeper understanding of the biology of T cells recognizing these known antigens is beginning to identify a path forward to avoid suppressive responses and broaden the array of shared targets with greater efficacy potential (*13*).

Malignant transformation of cells depends on accumulation of DNA damage. The immune system frequently responds to the neoantigens that arise as a consequence of this DNA damage. Recognition of neoantigens also appear to be an important driver of the clinical activity of both T cell checkpoint blockade and adoptive T cell therapy as cancer immunotherapies. The characterization of mutated tumor–specific neoantigens has revolutionized tumor antigen research in the past 5 years because these antigens can be readily identified through technological advances in tumor sequencing, which allows them to be clinically testable (*14*). The identification of the peptide epitopes derived from mutated tumor antigens remains an open area of experimentation because many groups use proprietary and unique algorithms for the selection of epitopes that can be presented by tumor. High-resolution mass spectrometry can also directly identify MHC-bound surface peptides, which are processed and presented to the immune system.

Global collaborations are helping advance the field of tumor neoantigen identification. The Parker Institute assembled a global consortium wherein each participant predicted immunogenic epitopes from shared tumor sequencing data. A total of 608 epitopes were subsequently assessed for T cell binding in patient-matched samples. By integrating peptide features associated with presentation and recognition, a model of tumor epitope immunogenicity was developed that filtered out 98% of nonimmunogenic peptides with a precision above 0.70. Pipelines prioritizing model features had superior performance, and pipeline alterations leveraging them improved prediction performance. This data resource enables identification of parameters underlying effective antitumor immunity and is available to the research community (*15*). Five traits were identified that determine epitope immunogenicity in an integrated model: Peptides that have strong MHC binding affinity and long half-life are expressed highly and have either low agretopicity or high foreignness.

Are mutated neoantigens more effective antigen targets because they both increase the specificity of tumor targeting and stimulate higher-avidity T cells? This is yet to be determined experimentally; however, it was shown recently that these T cells can still become phenotypically exhausted (*16*). An important variable that most neoantigen identification pipelines are unlikely to be able to account for yet is whether the mutated antigen is "truncal," occurring early in tumorigenesis and homogeneously expressed throughout tumor tissue, or a "branch" mutation, occurring later in tumor development (*17*). "Branches" may be more heterogeneously expressed but may be subject to less tumor-immune system cross-talk, thus potentially promoting more active T cells. As neoantigens have emerged as targets of effective tumor-directed T cell responses, studies have revealed that increased neoantigen load is associated with improved patient outcomes for checkpoint blockade (*18*). Three clinical trials of neoantigen-based vaccines in patients with melanoma, using dendritic cells (DCs **[Q9]**) loaded with short peptides, long peptides, or RNA, have shown the safety, feasibility, and robust immunogenicity of this approach (*14*, *19*, *20*).

TSA can also be undefined in the form of tumor lysates. It is challenging to analyze the impact of a vaccine with an undefined target, but these mixed antigen approaches can promote polyclonal responses and, in the autologous setting, can include neoantigens that do not require identification through **[Q10]** a detailed pipeline for use. These lysates not only include non–tumor-associated self-antigens but may contain immune suppressive factors that can limit efficacy, such as interleukin-10 (IL-10) and transforming growth factor–β. In the setting of hepatocellular cancer, α-fetoprotein (AFP) is commonly overexpressed. Whereas AFP is immunogenic in a cancer vaccination setting, we recently showed that it is also highly immune suppressive and inhibits metabolic function in DCs [(*21*) and **[Q11]** Munson *et al.*, submitted 2022]. Others have used similarly suppressive molecules as the target TAA (*22*).

Vaccine platforms

How should these tumor antigens be delivered? The most critical components of a cancer vaccine are the tumor antigen (signal 1), costimulation (signal 2), and cytokines (signal 3) (Fig. 1). Antigens are typically combined with adjuvants that can stabilize the immunogenic molecule and can stimulate responses mediated by APCs and T cells. Currently used adjuvants include oil and water emulsion (Montanide), cytokines (granulocyte-macrophage colony-stimulating factor), Toll-like receptor (TLR) signal–inducing molecules [polyICLC (Hiltonol)], CD40 costimulation triggers, or more complex adjuvants, such as viruses. RNA vaccines have become viable vaccine platforms and are formulated with RNA molecules packaged into lipid nanoparticles to optimize delivery and immune stimulatory properties (*23*). The ideal signals needed to maximize favorable immune responses are not fully elucidated, and optimal adjuvants to pair with specific antigen platforms are still needed.

Fig. 1.

Vaccine constituents and platforms.

