Progress in research on neoantigenic cancer vaccines

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Abstract: Tumors that are malignant are one of the most serious threats to human health today. As per the 2018 Global Cancer Statistics, published in the American Cancer Society's official journal, roughly 18.1 million additional cancer cases and 9.6 million cancer deaths will occur worldwide in 2018 [1]. Although surgery, radiation, chemotherapy, and targeted therapy are the most effective therapies for malignancies, each of these treatments has limitations. However, each of these treatments has distinct limits, and the overall therapy impact is poor, as is the patient's long-term survival percentage. Tumor immunotherapy is a relatively new form of tumor treatment that has made tremendous development in recent years. It is a way for controlling and eliminating tumors by beginning and sustaining the tumor-immune cycle and reestablishing the body's normal anti-tumor immune response. Neoantigens have arisen as a new study avenue in tumor immunotherapy in recent years, and current research findings indicate that neoantigens are particularly useful in immune checkpoint inhibitor therapy, TIL therapy, and the generation of therapeutic tumor vaccines. We discuss the advancement of personalized vaccines based on neoantigens in this article.

1. The definition and properties of neoantigens

Tumor neoantigens, also known as tumor-specific antigens, are new, unknown proteins or peptides encoded by somatic cell genes that are generated by physicochemical damage or spontaneous mutations, found on the surface of tumor cells, derived from tumor-specific mutations, expressed only in tumor cells, and are tumor-specific (TSAs). They are formed as a result of tumor cell DNA alterations and have been demonstrated to play a critical role in the detection and killing of tumor cells by T cells - specifically in the immunological response mediated mostly by $CD8^+$T cells.

Neoepitopes are peptides that are recognized by the T cell receptor (TCR) and have been shown to induce anti-tumor cell immune responses. These antigenic peptides are expressed on major histocompatibility complex (MHC) molecules, which are recognized, processed, and presented by antigen-presenting cells (APCs), and finally recognized and bound specifically by TCRs, activating T cells, causing them to proliferate and differentiate into effector T cells, and triggering anti-tumor immune responses [3]. T cells can detect tumor neoantigens and induce immunological responses, making them viable targets for immune-mediated tumor therapy.

2. Neoantigen screening and prediction

2.1 Screening

Due to the fact that tumor cell mutations differ between individuals, screening for neoplastic antigens requires knowledge of the genomes of individual tumor patients. However, the vast number of possible false-positive neoantigen peptides discovered between the detection of somatic mutations in tumors and the recognition of neoantigens by the TCR to elicit an immune response has complicated the construction of vaccines against neoantigens.

Individual or mutant cells utilized to screen for neoantigens must meet the following four criteria: (1) the mutant gene is expressed at the protein level; (2) the mutant protein can be processed by APC into a peptide suitable for presentation of its own MHCImolecule; (3) the mutant peptide has a high

affinity for the patient's own MHCImolecule; and (4) the antigenic peptide-MHCIcomplex has a high affinity for the effector T cell receptor (T cell receptor, TCR). [5]

2.2 Prediction

The present approach for screening individualized neoantigenic peptides can be broken into four steps: (1) using next-generation sequencing (NGS) technology, total RNA and genome sequencing of tumor cells and peripheral blood cells to find tumor-specific mutant peptides; (2) Prediction of MHC-peptide binding At the moment, the most frequently utilized methods for screening tumor neoantigens include exome sequencing in conjunction with transcriptome sequencing, tandem microgene sequencing, target gene sequencing, shared neoantigen peptide library screening, antigen ligandomics, and mass spectrometry. as well as mass spectrometry.

3. Neoantigen cancer vaccines

Neonatal antigen vaccines are peptide, RNA, and dendritic cell vaccines that specifically target neoantigens to promote the body's active immunity. In comparison to conventional vaccines, the primary advantage of individualized neoantigen vaccines is that conventional vaccines are constrained by both HLA and antigen expression, limiting the patients to whom they are applicable; whereas, because individualized neoantigen vaccines are "tailor-made" for each patient's tumor tissue mutation antigen, even though they are still constrained by the patient's HLA restriction.

