Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Pharmacology

Peptides as cancer vaccines

Marta Calvo Tardón[1], Mathilde Allard[1], Valérie Dutoit[2], Pierre-Yves Dietrich[2] and Paul R Walker[1]

Cancer vaccines based on synthetic peptides are a safe, well-tolerated immunotherapy able to specifically stimulate tumor-reactive T cells. However, their clinical efficacy does not approach that achieved with other immunotherapies such as immune checkpoint blockade. Nevertheless, major advances have been made in selecting tumor antigens to target, identifying epitopes binding to classical and non-classical HLA molecules, and incorporating these into optimal sized peptides for formulation into a vaccine. Limited potency of currently used adjuvants and the immunosuppressive tumor microenvironment are now understood to be major impediments to vaccine efficacy that need to be overcome. Rationally designed combination therapies are now being tested and should ultimately enable peptide vaccination to be added to immuno-oncology treatment options.

Addresses
[1] Center for Translational Research in Onco-Hematology, Division of Oncology, Geneva University Hospitals and University of Geneva, Geneva, Switzerland
[2] Center for Translational Research in Onco-Hematology, Department of Oncology, Geneva University Hospitals and University of Geneva, Geneva, Switzerland

Corresponding author: Walker, Paul R (Paul.Walker@unige.ch)

Current Opinion in Pharmacology 2019, **47**:20–26

This review comes from a themed issue on **Cancer**

Edited by **Laura Soucek** and **Jonathan Whitfield**

https://doi.org/10.1016/j.coph.2019.01.007

Introduction

Therapeutic cancer vaccines based on peptides have been envisaged and developed for almost 40 years and yet the approach remains in a status of 'potential' interest for cancer therapy rather than one with unequivocal clinical benefit. Notwithstanding this stark appraisal of the current situation and the absence of FDA-approval for peptide cancer vaccines, there have been major advances in the field. Peptide vaccines are able to elicit an immune response against a tumor [1,2], and hundreds of clinical trials [3•] are providing a wealth of information that is driving the field forward. A realistic roadmap for clinical development will take into account the lessons learned from suboptimal vaccination protocols, the resistance of tumor cells and the hostility of the tumor microenvironment, and the opportunities of combinations with other forms of immunotherapy such as immune checkpoint blockade (ICB).

Cancer vaccines targeting defined antigens aim to induce or expand cancer-specific T cells and rely on DNA, RNA, proteins or peptides. The latter offer the most direct way of targeting a specific epitope, the portion of the antigen that is recognized by the T-cell receptor in association with human leukocyte antigen (HLA) molecules, thus stimulating T cells with defined tumor specificity. This precision targeting contrasts with the broad immunity (including autoimmune responses) induced by immune checkpoint blocking antibodies and contributes to the excellent safety and tolerability profile of peptide vaccines. Moreover, synthesis of clinical grade peptides of virtually any specificity is achieved more rapidly and cost-effectively than a human or humanized therapeutic antibody. Nevertheless, these advantages are offset by the fact that a given peptide epitope will efficiently bind to only one or a few HLA alleles, thus limiting a particular peptide vaccine formulation to a subset of cancer patients. In many clinical trials using peptide vaccines in Europe and the USA, HLA-A2 binding peptides are used and inclusion criteria require expression of this allele, a condition satisfied by around one third of patients. Choice of the peptide sequence is the first essential requirement of a peptide vaccine, but this is not sufficient to elicit an effective immune response. Peptide length or other modifications, administration regimen, adjuvants and combinations with other therapies are all key in determining final clinical efficacy of therapeutic peptide vaccines.

Antigens to target

Many therapeutic vaccines have targeted non-mutated tumor-associated antigens (TAA), which are shared between healthy and tumor cells, but are overexpressed by cancer cells. The advantage of targeting TAA is their expression by cancers from many individuals. However, since these TAA are self-proteins, the repertoire of high avidity T cells with corresponding specificity can be restricted due to immunological tolerance. Whether this significantly impacts clinical vaccination has been difficult to directly assess, because immunomonitoring is often relatively insensitive and never exhaustive. More recent advances may address this issue more adequately, although with the limitations of clinical sampling in the

peripheral blood rather than at the tumor site [4,5]. Results of phase III trials of such TAA vaccines have been disappointing in the case of pancreatic cancer, non-small-cell lung cancer and renal cell carcinoma [6–8]. Nevertheless, the approach continues in other indications, including bladder cancer, prostate cancer, and glioma [9–11]. Although data are only reported for pilot studies and phase I/II trials to date, the results are promising as they show peptide-specific CD8 T cell-responses in several patients, which was correlated with longer survival.

