

# New emerging targets in cancer immunotherapy: the role of neoantigens



Leticia De Mattos-Arruda <sup>1,2</sup>, Juan Blanco-Heredia <sup>1,2</sup>,  
Carmen Aguilar-Gurrieri,<sup>1</sup> Jorge Carrillo,<sup>1,2</sup> Julià Blanco<sup>1,2,3</sup>

**To cite:** De Mattos-Arruda L, Blanco-Heredia J, Aguilar-Gurrieri C, *et al.* New emerging targets in cancer immunotherapy: the role of neoantigens. *ESMO Open* 2020;4:e000684. doi:10.1136/esmoopen-2020-000684

Received 15 January 2020  
Revised 19 February 2020  
Accepted 20 February 2020

## ABSTRACT

The success of cancer therapies with immune checkpoint inhibitors is transforming the treatment of patients with cancer and fostering cancer research. Therapies that target immune checkpoint inhibitors have shown unprecedented rates of durable long-lasting responses in patients with various cancer types, but only in a fraction of patients. Thus, novel approaches are needed to make immunotherapy more precise and also less toxic. The advances of next-generation sequencing technologies have allowed fast detection of somatic mutations in genes present in the exome of an individual tumour. Targeting neoantigens, the mutated peptides expressed only by tumour cells, may enable antitumour T-cell responses and tumour destruction without causing harm to healthy tissues. Currently, neoantigens can be identified in tumour clinical samples by using genomic-based computational tools. The two main treatment modalities targeting neoantigens that have been investigated in clinical trials are personalised vaccines and tumour infiltrating lymphocytes-based adoptive T-cell therapy. In this mini review, we discuss the promises and challenges for using neoantigens as emergent targets to personalise and guide cancer immunotherapy in a broader set of cancers.

## INTRODUCTION

Cancer immunotherapies prompting the immune system to attack tumours have mediated durable clinical responses in patients with metastatic melanoma, lung cancer, bladder cancer and other tumour types.<sup>1,2</sup>

The common goal of immunotherapies is to invigorate the immune system to destroy cancer cells. The activation of T-cell killing activity is a balance between positive signals provided by the specific recognition of tumour antigens, activating receptors and negative signals provided by immune checkpoint receptors.<sup>3</sup> Therefore, the therapeutic manipulation of this balance can be achieved inhibiting negative signals using immune checkpoint inhibitors (ie, antiprogrammed cell death protein 1 (PD-1), antiprogrammed cell death-ligand 1 (PD-L1) or anticytotoxic T-lymphocyte antigen 4 antibodies) intended to restore T-cell function in an immunosuppressed tumour environment.<sup>4,5</sup>

The actions of immune checkpoint inhibitors rely on the presence of tumour

infiltrating lymphocytes (TILs) on the tumour.<sup>6,7</sup> Previous data suggest that melanoma regression after therapeutic PD-1 inhibition requires pre-existing CD8 +T cells that are negatively regulated by the interaction between PD-1 and PD-L1.<sup>6</sup> More recently, PD-1-expressing neoantigen-specific T-cells have been identified in the peripheral blood of patients with melanoma and gastrointestinal cancers, correlating with the recently documented activity of PD-1 inhibitors in these populations.<sup>8,9</sup> Evidence suggests that clinical responses in patients with cancer after the administration of immune checkpoint inhibitors may also be mediated by neoepitope-reactive T-cells.<sup>10-12</sup>

Tumours with high TILs ('hot tumours') have, in general, better responses to checkpoint inhibitors than those lacking or having sparse TILs ('cold tumours').<sup>3</sup> Additionally, TILs can be isolated from tumour biopsies, expanded and activated *in vitro*, and reinfused to the same individual, showing antitumour activity *in vivo*.<sup>1</sup> However, the inhibition of immune checkpoint is not antigen-specific and may modify the global T-cell response causing immunological side effects.<sup>13</sup> In addition, and apart from a few exceptions (eg, microsatellite instability high cancers),<sup>14</sup> approximately one-third of patients will derive benefit from checkpoint inhibitors, while many patients will experience disease progression. More precise and specific therapeutic approaches to direct T-cell responses against the tumour are needed. Here, we review an emerging tool for cancer immunotherapy, the so-called neoantigens and discuss their role as targets for cancer vaccines and adoptive T-cell therapies.

## Neoantigens as targets for personalised immunotherapies

Neoantigens represent a class of tumour antigens generated by non-synonymous somatic mutations that can be identified by T-cells as non-self-proteins. Single-nucleotide variants (SNV), mutational frameshifts, splice variants

© Author (s) (or their employer(s)) 2020. Re-use permitted under CC BY-NC. No commercial re-use. Published by BMJ on behalf of the European Society for Medical Oncology.

