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Current Perspective

Neoepitopes-based vaccines: challenges and perspectives

Vincent Alcazer [a,b,c], Paola Bonaventura [c,d], Laurie Tonon [e], Sandrine Wittmann [c,d], Christophe Caux [c,d], Stéphane Depil [b,c,d,*]

[a] *Hospices Civils de Lyon, Service D'hématologie Clinique, Centre Hospitalier Lyon Sud, Pierre-Bénite, France*
[b] *Université Claude Bernard Lyon 1, Lyon, France*
[c] *Inserm U1052/CNRS 5286, Centre de Recherche en Cancérologie de Lyon, Lyon, France*
[d] *Centre Léon Bérard, Lyon, France*
[e] *Synergie Lyon Cancer, Centre Léon Bérard, Lyon, France*

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Abstract First generations of cancer vaccines using shared tumour antigens have been associated with disappointing clinical results. However, the paradigm shift introduced by immune checkpoint inhibitors has led to a renewed interest on anti-tumoural vaccination based on mutation-associated neoantigens. First clinical results are encouraging with some signs of clinical activity associated with induction of a specific immune response. In advanced or metastatic diseases, vaccination may either enhance the response to Programmed cell death 1 (PD-1/-L1) antagonists by increasing the number of effectors within the tumour or induce an anti-tumoural T-cell response in immunologically 'cold' tumours. There is also a strong rationale to use cancer vaccines in an adjuvant setting to induce a long-term control of the residual disease. Prediction of neoepitopes efficiently presented by Human Leukocyte Antigen (HLA) molecules remains a challenge, as well as identification of clonal neoantigens. Some mechanisms of resistance are already identified, such as tumour loss of neoepitopes-presenting HLA class I molecules. In this context, the role of CD4+ T cells induced by different cancer vaccines should be clarified. Finally, although studies have focused on mutated epitopes corresponding to single nucleotide variants, other neoantigens could be of strong interest such as those linked to tumour specific RNA-splicing abnormalities or associated with insertions-deletions.

* *Corresponding author*: Centre Léon Bérard, 28 Prom. Léa et Napoléon Bullukian, 69008 Lyon, France.
E-mail address: stephane.depil@lyon.unicancer.fr (S. Depil).

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1. Towards personalised vaccines

First generation cancer vaccines have shown relatively disappointing clinical results, with less than 7% objective clinical responses and an overall clinical benefit rate estimated around 20% [1]. The use of non–tumour-specific antigens, which elicit low affinity T-cell response due to elimination of the most reactive T cells by central tolerance, is one of the multiple factors that explain these results. Tumour-specific antigens (neoantigens), derived from tumour gene mutations or rearrangements, have been reported to induce stronger immune responses in the absence of central tolerance [2]. The abundant literature around mutation-associated neoantigens (MANA) together with the paradigm shift introduced by immune checkpoint inhibitors (ICIs) led to a renewed interest in the field of cancer vaccines. Indeed, multiple studies showed a link between clinical responses to ICIs and tumour mutational burden in melanoma [3], lung cancer [4] or colorectal cancer [5]. Furthermore, a linear relation was observed between anti-PD1/-L1 objective response rate and the median number of coding somatic mutations per megabase of DNA [6]. Because mutational burden is a reflection of the quantity of MANA [7], these latter may represent the main targets of activated T cells after ICI therapy.

2. Neoepitope-based vaccines: current challenges

2.1. Neoepitope selection

The identification of neoepitopes relies on different steps. First, a precise detection of tumour somatic mutations is performed by combined whole exome sequencing of tumour and normal cells [8]. A matched RNA expression analysis (such as RNA sequencing) of tumour cells is added to select expressed mutations. Prediction softwares are then used to identify potential neoepitopes with a high affinity for the individual's major histocompatibility complex molecules (MHCs) [9]. However, classical algorithms have a limited predictive value as they do not take into account the different steps of epitope processing. Moreover, the affinity threshold needed to elicit a cytotoxic T-cell response has been mostly validated on viral epitopes and may not be adapted for mutated self-peptides [10]. In a set of 448 potential CD8-T-cell epitopes identified in a patient with melanoma, van Rooij *et al.*[11] found that less than 1% were effectively recognised by the patient's T cells. The positive predictive value of such tools is, therefore, pretty low and different additional steps (such as proteasome cleavage/TAP transport predictions or self-proteins cross-reactivity verification) can be added to the prediction [12,13]. Recently, a quality-control step considering the probability of recognition of a neoepitope by a T-cell receptor has been proposed, using a model based on sequence similarity with that of known antigens [14]. It is also possible to verify predicted epitopes' MHC affinity by binding assay or to test their immunogenicity by *in vitro* stimulation assays.