Both neoantigens formed as a result of single nucleotide mutations and neoantigens generated as a result of insertion/deletion mutations Two distinct tailored RNA or peptide-based vaccination techniques have been studied recently in the field of vaccination, with the latter projected to be particularly immunogenic. The statistics gathered imply that vaccination can indeed both enhance and prevent the spread of disease. The data collected indicate that vaccination can actually both enhance pre-existing antigen-specific T cells and generate a larger pool of new T cells specific to cancer patients. According to their published statistics, the vaccine's upper limit for the peptide-based strategy is 20 peptides. According to their published data, the peptide-based strategy has a maximum of 20 peptides and is not effective in all patients due to the inability of some patients to synthesis peptides. significantly lower, as each vaccine contains only ten mutations. [6]

Neoantigen cancer vaccines are prepared in four fundamental steps: collecting tumor tissue and normal cells, conserving neoantigens, identifying neoepitopes, and manufacturing vaccinations [7]. The following summarizes the progress of research on neoantigen vaccinations for certain types of cancers.

3.1 Melanoma

CARRENO et al. [9] conducted the first clinical trial of a neo antigen-based vaccine in 2008. They used whole exon sequencing to analyze missense mutations in tumor tissues from three HLA-A2.1 positive melanoma patients at different sites, predicted and identified seven neo antigenic epitopes, loaded DCs in vitro, and reinfused once every six weeks with the initial dose of 1.5107 DCs/peptide. The results indicated that a robust neoantigen-specific T cell immune response was detected 2 weeks after the first DC infusion, peaking at 8-9 weeks; and that neoantigen-specific $CD8^{+}$T cells were detected in patients following vaccine infusion using tetrameric staining, while memory T cells remained detectable 4 months after the last DC infusion. Although nascent antigen-specific T lymphocytes recognized the corresponding wild-type antigens, it was discovered that they did not recognize the corresponding wild-type antigens.

The first clinical success of peptide vaccines targeting nascent antigens occurred in melanoma research, and two studies published in Nature in 2017 [8-9] confirmed that nascent antigen-based tumor vaccines could achieve promising efficacy in the treatment of malignant melanoma, garnering widespread attention and paving the way for personalized immunotherapy for cancer patients. OTT et al. [8] studied six patients with melanoma and predicted 20 neoplastic antigens with high immunogenicity and expression levels for each patient, from which a peptide vaccine was produced.

60% of neoplastic antigens generated $CD4^+$T-cell responses confined to MHC type II and 16% to MHC type I. The results indicated that four of the six vaccinated patients did not experience recurrence within 25 months of vaccination; the other two patients developed recurrence but achieved complete remission following additional treatment with PD-1 antibodies; specific immune cells directed against neoplastic antigens were detected in both patients. The researchers picked ten mutant RNA pieces for each patient's tumor vaccination, and eight patients saw complete tumor clearance with no evidence of recurrence within 12 months of treatment. The remaining five patients had metastases at the time of vaccination, two experienced a decrease in tumor size following vaccination, and one experienced complete remission following PD-1 antibody treatment [9].

Since then, clinical trials utilizing precision immunotherapy based on tailored vaccines have been conducted in various solid tumors with encouraging outcomes.

3.2 Glioma

The American and German research teams stated above published the results of their clinical trials of "personalized tumor vaccinations" in glioblastoma in Nature in 2018 [10-11]. Thus, it is critical to combine tailored vaccinations with other therapeutic approaches.