<sup>1</sup>IrsiCaixa AIDS Research Institute, Germans Trias i Pujol University Hospital, Badalona, Spain

<sup>2</sup>Germans Trias i Pujol Research Institute (IGTP), Badalona, Spain

<sup>3</sup>Universitat de Vic-Universitat Central de Catalunya (UVic-UCC), Vic, Spain

### Correspondence to

Dr Leticia De Mattos-Arruda; ldemattos@irsicaixa.es



or gene fusions can result in new peptide sequences (neoepitopes), which are strictly tumour specific and absent in healthy tissues.<sup>15–18</sup>

T-cells recognise neoantigens after they are processed into small peptides and presented by the major histocompatibility complex molecules (MHC or human leucocytes antigens, HLA, in humans) on the surface of the cells.<sup>19</sup> It has been shown that 1%–2% of tumour mutations result in neoantigens that bind to HLA and is recognised by T-cell repertoire.<sup>1</sup> While CD8 +T cells recognise peptides in the context of MHC-I molecules, which are expressed by all nucleated cells, CD4 +T cells recognise peptides presented by MHC-II molecules, which are only produced by a reduced number of immune cell subsets (mainly dendritic cells (DCs), but also B-cells and macrophages) called professional antigen presenting cells (APCs).<sup>20</sup>

Advances and widespread use of next-generation sequencing (NGS) has facilitated the rapid identification of non-synonymous somatic mutations in clinical specimens. Computational analyses of DNA sequencing (either whole exome or whole genome sequencing) and RNA sequencing detect expressed gene mutations.<sup>21</sup> NGS data can also be used for genotyping HLA alleles of each patient.<sup>21</sup> A number of computational pipelines for neoantigen prediction are available<sup>22–23</sup> and they are usually based on MHC class I and II processing and presentation. Most pipelines provide peptide-HLA binding affinity predictions, and have also incorporated features like variant allele fraction, gene expression and clonality of mutations. However, there is no standard universal workflow for neoantigen prediction yet.

The critical importance of neoantigens relies on their capability of being targets for antitumour-specific T-cell responses because they selectively target tumour relative to healthy tissues. Furthermore, the potential for expanding T-cell clones is intact for neoantigens, since they are completely new for the immune system, while this possibility is lower for unmutated tumour targets, such as tumour-associated antigens and cancer testis antigens, whose T-cell clones may be absent due to the mechanisms of central or peripheral tolerance. This raises the possibility that vaccines targeting specific individual mutated immunogenic epitopes may be more effective.

Robust work demonstrated that T-cells target neoantigens in patients that respond to immune checkpoint inhibition, adoptive T-cell therapies and therapeutic vaccines.<sup>10–11 24–30</sup> In general, cancers with high mutational burden and high number of predicted neoantigens exhibited better objective responses to checkpoint inhibitors.<sup>10–12 14</sup> Neoantigens can also be potentially predicted in cancers with low tumour mutation burden. In fact, non-SNV mutations (ie, frameshifts, fusions) can be sources of potent immunogenic neoantigens.<sup>16–18</sup>

To make immunotherapy more precise, two main treatment modalities targeting neoantigens have been investigated in clinical trials: (1) personalised vaccines<sup>27–28 30–32</sup> and, (2) TILs-based adoptive T-cell therapies<sup>24–26 33–35</sup> (figure 1). Both approaches have demonstrated early

promise in patients with advanced solid tumours, opening the gateway to new personalised immunotherapies against cancer.

### Personalised cancer vaccines

Therapeutic vaccines targeting tumour-specific neoantigens are intended not only to enhance pre-existing memory or effector T-cell responses, but also to expand new antitumour naïve T-cell clones against otherwise poorly immunogenic mutations, broadening T-cell responses and contributing to tumour destruction.<sup>36–37</sup> Because each patient shows a particular HLA type composition and a unique tumour genomic make-up, its specific neoantigen set can be identified, selected and then presented to the immune system as a personalised vaccine preparation.<sup>38</sup>