Cancer vaccines take many forms, including nucleic acid, peptides, proteins, and undefined mixtures (cell or tumor lysates). Multiple platforms exist for delivering target tumor antigens, including patient-derived DCs, viral vector, TLR agonist, pathogen signal adjuvants, and nanoparticle formulations. Cytokines, growth factors, and targeting antibodies can be included, and some vaccines can be delivered by electroporation. Peripheral blood mononuclear cells or tumors can be monitored for vaccine responses.

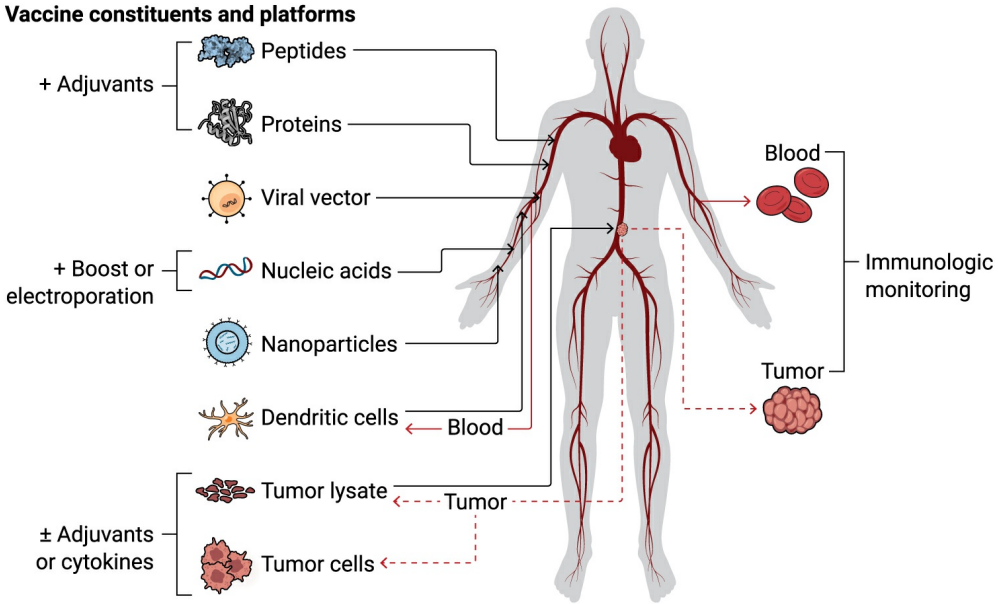


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Fig. 2.

[Q16]Epitope spreading.

Vaccine-induced, adoptively transferred, or spontaneously activated T cells initiate tumor cell killing through tumor antigen recognition. This leads to tumor lysis and the release of proteins, including self-antigens, TAA, and any expressed and mutated neoantigens. The released tumor antigens are taken up by endogenous APC, such as DC. These APCs can cross-present a new wave of tumor antigens to T cells and thus broaden the activated T cell population that can recognize tumor cells.

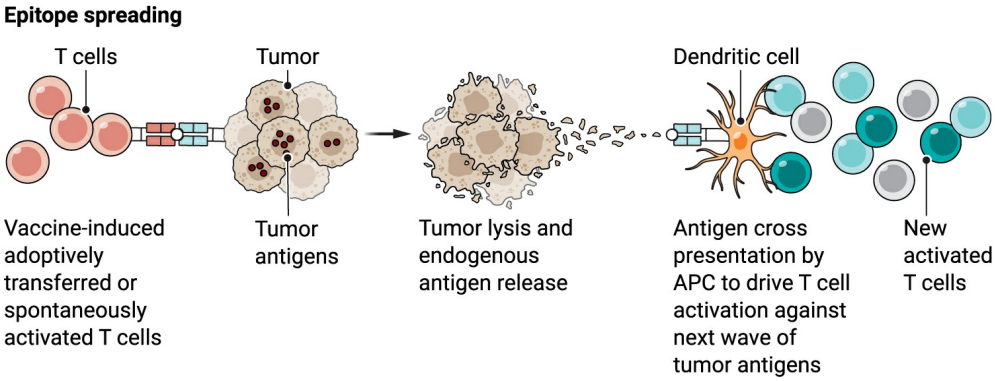

Table 1.

Hurdles for cancer vaccines in vivo and approaches to address them**[Q17]**.