Targeting epitopes expressed only in cancer cells and absent in healthy tissue, the so-called tumor-specific antigens (TSA), can obviate the limitations of a partially tolerant T-cell repertoire. These antigens can originate from viruses associated with certain cancers (e.g. HPV and HBV) or from mutated proteins, termed neoantigens. In the former category, several phase I and II clinical trials targeting HPV are underway or have been completed (as recently reviewed [12]). Although peptide vaccination alone may be insufficient for tumor regression, encouraging results from a phase II trial in patients with incurable HPV-16 related malignances point to the interest of long-peptide vaccination combined with ICB [13]. However, human cancers with a known viral etiology are the exception, and most TSA derive from mutated epitopes. These neoantigens can arise from point mutations, but other genetic rearrangements such as insertions and deletions can also be the underlying cause [14]. Some of these may be common to multiple tumors, such as the neoepitope expressed by many glioblastomas, EGFRvIII, as a result of a truncation in the wild-type EGFR. However, a phase III clinical trial targeting this epitope with rindopepimut vaccine in addition to chemotherapy did not improve survival over chemotherapy alone [15]. This study assessed humoral responses but did not address the role of vaccine-induced T cells. Since the best described mechanism of action of peptide vaccines for cancer is induction of tumor-specific T cells, it is difficult to judge whether failure of this trial was a result of an absence of such a cellular response.

An additional problem of targeting only one epitope, as performed in the previous study, is the heterogeneous antigen expression and the outgrowth of antigen-negative tumor cells. Multi-peptide vaccines are one solution to this, as long as sufficient tumor antigens are identified. For TAAs, this was achieved by peptide elution from tumor cells for the IMA901 vaccine for renal cell carcinoma [8] and the IMA950 vaccine for glioblastoma using as adjuvants GM-CSF [16] or poly-ICLC [17], and from *in vitro* predictions for other multi-peptide vaccines for pediatric glioma and multiple myeloma [11,18]. These studies showed immune responses against multiple peptides in several patients, encouraging further development of multiple TAA peptide vaccines. However, the magnitude and/or therapeutic efficacy of these responses still need to be improved, as shown by the IMA901 phase III clinical trial that showed no improvement in overall survival [8]. For TSA, there have been major advances in genome mapping technologies to identify neoepitopes even in cancers from individual patients [19,20••,21], thus opening the way to personalized peptide vaccines [22•], which has yielded particularly encouraging results in a phase I trial for melanoma, in which up to 20 personalized long peptides were administered to patients [20••]. Other studies in glioblastoma are following the same approach, such as the phase I GAPVAC trial and the phase I/Ib trial of a personalized neoantigen vaccine; both showing sustained CD8 and CD4 T cell responses [23•,24•]. Although multi-peptide vaccines are the most direct way to broaden anti-tumor immunity and avoid immune escape, significant tumor cell killing can liberate additional tumor antigens, promote epitope spreading, and expand T cells of different specificities to that induced by the vaccine or other immunotherapy [25,26].

HLA binding and peptide length

Minimal peptide epitopes of 8–11 amino acids with appropriate binding motifs can associate with certain HLA class I (HLA-I) alleles without further processing, thereby forming ligands for CD8 T cells. Similarly, longer peptides of 13–18 amino acids can directly bind to HLA class II (HLA-II) alleles and stimulate CD4 T cells. However, the simplicity of administering peptide vaccines based on minimal peptide epitopes must be balanced with the risk that most injected peptides will exogenously bind to HLA expressing cells that do not express costimulatory molecules and do not, therefore, efficiently stimulate T cells [27,28]. This is principally a problem for HLA-I, which is expressed by most nucleated cells of the body. The implications of this may even lead to tolerance induction rather than activation [29]. Synthetic long peptides are now routinely employed in many clinical trials; they are generally more than 20 amino acids long, require processing and so favor presentation by professional antigen presenting cells such as dendritic cells, ideally suited for T-cell priming. Judicious choice of long peptide sequences can select regions encompassing both HLA-I and HLA-II binding epitopes; moreover, binding motifs for multiple HLA alleles may be present, which can be further increased by using multiple long peptides in individual patients, as recently described in the previously mentioned phase I trial for melanoma [20••]. Nevertheless, generation of HLA-I binding peptides requires processing of peptides that enter the cytosol, which may not occur efficiently for all peptides. Future trials may employ long peptides modified by the addition of a cell penetrating peptide sequence, shown to induce superior CD8 T-cell responses to long peptides alone in animal models [30,31]. Interestingly, although this approach promoted CD8 T-cell induction, this was not at the expense of CD4 T-cell immune responses, which are increasingly recognized as being an essential component of

anti-cancer immunity [32•,33]. Indeed, CD4 T cells, particularly Th1 cells, are not only important for efficient CD8 T-cell priming, recruitment at the tumor site and establishing memory, but they may also exert CD8-independent anti-tumor effect functions, justifying CD4-inducing approaches in peptide vaccination [22•,34,35].

