COMMENTARY

Modified Vaccinia virus Ankara-based vaccines in the era of personalized immunotherapy of cancer

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ABSTRACT

While interest in immunotherapies is renewed by the successful introduction of immune checkpoint blocking agent in the clinic, advances in genome sequencing are opening new possibilities in the design of increasingly personalized vaccines. Personalization of medicine can now be realistically contemplated at the single patient level. Unlike the previous generation of cancer vaccines, neoantigen directed vaccines would target truly specific tumor antigens resulting from acquired tumor genome mutations. Immune response induced by this next generation vaccine would not be subject to self-tolerance and will likely result to enhanced efficacy. Nevertheless, this new technologies can hold to their promises only if sponsors manage to meet several scientific, technical, logistical and regulatory challenges. In particular manufacturers will have to design, manufacture, and deliver to the patient a new pharmaceutical grade in a matters of weeks. In this paper, we briefly review current technologies currently tried at the translation of personalized vaccines and explore the possibilities offered by the Modified Vaccinia virus Ankara in this next wave of cancer vaccines.

ARTICLE HISTORY

Received 3 May 2017
Accepted 22 May 2017

KEYWORDS

cancer immunotherapy;
neoantigens; Personalized
vaccines

Introduction

Harnessing the immune system to eradicate tumor cells has become an established therapeutic strategy as demonstrated by FDA approval of sipuleucel-T (Provenge), ipilimumab (Yervoy, anti-CTLA-4), and more recently, the programmed cell death (PD)-1 antibody (pembrolizumab, Keytruda) for the treatment of multiple types of cancer. Despite the great success of checkpoint blockade therapies, more than 50% of cancer patients still fail to respond to immunotherapy. Meanwhile, technological breakthroughs in the field of DNA sequencing, genomics, and bioinformatics have paved the way to the concept of personalized medicine. These advances have hence enhanced our ability to detect new immune targets and tumor-specific neoantigens (mutation-derived antigens), and have accelerated the development of immunotherapies with potentially broader coverage of cancer patients by providing an unprecedented opportunity for the design of mutation targeted immunotherapy. While CAR T-cells constitute an efficient tool to target surface antigens, cancer vaccines provide a mean to target the whole cancer mutanome.¹ In this paper, we will discuss the impact of these recent progresses on the role of a well-established cancer vaccine platform: the MVA (Modified Vaccinia virus Ankara).

Designing personalized cancer vaccines would translate into a major paradigm shift. In spite of generating a common vaccine against a known antigen, and then assess if individual patient tumors would express this antigen, the customized approach implies the prior identification of specific antigens

for each patient and the subsequent generation of a vaccine targeting these mutations. Although this strategy has been proposed for more than a decade,² the development of an individualized cancer vaccine faces major challenges in several domains before delivering to its promise.

Personalized cancer vaccines will have to be designed, manufactured and made available quickly after the diagnosis. While needs can vary considerably from one clinical indication to the other, it can be anticipated that the vaccine would need to be available from 4 to 6 weeks after diagnosis to allow a realistic integration in the current workflow of patient care. Within this time frame, vaccine manufacturers will have to identify the most relevant antigens to elicit an efficient T cell response, and manage various issues relating to the generation of a pharmaceutical grade product within acceptable costs of manufacturing. These difficulties may be exacerbated by the absence of appropriate regulatory guidelines. Last, their adoption in routine care will be subordinated to their seamless integration in an ever increasingly complex therapeutic armamentarium.

Several technologies are currently assessed as potential platforms for custom-designed vaccines: nucleic acids, peptides, live bacterial or viral vectors or human cells; all presenting loaded neoantigens to the patient's immune system. In our vision, the combination of technical, operational, and regulatory constraints might represent an opportunity for well-established technologies such as viral-based cancer vaccines. In this respect, the Modified Virus Ankara (MVA), an attenuated vaccinia virus, displays several advantages. It has served as an

effective vector in previous attempts to develop cancer vaccines, some of which had demonstrated significant clinical efficacy in phase 2 trials. In this commentary, we will shortly highlight some of the MVA features that may be of added value in the implementation of an individualized vaccination approach.