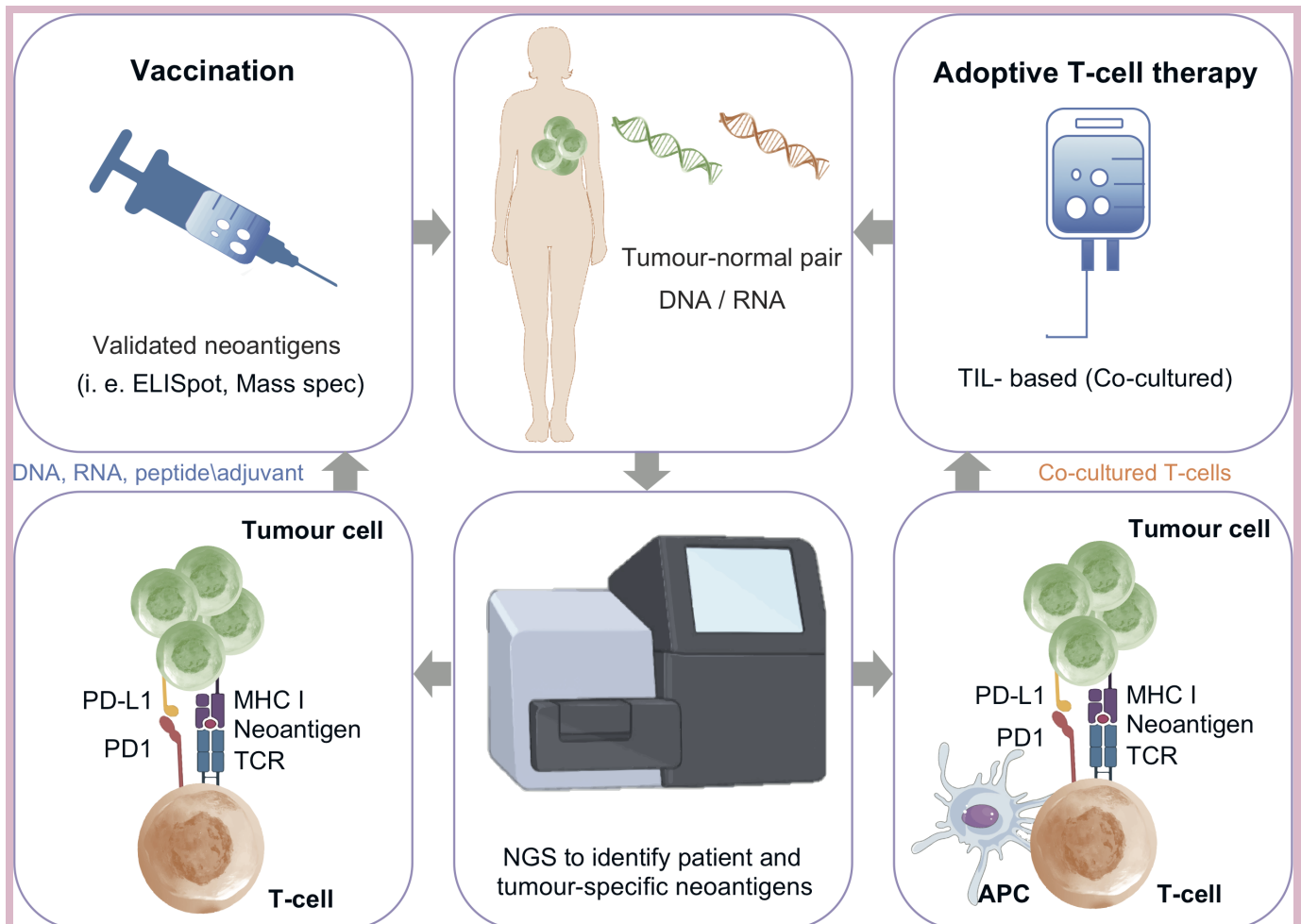
The genome-based identification of immunogenic neoantigens via NGS and further computation analyses, coupled with mass spectrometry or T-cell reactivity assays are relevant and need to be robust to maximise the immunogenicity of neoantigens loaded into the vaccine. However, for the design of cancer vaccines, several aspects beyond tumour-specific neoantigens have to be taken under consideration. Among them, formulation, immune adjuvants and the delivery system are specially relevant because they may strongly impact on the immunogenicity and vaccination outcome.<sup>38</sup>

Neoantigen vaccines can be formulated as DNA or RNA coding for neoantigens, as synthetic peptides, as virus-based systems and also as cellular preparations of DC-loaded with neoantigens or tumour cell lysates.<sup>36</sup> However, more sophisticated presentation strategies are under development, including DC targeted recombinant immunogens,<sup>39</sup> viral vectors or polymeric multivalent neoantigen preparations.<sup>40</sup>

Following on from encouraging neoantigen vaccine studies in mouse models,<sup>41–44</sup> the first-in-human clinical trials testing vaccines in melanoma and glioblastoma patients have shown safety and feasibility.<sup>27–32</sup> In a pivotal study, in vitro generated DCs loaded with neoepitopes improved pre-existing anti-tumour T-cell responses and induced responses to neoepitopes that were undetectable prior to vaccination in metastatic melanoma patients.<sup>31</sup>

In a phase I study, six patients with high-risk melanoma received long peptide neoantigen vaccines, two of them (stage IV) achieved complete responses after receiving subsequent PD-1 inhibitor and the other four (high-risk stage IIIB-C) showed no recurrence at follow-up.<sup>28</sup> In another phase I study, 13 patients with high-risk or advanced melanoma received RNA vaccines encoding neoantigens derived from expressed mutations.<sup>27</sup> Two of the five patients with advanced disease experienced vaccine-related objective responses; one patient developed a complete response to vaccination in combination with PD-1 inhibitor.