Epitope prediction

Approaches to select peptide vaccine epitopes differ according to whether the epitope is a TSA derived from a mutated gene, or a non-mutated TAA. For the latter, it is essential to determine preferential expression of the protein by the tumor, and ideally (as for TSA) presentation of the peptide on tumor cell HLA molecules. This is most directly determined by elution of peptide bound to HLA from tumor cells, with subsequent detection and characterization by mass spectrometry [36–38]. For mutated epitopes, the development of faster and cheaper deep-sequencing techniques has revolutionized identification of putative neoepitopes [39], even at the single-cell level [40]. This can be followed by bioinformatics algorithms to predict peptide–HLA binding [41], which can be combined with peptide characterization [37,38,42]. Regardless of the sophistication of epitope prediction from TAA or TSA, it is also essential to prove T-cell recognition. Here, the original techniques of reverse immunology that opened the era of tumor immunotherapy have been brought up to date with 21st century technology. Culture of fastidious T-cell clones from cancer patients is no longer a bottleneck, with TCR transduction, or healthy donor T cells being used to validate epitope recognition [43,44]. Furthermore, the relationship between TCR sequences and epitope specificity is becoming progressively unraveled [45], opening future possibilities for combining *in silico* approaches with cellular immunology to determine whether predicted epitopes should be targeted by vaccines [21].

HLA-E-binding peptides as potential universal tumor epitopes

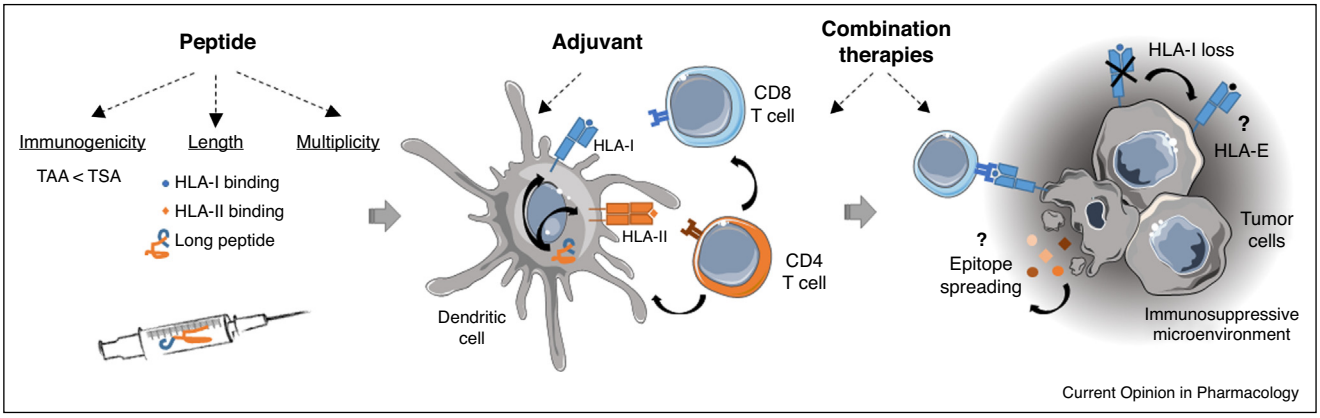
To date therapeutic cancer vaccines have mostly focused on antigenic peptides presented by classical HLA-I molecules. However, the existence of unconventional CD8 T-cell responses restricted by the non-classical HLA-I molecule HLA-E has recently emerged, offering the opportunity to identify alternative peptide targets in cancer patients [46•]. As for classical HLA-I, HLA-E is broadly expressed and assembles with β2-microglobulin to present intracellular-derived peptides at the cell surface [47]. However, whereas classical HLA-I has thousands of allotypes, HLA-E shows little polymorphism, with only two alleles that differ outside the peptide-binding groove [48]. Thus, while the highly polymorphic classical HLA-I molecules imposes diverse peptide repertoires among patents, HLA-E–peptide complexes could provide universal antigenic targets. Furthermore, while classical HLA-I alleles are frequently down modulated in cancer cells, promoting immune escape from CD8 T cells [49•], HLA-E expression is retained in numerous hematopoietic and solid malignancies, and for certain of these, levels are correlated with prognosis and/or immune infiltration [50]. Hence, HLA-E binding peptides may represent attractive therapeutic targets, especially when classical HLA-I expression is lost. However, an HLA-E-restricted anti-tumor T-cell response remains unexplored.