In a phase I/II trial involving six European centers [11], HILF et al. of Immatics Biotechnologies evaluated two vaccine strategies: one was similar to the personalized neo antigen vaccine used by KESKIN et al. [10]; the other was a non-mutated protein fragment corresponding to a tumor-associated protein present on cancer cells. The latter vaccine was not customized for each patient, and the tumor-related proteins were identified through an analysis of 30 glioblastomas utilizing methodologies for identifying proteins highly associated with this type of tumor. This trial enrolled 15 patients newly diagnosed with glioblastoma following surgical resection and chemotherapy. Four patients received only the non-personalized vaccine, while 11 received both vaccines consecutively. Thirteen patients who received the non-personalized vaccine demonstrated an evaluable immunological response, and twelve of these thirteen patients exhibited $CD8^+$T cells that identified at least one protein in the non-personalized vaccine. Eight of ten patients who received the tailored vaccine and developed an evaluable immune response demonstrated development of a $CD4^+$T cell response against the tumor antigen.

They immunized eight patients newly diagnosed with glioblastoma following surgical resection and conventional radiotherapy in a phase I/Ib study and analyzed blood samples from vaccinated patients to determine whether helper $CD4^+$T cells and killer $CD8^+$T cells responded to these neoplastic antigens. The vaccine failed to generate a robust T-cell response in individuals treated with the potent immunosuppressant dexamethasone (used to minimize brain swelling), whereas two patients who did not receive dexamethasone developed a neoplastic T-cell response. Notably, these immature antigen-specific T lymphocytes produced cytokines involved in tumor cell death. However, all of the vaccinated patients eventually died of cancer.

In a phase I/Ib study, they immunized eight patients newly diagnosed with glioblastoma after surgical resection and conventional radiotherapy and analyzed blood samples from vaccinated patients in order to verify whether T cell types called helper $CD4^+$T cells and killer $CD8^+$T cells responded to these neoplastic antigens. The vaccine failed to elicit a strong T-cell response in participants treated with the potent immunosuppressant dexamethasone (used to reduce swelling around the brain), and two patients who did not receive dexamethasone showed a neoplastic T-cell response. Notably, these nascent antigen-specific T cells secreted cytokines that were involved in killing tumor cells. However, all of the vaccinated patients eventually died of cancer.

3.3 Lung cancer

Lung cancer is the malignant tumor with the fastest growing incidence and mortality rate, and the most threatening to the health and life of the population. Targeted therapies that trigger drug resistance and immune checkpoint inhibitor therapy do not provide long-term benefit to patients, and no effective treatment options are available for the vast majority of patients.

In a patient with squamous cell carcinoma of the lung who showed significant disease progression after chemotherapy and epidermal growth factor receptor (EGFR) inhibitor treatment, an individualized tumor neoantigen peptide vaccine was designed and applied to the patient based on tumor mutation profile and HLA typing. Immunomonitoring of peripheral blood showed that specific cytotoxic T lymphocytes (CTL) were primarily targeted to peptide targets containing the widely shared EGFRL858R mutation, particularly restricted to HLA-A∗3101. this immune targeting of driver mutations may be particularly beneficial for Asian lung cancer patients , as it has a relatively high mutation rate in this patient population.

It has been reported that the State Key Laboratory of Cell Therapy, Sichuan University, in collaboration with the Cancer Center of Tongji University, the Clinical Research Center for Tumor Biotherapy of Tongji University, and the Department of Oncology, West China Hospital, Sichuan University, completed several cases of individualized DC vaccine based on tumor nascent antigens for advanced small cell lung cancer [13].This therapy was first predicted and analyzed by second-generation sequencing (NGS), bioinformatic analysis, and in vitro experiments of patients' tumor The patients with advanced multinodular metastatic small cell lung cancer had no significant adverse effects after treatment, and later review showed that the primary lesions, such as lung, were stable and the metastatic lesions, such as cranial, were smaller than before treatment. A clinical study of neo antigen immunotherapy by a research team from Tianjin, China [14] attracted a lot of attention from experts at home and abroad. The primary endpoint was feasibility and safety, and the secondary endpoints were PPV immunogenicity, progression-free survival (PFS) and overall survival (OS). There were no treatment-related serious and fatal adverse events, except for transient rash, fatigue or fever in three patients, and no patients discontinued treatment because of adverse events. Such risk exists).