In these first published studies, vaccines induced both CD8+ and CD4+T cell responses, probably due to the use of long peptides that may also bind to MHC-II.<sup>27–28 36–45</sup> This



**Figure 1** Two strategies targeting neoantigens as cancer immunotherapy. On the left: Neoantigen vaccination; from the tumour genome a computational pipeline is ran to identify neoantigens and after in vitro validation, a vaccine is designed and administrated to the patient. On the right: TIL-based adoptive T-cell therapy, T-cells removed from the patient are expanded and reinfused back to the patient (neoantigen-specific TIL based). APC, antigen presenting cells; MHC, major histocompatibility complex; NGS, next-generation sequencing; PD1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TIL, tumour infiltrating lymphocyte; TCR, T-cell receptor.

fact could be relevant as effective antitumour responses seem to require both CD8 and CD4 tumour-specific T-cells, even in tumours that do not express class-II MHC molecules.<sup>45</sup> However, the prediction of specific neoantigen to MHC-II is not yet standardised.

These clinical trials showed the feasibility and safety of single agent personal vaccination. The adverse events were, in general, mild including injection site reactions, flu-like symptoms, rash and fatigue.<sup>27 28 36</sup> The response assessment used diverse criteria. Either standard RECIST 1.1 criteria or immune-related response criteria guidelines.<sup>46 47</sup> In the response assessment of gliomas, the Response Assessment in Neuro-oncology (RANO) criteria and the Immunotherapy Response Assessment in Neuro-Oncology criteria were applied.<sup>48</sup> These studies evidenced that clinical responses with single agent vaccine were observed in a minority of cases, and highlighted the potential to combine vaccine with checkpoint inhibitors.<sup>27 28 36</sup> How these results will be translated into

clinical benefit of cancer patients remains to be demonstrated by future clinical trials.

Personalised neoantigen vaccines in combination with immune checkpoint inhibitors or other therapies are being tested in clinical trials for a variety of solid tumours (table 1). An alternative to personalised vaccines is based on the observation that some cancer types share tumour somatic mutations among affected individuals. Although a significant minority of patients with certain common cancers may have HLA class I shared neoantigens (eg, *KRAS* mutation in up to 15% of colon and lung cancers), those common neoantigen specificities can be exploited to define ‘off-the-shelf’ vaccines across cancer types. For example, current clinical trials are investigating off-the-shelf neoantigen vaccines for patients with metastatic colorectal cancer with microsatellite instability-high status (NCT04041310), or neoantigens derived from *KRAS* mutation among non-small-cell lung cancer, pancreatic ductal adenocarcinoma and microsatellite-stable

**Table 1** Selected clinical trials targeting neoantigens as targets for personalised or off-the-shelf vaccines

Strategy	ClinicalTrials.gov identifier	Tumour type	Setting	Phase	Treatment	Target accrual
Personalised neoantigen vaccine	NCT03558945	Pancreatic tumour	Advanced	Phase 1	Personalised neoantigen vaccine	60
	NCT04087252	Solid tumour	Advanced	Phase 1	Personalised neoantigen vaccine	30
	NCT03715985	Melanoma, lung cancer, kidney cancer	Advanced	Phase 1	EVAX-01-CAF09b	25
Personalised vaccine with checkpoint inhibitors	NCT03606967	Breast cancer (oestrogen receptor negative, HER2/Neu negative, triple-negative)	Advanced	Phase 2	Carboplatin, durvalumab gemcitabine, nab-paclitaxel, personalised synthetic long peptide vaccine, poly ICLC	70
	NCT02950766	Kidney cancer	Advanced	Phase 1	NeoVax, Ipilimumab	15
	NCT03359239	Urothelial, bladder cancer	Advanced	Phase 1	Atezolizumab, PGV001, Poly ICLC	15
	NCT02287428	Glioblastoma	Advanced	Phase 1	Radiation Therapy, personalised neoantigen vaccine, pembrolizumab	46
	NCT03289962	Melanoma, lung cancer, bladder cancer, colorectal cancer, triple negative breast cancer, renal cancer, head and neck cancer, other solid cancers	Advanced	Phase 1	RO7198457/mRNA+atezolizumab	770
Off-the-shelf neoantigen vaccine	NCT03532217	Prostate cancer	Advanced	Phase 1	PROSTVAC-V, PROSTVAC-F, Nivolumab, Ipilimumab, Neoantigen DNA vaccine	20
	NCT03639714	Lung cancer, microsatellite stable colorectal cancer, gastro-oesophageal adenocarcinoma, urothelial carcinoma	Advanced	Phase 1/2	GRT-C901/GRT-R902 +nivolumab/ ipilimumab	241
	NCT02897765	Melanoma, lung cancer, bladder cancer	Advanced	Phase 1b	NEO-PV-01 +nivolumab	55
	NCT03391232	Colorectal cancer	Advanced	Phase 1/2	PolyPEP11018 colorectal cancer vaccine	15
Off-the-shelf vaccine with checkpoint inhibitors	NCT03953235	Lung cancer, colorectal cancer, pancreatic cancer, and other mutation-positive tumours	Advanced	Phase 1/2	A fixed set of neoantigens that are shared across a subset of cancer patients+nivolumab	144
	NCT04041310	Mismatch repair deficient or microsatellite instability high colorectal cancer, gastric, gastro-oesophageal junction and endometrial tumours	Advanced	Phase 1	Nous-209 (FrameShift Peptides neoantigen-encoding genetic vaccines)+pembrolizumab	34
	NCT03893903	Glioma	Advanced	Phase 1	3 arms: IDH1R132H peptide vaccine, IDH1R132H peptide vaccine and avelumab, avelumab alone.	60