The role of HLA-E is best characterized as an NK receptor ligand; a restricted peptide-set derived from the signal sequences of others HLA-I molecules is presented and protects healthy cells from NK cytotoxicity through interaction with the inhibitory CD94/NKG2A receptor. Nonetheless, during cellular stress, infection or malignant transformation, HLA-E can present a more diverse repertoire of peptides recognized by CD8 T cells and can contribute to immunity in various infections (reviewed in Ref. [51]). Indeed, HLA-E-restricted pathogen-specific CD8 T cells can display polyclonality, polyfunctionality, and long-term persistence, that is, features that would be appropriate for anti-tumor immunity. In mice, *in vivo* studies convincingly demonstrated immune surveillance of tumors with TAP [52,53•] or ERAPP [54] deficiencies by T cells restricted by the functional homolog of HLA-E, Qa-1 [49•]. Moreover, Qa-1 restricted CD8 T cells could be induced by peptide vaccination [52,53•]. In human *in vitro* studies using classical HLA-I negative cells, HLA-E was shown to bind a set of self-derived peptides related to heat shock responses [55,56] and defective antigen-processing [57]. Collectively, these data encourage future efforts to identify and address immunogenicity of the HLA-E–peptidome naturally presented in human tumors, and to test the feasibility of therapeutic vaccination. Finally, while HLA-E binding-peptide may represent potent therapeutic targets when expressed at the surface of malignant cells, their self-origin mandates vigilance; any on-target autoimmune side effects must be assessed.

Adjuvants and vaccine formulation

The formulation of a peptide vaccine and the choice of adjuvant are critical for vaccine efficacy, with no consensus concerning what is optimal for therapeutic vaccination in cancer. The primary role of the adjuvant in any vaccine is to ensure sufficient costimulation by the antigen presenting cells that prime T cells. There are additional requirements for a therapeutic peptide vaccine: facilitating cross-presentation of the vaccine peptides to stimulate CD8 T cells, protecting the peptides from too rapid degradation, and promoting effector T-cell homing to the tumor site. Current vaccines have mostly employed a restricted range of adjuvants, including Montanide ISA-51 (IFA), TLR agonists, and GM-CSF. Caution in clinical trials has generally resulted in the use of single

Figure 1



The figure is an original scheme using modified images from smart Servier Medical Art (using license Creative Commons Attribution 3.0 France https://creativecommons.org/licenses/by/3.0/).

adjuvants, but multiple adjuvants may ultimately be necessary, as recently discussed [58,59]. Future developments will also need to consider modulating the duration of antigen presentation [60], and minimizing the retention and inactivation of activated T cells in water-in-oil depots (Montanide, IFA) at the injection site [61,62•].

Synergistic combination therapies

High magnitude, highly functional, tumor-specific T cells induced by the most optimal peptide vaccine that can be envisaged still face a final formidable hurdle: the tumor microenvironment. Tumor cells, myeloid cells, regulatory T cells, an aberrant vasculature and physicochemical features of the tumor microenvironment such as hypoxia and lactate accumulation, all contribute to inhibit T-cell infiltration or function. Fortunately, the revolution in clinical cancer immunotherapy offers a multitude of opportunities for rational combinations with peptide vaccination, many of which are already under clinical trial [3•]. These can use peptide vaccination to sensitize to the immunomodulator (e.g. ICB), or use ICB antibodies to maintain the functionality of vaccine-induced T cells. Although the end result, clinical efficacy, might be the same, the underlying mechanism will influence the choice and sequence of administering the different therapies. Combinations are not only restricted to immunotherapy, but can include radiotherapy, targeted therapy, anti-angiogenic therapy and chemotherapy. Certain chemotherapeutic agents, when used in the right sequence, can promote anti-tumor immunity by eliciting immunogenic tumor cell death [63], and anti-angiogenic strategies can enhance T-cell infiltration [64].

Perspectives

The future for therapeutic peptide vaccines is encouraging, because we have tools to identify target antigens, adjuvants to potentially combine for enhanced immunogenicity and a multitude of clinically relevant immunomodulators (Figure 1). A major challenge of this cornucopia of opportunities is how to rationally combine and test a multimodal cancer therapy in a clinical context. Tumor immunity requires investigation *in vivo*, which obligates uses of immunocompetent animals in preclinical testing, and yet the targeted antigens will be of human origin in the clinical vaccine. Despite advances in using humanized animals and more sophisticated *in vitro* cultures, these must be used in addition to biological and clinical information, with improved immunomonitoring from clinical trials. We should be inspired by the cancer immunotherapy revolution of ICB that was built on deciphering conserved immune mechanisms between mice and humans, to develop a next generation of potent peptide vaccines to incorporate into new multimodality treatments for cancer patients.

Funding

This work was supported by the Association Frédéric Fellay and Fond'action contre le cancer.

Conflict of interest statement

P.R.W. and P.-Y.D. have ownership interest in patents related to cell penetrating peptides and are consultant/advisory board members for Amal Therapeutics.

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. Kumai T, Fan A, Harabuchi Y, Celis E: **Cancer immunotherapy: moving forward with peptide T cell vaccines**. *Curr Opin Immunol* 2017, **47**:57-63.

2. Van der Burg SH: **Correlates of immune and clinical activity of novel cancer vaccines**. *Semin Immunol* 2018, **39**:119-136.

3. • Bezu L, Kepp O, Cerrato G, Pol J, Fucikova J, Spisek R, Zitvogel L, Kroemer G, Galluzzi L: **Trial watch: peptide-based vaccines in anticancer therapy**. *Oncoimmunology* 2018, **7**:e1511506.
Useful and comprehensive review of clinical trials (and some preclinical studies) using peptide vaccines from 2015 until mid 2018.

