

# PERSONALIZED NEOANTIGEN VACCINE AGAINST CANCER

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## SUMMARY

No significant therapeutic solutions for advanced cancer are available to date. However, an old idea based on usage of tumor-specific antibodies as the basis for a new vaccination form in patients with advanced tumors, has attracted recent research and is possible due to development of personalized neoantigenic vaccines. Each tumour has indeed, its own mutation content, only a small percentage are shared between patients. Technological advances and the established genome databases, allowed for a rapid mapping of mutations in the genome. The latter allows for a rational selection of targets for personalized vaccines and on-demand production of customized therapy according to the patient's individual tumour properties. This article accordingly, summarizes basic principles of vaccines for personalized neoantigens, translational research and clinical studies related to these vaccines. Finally, the future of such therapy with personalized vaccines is discussed.

**Key words:** personalized vaccines, cancer, mutations, neoantigens, immunity

## INTRODUCTION

Vaccines are a hot topic both in science and in the clinical practice. Currently, vaccines are heavily discussed within the public these days as well, primarily as a consequence of the COVID-19. The reason for this is the speed with which products aimed to prevent COVID-19 have stepped into the market. Indeed, these products are supported along with a huge propaganda stating them as 'new types of vaccines' with still unknown effects on human health and not always convincing effects required for the vaccines. Previously, some preventative vaccines like those against childhood diseases have had their ups and downs depending on the target population of users. Cancer vaccines are a therapeutic tool while standard vaccines against, for example, measles and tetanus are preventive products applied in healthy people to incite the immune system to produce antibodies

against the particular infection agent. In the case of cancer vaccines, the body helps to fight the disease that is already present. However, such vaccines may also have preventive effects.

Somehow in the shadow of developments of preventive vaccines in the arena of infective disease, personalized vaccines against tumours, especially those based on tumour-specific neoantigenic properties, are highly promising and potentially useful in cancer treatment. The idea of tumour-specific antigens is not new and provides hope for decades in solving the cancer problem. Those expectations were unfortunately unrealistic so far. The main reason was that these previously studied cancer vaccines were not personalized and their development was accordingly hampered by high tumor heterogeneity. In particular, first efforts were based on the wrong assumption that tumour-specific antigens are unique for all patients. Over time, the clinical practice pro-

vided evidence on unique tumour-specific antigens and cancer vaccines should be designed on these personalized properties.

In the 1980s, several laboratories studying cancer immunology began with research based on the idea that proteins encoded by genes mutated in tumours could serve as immune targets on cancer cells. The hypothesis was that such neoantigens, upon binding by the major histocompatibility complex (MHC) protein, would be present on the outer surface of the cancer cell membrane where T cells could detect them with appropriate receptors. This first personalized cancer vaccines grew out of a joint effort in the field of unique tumor mutations identification by use of a computer source aimed to trigger an immune response in cancer patients, helping them fight disease. For many years, Schreiber studied mice that developed sarcomas after exposure to a chemical carcinogen as a model for characterizing interactions between cancer and the immune system. In 2011, he proposed the possibility of sequencing the DNA of these cancer cells to identify unique cancer peptides or neoantigens, in order to stimulate the immune system against cancer. In contrast to the cellular immune therapies, which provide tumor-attacking T cells directly to the patient, the idea was to use such neoantigens to create cancer vaccines that promote differentiation of endogenous T-cells (Schreiber et al. 2011).