3.4 Colon and Pan-cancerous Solid Tumors

In 2020, KLOOR et al. published the results of a phase I/IIa cancer vaccine study, which successfully demonstrated the safety and immunogenicity (i.e., the ability to elicit an immune response) of an FSP neoantigen-based vaccine in patients with micro satel-liteinstability (MSI) colorectal cancer. Researchers vaccinated MSI colon cancer patients with FSP neoantigen vaccine derived from mutated AIM2, HT001, and TAF1B genes and showed that the vaccine was well tolerated systemically, induced sustained humoral and cellular immune responses, and improved patient survival.

A single-arm open-label clinical trial of iNeoVac-P01, a peptide-based neoplastic antigen vaccine, was designed by a research team at Zhejiang Run Runyiff Hospital [18]. 22 patients with advanced solid tumors of pan-cancerous species who failed standard therapy were prospectively enrolled in the study, and the iNeo artificial intelligence vaccine design platform, individualized peptide drug preparation and quality control system were used based on high-throughput sequencing data. The individualized peptide vaccine iNeoVac-P01 was designed for each patient and evaluated for safety and efficacy by immune response, tumor load, adverse events, progression-free survival (PFS), and overall survival (OS). Disease control rate (DCR) was 71.4%, median PFS was 4.6 months, and median OS was not reached (12-month OS=55.1%). The study suggests that iNeoVac-P01 monotherapy is feasible and safe for patients with advanced solid tumors. it induces a T-cell-mediated immune response against tumor neoplastic antigens and may have good antitumor efficacy. in addition, the findings reveal several potential biomarkers that predict better response.

3.5 Pancreatic cancer

The incidence of pancreatic cancer is rapidly increasing in recent years, therefore, it is urgent to conduct in-depth research on the pathogenesis of pancreatic cancer to find effective treatment methods, and with the rapid development of tumor immunotherapy, neo antigen vaccine has become a hot spot for pancreatic cancer treatment in recent years.

In a patient with metastatic pancreatic ductal carcinoma who was 62 years old and diagnosed with pancreatic ductal carcinoma (stage IIb, pT3pN1M0) in October 2011, SONNTAG et al [15] treated a

patient with metastatic pancreatic ductal carcinoma with an individualized neoantigen peptide vaccine on days 1, 3, 7, 14 and 28 after achieving the second complete remission with chemotherapy. The patient survived 6 years after the first diagnosis and 4 years in complete remission; no vaccine-related adverse effects were observed at follow-up, and vaccine-specific TCR sequences were detected in the patient's peripheral blood after the 12th, 17th, and 34th vaccine doses.

Swiss BASSANI-STERNBERG et al [16] conducted a phase Ib clinical trial of an individualized neo antigenic dendritic cell vaccine for pancreatic cancer and showed that the vaccine used in pancreatic cancer patients could restructure important immunosuppressive factors in the tumor microenvironment, enhance tumor immune recognition, and thus enhance the response to PD-1/PD-L1 blockade.

4. Extensions and Improvements

4.1 Field Stretching

Researchers at the Johns Hopkins-Kimmel Cancer Center found that combining the neoantigen tumor vaccine PancVAX with two drugs, an anti-PD-1 antibody and an agonist OX40 antibody, resulted in a better response to treatment by converting T-cell-depleted tumors into tumors rich in specific T cells. depleted tumors transformed into tumors rich in specific T cells that responded better to treatment, resulting in smaller pancreatic cancer masses in mice, demonstrating that pancreatic cancers that respond poorly to PD-1 immunotherapy can benefit from this combination [19].

Recent studies have combined neo antigenic cancer vaccines with pericytes and achieved significant therapeutic effects.