4. Muller S, Agnihotri S, Shoger KE, Myers MI, Smith N, Chaparala S, Villanueva CR, Chattopadhyay A, Lee AV, Butterfield LH *et al.*: **Peptide vaccine immunotherapy biomarkers and response patterns in pediatric gliomas**. *JCI Insight* 2018, **3**.

5. Dimitrov S, Gouttefangeas C, Besedovsky L, Jensen ATR, Chandran PA, Rusch E, Businger R, Schindler M, Lange T, Born J *et al.*: **Activated integrins identify functional antigen-specific CD8(+) T cells within minutes after antigen stimulation**. *Proc Natl Acad Sci U S A* 2018, **115**:E5536-E5545.

6. Middleton G, Silcocks P, Cox T, Valle J, Wadsley J, Propper D, Coxon F, Ross P, Madhusudan S, Roques T *et al.*: **Gemcitabine and capecitabine with or without telomerase peptide vaccine GV1001 in patients with locally advanced or metastatic pancreatic cancer (TeloVac): an open-label, randomised, phase 3 trial**. *Lancet Oncol* 2014, **15**:829-840.

7. Mitchell P, Thatcher N, Socinski MA, Wasilewska-Tesluk E, Horwood K, Szczesna A, Martin C, Ragulin Y, Zukin M, Helwig C *et al.*: **Tecemotide in unresectable stage III non-small-cell lung cancer in the phase III START study: updated overall survival and biomarker analyses**. *Ann Oncol* 2015, **26**:1134-1142.

8. Rini BI, Stenzl A, Zdrojowy R, Kogan M, Shkolnik M, Oudard S, Weikert S, Bracarda S, Crabb SJ, Bedke J *et al.*: **IMA901, a multipeptide cancer vaccine, plus sunitinib versus sunitinib alone, as first-line therapy for advanced or metastatic renal cell carcinoma (IMPRINT): a multicentre, open-label, randomised, controlled, phase 3 trial**. *Lancet Oncol* 2016, **17**:1599-1611.

9. Obara W, Eto M, Mimata H, Kohri K, Mitsuhata N, Miura I, Shuin T, Miki T, Koie T, Fujimoto H *et al.*: **A phase I/II study of cancer peptide vaccine S-288310 in patients with advanced urothelial carcinoma of the bladder**. *Ann Oncol* 2017, **28**:798-803.

10. Obara W, Sato F, Takeda K, Kato R, Kato Y, Kanehira M, Takata R, Mimata H, Sugai T, Nakamura Y *et al.*: **Phase I clinical trial of cell division associated 1 (CDCA1) peptide vaccination for castration resistant prostate cancer**. *Cancer Sci* 2017, **108**:1452-1457.

11. Pollack IF, Jakacki RI, Butterfield LH, Hamilton RL, Panigrahy A, Normolle DP, Connelly AK, Dibridge S, Mason G, Whiteside TL *et al.*: **Antigen-specific immunoreactivity and clinical outcome following vaccination with glioma-associated antigen peptides in children with recurrent high-grade gliomas: results of a pilot study**. *J Neurooncol* 2016, **130**:517-527.

12. Chabeda A, Yanez RJR, Lamprecht R, Meyers AE, Rybicki EP, Hitzeroth II: **Therapeutic vaccines for high-risk HPV-associated diseases**. *Papillomavirus Res* 2018, **5**:46-58.

13. Massarelli E, William W, Johnson F, Kies M, Ferrarotto R, Guo M, Feng L, Lee JJ, Tran H, Kim YU *et al.*: **Combining immune checkpoint blockade and tumor-specific vaccine for patients with incurable human papillomavirus 16-related cancer: a phase 2 clinical trial**. *JAMA Oncol* 2018, **5**:67-73.

14. 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.

15. Weller M, Butowski N, Tran DD, Recht LD, Lim M, Hirte H, Ashby L, Mechtler L, Goldlust SA, Iwamoto F *et al.*: **Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial**. *Lancet Oncol* 2017, **18**:1373-1385.

16. Rampling R, Peoples S, Mulholland PJ, James A, Al-Salihi O, Twelves CJ, McBain C, Jefferies S, Jackson A, Stewart W *et al.*: **A cancer research UK first time in human phase I trial of IMA950 (novel multipeptide therapeutic vaccine) in patients with newly diagnosed glioblastoma**. *Clin Cancer Res* 2016, **22**:4776-4785.

17. Migliorini D, Dutoit V, Allard M, Grandjean Hallez N, Marinari E, Widmer V, Philippin G, Corlazzoli F, Gustave R, Kreutzfeldt M *et al.*: **Phase I/II trial testing safety and immunogenicity of the multipeptide IMA950/poly-ICLC vaccine in newly diagnosed adult malignant astrocytoma patients**. *Neuro-Oncology* 2019: noz040 http://dx.doi.org/10.1093/neuonc/noz040.