The fact that a majority of predicted class I epitopes may not be effectively presented by MHC class I molecules on tumour cells remains a major concern. A validation step has been added by some authors, using mass spectrometry for identification of peptides eluted from tumour MHC class I molecules [15]. The sensitivity of such approach is, however, limited to neoepitopes with relatively high expression, preventing the detection of less abundant but still immunogenic peptides [16]. The optimisation of MHC class II neoepitopes prediction is another challenge (MHC class II epitopes harbour a high diversity with variables sizes rendering prediction more complicated than for MHC class I epitopes), considering the potential role of CD4+ T-cell response after neoepitope-based vaccine [17,18].

2.2. Formulation

Different formulations or antigen sources currently exist. DNA vaccines consist in transfecting a DNA sequence encoding for the neoepitopes of interest. Although unmethylated CG-rich DNA was supposed to provide an immune adjuvant by Toll-like receptor (TLR) stimulation, poor immunogenicity and clinical activity were observed [19]. In the opposite, RNA vaccines harbour a convincing profile with a potential adjuvant effect (by TLR 7/8 stimulation) and multiple epitope encoding possibility [20]. However, this TLR stimulation precludes the combination with other adjuvants. Conversely, peptide-based vaccine can be combined with any adjuvant. Of note, the diversity of possible adjuvants has already been reviewed elsewhere and is beyond the scope of this article [2,21]. As single peptide formulation is associated with a higher risk of immune escape, multipeptides approaches are usually developed [22]. With their potential to stimulate both CD4+ and CD8+ T-cell response, long peptides (20–30 mer) are indeed being more and more considered [23]. Other formulations such as viral vectors, *ex vivo*–generated dendritic cells or whole cell have also being developed. The impressive results obtained in infectious diseases with viral vectors, eliciting a strong cytotoxic T-cell response in terms of quality and number, merit a particular attention [24]. Considering the importance of T-cell numbers in clinical responses after adoptive T-cell therapies, there is no doubt that vectors inducing a higher number of effector T cells compared to peptides or mRNA approaches would be of a great interest.

2.3. Tumour heterogeneity

Given tumour heterogeneity, especially at an advanced stage, there is a high risk of selecting epitopes present in

certain tumour clones only ('subclonal' epitopes). In Carreno's study, a majority of the identified neoepitopes were not found at the different tumour sites [25]. Assigning a clonal (shared by all the tumour clones) or subclonal (specific to a subclone) status to a mutation remains difficult and requires data of excellent quality. Moreover, as they do not confer any survival advantages, passenger mutations could be lost without any counterpart for tumour cells.

As most mutations occur randomly in passenger-type genes, the frequency of mutated neoantigens shared between different tumours is very low. It was found that out of a total of 911,548 mutated neoantigens, only 24 were shared in at least 5% of patients [7]. A recent study found that, among 3760 predicted neoantigens, only 0.42% were found in more than one tumour [13]. It is, therefore, necessary to define for each patient a panel of neoepitopes in a purely personalised approach. Considering the impact of the presence of clonal versus subclonal neoepitopes in the quality of the anti-tumour immune response [26], it will theoretically be appropriate to favour clonal neoepitopes or a mixture of neoepitopes representative of the main subclones, prioritising when possible neoepitopes derived from driver mutations, which adds complexity to the neoepitopes selection method. The delay related to this complex process of production may be an issue for the use in advanced progressive diseases. One possibility is to start with a vaccine containing shared tumour antigens before combining the personalised vaccine [27].

2.4. Mechanisms of resistance

A possible limitation of this approach is the availability of the patient's T repertoire. It was observed that the T-cell repertoire of healthy donors contained T cells recognising MANA for which specific intra-tumour T lymphocytes were not found in the corresponding tumours [28].

A resistance mechanism already identified in Sahin's study is the β2-microglobulin loss leading to the absence of MHC class I molecules on tumour cells [27]. This mechanism has also been identified in acquired resistance to PD1/-L1 antagonists [29]. More subtly, the loss of heterozygosity (LOH) of HLA alleles corresponding to MHCs presenting mutated neoepitopes could represent an initial or acquired resistance mechanism. HLA LOH has been shown in 40% of non-small cell lung cancers and is associated with a large amount of subclonal neoantigens [30]. It will, therefore, be necessary to verify the quality of MHC expression and ideally to select neoepitopes in accordance with the corresponding MHC expression. Optimisation of high affinity MHC class II neoepitopes prediction will also help mounting a diversified response by adding CD4+ T-cell anti-tumoural effects.