This approach finds a strong rationale in the basis of cancer genetics. Cancer is indeed, characterized by accumulation of genetic alterations. Somatic mutations can generate a spectre of cancer neoepitopes that may be then recognized by the autologous T-lymphocyte system. T-cells recognize such antigens as foreign and this fact is in the basis for ideal vaccine development. Each tumour has its own mutation content, of which only a small percentage is shared among patients with same cancer type. The molecular nature of tumor-specific antigens however, remained unknown until the discovery and application of cloning techniques (van der Bruggen et al. 1991). Screening of patient tumor-derived expression libraries with autologous tumor-reactive CD4<sup>+</sup> or CD8<sup>+</sup> T-cells revealed two categories of spontaneously recognized T cell antigens: (i) nonmutated proteins with tumor-associated expression and (ii) mutated gene products (Coulie et al. 2014). Now the rational selection of targets for vaccines as well as on-demand produc-

tion of a therapy customized to a patient's individual tumour is possible due to advances in sequencing and genome databases along with immunotherapy (Hundal et al. 2016). Both types of mutations that accumulate in cancer, namely the driver and passenger mutations, can alter sequence proteins and create new epitopes. These new epitopes can be processed and presented on major histocompatibility complex (MHC). Sequence altered complexes are termed neoantigenes. The new mutated epitopes recognized by T-lymphocytes are called neoepitopes (Schumacher & Schreiber 2015). It is important to note that neoepitopes are absent in normal cells but are exclusively present in cancer cells. As the vast majority of mutated antigens detected in these pioneering studies were found to be unique for each individual patient, immunological recognition of neoepitopes was quickly established as clinically highly relevant. Polymerase chain reaction (PCR) sequencing methods prompted the discovery of mutations back in 2008 to identify cancer neoantigens (Segal et al. 2008). A series of 11 breast cancers and 11 colorectal cancers, neoantigens were obtained identifying missense mutations in cancer-related genes from Sanger sequencing data and using available neural networks to evaluate the binding of the encoded proteins to the most common MHC type. Authors predicted that these cancers average between 7 (breast) and 10 (colorectal) novel epitopes per patient. The immune system could, by a variety of approaches including personalized vaccines, be stimulated to respond to these novel peptides (Segal et al. 2008).

## CREATION OF CANCER VACCINE

Creation of individualized cancer vaccines require identification of cancer-specific peptides -neoantigens. Afterwards, a platform based on cells, proteins, or nucleic acid should be employed to deliver neoantigens to patients. The patient immune system will then attack the tumor. Antigen-presenting cells, such as dendritic cells, internalize cancer-specific peptides and display them on their surface using major histocompatibility complex (MHC) proteins. This triggers T-cells with receptors that bind these neoantigens to differentiate into effector or killer T cells that mobilize an immune response against cancer cells.

Designing of such vaccines may be based on three options: 1. *a vaccine based on dendritic cells*. In this pro-



cess, monocytes are extracted from patients' blood and cultured with synthetic versions of selected cancer neoantigens. The goal within such approach is creation of mature dendritic cells that carry these neoantigens. These cells are then re-inoculated into the patient; 2. *Long synthetic peptides* - synthetic peptides containing neoantigen sequences are injected into the body, where they are picked up by cells that present the antigen. 3. *DNA and RNA vaccines* - nucleic acids that encode neoantigens are introduced into the body, where they are translated into proteins and collect cells that present the antigen. The cancer vaccine is meant to be inoculated subcutaneously, after which the vaccine travels to the lymph nodes, *i.e.* the immune cells. Once in the area, the vaccine penetrates dendritic cells that present a specific T-cell vaccine antigen. These cells directly fight cancer cells.

One of the problems in cancer vaccines is in the presence of extremely small ingredients. To prevent those small ingredients from being dispersed throughout the body through the bloodstream, a system has been developed that chemically binds together small cancer vaccine particles. Thus, new vaccines called Polycondensate Neoepitope (PNE) have been developed, which consist of a neoantigen that is a mutated specific antigen characteristic of the tumor and of the adjuvant in question. These two ingredients bind together and form a larger entity that the blood vessels cannot absorb. This is how they get to the lymph nodes. Only when they reach the interior of the dendritic cells, the vaccine components separate again allowing the dendritic cells to present T-lymphocyte antigen. An important part of this personalized strategy is a careful analysis of the neoantigens in each individual patient. Such a personally activated immune system enables an effective fight against tumors. It is important to emphasize that neoantigens are not present in normal cells.