Hurdle	Approaches
Quality of vaccine-induced T cells	-Optimize priming signals by including costimulation and cytokines
	-Evaluate neoantigen targets
T cell polyfunctionality and exhaustion	-Evaluate neoantigens and more recently expressed "branch" antigen targets
	-Optimize priming and/or boosting signals by including costimulation and cytokines
Vaccine trafficking to tumor and tumor tissue penetration	-Inject a tumor with an activating signal (chemokine, oncolytic virus, and nonsuppressive tumor killing agent) to optimize tumor targeting
Heterogeneity of antigen expression	-Include multiple antigens in vaccine
	-Promote epitope spreading
Antigen loss or MHC loss	-Include multiple antigens presented by multiple human lymphocyte antigen**[Q18]** molecules
	-Promote epitope spreading
	-Provide intratumoral IFN-γ signal to up-regulate MHC class I

DCs are the professional APCs responsible for inhibiting unwanted responses (tolerance) and activating pathogen-driven responses. As "nature's adjuvant," they can provide antigen presentation with multiple layers of costimulation and also secrete key cytokines, as well as provide signals that are not yet well understood. There are many different types of DC (*24*), but most cancer vaccine clinical trials use in vitro differentiated myeloid DCs because they are a reliable platform for promoting T cell responses.

Standard-of-care (SOC) approaches have shown vaccine-like "effects" mediated by in vivo tumor lysis, including radiofrequency ablation, embolization (*25*), chemotherapy, radiotherapy, and small-molecule signaling pathway inhibitors in a subset of patients (Fig. 1A). Some agents may provide additional stimulatory signals; gemcitabine promotes chromatin demethylation, and radiation induces DNA damage. Recombinant viruses carrying tumor antigens can also provide strong immune stimulation signals. Many challenges face researchers trying to harness and optimize regimens to promote antigen release and immune stimulation in addition to facilitating tumor cell growth inhibition and lysis.

Rational combinations: Building on success to improve efficacy

How can the activation of T cells and antitumor activity and efficacy be improved? For DC vaccines, a number of

combinations have been tested, including addition of cytotoxic T lymphocyte–associated protein 4 (CTLA-4) (*26*) and/or programmed cell death protein 1 (PD-1)/PD ligand 1**[Q12]** blockade, adoptive transfer of T cells (*27*), low dose IL-2 (*28*), interferon-α (IFN-α) (*29*), and chemotherapy (*30*), which have yielded very modest improvements. Strategies to improve the efficacy of sipuleucel-T are also being investigated, including improving activated T cell trafficking to tumor with anti–CTLA-4 and supporting T cell activation and proliferation with IL-7 (*31*). The initial clinical success of HPV16 peptide encoding SLP**[Q13]** vaccines in early-stage cervical cancer did not yield the same efficacy in later-stage patients, leading to combination testing with cisplatin (*32*) and testing SLP combined with checkpoint blockade (*33*).

A critical issue is timing and sequencing of vaccine inclusion. Murine studies have shown that a vaccine before checkpoint blockade is more efficacious than the opposite order (*34*), which was also supported by data in human patients (*35*). Further examination of checkpoint combinations is complicated by FDA approvals such that patients eligible for experimental vaccine trials may have already received checkpoint blockade as a SOC.

Immune monitoring to understand mechanism of action

How do we get cancer vaccines to the next stage of success? We need a better mechanistic understanding of their immune and clinical impacts. Dissection of immune responses induced by vaccination has led to important insights. Toxicity has not limited broad vaccine platform testing and dose escalation within a platform to the extent that dose increases have an effect, which is not always the case. Widely variable responses to cancer vaccines highlights the critical need for strategies that identify patients who are likely to benefit from this treatment. Measuring frequencies of vaccine peptide–specific cells allows for both determination of cell numbers and detailed phenotypic characterizations. Such analysis has defined T cell differentiation and exhaustion levels associated with different cancers and how vaccination affects these cells. Functional testing is also essential for understanding how vaccines skew immune responses and is carried out preferably by ex vivo analysis to avoid potential skewing of in vitro stimulation. IFN-γ ELISPOT**[Q14]** and intracellular cytokine staining are common functional assays that can provide evidence of potentially successful vaccination but often fall short of insightful correlations with clinical outcomes.

Epitope spreading has emerged as a key indicator of vaccine efficacy (Fig. 1B). This involves promotion of successive waves of T cell activation against target tissue antigenic specificities (*2*, *3*, *36*–*39*), which diversifies the T cell repertoire. Such repertoire diversification facilitates a greater breadth of cellular immune responses such that the expansion of T cell clones in the tumor correlates with better outcomes. Epitope spreading also has obvious implications for targeting antigenically diverse tumors and overcoming the effects of loss of antigen expression by some tumors (*14*). Cancer vaccines must also be able to overcome tumor-induced immune suppression. Preventative cancer vaccination trials in high-risk patients have been severely constrained even at premalignant stages because of the presence of highly suppressive myeloid-derived suppressor cells (MDSCs) (*40*, *41*). High circulating frequencies of functionally suppressive T_{regs} and MDSCs are known to correlate with reduced immune and clinical responses in many tumor types (*29*, *42*), suggesting that targeted reduction of these suppressive cells before vaccination may improve outcomes.