Finally, advanced tumour-associated immunosuppression represents a general resistance mechanism to immunotherapy. In this context, there is a strong rationale for combining vaccine approaches with anti-PD-1/-L1 from the outset, so as to inhibit the resistance induced by IFN-γ response [31] or even favour a *de novo* immune response and initial T-cell activation [32].

3. Perspectives

3.1. Addition of other tumour antigens

Although ongoing studies have focused on mutated epitopes corresponding to single nucleotide variants (SNVs), other neoantigens could be of great interest such as those derived from tumour specific RNA splicing abnormalities [33] or from insertions-deletions (indels). Indels could be a major source of neoantigens, and a recent study suggests a higher frequency of epitopes with high MHC affinity for epitopes derived from indels compared with SNVs. In addition, the localisation of indels in tumour suppressor genes could also be associated with a higher probability of obtaining shared antigens [34]. The combination with PD-1/-L1 antagonists also highlights the possibility of using some cancer germline antigens [35].

3.2. Improving T-cell response

Targeting multiple neoepitopes, together with stimulating a CD4+ T-cell response, is a promising way to induce an efficient immune response against the tumour. Sahin *et al* [27]. reported the feasibility and immunogenicity of an mRNA-based vaccine targeting multiple selected neoepitopes in melanoma. Responses were detected against 60% of the predicted neoepitopes, with 57% of isolated CD4+, 17% of CD8+ and 26% of combined CD4+ and CD8+ responses. Ott *et al.* [36] also demonstrated the immunogenicity and feasibility of a vaccine that targets up to 20 predicted neoantigens in patients with melanoma. Using long synthetic 15–30 mer peptides with polyinosinic:polycytidylic acid and poly-L-lysine double-stranded RNA (poly-ICLC) (Hiltonol) as an adjuvant injected subcutaneously, the authors reported both CD4+ and CD8+ T-cell responses, respectively targeting 60% and 16% of the vaccine neoantigens. These results underline the importance of the CD4+ T cells in supporting CD8+ response and providing additional anti-tumoural effects. Of note, CD8+ T-cell response was evaluated after 2 weeks of *in vitro* restimulation in Ott's study. Thus, it is not possible to conclude that the vaccine really induced a specific response in patients because an *in vitro* priming against the tested antigens cannot be formally excluded. Still, four of six vaccinated patients had no recurrence 25 months after vaccination. The two others with recurrent diseases were successfully treated with anti-PD-1 therapy inducing a complete tumour regression.

Combination of cancer vaccine with ICI can also help improving T-cell response by promoting T-cell activation and epitope spreading [37,38]. In Sahin's study, the only complete response was observed in combination with PD1 blockade [27]. Several studies are currently underway based on this scheme; for instance, NCT02897765 study adds a personalised vaccine after 3 months of nivolumab in different types of cancers. The advantage in this context of advanced disease is the possibility of performing sequential tumour biopsies to evaluate changes in the immune infiltrate after vaccination, in addition to demonstrate the induction of a specific immune response detected in blood. Furthermore, neoepitope-based vaccines could be an interesting approach in combination with ICIs for diseases with low or no response to ICIs alone despite a significant mutational burden. This is the case for microsatellite stable colorectal cancer, where response to immunotherapeutic approaches (eg T-cell recruiting bispecific antibody with atezolizumab) have shown clinical responses in favour of a functional immune system [39]. This may represent an interesting proof of concept of efficacy.

Depleting the immunosuppressive milieu (by using cyclophosphamide for regulatory T-cell depletion or gemcitabine for myeloid-derived suppressor cells) or promoting T-cell expansion by the use of cytokines such as IL-7 are other ways for improving T-cell response [40,41].

Finally, combination of different immunotherapies will probably be required to eradicate advanced tumours. Moynihan *et al.* evaluated in a murine syngeneic tumour model, a combination including a tumour-targeting antibody, recombinant IL-2, anti-PD-1 antibody and specific vaccine [42]. This combination induced tumour infiltration of both innate and adaptive immune cells, mediating a strong anti-tumoural effect with eradication of large established tumours. Although the tolerability of such combination must be confirmed in humans, it makes no doubt that enhancing the vaccinal response by different simultaneous ways is a promising approach.