Finding the most appropriate antigens

One first issue in the design of the cancer vaccine is the selection of relevant target antigens. Current designs have been based on either tumor-associated antigens (TAA), that are highly expressed in tumor cells compared with normal cells (e.g. Her-2, Muc-1), on onco-fetal antigens (e.g., AFP, CEA, 5T4), or on cancer/testis antigens (NY-ESO1, MAGE-A3, CT83). TAAs have been subject to intense effort of research as cancer vaccine targets in the previous generations of cancer vaccines.³ However, all these self antigens may bear the risk of clinical inefficacy due to central immunotolerance or autoimmunity. Hence, non self antigens exhibit lower risk of failure. They are either viral oncogenes (e.g., HPV E6/E7, EBNA-1) or tumor-specific neoantigens (TSNA) resulting from DNA misrepair or chromosomal rearrangement. These non-self antigens are now considered as more efficient than their self counterparts.⁴⁻⁶

Because of high mitotic rates and deficient repair mechanisms, tumor are characterized by the accumulation of somatic mutations from the earliest stage of tumorigenesis.⁷ The rate of mutation in the tumor exome varies considerably from one cancer to the other from a median of less than one mutation per million of bases in rhabdoid tumors and Ewing sarcoma to more than 10 in a million bases in melanoma or lung cancer.⁸ The onset of non-synonymous mutations in the coding region of DNA may results in the synthesis of aberrant protein species.

Current, sequencing technologies can reliably identify mutations in tumor material, however, the number of mutations can be relatively high – up to several hundreds – and a selection must be performed to select a panel of vaccine targets. Criteria entering in the selection of most relevant antigens are level of antigen expression, conservation across tumor cells, presentation by the MHC, and potential immunogenicity.⁹ Noteworthy, neoantigenic landscape of tumors can prove relatively heterogeneous and evolve during time, in particular when under immune selective pressure.^{10,11}

Several authors have attempted to identify such neoantigens that are recurrent in patients so that target libraries can be built to develop vaccines.¹² These studies did not results in the identification of recurrent somatic mutations but identified mutational hotspots¹³ associated with increased probability of mutation. Consequently, development of cancer vaccines requires the identification of neoantigen on a patient by patient basis and consequently, the vaccine design should be tailored and manufactured for individual patient. These considerations require the definition of a robust workflow for the design of the vaccine. Selection of antigens can be assisted by *in silico* prediction or bioanalytic approaches. *In silico* predictors are built using machine learning algorithms such as neural networks and trained to identify highly immunogenic sequences.¹⁴ The algorithm is then used to classify epitopes for their affinity for HLA class I protein as an index of their potential presentation.

Additional filtering can be added by factoring in levels of expression at RNA level using RNAseq. While *in silico* based approaches have been shown to have some level of predictivity on the immunogenicity of a library of putative epitopes, their current level of validation is unlikely to permit to have a vaccine targeted only to a very restricted number of predicted epitopes as there is a significant risk that these epitopes are not associated with an actual response *in vivo*.^{14,15} Also, the reliability of these approaches remains questionable when predicting CD4 epitopes. Last, it should be mentioned that several collaborative consortia, such as TESLA (Tumor Neoantigen Selection Alliance), supported by the Parker Institute,¹⁶ or the European APERIM (Advanced bioinformatic platform for personalized cancer immunotherapy),¹⁷ are underway to achieve a higher level of validation of these predictive tools. Alternatively to *in silico* approaches, some authors have proposed the direct identification of presented peptides using LC/MS approaches, potentially after immunoprecipitation of tumor MHC. This approach allows for an unbiased identification of peptides presented by a patient tumor cells. However, it is likely that improvement in analytical sensitivity will be required to allow routine use of this technology.^{18,19}

In practice, the uncertainty around the best method for predicting relevant epitopes and the dynamic nature of the tumor mutational landscape result in the conclusion that, whatever the technology, a personalized vaccine will embark a relatively high number of distinct neoantigens. Thus, the capacity of the vector will a key decision factor.

Manufacturing and regulatory challenges

In complement to antigen discovery and vaccine design, significant efforts are still required to manufacture the product according to pharmaceutical regulations. Quality control and regulatory reviews in the pharmaceutical industry were historically based on extensive chemical and biological characterization of the drug product as well as tight control thereof during routine manufacturing. However, given the time constraints in cancer care, manufacturers of unique personalized vaccines must invent new ways to deliver a product matching FDA/EMA requirements, without engaging into lengthy analytical characterization of product properties and stability. While needs may vary from one indication to the other, a product should be made available within weeks. Necessity for fast manufacturing is certainly the major challenge that this emerging field will face.

When presented with increasingly personalized products, regulators responded with specialized organizations, such as the FDA's Personalized Medicine teams at CDRH and CBER,²⁰ or guidelines, such as the ATMP (advanced therapeutic medicinal product) regulation of the European Medicinal Agency (EMA).²¹ These guidelines have taken in account some specificities of the next generation of products by ensuring compliance through a “*quality by design*” and risk-based approach.