Lessons learned and future prospects

Greater success is being seen from new formulations. FixVac is an intravenously administered liposomal RNA vaccine that targets multiple antigens alone or in combination with PD-1 blockade. FixVac can mediate durable objective responses in checkpoint inhibitor–experienced patients with unresectable melanoma, and individuals given more doses showed improved responses (*19*). Vaccine-induced T cell infiltration and neoepitope-specific killing of autologous tumor cells were shown in postvaccination resected metastases in vaccinated individuals (*43*).

In addition, a neoantigen vaccine was able to promote intratumoral T cell responses in a small glioblastoma (GBM) trial, and neoantigen-specific T cells from the peripheral blood were detected in intracranial GBM tumor tissue. Neoantigen-targeting SLP vaccines thus have the potential to alter the immune milieu of a cold and weakly mutated tumor such as GBM. The best responses were in patients not receiving dexamethasone, shedding light on SOC medications that may limit efficacy of combination therapies (*44*).

CONCLUSIONS

Cancer vaccination is an approach that can safely promote antitumor T cell responses in vivo and can have therapeutic effects in patients with cancer at many stages of disease. Whereas the basic structure of a successful vaccine is well understood, the optimization for each component remains under development [Table 1] **[Q15]**. Many logical combinations have been tested, including cytokines and checkpoint blockade, but few have provided significant improvements. Immune

monitoring has revealed key mechanisms of efficacy that can be applied to new cancer vaccine approaches, particularly epitope spreading induction. Optimized treatment schedules and a better mechanistic understanding of how to induce effective responses will be critical for the future success of cancer vaccines.

REFERENCES AND NOTES

1 **D. S. Chen, I. Mellman**, Oncology meets immunology: The cancer-immunity cycle. *Immunity* 39, 1–10 (2013).

2 **L. H. Butterfield, B. Comin-Anduix, L. Vujanovic, Y. Lee, V. B. Dissette, J.-Q. Yang, H. T. Vu, E. Seja, D. K. Oseguera, D. M. Potter, J. A. Glaspy, J. S. Economou, A. Ribas**, Adenovirus MART-1–engineered autologous dendritic cell vaccine for metastatic melanoma. *J. Immunother.* 31, 294–309 (2008).

3 **L. H. Butterfield, A. Ribas, V. B. Dissette, S. N. Amarnani, H. T. Vu, D. Oseguera, H.-J. Wang, R. M. Elashoff, W. H. McBride, B. Mukherji, A. J. Cochran, J. A. Glaspy, J. S. Economou**, Determinant spreading associated with clinical response in dendritic cell-based immunotherapy for malignant melanoma. *Clin. Cancer Res.* 9, 998–1008 (2003).

4 **A. A. van de Loosdrecht, S. van Wetering, S. J. A. M. Santegoets, S. K. Singh, C. M. Eeltink, Y. den Hartog, M. Koppes, J. Kaspers, G. J. Ossenkoppele, A. M. Kruisbeek, T. D. de Gruijl**, A novel allogeneic off-the-shelf dendritic cell vaccine for post-remission treatment of elderly patients with acute myeloid leukemia. *Cancer Immunol. Immunother.* 67, 1505–1518 (2018).

5 **V. Leko, S. A. Rosenberg**, Identifying and targeting human tumor antigens for T cell-based immunotherapy of solid tumors. *Cancer Cell* 38, 454–472 (2020).

6 **L. H. Butterfield**, Lessons learned from cancer vaccine trials and target antigen choice. *Cancer Immunol. Immunother.* 65, 805–812 (2016).

7 **M. A. Cheever, C. S. Higano**, PROVENGE (Sipuleucel-T) in prostate cancer: The first FDA-approved therapeutic cancer vaccine. *Clin. Cancer Res.* 17, 3520–3526 (2011).

8 **M. A. Cheever, J. P. Allison, A. S. Ferris, O. J. Finn, B. M. Hastings, T. T. Hecht, I. Mellman, S. A. Prindiville, J. L. Viner, L. M. Weiner, L. M. Matrisian**, The prioritization of cancer antigens: A national cancer institute pilot project for the acceleration of translational research. *Clin. Cancer Res.* 15, 5323–5337 (2009).

9 **S. A. Rosenberg, J. C. Yang, N. P. Restifo**, Cancer immunotherapy: Moving beyond current vaccines. *Nat. Med.* 10, 909–915 (2004).

10 **J. Quandt, C. Schlude, M. Bartoschek, R. Will, A. Cid-Arregui, S. Schölch, C. Reissfelder, J. Weitz, M. Schneider, S. Wiemann, F. Momburg, P. Beckhove**, Long-peptide vaccination with driver gene mutations in p53 and Kras induces cancer mutation-specific effector as well as regulatory T cell responses. *Onco. Targets. Ther.* 7, e1500671 (2018).