For now, all of these vaccine platforms are optional as researchers continue to study the immune system's response to certain neoantigens. Questions include the minimum number and types of neoantigens required to elicit a strong cancer-targeted immune response and whether all neoantigens are encoded by all cancer cells (clonal mutation) or only by a portion of them (sub clonal mutation). Rare types of mutations that dramatically alter protein sequences, such as those resulting from insertion or deletion of frame shifts or fusion peptides due to chromosome structural changes, are likely to yield more potent

neoantigens with strong MHC binding potential because they are more obviously selfless to the immune system (Hundal et al. 2018).

## TRANSLATIONAL RESEARCH AND CLINICAL STUDIES

Positive results with personalized neoantigenic cancer vaccines have fostered faith in the successful treatment of patients. These vaccines have so far shown highly specific immune responses to cancer cells without serious adverse effects in patients. This initial enthusiasm needs to be confronted with the challenges of clinical trials in order for the concept of personalized vaccines to be routinely applied. (Sahin & Türeci 2018). Dozens of clinical studies with personalized vaccines are currently underway. They are using different vaccine platforms: RNA, DNA, synthetic long peptide, dendritic cells and are mainly in the 1st or 2nd phase of clinical trials. Let us mention just a few of them. One phase 2 clinical trial study is conducted at the National Institute of Health in the United States. The goal is to cure patients with multiple solid tumours using dendritic cells as a vaccine platform (Tral ID NCT03300843). Another study on multiple solid tumours is in Phase 1 clinical trials (NCT03289962) using the RNA vaccine platform. The aim of the study is ambitious and for example aims to collect 567 patients for examination. One study uses the DNA vaccine platform and is also in Phase 1 (NCT02348320) conducted on 30 breast cancer patients (Washington University School of Medicine). A study on follicular lymphomas (NCT03121677) is being conducted at the same University in association with Bristol-Myers Squibb. For this study, a synthetic long peptide is used as the vaccine platform, and the patient growth target is 20.

The choice of the best approach to personalized cancer vaccines will depend on the determined and validated number and types of neoantigens needed to best improve patient outcomes, but also on vaccine platforms that are most measurable, cost-effective, efficient, and rapid to produce. Dendritic cell vaccines seem to be very effective in eliciting an immune response because the role of dendritic cells in the immune system is to present antigens. However, their preparation requires careful cultivation of blood-derived immune cells to mature dendritic cells prior to incubation with selected tumour peptides. Scaling this process would require significant auto-

mation of laboratory steps to produce vaccines for multiple patients simultaneously (Keskin et al. 2019, Ma et al. 2011.).

Another apparently promising approach is based on cocktails made of synthetic long peptides that are injected intramuscularly. Peptides are typically about 20 amino acids in length and each contains a neoantigen sequence of 8 to 11 amino acids. Two safety studies using this approach have been published so far, one in patients with melanoma (Ott et al. 2017) and one in patients with glioblastoma (Keskin et al. 2019). Because they are synthesized, long peptide production is scalable, but their use in human therapy requires specific process controls to ensure good manufacturing practice (GMP). Neoantigen peptides of each patient must be synthesized in isolation to ensure purity, and certain peptide sequences may be challenging to put them in solution before injection. All of these aspects may limit their widespread use in personalized vaccines in the future.

Another way to elicit a neoantigen-induced immune response is by using DNA or RNA vaccines that encode predicted neoantigens. These vaccines have been shown to be effective in preclinical models and in the early stages of human RNA vaccine trials. These vehicles are relatively inexpensive to manufacture. The final sequences of DNA or RNA-encoded neoantigens are easier to confirm after vaccine synthesis than peptide sequences, which require basic sequencing technology instead of less sensitive mass spectrometry. However, one should be very careful while introducing nucleic acids that contain neoantigen coding sequences into cells to enable their conversion into peptides, systemic effects on the genome and the organism remain indeed unknown (Sahin et al. 2017).