3.3. Optimisation of the timing of vaccination

Most of studies have used cancer vaccines in an advanced or metastatic setting. Considering the risk of higher immunosuppression and of higher resistance associated to clonal heterogeneity in case of high tumour burden, it has been suggested that cancer vaccination should be used in an adjuvant setting in a context of low residual disease [1]. Vaccination could be also used in a neoadjuvant setting. In this context, it has been shown in murine models that T-cell stimulation by ICI was more efficient in preventing metastasis when applied before tumour resection [43]. In fact, more and more studies are currently being performed in an adjuvant situation (Table 1). Triple negative breast cancer, in

Table 1
Ongoing recruiting studies using neoepitopes for personalised therapeutic vaccine.

Study number	Promotor	Cancer	Vaccine type	Position
NCT02301611	Cancer Insight, LLC	Melanoma	DC	Adjuvant
NCT02348320	Washington University School of Medicine	Breast (TN)	DNA	Adjuvant
NCT02600949	M.D. Anderson Cancer Center	Pancreatic, colorectal	Long peptide	Advanced/metastatic
NCT02721043	Nina Bhardwaj, Icahn School of Medicine at Mount Sinai	Solid tumours	Long peptide	Adjuvant
NCT02808364	Guangdong 999 Brain Hospital	Glioblastoma	DC	Adjuvant
NCT02808416	Guangdong 999 Brain Hospital	Solid tumours with brain metastases	DC	Adjuvant
NCT02933073	UConn Health	Ovarian	Long peptide	Neoadjuvant or adjuvant
NCT02956551	Sichuan University	Carcinoma, non-small cell lung	DC	Advanced/metastatic
NCT02933073	UConn Health	Ovarian	Long peptide	Adjuvant
NCT03122106	Washington University School of Medicine	Pancreatic	DNA	Adjuvant
NCT03289962	Genentech, Inc.	Melanoma, non-small cell lung, bladder, colorectal, breast (TN), renal, head and neck	RNA	Advanced/metastatic
NCT03480152	National Cancer Institute (NCI)	Melanoma, colon, gastrointestinal, genitourinary, hepatocellular	RNA	Advanced/metastatic
NCT03552718	NantBioScience, Inc.	Colorectal, breast, head and neck, melanoma, non-small cell lung, pancreatic, liver	DC	Adjuvant
NCT03633110	Genocea Biosciences, Inc.	Melanoma, non-small cell lung, head and neck, urothelial carcinoma, renal cell carcinoma	Long peptide	Adjuvant
NCT03558945	Changhai Hospital	Pancreatic	NA	Adjuvant
NCT03645148	Zhejiang Provincial People's Hospital and Hangzhou Neoantigen Therapeutics Co., Ltd	Pancreatic	Long peptide	Advanced/metastatic

DC: dendritic cells, NA: not available, TN: triple negative

non-complete pathological response following neoadjuvant chemotherapy, is well suited to this type of approach. The risk of relapse is high, and the vaccine is set up in a context of low tumoural mass after conventional treatment combining neoadjuvant chemotherapy, surgery and radiotherapy. Several phase I trials are underway in this context, such as NCT02348320 study. It will be necessary to set up control groups in this setting to demonstrate a benefit in terms of survival. Furthermore, the absence of biopsy-accessible tumours restrains the immunological analyses to the blood level. Nevertheless, it is likely that the adjuvant setting in pathologies at high risk of relapse represents a positioning of choice for vaccine approaches, used alone or in combination with an anti-PD-1/-L1.

4. Conclusion

Vaccination based on specific neoantigens in a personalised approach opens new therapeutic perspectives in oncology. Recent studies have indeed provided a clinical proof of concept in melanoma [25,36]. Immunogenicity is, however, too often considered as a surrogate marker of efficacy, and objective responses remain low. In advanced metastatic diseases, vaccination may either enhance the response to PD-1/-L1 antagonists by increasing the number of effectors within the tumour or induce an anti-tumoural T response in immunologically 'cold' tumours (characterised by the absence of T infiltration), a prerequisite for the activity of an anti-PD-1/-L1. The lack of HLA class I expression by tumour cells represent an important limitation of this approach. Nevertheless, $CD4^+$ T cells induced by vaccination may promote an effective anti-tumoural response by interferon-γ secretion and stimulation of other cells from the immune environment. This aspect is barely known and needs to be clarified given the importance of the $CD4^+$ T-cell response generated during the first neoepitope-based vaccination trials [17]. Finally, adjuvant therapy represents a privileged positioning for vaccination, so as to allow long-term control of residual disease following the generation of an immune response.

Conflict of interest statement

S.D. is an employee for Cellectis and reports personal fees from AstraZeneca, Elsalys, Erytech Pharma and Netris Pharma. The other authors declare no potential conflict of interest.

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