11 **D. Hoyos, R. Zappasodi, I. Schulze, Z. Sethna, K. César de Andrade, D. F. Bajorin, C. Bandlamudi, M. K. Callahan, S. A. Funt, S. R. Hadrup, J. S. Holm, J. E. Rosenberg, S. P. Shah, I. Vázquez-García, B. Weigelt, M. Wu, D. Zamarin, L. F. Campitelli, E. J. Osborne, M. Klinger, H. S. Robins, P. P. Khincha, S. A. Savage, V. P. Balachandran, J. D. Wolchok, M. D. Hellmann, T. Merghoub, A. J. Levine, M. Łuksza, B. D. Greenbaum**, Fundamental immuneoncogenicity trade-offs define driver mutation fitness. *Nature* 606, E5 (2022).

12 **L. G. de Sousa, K. Rajapakshe, J. R. Canales, R. L. Chin, L. Feng, Q. Wang, T. Z. Barrese, E. Massarelli, W. William, F. M. Johnson, R. Ferrarotto, I. Wistuba, C. Coarfa, J. Lee, J. Wang, C. J. M. Melief, M. A. Curran, B. S. Glisson**, ISA101 and nivolumab for HPV-16+ cancer: Updated clinical efficacy and immune correlates of response. *J. Immunother. Cancer* 10, e004232 (2022).

13 **C. S. Eberhardt, H. T. Kissick, M. R. Patel, M. A. Cardenas, N. Prokhnevska, R. C. Obeng, T. H. Nasti, C. C. Griffith, S. J. Im, X. Wang, D. M. Shin, M. Carrington, Z. G. Chen, J. Sidney, A. Sette, N. F. Saba, A. Wieland, R. Ahmed**, Functional HPV-specific PD-1+ stem-like CD8 T cells in head and neck cancer. *Nature* 597, 279–284 (2021).

14 **B. M. Carreno, V. Magrini, M. Becker-Hapak, S. Kaabinejadian, J. Hundal, A. A. Petti, A. Ly, W.-R. Lie, W. H. Hildebrand, E. R. Mardis, G. P. Linette**, A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. *Science* 348, 803–808 (2015).

15 **D. K. Wells, M. M. van Buuren, K. K. Dang, V. M. Hubbard-Lucey, K. C. F. Sheehan, K. M. Campbell, A. Lamb, J. P. Ward, J. Sidney, A. B. Blazquez, A. J. Rech, J. M. Zaretsky, B. Comin-Anduix, A. H. C. Ng, W. Chour, T. V. Yu, H. Rizvi, J. M. Chen, P. Manning, G. M. Steiner, X. C. Doan; Tumor Neoantigen Selection Alliance, T. Merghoub, J. Guinney, A. Kolom, C. Selinsky, A. Ribas, M. D. Hellmann, N. Hacohen, A. Sette, J. R. Heath, N. Bhardwaj, F. Ramsdell, R. D.**

Schreiber, T. N. Schumacher, P. Kvistborg, N. A. Defranoux, Key parameters of tumor epitope immunogenicity revealed through a consortium approach improve neoantigen prediction. *Cell* 183, 818–834.e13 (2020).

16 S. Li, Y. Simoni, S. Zhuang, A. Gabel, S. Ma, J. Chee, L. Islas, A. Cessna, J. Creaney, R. K. Bradley, A. Redwood, B. W. Robinson, E. W. Newella, Characterization of neoantigen-specific T cells in cancer resistant to immune checkpoint therapies. *Proc. Natl. Acad. Sci. U.S.A.* 118, e2025570118 (2021).

17 N. McGranahan, A. J. S. Furness, R. Rosenthal, S. Ramskov, R. Lyngaa, S. K. Saini, M. Jamal-Hanjani, G. A. Wilson, N. J. Birkbak, C. T. Hiley, T. B. K. Watkins, S. Shafi, N. Murugaesu, R. Mitter, A. U. Akarca, J. Linares, T. Marafioti, J. Y. Henry, E. M. V. Allen, D. Miao, B. Schilling, D. Schadendorf, L. A. Garraway, V. Makarov, N. A. Rizvi, A. Snyder, M. D. Hellmann, T. Merghoub, J. D. Wolchok, S. A. Shukla, C. J. Wu, K. S. Peggs, T. A. Chan, S. R. Hadrup, S. A. Quezada, C. Swanton, Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* 351, 1463–1469 (2016).

18 K. Litchfield, J. L. Reading, C. Puttick, K. Thakkar, C. Abbosh, R. Bentham, T. B. K. Watkins, R. Rosenthal, D. Biswas, A. Rowan, E. Lim, M. A. Bakir, V. Turati, J. A. Guerra-Assunção, L. Conde, A. J. S. Furness, S. K. Saini, S. R. Hadrup, J. Herrero, S.-H. Lee, P. V. Loo, T. Enver, J. Larkin, M. D. Hellmann, S. Turajlic, S. A. Quezada, N. McGranahan, C. Swanton, Meta-analysis of tumor- and T cell-intrinsic mechanisms of sensitization to checkpoint inhibition. *Cell* 184, 596–614.e14 (2021).