The last critical point is cost control. It is of particular relevance in an environment where high drug costs are facing an ever increasing scrutiny. While the final pricing of personalized vaccines will obviously be discussed on the basis of their medical utility, manufacturers should make all possible efforts to

control costs of treatment and think of innovative business models. Failure to do so will drastically limit the adoption of this emerging class of therapy. A notorious example is the bankruptcy of Dendreon, the manufacturer of the first personalized CAR-T cell therapy to hit the market.²²

Suitable vectors for personalized cancer vaccines

Considering the aforementioned challenges, selecting the best vaccine platform among the multiple possibilities has been a conundrum. A number of technological options are now being considered for the development of a neoantigen-based vaccine (Table 1). They can be categorized in 5 major families: peptide-based vaccines, nucleic acids (DNA, mRNA)-based vaccines, human cell-based approaches, live vector vaccines based on engineered viruses or bacteria. Unfortunately, no systematic experiment was performed to compare different formats displaying the same TAA, in terms of intensity or quality of the immune response. Moreover, it is likely that similar antigens displayed by diverse platforms in the same preclinical model resulted in different immunogenicity profiles.^{23,24}

Peptide vaccines, based on either whole antigens or short peptides (9-mers), have been largely studied but they generally elicited transient and relatively weak T cell responses, as exemplified by the gp100₂₈₀₋₂₈₈ peptide in melanoma²⁵ which showed limited clinical efficacy. This disappointing outcome was attributed to poor *in vivo* stability or to the lack of specificity of peptides for “professional” antigen presenting cells. The use of long peptides (30-mers), adjuvanted with biologically active factors was promoted to circumvent these limitations in several indications (breast,²⁶ pancreatic,²⁷ lung²⁸ and colorectal²⁹ cancers). However, these products did not reach clinical registration and are currently improved in the setting of neoantigen vaccination by different companies.

The demonstration that naked foreign nucleic acids injected *in vivo* can penetrate somatic cells and can result in the expression of the encoded sequence opened the door to use of DNA or mRNA vaccines.³⁰ Building upon the experience

accumulated in the field of gene therapy, DNA plasmid vaccines were designed in conjunction with various technologies intended to increase the rate of transfection such as electroporation or high pressure needleless injection devices.³¹ Despite promising clinical results, these vaccines proved clinically disappointing. Studies administrating up to 2 mg of plasmidic DNA intramuscularly or intranodally in advanced melanoma or colorectal cancer did not provide anti-tumor activity nor clinical benefit. Furthermore, possible integration of exogenous DNA remains a concern. So further improvement of vectors and administration routes are still needed for a DNA based approach to be used routinely; one promising approach is developed by Vaccibody (Oslo, Norway): a DNA plasmid encoding for a multidomain protein comprising a targeting factor, a stimulating cytokine complex and strings of antigens, the plasmid is being delivered using a needleless high pressure device.³² Similarly, RNA vaccines utilization has been hampered by difficulties to address sufficient amount of RNA to the target cells to achieve a consistent expression of the antigen. Recent developments of advanced formulations targeting RNA to the spleen, and compatible with repeated administrations, have renewed the interest in RNA vaccines and opened up opportunities to target neoantigens using these approaches.³³

One alternative modality of use of peptides and nucleic acids is the use of dendritic cells as vector after *in vitro/ex vivo* exposure to the antigen. Autologous dendritic cells are pulsed with peptide, sequences of RNA encoding for the antigenic sequence or with tumor lysates prior to administration to the patient. However, most initiatives in the field did not reach late stage clinical development and have been limited by logistical hurdles and excessively high costs. Current efforts aim at tackling these limitations using off the shelf dendritic cells.³⁴

Last, live vectors such as bacteria or viruses constitute an attractive option to overcome limitations of the technologies described above. Live vector vaccines may be based on viral or bacterial platforms. The immune environment elicited by infection of cells by such live vectors has been shown to promote an enhanced immune response in terms of frequency and avidity

Table 1. List of principal technological platforms for the development of neoantigen-directed vaccines.