In addition, a good clinical example of cancer vaccines in metastatic melanoma treatment. Until recently, metastatic melanoma was an incurable and deadly disease. New developments in therapy have significantly improved prognosis. For example, personalized melanoma vaccines that elicit a long-lasting antitumor response in patients even 4 years after immunization have been developed. This vaccine was injected into patients on average approximately 18 weeks after vaccination. Four years after vaccination, all 8 patients were alive and 6 showed no signs of active disease. Interestingly, the patient's immune cells did not recognize only specific tumour epitome on which the vaccine was

made has already been recognized by other melanoma related epitopes as well. Analysis of two patients in whom the tumour has spread to the lungs showed that they were treated with an immune checkpoint inhibitor (Hu et al. 2021). These are drugs that loosens some of the restrains of immune response to cancer. However, their T cells found ways to fight lethal melanoma cells.

Using the so-called. polymerization technique called polycondensation researchers have addressed one of the key problems of vaccine delivery. In a paper published in ACS Central Science (Wei et al. 2020) the authors show results of a prototype vaccine that can travel automatically to a desired location and activate immune cells at site. Also, the researchers worked in parallel on development of an algorithm for fast and accurate prediction of mutated tumor antigens (Ott et al. 2017). Based on such encouraging results and long-term activity of specific antitumor T lymphocytes, it can be concluded that personalized neoantigen peptide vaccination can help control metastatic tumours, even when combined with specific immune checkpoint inhibition. (Carreno et al. 2015).

## INSTEAD OF A CONCLUSION

The results of early preclinical and clinical research on the so-called neoantigen vaccines are extremely encouraging. The end result of this type of therapy will depend on methodological improvements that ensure the reliability of neoantigen prediction and scalability of the vaccine platform. A number of issues remain to be addressed to make personalized neoantigen-based cancer vaccines the ultimate solution. This requires a multidisciplinary approach: specialist in cancer genomics, for cancer immunology, for vaccine development for oncology and diagnostics. One of the limitations in research could be the relatively difficult access of patients for testing given the intensity of new immune treatments and the growing need for randomized clinical trials. This may limit a scrutinized testing of neoantigenic personalized vaccines where preference may be given to therapies that are less personalized and therefore more susceptible to mass production. It will be interesting to see if neoantigen vaccines find their niche and how wide that niche will be. Nonetheless, efforts on this issue will improve our understanding and clarify the next steps towards more effective anti-cancer immunotherapies.



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## SAŽETAK

### Personalizirana neoantigenaska cjepiva protiv raka

Do danas nisu bila dostupna značajna terapijska rješenja za uznapredovali rak. Međutim, stara ideja zasnovana na korištenju antitijela specifičnih za tumor, ali koja se temelje na personaliziranim neoantigenaskim cjepivima, daje osnovu za novi oblik cijepjenja pacijenata s uznapredovalim tumorima. Svaki tumor sadrži vlastite tipove mutacija, a samo mali postotak tih mutacija zajednički je za tumore ostalih pacijenata. Tehnološki znanstveni napredak i uspostavljene genomske baze podataka omogućili su brzo mapiranje mutacija u genomu što omogućuje racionalan odabir ciljeva za personalizirana cjepiva i njihovu proizvodnju. Takav pristup prilagođene terapije za specifične mutacije u skladu je s individualnim svojstvima tumora pacijenta. U članku se sažeto prikazuju osnovna načela personaliziranih neoantigenaskih cjepiva, translacijska istraživanja te kliničke studije povezane s tim cjepivima. Konačno, raspravlja se o budućnosti terapije oboljelih s uznapredovalim tumorima primjenom personaliziranih cjepiva.

**Ključne riječi:** personalizirana cjepiva, rak, mutacije, neoantigeni, imunitet