19 U. Sahin, P. Oehm, E. Derhovanessian, R. A. Jabulowsky, M. Vormehr, M. Gold, D. Maurus, D. Schwarck-Kokarakis, A. N. Kuhn, T. Omokoko, L. M. Kranz, M. Diken, S. Kreiter, H. Haas, S. Attig, R. Rae, K. Cuk, A. Kemmer-Brück, A. Breitkreuz, C. Tolliver, J. Caspar, J. Quinkhardt, L. Hebich, M. Stein, A. Hohberger, I. Vogler, I. Liebig, S. Renken, J. Sikorski, M. Leierer, V. Müller, H. Mitzel-Rink, M. Miederer, C. Huber, S. Grabbe, J. Utikal, A. Pinter, R. Kaufmann, J. C. Hassel, C. Loquai, Ö. Türeci, An RNA vaccine drives immunity in checkpoint-inhibitor-treated melanoma. *Nature* 585, 107–112 (2020).

20 Z. Hu, P. A. Ott, C. J. Wu, Towards personalized, tumour-specific, therapeutic vaccines for cancer. *Nat. Rev. Immunol.* 18, 168–182 (2018).

21 P. M. Santos, A. V. Menk, J. Shi, A. Tsung, G. M. Delgoffe, L. H. Butterfield, Tumor-derived α-fetoprotein suppresses fatty acid metabolism and oxidative phosphorylation in dendritic cells. *Cancer Immunol. Res.* 7, 1001–1012 (2019).

22 J. W. Kjeldsen, C. L. Lorentzen, E. Martinenaite, E. Ellebaek, M. Donia, R. B. Holmstroem, T. W. Klausen, C. O. Madsen, S. M. Ahmed, S. E. Weis-Banke, M. O. Holmström, H. W. Hendel, E. Ehrnrooth, M.-B. Zocca, A. W. Pedersen, M. H. Andersen, I. M. Svane, A phase 1/2 trial of an immune-modulatory vaccine against IDO/PD-L1 in combination with nivolumab in metastatic melanoma. *Nat. Med.* 27, 2212–2223 (2021).

23 S. Tahtinen, A.-J. Tong, P. Himmels, J. Oh, A. Paler-Martinez, L. Kim, S. Wichner, Y. Oei, M. J. McCarron, E. C. Freund, Z. A. Amir, C. C. de la Cruz, B. Haley, C. Blanchette, J. M. Schartner, W. Ye, M. Yadav, U. Sahin, L. Delamarre, I. Mellman, IL-1 and IL-1ra are key regulators of the inflammatory response to RNA vaccines. *Nat. Immunol.* 23, 532–542 (2022).

24 G. Schreibelt, K. F. Bol, H. Westdorp, F. Wimmers, E. H. J. G. Aarntzen, T. D. Boer, M. W. M. M. van de Rakt, N. M. Scharenborg, A. J. de Boer, J. M. Pots, M. A. M. O. Nordkamp, T. G. M. van Oorschot, J. Tel, G. Winkels, K. Petry, W. A. M. Blokx, M. M. van Rossum, M. E. B. Welzen, R. D. M. Mus, S. A. J. Croockewit, R. H. T. Koornstra, J. F. M. Jacobs, S. Kelderman, C. U. Blank, W. R. Gerritsen, C. J. A. Punt, C. G. Figdor, I. J. M. de Vries, Effective clinical responses in metastatic melanoma patients after vaccination with primary myeloid dendritic cells. *Clin. Cancer Res.* 22, 2155–2166 (2016).

25 L. Ayaru, S. P. Pereira, A. Alisa, A. A. Pathan, R. Williams, B. Davidson, A. K. Burroughs, T. Meyer, S. Behboudi, Unmasking of α-fetoprotein-specific CD4+ T cell responses in hepatocellular carcinoma patients undergoing embolization. *J. Immunol.* 178, 1914–1922 (2007).

26 A. Ribas, B. Comin-Anduix, B. Chmielowski, J. Jalil, P. de la Rocha, T. A. McCannel, M. T. Ochoa, E. Seja, A. Villanueva, D. K. Oseguera, B. R. Straatsma, A. J. Cochran, J. A. Glaspy, L. Hui, F. M. Marincola, E. Wang, J. S. Economou, J. Gomez-Navarro, Dendritic cell vaccination combined with CTLA4 blockade in patients with metastatic melanoma. *Clin. Cancer Res.* 15, 6267–6276 (2009).