IDH, isocitrate dehydrogenase; Poly ICLC, Polyinosinic-Polycytidylic acid stabilized with polylysine and carboxymethylcellulose.

**Table 2** Selected clinical trials for using TIL-based adoptive T-cell therapy

Strategy	ClinicalTrials.gov identifier	Tumour type	Setting	Phase	Treatment	Target accrual
TIL-based adoptive T-cell therapy	NCT04072263	Ovarian cancer	Advanced	Phase 1, Phase 2	TILs, interferon alfa 2A, carboplatin, paclitaxel	12
	NCT03992326	Solid tumour	Advanced	Phase 1	TILs, cyclophosphamide, fludarabine, IL-2, radiotherapy	60
	NCT03412526	Ovarian cancer	Advanced	Phase 2	Fludarabine, radiation, TIL administration, IL-2	15
TIL-based adoptive T-cell therapy with checkpoint inhibitors	NCT03296137	Cancer	Advanced	Phase 1/2	Autologous TILs, ipilimumab, nivolumab, IL-2, cyclophosphamide, fludarabine	25
	NCT03158935	Ovarian cancer, melanoma	Advanced	Phase 1	Cyclophosphamide, fludarabine, pembrolizumab, TILs, IL-2	24
	NCT02652455	Melanoma	Advanced	Early Phase 1	Nivolumab, surgery to remove tumour for growth of TIL, CD137 cyclophosphamide, fludarabine, TIL Infusion, IL-2	11
	NCT03935347	Urothelial	Advanced	Phase 2	Cyclophosphamide, fludarabine, pembrolizumab, autologous TILs, LN-145, IL-2	12
	NCT02621021	Melanoma	Advanced	Phase 2	Cyclophosphamide, fludarabine, IL-2, pembrolizumab, young TIL	170
Clonal neoantigen adoptive T-cell therapy	NCT04032847	Lung cancer	Advanced	Phase I/IIa	ATL001, autologous clonal neoantigen T- cells	50
	NCT03997474	Melanoma	Advanced	Phase I/IIa	ATL001, autologous clonal neoantigen T-cells	20

IL-2, interleukin-2; TILs, tumour infiltrating lymphocytes.

colorectal cancer in combination with an anti-PD-1 therapy (NCT03953235).

### TILs-based adoptive T-cell therapies

The enhancement of T-cell responses can be also achieved by expanding or generating tumour reactive T-cells ex vivo and using them as cellular therapeutic products that, once infused into cancer patients, can kill tumour cells.<sup>24–26 33 34</sup>

TIL-based adoptive cell transfer has shown the most encouraging clinical activity to date. Most strategies use bulk, randomly isolated TILs from the tumour tissue for ex-vivo expansion and infusion.<sup>49 50</sup> However, targeting unique, tumour-specific neoantigens have been pursued as an attractive cell therapy strategy. Current methodology employed to identify and expand neoantigen-reactive TILs involve NGS of tumour derived DNA and RNA, tumour culture in high-dose interleukin-2 (IL-2) to expand antitumour TILs in the presence of neoantigens and APCs.<sup>1</sup> The T-cells are then analysed for upregulation

of activation markers to identify neoantigen-reactive T-cells. The resulting cell preparation can be then infused as a cellular therapeutic product (figure 1).