CHEN et al [20] in Nanjing, China, established two efficient neo antigen screening strategies and built a neo antigen peptide library for common solid tumors (gastric cancer, colorectal cancer, etc.) Subsequently, a loaded neo antigen peptide library was used in six patients (thymoma, pancreatic cancer, uterine cancer) July 15, 2019 Neon Therapeutics, Inc. announced:NT-001 Clinical trial demonstrates for the first time that NEOPV-01 individualized cancer vaccine in combination with Bristol-Myers Squibb PD-1 tumor immunotherapy OPDIVO (nivolumab) in patients with advanced or metastatic melanoma, smoking-associated non-small cell lung cancer (NSCLC) and bladder cancer significantly prolongs progression-free survival.NEOPV-01 is These top-line data are from the Phase Ib NT-001 trial, in which 82 enrolled patients received at least one dose of OPDIVO (nabumab) in all three different tumor types, the vaccine combined with PD-1 significantly prolonged progression-free survival compared with historical single immune checkpoint inhibitors.

As a result, one patient with metastatic thymoma achieved complete remission after 29 months of treatment, another patient with metastatic pancreatic cancer achieved immune-related partial remission, and the remaining four patients achieved long-term disease stabilization with a mean progression-free survival (PFS) of 8.6 months.

The above clinical trials showed that neoantigen cancer vaccine can effectively induce specific T-cell immune response, and patients with refractory and progressive tumors can achieve remission after receiving neoantigen vaccine with minimal adverse effects, suggesting that neoantigen vaccine is feasible for clinical application and laying a solid foundation for the expansion of clinical trials of neoantigen vaccine.

4.2 Improvements in antigen detection and T-cell screening

One possibility is to induce different populations of immune T cells capable of recognizing and destroying as many cancer cells as possible at one time, in order to reduce the chances of cancer cells "escaping" the T cell response and not being recognized by the immune response. It is therefore desirable that vaccines encode a large number of cancer-specific antigens. This is particularly relevant for personalized genetic vaccine approaches based on individual cancer-specific nascent antigens. To optimize the probability of success, vaccines should target as many nascent antigens as possible.

Alfred et al. from Nouscom [21] explored a series of methods for cancer nascent antigens for personalized vaccines, improving the process of screening nascent antigens by parallel DNA

sequencing. The approach is also related to the construction of vectors or vector collections carrying nascent antigens for personalized vaccines.

In terms of T-cell epitopes, a patent by Biotech RNA Pharmaceuticals [22] shows a method for predicting T-cell epitopes that can be used for vaccination. The method is also applicable to predict whether modifications in peptides or polypeptides (e.g., tumor-associated neoantigens) are immunogenic and, in particular, whether they can be used for vaccination.

The patent performs mutation detection by exome sequencing, selects vaccine targets by individual bioinformatic prioritization of mutant epitopes predicted to be abundantly expressed and good MHC class II binders, and rapidly generates synthetic mRNA vaccines encoding multiple of these mutant epitopes. Vaccination with such multimeric neoepitope mRNA vaccines induced robust tumor control in mice and complete rejection of established aggressive growth tumors. Furthermore, we demonstrate that $CD4^+$T cell neoepitope vaccination induces CTL responses against independent immunodominant antigens in tumor-bearing mice, indicating a combined effect of antigen spreading (orchestration). Finally, by analyzing the corresponding human cancer types using the same bioinformatics algorithm, the technology also shows the predicted abundance of mutants that bind to MHC class II in human cancers. The customized immunotherapy regimens in this patent may be considered as a universally applicable blueprint for synthesizing the vast new epitope target pool of cancers to be able to target each patient's tumor with a "just in time" generated vaccine.

5. Problems and Prospects

Despite the current breakthroughs in cancer vaccines against tumor neoantigens, which hold significant promise for use alone or in combination with other therapies, there are still many challenges to their widespread use in clinical cancer therapy: (1) the differences in the predictive power of existing sequencing technologies for neoantigens, how to rapidly and accurately identify neoantigens with strong immunogenicity from the large number of mutations identified by tumorigenomic technologies, and how to identify neoantigens with strong immunogenicity from the large number of mutations identified by tumorigenomic technologies. (2) The time-consuming and complicated process of individualized neoantigen vaccine preparation may lead to the loss of the optimal timing of vaccine treatment for tumor patients, which severely limits the application of vaccines, and therefore the time of therapeutic vaccine or cell preparation needs to be shortened to be applied to patients as soon as possible. It is believed that in the near future, neoantigen-based immunotherapy will bring a milestone breakthrough in the treatment of patients with malignant tumors.