18. Nooka AK, Wang ML, Yee AJ, Kaufman JL, Bae J, Peterkin D, Richardson PG, Raje NS: **Assessment of safety and immunogenicity of PVX-410 vaccine with or without lenalidomide in patients with smoldering multiple myeloma: a nonrandomized clinical trial**. *JAMA Oncol* 2018:e183267.

19. Gubin MM, Artyomov MN, Mardis ER, Schreiber RD: **Tumor neoantigens: building a framework for personalized cancer immunotherapy**. *J Clin Invest* 2015, **125**:3413-3421.

20. •• 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.
A landmark study in personalized peptide vaccination, showing the feasibility of targeting multiple neoantigens from melanoma patients with a multiple long peptide vaccine. The vaccine was safe and immunogenic, with induction of both CD4 and CD8 T cells, and showed clinical efficacy in some patients. Moreover, subsequent anti-PD1 treatment in 2 patients with tumor recurrence resulted in broadening of the anti-tumor response and tumor regression.

21. Schumacher TN, Schreiber RD: **Neoantigens in cancer immunotherapy**. *Science* 2015, **348**:69-74.

22. • Sahin U, Tureci O: **Personalized vaccines for cancer immunotherapy**. *Science* 2018, **359**:1355-1360.
An up to date review of the cutting edge technological advances for personalized vaccines (including peptide vaccines) targeting tumor mutations from individual patients.

23. • 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.
A technologically sophisticated personalized peptide vaccine trial for glioblastoma targeting sequentially non-mutated antigens from a 'warehouse' of previously defined antigens, and then mutated neoantigens for each patient. The former antigens induced CD8 T cell responses, the latter category induced CD4 T cell responses. In view of the limited number of mutations and neoantigens in glioblastoma and the apparent CD4 T cell bias of the vaccine induced responses, this study revives the interest and necessity of targeting both non-mutated antigens (TAA) and mutated neoantigens (TSA).

24. • 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.
A personalized peptide vaccine phase I/Ib trial for glioblastoma targeting only neoantigens. Immunogenicity of the vaccine was validated, but principally in patients that did not require dexamethasone (which was required to limit brain edema) during priming. In these patients there was a CD4 T cell dominance of the induced response, despite epitope identification based on HLA class I binding algorithms. Importantly, there was evidence for vaccine-induced T-cell infiltration of the tumor site, but also signs of an exhaustion phenotype.

25. Gulley JL, Madan RA, Pachynski R, Mulders P, Sheikh NA, Trager J, Drake CG: **Role of antigen spread and distinctive characteristics of immunotherapy in cancer treatment**. *J Natl Cancer Inst* 2017, **109**.

26. Verdegaal EM, de Miranda NF, Visser M, Harryvan T, van Buuren MM, Andersen RS, Hadrup SR, van der Minne CE, Schotte R, Spits H *et al.*: **Neoantigen landscape dynamics during human melanoma-T cell interactions**. *Nature* 2016, **536**:91-95.

27. Eisen HN, Hou XH, Shen C, Wang K, Tanguturi VK, Smith C, Kozyrytska K, Nambiar L, McKinley CA, Chen J *et al.*: **Promiscuous binding of extracellular peptides to cell surface class I MHC protein**. *Proc Natl Acad Sci U S A* 2012, **109**:4580-4585.

28. Santambrogio L, Sato AK, Fischer FR, Dorf ME, Stern LJ: **Abundant empty class II MHC molecules on the surface of**

immature dendritic cells. *Proc Natl Acad Sci U S A* 1999, **96**:15050-15055.

29. Melief CJ, van Hall T, Arens R, Ossendorp F, van der Burg SH: **Therapeutic cancer vaccines**. *J Clin Invest* 2015, **125**:3401-3412.

30. Derouazi M, Di Berardino-Besson W, Belnoue E, Hoepner S, Walther R, Benkhoucha M, Teta P, Dufour Y, Yacoub Maroun C, Salazar AM *et al.*: **Novel cell-penetrating peptide-based vaccine induces robust CD4+ and CD8+ T cell-mediated antitumor immunity**. *Cancer Res* 2015, **75**:3020-3031.

31. Belnoue E, Di Berardino-Besson W, Gaertner H, Carboni S, Dunand-Sauthier I, Cerini F, Suso-Inderberg EM, Walchli S, Konig S, Salazar AM *et al.*: **Enhancing antitumor immune responses by optimized combinations of cell-penetrating peptide-based vaccines and adjuvants**. *Mol Ther* 2016, **24**:1675-1685.

32. • Marty R, Thompson WK, Salem RM, Zanetti M, Carter H: **Evolutionary pressure against MHC class II binding cancer mutations**. *Cell* 2018, **175**:416-428 e413.
This study uses sophisticated modelling to help explain the importance of CD4 T cells in anti-tumor immunity in human populations. The data suggest that mutations influencing antigens able to bind to HLA-II shape tumor evolution.