Adoptive transfer of autologous TILs that specifically target proteins encoded by somatic mutations have mediated objective clinical regressions in patients with metastatic melanoma,<sup>34 35</sup> bile duct,<sup>26</sup> colon<sup>24</sup> and breast cancers<sup>25</sup> demonstrating that treatment enhances T-cell recognition of tumour-specific neoantigens.

A patient with metastatic cholangiocarcinoma was treated with *ERBB2IP* mutation-reactive T-cells isolated from TILs (containing 25% of mutation-specific T-cells),<sup>26</sup> resulting in reduction in size of target lesions of 30% at 7 months post-treatment with prolonged stabilisation of the disease. After disease progression, patient was retreated with a >95% pure population of mutation-reactive CD4 +T cells, showing again a reduction in size of target lesions in lung and liver. These results provide evidence that a CD4 +T cell response against a mutated antigen can be



employed to mediate regression of a metastatic epithelial cancer.

A metastatic colorectal cancer patient that showed the *KRAS* mutation G12D and the HLA-C\*08:02 was treated with mutant *KRAS*-specific CD8<sup>+</sup>T cells.<sup>24</sup> After T-cell infusion, an objective regression of all lung metastases of the patient was observed. After a 9-month period of partial response, one lung metastasis showed clinical progression associated with the loss of the HLA-C\*08:02 locus in the chromosome 6. The loss of expression of this molecule provided a direct mechanism of tumour immune evasion and T-cell-mediated selection pressure.

A third case report revealed a chemorefractory hormonal receptor-positive metastatic breast cancer patient who was treated with TILs reactive against mutant versions of four proteins—*SLC3A2*, *KIAA0368*, *CADPS2* and *CTSB*. Following an infusion of TILs with high levels of neoantigen-specific T-cell reactivity in conjunction with interleukin-2 and PD-1 inhibitor, a complete durable regression of the metastatic breast cancer was observed. The patient's complete tumour regression did not seem consistent with a response to a short course of single-agent pembrolizumab.<sup>25</sup>

In general, the most common toxicities during TIL therapy are due to the effects of the lymphodepleting preparative regimens and the subsequent IL-2 after TIL infusion.<sup>51–52</sup> TIL-related toxicity is less common, but patients may develop, mostly transient, dyspnoea, chills and fever shortly after infusion of TIL.<sup>51–52</sup> Autoimmune-like toxicity such as uveitis, hearing loss and vitiligo after TIL therapy can also occur.

Alternatively, targeting neoantigens derived from clonal mutations (ie, present in all cancer cells) are expected to effectively enhance the ability of the immune system to attack all of the tumour cells in the body.<sup>53</sup> Previous data showed that clonal neoantigens elicit T-cell immunoreactivity and sensitivity to immune checkpoint inhibition.<sup>54</sup> This strategy is being explored in clinical trials. Clonal neoantigens are identified, TILs are *ex vivo* primed to recognise them and patients receive their own expanded clonal neoantigen-reactive T-cell product.<sup>53</sup>

Several clinical trials are ongoing to explore adoptive cellular therapy as monotherapy or in combination with checkpoint inhibitors (table 2).

## CONCLUSIONS

Vaccines and TILs-based adoptive T-cell therapies hold promise to make individually tailored medicines to a wide range of patients while targeting individual neoepitopes. They have shown to be safe, feasible and capable of eliciting strong T-cell responses. However, it should be noted that T-cell recognition may not be necessarily translated into long-term clinical objective responses.

The use of a personal neoantigen vaccine is anticipated to help address two major challenges for effective cancer immunotherapy. First, addressing tumour heterogeneity and clonal evolution when analysing clinical

specimens.<sup>54–55</sup> A single resected metastasis might not reflect the most up to date landscape of tumour neoantigens. Targeting highly heterogeneous tumours might likely need to target a diversity of malignant clones per patient, as well as minimising the chance of tumour escape by loss of antigen. Second, these therapies are selectively targeting tumours relative to healthy tissues, potentially reducing side effects.

The selection of ideal antigens is still deficient and lacks validation. Such a validation will need the definition of the role of elicited immune responses in clinical efficacy. However, classical technologies to quantify T-cell responses (ELISPOT, tetramer) require large amount of blood, thus limiting their use in large clinical trials. Therefore, high-throughput and unbiased computational strategies for prediction and new single cell sequencing techniques for *in vivo* measurements will be required to definitively validate, understand and improve neoantigen-based immunotherapies.

Expansion of T-cell responses (either by vaccination or cellular therapy) and checkpoint inhibition represent synergic strategies to drive immune control of tumours, and therefore, it is plausible that their combination may enhance efficacy. It is currently unknown whether neoantigen-based immunotherapy should be given before, after or concurrently with checkpoint inhibitors.