27 T. Chodon, B. Comin-Anduix, B. Chmielowski, R. C. Koya, Z. Wu, M. Auerbach, C. Ng, E. Avramis, E. Seja, A. Villanueva, T. A. McCannel, A. Ishiyama, J. Czernin, C. G. Radu, X. Wang, D. W. Gjertson, A. J. Cochran, K. Cornetta, D. J. L. Wong, P. Kaplan-Lefko, O. Hamid, W. Samlowski, P. A. Cohen, G. A. Daniels, B. Mukherji, L. Yang, J. A. Zack, D. B. Kohn, J. R. Heath, J. A. Glaspy, O. N. Witte, D. Baltimore, J. S. Economou, A. Ribas, Adoptive transfer of MART-1 T-cell

receptor transgenic lymphocytes and dendritic cell vaccination in patients with metastatic melanoma. *Am. Assoc. Cancer Res.* 20, 2457–2465 (2014).

28 J. M. Greene, E. J. Schneble, D. O. Jackson, D. F. Hale, T. J. Vreeland, M. Flores, J. Martin, G. S. Herbert, M. O. Hardin, X. Yu, T. E. Wagner, G. E. Peoples, A phase I/IIa clinical trial in stage IV melanoma of an autologous tumor–dendritic cell fusion (dendritoma) vaccine with low dose interleukin-2. *Cancer Immunol. Immunother.* 65, 383–392 (2016).

29 L. H. Butterfield, L. Vujanovic, P. M. Santos, D. M. Maurer, A. Gambotto, J. Lohr, C. Li, J. Waldman, U. Chandran, Y. Lin, H. Lin, H. A. Tawbi, A. A. Tarhini, J. M. Kirkwood, Multiple antigen-engineered DC vaccines with or without IFNα to promote antitumor immunity in melanoma. *J. Immunother. Cancer* 7, 113 (2019).

30 P. Kongsted, T. H. Borch, E. Ellebaek, T. Z. Iversen, R. Andersen, Ö. Met, M. Hansen, H. Lindberg, L. Sengeløv, I. M. Svane, Dendritic cell vaccination in combination with docetaxel for patients with metastatic castration-resistant prostate cancer: A randomized phase II study. *Cytotherapy* 19, 500–513 (2017).

31 L. Fong, P. Carroll, V. Weinberg, S. Chan, J. Lewis, J. Corman, C. L. Amling, R. A. Stephenson, J. Simko, N. A. Sheikh, R. B. Sims, M. W. Frohlich, E. J. Small, Activated lymphocyte recruitment into the tumor microenvironment following preoperative Sipuleucel-T for localized prostate cancer. *J. Natl. Cancer Inst.* 106, dju268 (2014).

32 G. G. Kenter, M. J. P. Welters, A. R. P. M. Valentijn, M. J. G. Lowik, D. M. A. B. der Meer, A. P. G. Vloon, F. Essahsah, L. M. Fathers, R. Offringa, J. W. Drijfhout, A. R. Wafelman, J. Oostendorp, G. J. Fleuren, S. H. van der Burg, C. J. M. Melief, Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. *N.Engl. J. Med.* 361, 1838–1847 (2009).

33 E. Massarelli, W. William, F. Johnson, M. Kies, R. Ferrarotto, M. Guo, L. Feng, J. J. Lee, H. Tran, Y. U. Kim, C. Haymaker, C. Bernatchez, M. Curran, T. Z. Barrese, J. R. Canales, I. Wistuba, L. Li, J. Wang, S. H. van der Burg, C. J. Melief, B. Glisson, Combining immune checkpoint blockade and tumor-specific vaccine for patients with incurable human papillomavirus 16–related cancer: A phase 2 clinical trial. *JAMA Oncol.* 5, 67–73 (2019).

34 V. Verma, R. K. Shrimali, S. Ahmad, W. Dai, H. Wang, S. Lu, R. Nandre, P. Gaur, J. Lopez, M. Sade-Feldman, K. Yizhak, S. L. Bjorgaard, K. T. Flaherty, J. A. Wargo, G. M. Boland, R. J. Sullivan, G. Getz, S. A. Hammond, M. Tan, J. Qi, P. Wong, T. Merghoub, J. Wolchok, N. Hacohen, J. E. Janik, M. Mkrtichyan, S. Gupta, S. N. Khleif, PD-1 blockade in subprimed CD8 cells induces dysfunctional PD-1+CD38hi cells and anti-PD-1 resistance. *Nat. Immunol.* 20, 1231–1243 (2019).

35 P. M. Santos, J. Adamik, T. R. Howes, S. Du, L. Vujanovic, S. Warren, A. Gambotto, J. M. Kirkwood, L. H. Butterfield, Impact of checkpoint blockade on cancer vaccine–activated CD8+ T cell responses. *J. Exp. Med.* 217, e20191369 (2020).