33. 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.

34. Tran E, Turcotte S, Gros A, Robbins PF, Lu YC, Dudley ME, Wunderlich JR, Somerville RP, Hogan K, Hinrichs CS *et al.*: **Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer**. *Science* 2014, **344**:641-645.

35. Platten M, Schilling D, Bunse L, Wick A, Bunse T, Riehl D, Karapanagiotou-Schenkel I, Harting I, Sahm F, Schmitt A *et al.*: **A mutation-specific peptide vaccine targeting IDH1R132H in patients with newly diagnosed malignant astrocytomas: a first-in-man multicenter phase I clinical trial of the German Neurooncology Working Group (NOA-16)**. *J Clin Oncol* 2018, **36** 2001-2001.

36. 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.

37. Dutoit V, Herold-Mende C, Hilf N, Schoor O, Beckhove P, Bucher J, Dorsch K, Flohr S, Fritsche J, Lewandrowski P *et al.*: **Exploiting the glioblastoma peptidome to discover novel tumour-associated antigens for immunotherapy**. *Brain* 2012, **135**:1042-1054.

38. Fritsche J, Rakitsch B, Hoffgaard F, Romer M, Schuster H, Kowalewski DJ, Priemer M, Stos-Zweifel V, Horzer H, Satelli A *et al.*: **Translating immunopeptidomics to immunotherapy-decision-making for patient and personalized target selection**. *Proteomics* 2018, **18**:e1700284.

39. Pritchard AL, Burel JG, Neller MA, Hayward NK, Lopez JA, Fatho M, Lennerz V, Wolfel T, Schmidt CW: **Exome sequencing to predict neoantigens in melanoma**. *Cancer Immunol Res* 2015, **3**:992-998.

40. Gee MH, Han A, Lofgren SM, Beausang JF, Mendoza JL, Birnbaum ME, Bethune MT, Fischer S, Yang X, Gomez-Eerland R *et al.*: **Antigen identification for orphan T cell receptors expressed on tumor-infiltrating lymphocytes**. *Cell* 2018, **172**:549-563 e516.

41. Liu XS, Mardis ER: **Applications of immunogenomics to cancer**. *Cell* 2017, **168**:600-612.

42. Yadav M, Jhunjhunwala S, Phung QT, Lupardus P, Tanguay J, Bumbaca S, Franci C, Cheung TK, Fritsche J, Weinschenk T *et al.*: **Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing**. *Nature* 2014, **515**:572-576.

43. Pasetto A, Gros A, Robbins PF, Deniger DC, Prickett TD, Matus-Nicodemos R, Douek DC, Howie B, Robins H, Parkhurst MR *et al.*: **Tumor- and neoantigen-reactive T-cell receptors can be identified based on their frequency in fresh tumor**. *Cancer Immunol Res* 2016, **4**:734-743.

44. Stronen E, Toebes M, Kelderman S, van Buuren MM, Yang W, van Rooij N, Donia M, Boschen ML, Lund-Johansen F, Olweus J *et al.*: **Targeting of cancer neoantigens with donor-derived T cell receptor repertoires**. *Science* 2016, **352**:1337-1341.

45. Dash P, Fiore-Gartland AJ, Hertz T, Wang GC, Sharma S, Souquette A, Crawford JC, Clemens EB, Nguyen THO, Kedzierska K *et al.*: **Quantifiable predictive features define epitope-specific T cell receptor repertoires**. *Nature* 2017, **547**:89-93.

46. • Godfrey DI, Le Nours J, Andrews DM, Uldrich AP, Rossjohn J: **Unconventional T cell targets for cancer immunotherapy**. *Immunity* 2018, **48**:453-473.
Review covering the role and therapeutic potential of unconventional T cells (including HLA-E-restricted, but also CD1-restricted and MR1-restricted, as well as $\gamma\delta$ T cells) in tumor immunity.

47. Boegel S, Lower M, Bukur T, Sorn P, Castle JC, Sahin U: **HLA and proteasome expression body map**. *BMC Med Genomics* 2018, **11**:36.

48. Ramalho J, Veiga-Castelli LC, Donadi EA, Mendes-Junior CT, Castelli EC: **HLA-E regulatory and coding region variability and haplotypes in a Brazilian population sample**. *Mol Immunol* 2017, **91**:173-184.

49. • Marijt KA, Doorduijn EM, van Hall T: **TEIPP antigens for T-cell based immunotherapy of immune-edited HLA class I(low) cancers**. *Mol Immunol* 2018. (in press).
Review covering the work of van Hall and others on tumor epitopes associated with antigen processing defects (including Qa-1-restricted T cells and tumor escape mechanisms associated with HLA-I downregulation).