The combination of vaccination with adoptive T-cell therapy might cause a sustained immune response that could be coupled to enhance the efficacy of transferred T-cells, and if feasible, should be tested in clinical trials.

The field is expected to advance in the next few years in terms of better detection of immunogenic neoantigens, standardisation of techniques and delivery platforms, in addition to have trained staff personal in centres with high expertise.

**Twitter** Leticia De Mattos-Arruda @demattosarruda

**Contributors** All authors contributed to the review. All authors have read and approved the final version of the manuscript.

**Funding** The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

**Competing interests** LDM-A has received honoraria for participation in a speaker's bureau/ consultancy from Roche. JBH is CEO and cofounder and JC is CSO and cofounder of AlbaJuna Therapeutics.

**Patient consent for publication** Not required.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, any changes made are indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

## ORCID iDs

Leticia De Mattos-Arruda <http://orcid.org/0000-0002-4992-3645>  
Juan Blanco-Heredia <http://orcid.org/0000-0001-9526-7450>

## REFERENCES

- 1 Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. *Science* 2015;348:62–8.
- 2 Sharma P, Allison JP. The future of immune checkpoint therapy. *Science* 2015;348:56–61.
- 3 Galon J, Bruni D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. *Nat Rev Drug Discov* 2019;18:197–218.
- 4 Hargadon KM, Johnson CE, Williams CJ. Immune checkpoint blockade therapy for cancer: an overview of FDA-approved immune checkpoint inhibitors. *Int Immunopharmacol* 2018;62:29–39.
- 5 Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell* 2015;161:205–14.
- 6 Tumei PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014;515:568–71.
- 7 Hendry S, Salgado R, Gevaert T, et al. Assessing tumor-infiltrating lymphocytes in solid tumors. *Adv Anat Pathol* 2017;24:235–51.
- 8 Gros A, Parkhurst MR, Tran E, et al. Prospective identification of neoantigen-specific lymphocytes in the peripheral blood of melanoma patients. *Nat Med* 2016;22:433–8.
- 9 Gros A, Tran E, Parkhurst MR, et al. Recognition of human gastrointestinal cancer neoantigens by circulating PD-1+ lymphocytes. *J Clin Invest* 2019;129:4992–5004.
- 10 Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med* 2014;371:2189–99.
- 11 Van Allen EM, Miao D, Schilling B, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* 2015;350:207–11.
- 12 Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology. mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015;348:124–8.
- 13 Martins F, Sofiya L, Sykietis GP, et al. Adverse effects of immune-checkpoint inhibitors: epidemiology, management and surveillance. *Nat Rev Clin Oncol* 2019;16:563–80.
- 14 DT L, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch repair deficiency. *J. Clin. Oncol* 2015;33:LBA100.
- 15 Schumacher TN, Scheper W, Kvistborg P. Cancer neoantigens. *Annu Rev Immunol* 2019;37:173–200.
- 16 Yang W, Lee K-W, Srivastava RM, et al. Immunogenic neoantigens derived from gene fusions stimulate T cell responses. *Nat Med* 2019;25:767–75.
- 17 Smith CC, Selitsky SR, Chai S, et al. Alternative tumour-specific antigens. *Nat Rev Cancer* 2019;19:465–78.
- 18 Turajlic S, Litchfield K, Xu H, et al. Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: a pan-cancer analysis. *Lancet Oncol* 2017;18:1009–21.
- 19 Blum JS, Wearsch PA, Cresswell P. Pathways of antigen processing. *Annu Rev Immunol* 2013;31:443–73.
- 20 Kambayashi T, Laufer TM. Atypical MHC class II-expressing antigen-presenting cells: can anything replace a dendritic cell? *Nat Rev Immunol* 2014;14:719–30.
- 21 Richters MM, Xia H, Campbell KM, et al. Best practices for bioinformatic characterization of neoantigens for clinical utility. *Genome Med* 2019;11:56.
- 22 Hundal J, Kiwala S, McMichael J, et al. pVACtools: a computational toolkit to identify and visualize cancer neoantigens. *bioRxiv* 2019.
- 23 Jurtz V, Paul S, Andreatta M, et al. NetMHCpan-4.0: improved peptide-MHC class I interaction predictions integrating eluted ligand and peptide binding affinity data. *J Immunol* 2017;199:3360–8.
- 24 Tran E, Robbins PF, Lu Y-C, et al. T-Cell transfer therapy targeting mutant KRAS in cancer. *N Engl J Med* 2016;375:2255–62.