Type	Name	Description	Sponsor
Viral vaccines	xZVex™-Neo	Lentiviral-based vaccine	ImmuneDesign / Gritstone (USA)
	Unknown	Chimp adenovirus	Gritstone (USA)
Bacterial vaccines	Exovax Vaccine	Chimp adenovirus vaccine	Nouscom (CH, I)
	MINE™	Modified Listeria vectored vaccine	Advaxis (USA)
Peptide vaccines	NEO-PV-01	Peptides adjuvanted with poly ICLC	NEON Therapeutics (USA)
	Prophage	Peptide vaccine adjuvanted with HSPPC-96	Agenus (USA)
	DPX-Neo	Liposome encapsulated adjuvanted peptides	IMV Immunovaccine (CND)
	Personalized SLP®	Long peptide adjuvanted by modified TLR 1/2 ligand	ISA Pharmaceuticals (NL)
	Immunotherapy		
Dendritic cell vaccines	ET-08	Dendritic cells transfected with patient derived tumor exosomes	Exocyte Therapeutics (SGP)
	DC Cancer Vaccine	Dendritic cells	OncoTherapy Science, tella Inc. (JP)
	Percellvac	Allogenic Dendritic cell vaccine	Tricision therapeutics
Nucleic acid vaccines	Vaccibody	DNA plasmid encoding for a protein complex encoding for a targeting factor, an adjuvant and antigens; administered using a high pressure needle-free device.	Trinomab Biotech (CN)
	IVAC Mutanome	mRNA delivered in spleen targeted nanoparticles	Vaccibody (NO)
	mRNA Therapeutics™	mRNA vaccine	BioNtech (D) Caperna / Moderna(USA)

of cytotoxic T cells.³⁵ Additionally, live vectors constitute an attractive mean to target professional antigen presenting cells with a relatively easier manufacturing and logistical process than dendritic cell vaccines.³⁶ Attenuated forms of *Listeria monocytogenes* have been used for the development of cancer vaccines.³⁷ This technology is currently applied by Advaxis (Princeton, NJ, US) as a platform for neoantigen vaccine delivery. Besides, the large majority of live vaccine vectors are viruses. α viruses, Adenoviruses, viruses from the pox family or lentiviral systems such as ImmuneDesign's ZVex[®] (Seattle, WA, US) are used as cancer vaccines.³⁶ Each platform has its advantages and limitations, but generally may be limited by safety concerns originating from risks of genome integration and uncontrolled replication in immunocompromised patients. To circumvent these limitations, the Modified Virus Ankara (MVA) has served as a safe and effective delivery platform for cancer antigens in numerous cancer trials.

Advantages of the MVA platform

The Modified Virus Ankara (MVA) has been generated through serial passages in chicken embryo fibroblasts.³⁸ It has been used safely and effectively for smallpox vaccination in more than a hundred thousand individuals.³⁹ The MVA has lost the pathogenicity of its parental virus, the Chorioallantois Vaccinia virus Ankara, through alterations of its genome, i.e. 15% of genome loss. The virus is replication defective in human cells, ensuring its safety and tolerability in clinical use. Furthermore, it provides an inexpensive cellular system to produce large quantities of the virus in egg-based manufacturing processes.

Subsequently to its use as a smallpox vaccine, MVA was largely used as a genetic vaccine vector in several trials, targeting several cancer antigens in various indications including melanoma, prostate, breast, kidney and lung cancer.⁴⁰ MVA-based vaccines have achieved good clinical results when given either as a monotherapy or in combination with chemotherapy, radiotherapy or immune checkpoint inhibitor.⁴¹

The large variety of antigens used in the MVA platform confirms the robustness and flexibility of the vector. Inserts of up to 11 kilobases can reliably be cloned in the vector, as demonstrated by our experience with the full length cDNA of dystrophin, one of the largest human proteins. This high cloning capacity enables the insertion of a large number of protein antigens and also leaves room for additional material intended to increase the immunogenicity of the vaccine. Practically, one single vector can be used to generate a response against different antigens and poly-epitopes.

MVA vaccines act on several key points in the tumor immunity cycle (Fig. 1). After targeted expression of the cancer antigen at the site of injection, the viral infection triggers the release of immune alarm signals, then stimulating the intervention of professional APC that will present both encoded antigens and viral components. Noteworthy, all these steps take place outside of the immunosuppressive environment of the tumor. MVA vaccination has the potential to elicit both CD4 and CD8 responses.⁴² The diversity of these responses leverages an efficient anti-tumor response, and decreases the risks of immune escape by counterselection of negative tumor cell

Figure 1. Mechanism of action of MVA vaccines at cellular levels. Infection of cell (1), followed by intervention of professional APC (2) and presentation of viral components including antigenic transgene (3). After priming and maturation, effectors T cells home into the site of the tumor and (exert their cytolytic action on tumor cells presenting the targeted antigen (4). As a consequence, tumor cell death might release other cancer antigens that then lead to spreading of the response (5). We could demonstrate this phenomenon in the case of TG4010, a MVA-based vaccine used in patients with NSCLC.