50. Wieten L, Mahaweni NM, Voorter CE, Bos GM, Tilanus MG: **Clinical and immunological significance of HLA-E in stem cell transplantation and cancer**. *Tissue Antigens* 2014, **84**:523-535.

51. Joosten SA, Sullivan LC, Ottenhoff TH: **Characteristics of HLA-E restricted T-cell responses and their role in infectious diseases**. *J Immunol Res* 2016, **2016**:2695396.

52. Oliveira CC, van Veelen PA, Querido B, de Ru A, Sluijter M, Laban S, Drijfhout JW, van der Burg SH, Offringa R, van Hall T: **The nonpolymorphic MHC Qa-1b mediates CD8+ T cell surveillance of antigen-processing defects**. *J Exp Med* 2010, **207**:207-221.

53. • Doorduijn EM, Sluijter M, Querido BJ, Seidel UJE, Oliveira CC, van der Burg SH, van Hall T: **T cells engaging the conserved MHC class Ib molecule Qa-1(b) with TAP-independent peptides are semi-invariant lymphocytes**. *Front Immunol* 2018, **9**:60.
This study highlights that Qa-1(the murine homolog of HLA-E) can present immunogenic self-derived peptides and contribute to the immune surveillance of tumors with antigen processing defects. Also evidenced *in vivo* priming of tumor-reactive Qa-1-restricted CD8 T cells upon prophylactic peptide vaccination.

54. Nagarajan NA, Gonzalez F, Shastri N: **Nonclassical MHC class Ib-restricted cytotoxic T cells monitor antigen processing in the endoplasmic reticulum**. *Nat Immunol* 2012, **13**:579-586.

55. Michaëlsson J, Teixeira de Matos C, Achour A, Lanier LL, Kärre K, Söderström K: **A signal peptide derived from hsp60 binds HLA-E and interferes with CD94/NKG2A recognition**. *J Exp Med* 2002, **196**:1403-1414.

56. Wooden SL, Kalb SR, Cotter RJ, Soloski MJ: **Cutting edge: HLA-E binds a peptide derived from the ATP-binding cassette transporter multidrug resistance-associated protein 7 aSnd inhibits NK cell-mediated lysis**. *J Immunol* 2005, **175**:1383-1387.

57. Lampen MH, Hassan C, Sluijter M, Geluk A, Dijkman K, Tjon JM, de Ru AH, van der Burg SH, van Veelen PA, van Hall T: **Alternative peptide repertoire of HLA-E reveals a binding motif that is strikingly similar to HLA-A2**. *Mol Immunol* 2013, **53**:126-131.

58. Gouttefangeas C, Rammensee HG: **Personalized cancer vaccines: adjuvants are important, too**. *Cancer Immunol Immunother* 2018, **67**:1911-1918.

59. Temizoz B, Kuroda E, Ishii KJ: **Combination and inducible adjuvants targeting nucleic acid sensors**. *Curr Opin Pharmacol* 2018, **41**:104-113.

60. Khong H, Volmari A, Sharma M, Dai Z, Imo CS, Hailemichael Y, Singh M, Moore DT, Xiao Z, Huang XF *et al.*: **Peptide vaccine formulation controls the duration of antigen presentation and magnitude of tumor-specific CD8(+) T cell response**. *J Immunol* 2018, **200**:3464-3474.

61. Hailemichael Y, Dai Z, Jaffarzad N, Ye Y, Medina MA, Huang XF, Dorta-Estremera SM, Greeley NR, Nitti G, Peng W *et al.*: **Persistent antigen at vaccination sites induces tumor-specific CD8(+) T cell sequestration, dysfunction and deletion**. *Nat Med* 2013, **19**:465-472.

62. • Hailemichael Y, Woods A, Fu T, He Q, Nielsen MC, Hasan F, Roszik J, Xiao Z, Vianden C, Khong H *et al.*: **Cancer vaccine formulation dictates synergy with CTLA-4 and PD-L1 checkpoint blockade therapy**. *J Clin Invest* 2018, **128**:1338-1354.

The authors observe a synergistic effect on survival of mice with melanoma upon treatment with a gp100 peptide vaccine in combination with immune checkpoint inhibitors. Of note, the peptide formulation was critical– IFA had a negative impact whereas vaccination with gp100 peptide in saline resulted in better survival.

63. Pfirschke C, Engblom C, Rickelt S, Cortez-Retamozo V, Garris C, Pucci F, Yamazaki T, Poirier-Colame V, Newton A, Redouane Y *et al.*: **Immunogenic chemotherapy sensitizes tumors to checkpoint blockade therapy**. *Immunity* 2016, **44**:343-354.

64. Allen E, Jabouille A, Rivera LB, Lodewijckx I, Missiaen R, Steri V, Feyen K, Tawney J, Hanahan D, Michael IP *et al.*: **Combined antiangiogenic and anti-PD-L1 therapy stimulates tumor immunity through HEV formation**. *Sci Transl Med* 2017, **9**.