- 25 Zacharakis N, Chinnasamy H, Black M, et al. Immune recognition of somatic mutations leading to complete durable regression in metastatic breast cancer. *Nat Med* 2018;24:724–30.
- 26 Tran E, Turcotte S, Gros A, et al. Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. *Science* 2014;344:641–5.
- 27 Sahin U, Derhovanessian E, Miller M, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature* 2017;547:222–6.
- 28 Ott PA, Hu Z, Keskin DB, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature* 2017;547:217–21.
- 29 Hilf N, Kuttruff-Coqui S, Frenzel K, et al. Actively personalized vaccination trial for newly diagnosed glioblastoma. *Nature* 2019;565:240–5.
- 30 Keskin DB, Anandappa AJ, Sun J, et al. Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. *Nature* 2019;565:234–9.
- 31 Carreno BM, Magrini V, Becker-Hapak M, et al. Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. *Science* 2015;348:803–8.
- 32 Tanyi JL, Bobisse S, Ophir E, et al. Personalized cancer vaccine effectively mobilizes antitumor T cell immunity in ovarian cancer. *Sci Transl Med* 2018;10:eaao5931.
- 33 Stevanović S, Pasetto A, Helman SR, et al. Landscape of immunogenic tumor antigens in successful immunotherapy of virally induced epithelial cancer. *Science* 2017;356:200–5.
- 34 Zhou J, Dudley ME, Rosenberg SA, et al. Persistence of multiple tumor-specific T-cell clones is associated with complete tumor regression in a melanoma patient receiving adoptive cell transfer therapy. *J Immunother* 2005;28:53–62.
- 35 Robbins PF, Lu Y-C, El-Gamil M, et al. Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. *Nat Med* 2013;19:747–52.
- 36 Hu Z, Ott PA, Wu CJ. Towards personalized, tumour-specific, therapeutic vaccines for cancer. *Nat Rev Immunol* 2018;18:168–82.
- 37 Schumacher TN, Hacohen N. Neoantigens encoded in the cancer genome. *Curr Opin Immunol* 2016;41:98–103.
- 38 Sahin U, Türeci Özlem. Personalized vaccines for cancer immunotherapy. *Science* 2018;359:1355–60.
- 39 Krauss J, Krackhardt A, Jäger E, et al. No title. VB10.NEO, an Individ. neoepitope cancer vaccine, induces Posit. Clin. responses patients with local. Adv. or metastatic solid tumours, National harbor, Maryland 2019.
- 40 Lynn GM, Laga R, Jewell CM. Induction of anti-cancer T cell immunity by in situ vaccination using systemically administered nanomedicines. *Cancer Lett* 2019;459:192–203.
- 41 Castle JC, Kreiter S, Diekmann J, et al. Exploiting the mutanome for tumor vaccination. *Cancer Res* 2012;72:1081–91.
- 42 Gubin MM, Zhang X, Schuster H, et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature* 2014;515:577–81.
- 43 Matsushita H, Vesely MD, Koboldt DC, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoevasion. *Nature* 2012;482:400–4.
- 44 Yadav M, Jhunjunwala S, Phung QT, et al. Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. *Nature* 2014;515:572–6.
- 45 Alspach E, Lussier DM, Miceli AP, et al. MHC-II neoantigens shape tumour immunity and response to immunotherapy. *Nature* 2019;574:696–701.
- 46 Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin Cancer Res* 2009;15:7412–20.
- 47 Schwartz LH, Litière S, de Vries E, et al. RECIST 1.1-Update and clarification: from the RECIST Committee. *Eur J Cancer* 2016;62:132–7.
- 48 Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology Working group. *J Clin Oncol* 2010;28:1963–72.
- 49 Yamamoto TN, Kishton RJ, Restifo NP. Developing neoantigen-targeted T cell-based treatments for solid tumors. *Nat Med* 2019;25:1488–99.
- 50 Yossef R, Tran E, Deniger DC, et al. Enhanced detection of neoantigen-reactive T cells targeting unique and shared oncogenes for personalized cancer immunotherapy. *JCI Insight* 2018;3:122467.
- 51 Yang JC. Toxicities associated with adoptive T-cell transfer for cancer. *Cancer J* 2015;21:506–9.
- 52 Rohaan MW, van den Berg JH, Kvistborg P, et al. Adoptive transfer of tumor-infiltrating lymphocytes in melanoma: a viable treatment option. *J. Immunother. Cancer* 2018;6:102.
- 53 Robertson J, Salm M, Dangi M. Adoptive cell therapy with tumour-infiltrating lymphocytes: the emerging importance of clonal neoantigen targets for next-generation products in non-small cell lung cancer. *Immuno-Oncology Technology* 2019;3:1–7.
- 54 McGranahan N, Furness AJS, Rosenthal R, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* 2016;351:1463–9.
- 55 De Mattos-Arruda L, Sammut S-J, Ross EM, et al. The genomic and immune landscapes of lethal metastatic breast cancer. *Cell Rep* 2019;27:2690–708.