36 E. Ranieri, L. S. Kierstead, H. Zarour, J. M. Kirkwood, M. T. Lotze, T. Whiteside, W. J. Storkus, Dendritic cell/peptide cancer vaccines: Clinical responsiveness and epitope spreading. *Immunol. Invest.* 29, 121–125 (2000).

37 M. L. Disis, T. A. Gooley, K. Rinn, D. Davis, M. Piepkorn, M. A. Cheever, K. L. Knutson, K. Schiffman, Generation of T-cell immunity to the HER-2/neu protein after active immunization with HER-2/neu peptide–based vaccines. *J. Clin. Oncol.* 20, 2624–2632 (2002).

38 A. Ribas, J. A. Glaspy, Y. Lee, V. B. Dissette, E. Seja, H. T. Vu, N. S. Tchekmedyian, D. Oseguera, B. Comin-Anduix, J. A. Wargo, S. N. Amarnani, W. H. McBride, J. S. Economou, L. H. Butterfield, Role of dendritic cell phenotype, determinant spreading, and negative costimulatory blockade in dendritic cell-based melanoma immunotherapy. *J. Immunother.* 27, 354–367 (2004).

39 J. Wierecky, M. R. Müller, S. Wirths, E. Halder-Oehler, D. Dörfel, S. M. Schmidt, M. Häntschel, W. Brugger, S. Schröder, M. S. Horger, L. Kanz, P. Brossart, Immunologic and clinical responses after vaccinations with peptide-pulsed dendritic cells in metastatic renal cancer patients. *Cancer Res.* 66, 5910–5918 (2006).

40 T. Kimura, J. R. McKolanis, L. A. Dzubinski, K. Islam, D. M. Potter, A. M. Salazar, R. E. Schoen, O. J. Finn, MUC1 vaccine for individuals with advanced adenoma of the colon: A cancer immunoprevention feasibility study. *Cancer Prev. Res.* 6, 18–26 (2013).

41 P. Ma, P. L. Beatty, J. McKolanis, R. Brand, R. E. Schoen, O. J. Finn, Circulating myeloid derived suppressor cells (MDSC) that accumulate in premalignancy share phenotypic and functional characteristics with MDSC in cancer. *Front. Immunol.* 10, 1401 (2019).

42 J. Retseck, R. VanderWeele, H.-M. Lin, Y. Lin, L. H. Butterfield, A. A. Tarhini, Phenotypic and functional testing of circulating regulatory T cells in advanced melanoma patients treated with neoadjuvant ipilimumab. *J. Immunother. Cancer* 4, 38 (2016).

43 **U. Sahin, E. Derhovanessian, M. Miller, B.-P. Kloke, P. Simon, M. Löwer, V. Bukur, A. D. Tadmor, U. Luxemburger, B. Schrörs, T. Omokoko, M. Vormehr, C. Albrecht, A. Paruzynski, A. N. Kuhn, J. Buck, S. Heesch, K. H. Schreeb, F. Müller, I. Ortseifer, I. Vogler, E. Godehardt, S. Attig, R. Rae, A. Breitkreuz, C. Tolliver, M. Suchan, G. Martic, A. Hohberger, P. Sorn, J. Diekmann, J. Ciesla, O. Waksmann, A.-K. Brück, M. Witt, M. Zillgen, A. Rothermel, B. Kasemann, D. Langer, S. Bolte, M. Diken, S. Kreiter, R. Nemecek, C. Gebhardt, S. Grabbe, C. Höller, J. Utikal, C. Huber, C. Loquai, Ö. Türeci**, Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature* 547, 222–226 (2017).

44 **D. B. Keskin, A. J. Anandappa, J. Sun, I. Tirosh, N. D. Mathewson, S. Li, G. Oliveira, A. Giobbie-Hurder, K. Felt, E. Gjini, S. A. Shukla, Z. Hu, L. Li, P. M. Le, R. L. Allesøe, A. R. Richman, M. S. Kowalczyk, S. Abdelrahman, J. E. Geduldig, S. Charbonneau, K. Pelton, J. B. Iorgulescu, L. Elagina, W. Zhang, O. Olive, C. McCluskey, L. R. Olsen, J. Stevens, W. J. Lane, A. M. Salazar, H. Daley, P. Y. Wen, E. A. Chiocca, M. Harden, N. J. Lennon, S. Gabriel, G. Getz, E. S. Lander, A. Regev, J. Ritz, D. Neuberg, S. J. Rodig, K. L. Ligon, M. L. Suvà, K. W. Wucherpfennig, N. Hacohen, E. F. Fritsch, K. J. Livak, P. A. Ott, C. J. Wu, D. A. Reardon**, Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. *Nature* 565, 234–239 (2019).

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