clones. The large cloning capacity can also be used to select mutations targeting respectively CD4 or CD8 responses as both response types are demonstrated to play a role in the development of a durable anti-tumor response.⁴³

Two recent examples from our portfolio confirm the clinical efficacy of MVA-based vaccines. The first one is TG4001 (Tipapkinogene sovavivec), targeting E6 and E7 antigens from human papilloma virus, was used to treat high grade cervical intraepithelial neoplasia. In a phase I study, 9 out of 10 patients displayed eradication of HPV DNA 6 months after initiation of treatment and lesions regressed in 7 out of 10 patients.⁴⁴ These results were confirmed in a randomized, controlled phase II study (unpublished data). The second one is TG4010 (Mesmulogene ancovavivec, MVA-Muc1-IL2). It demonstrated a remarkable efficacy in non-small cell lung cancer patients; with doubling of both progression free survival (12 to 30% at 12 months) and overall survival (19 to 40% at 24 months), compared with placebo.⁴⁵ Immunomonitoring of patient samples from this TIME study demonstrated that clinical benefit was associated to epitope spreading to other cancer associated antigens.⁴⁶ Similarly, Prostavac[®] (Rilimogene galvacirepvec), an MVA based vaccine expressing PSA (Prostate specific antigen) and stimulating cytokines (LFA-3, ICAM-1, and B7.1) has shown encouraging results in prostate cancer.⁴⁷

It is frequently argued that the activity of viral vaccine might be impeded by neutralizing antibodies. In our experience, neither prior vaccination against smallpox, nor repeated administration negatively affected immunogenicity of the product. Hence the platform would be fit for repeated treatment of patients in case of evolution of the tumor mutational landscape.

In addition to these mechanistic advantages, MVA also deserves consideration from a pharmacokinetic et

pharmacodynamic point of view. DNA, RNA or peptide vaccines rely on the use of different sequences, which different biophysical behaviors which might potentially lead to product heterogeneity, affect in vivo stability, and thus impair biodistribution properties. In the case of live vectors, one can reasonably assume that biodistribution will be independent of the nature of the neoantigenic cassette, just as it remained constant across the different existing vaccines directed at various TAA.

There is a strong track record for design and manufacturing of MVA-based vaccines. The recent integration of novel genome editing technologies has greatly improved the cloning yields, and thus allowed significant reduction of manufacturing times from 2–3 months to a few weeks. This disruption should favorably impact the economics of the global manufacturing process. In our analysis, the recent progress on MVA manufacturing makes it compatible with the following cost and time targets for a pharmaceutical-grade personalized vaccine: total treatment cost $\leq$ \$15 000 with a lead time from tumor sequencing to vaccine delivery of 6 to 8 weeks.

The future of personalized vaccines and their integration into therapeutic schemes

Neoantigens vaccination is a promising treatment modality, in particular with respect to its capacity to embrace the entire cancer mutanome. Implementation of this approach is however contingent to the successful resolution of technical challenges associated with the *one-patient one-product* paradigm and integration of this new therapeutic class into the standards of care.

Among them, one should obviously consider the arrival of immune checkpoint inhibitors (ICI) in various oncology indications, either as standalone therapy or in combination with other immunotherapy products, leading to a paradigm shift in the treatment of cancer. We assume neoantigenic vaccine will be used in combination with ICI, taking advantage of potential synergy between these 2 therapeutic classes. For instance, combination with checkpoint blockade is a particularly appealing approach to attempt to increase response rates.⁴⁸ Among the several clinical trials in progress in this area, we wish to outline the combination trial between the MVA-based vaccine TG4010 and the anti-PD1 Nivolumab in NSCLC patients (NCT02823990). Nevertheless, heterogeneity of response across patients reflects a large diversity of immune mechanisms and highlights the importance to adapt the therapeutic intervention based on tumor immune profile.⁴⁹ Personalized vaccination will thus be associated with the development of precision medicine and related bioanalytical methods.

Third, the diversity of vaccine platforms also opens up the possibility to act sequentially on different immune mechanisms. This so called heterologous prime/boost vaccination has been shown to leverage synergy between different adaptive response mechanisms.^{50,51} Given their unique features, it is a reasonable assumption that MVA based platforms will be central in this upcoming developments.

Disclosure of potential conflicts of interest

KB and EQ are full time employees of Transgene SA.

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