References

[1] Ferlay, J., et al. "Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018." European Journal of Cancer 103(2018):356-387.

[2] Liu YT et al. "Advances in the study of neo antigens in tumor immunotherapy." Oncology 39.04 (2019):312-316.

[3] Joyce, et al. "T cell exclusion, immune privilege, and the tumor microenvironment." Science (2015).

[4] [4] Fang, C., et al. "microRNA-193a stimulates pancreatic cancer cell repopulation and metastasis through modulating TGF-β2/TGF-βRIII signalings." J Exp Clin Cancer Res 37.1(2018):25.

[5] Be-Tech Pharmaceutical (Suzhou) Co. A screening method for tumor neoantigens." CN109021062B.2021-08-20.

[6] ZHOU Weijun, and LI Yuhua. "Opportunities and challenges for clinical translation of individualized neoantigenic vaccines." Chinese Journal of Oncology Biotherapy 26.001(2019):16-21.

[7] Wolfel, T., et al. "A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma." Science 269.5228(1995):1281-1284.

[8] Beatriz, et al. "Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. " Science (2015).

[9] Ott, P. A., et al. "An immunogenic personal neoantigen vaccine for patients with melanoma." Nature 547.7662:217-221.

[10] "Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. " Nature 547.7662(2017):222-226.

[11] "Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial." Nature (2019).

[12] Hilf, N., et al. "Abstract 2654: GAPVAC-101 phase I trial: First data of an innovative actively personalized peptide vaccination trial in patients with newly diagnosed glioblastoma." Cancer Research 76.14 Supplement (2016):2654-2654.

[13] Li, F., et al. "Rapid tumor regression in an Asian lung cancer patient following personalized neoepitope peptide vaccination." Oncoimmunology 5.12(2016):

[14] Du, X., et al. "1177PDClinical study of personalized neoantigen peptide vaccination in advanced NSCLC patients." Annals of Oncology 30. Supplement_5(2019).

[15] Sonntag, K., et al. "Immune monitoring and TCR sequencing of CD4 T cells in a long term responsive patient with metastasized pancreatic ductal carcinoma treated with individualized, neoepitope-derived multipeptide vaccines: a case report." Journal of Translational Medicine 16.1(2018):23.

[16] Raja, R., et al. "Fostriecin potentiates genome instability and anti-tumor immunity in ovarian cancer." Journal for ImmunoTherapy of Cancer 8.Suppl 3(2020):A593-A593.

[17] Kloor, M., et al. "A Phase I/IIa trial of a frameshift peptide neoantigen-based vaccine for mismatch repair-deficient cancers." Clinical Cancer Research 26.17(2020):clincanres.3517.2019.

[18] Fang, Yong, et al. "A pan-cancer clinical study of personalized neoantigen vaccine monotherapy in treating patients with various types of advanced solid tumors." Clinical Cancer Research 26.17(2020): clincanres.2881.2019.

[19] Kinkead, H. L., et al. "Combining STING-based neoantigen-targeted vaccine with checkpoint modulators enhances antitumor immunity in murine pancreatic cancer." JCI Insight, 3.20(2018).

[20] Fangjun, et al. "Neoantigen identification strategies enable personalized immunotherapy in refractory solid tumors. " The Journal of clinical investigation (2019).

[21] NOUSCOM AG." Cancer mutation selection for the generation of personalized cancer vaccines." CN113424264A.2021-09-21.

[22] Biotech RNA Pharmaceuticals, Inc. and Translational Oncology, Johann Gutenberg University Mainz Medical University." Prediction of vaccine-ready T-cell epitopes." CN113484523A.2021-10-08.