EDITORIAL

Future ONCOLOGY

Neoantigens in the immuno-oncology space

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"High-throughput sequencing and computational analysis has identified neoantigens as potential antigenic source for cancer vaccine strategies."

First draft submitted: 21 July 2017; Accepted for publication: 11 August 2017; Published online: 4 October 2017

KEYWORDS
• antibodies • cancer
• immunotherapy • monoclonal
• neoantigens • vaccines

Scientific advances over the past decade have led to numerous breakthroughs in developing novel agents for the treatment of a wide variety of tumors. These have included improvements in traditional cytotoxic chemotherapy approaches, the development of agents targeting growth factor and multi-kinase pathways, as well as angiogenesis inhibitors designed to starve tumors through inhibition of blood vessel development [1–3]. In the past several years, immunotherapy has garnered a tremendous amount of interest based on both exciting preclinical as well as recent clinical results. Immune therapy is not only viable, but has become an important therapeutic option for the treatment of patients with cancer.

One exciting area in tumor immunotherapy that has demonstrated positive results in treating individuals with cancer is the use of immune checkpoint inhibitors. Checkpoint inhibitors block proteins on cancer cells, or the proteins on T cells that respond to them. This allows for T cells to recognize abnormal cells as cancerous allowing for the immune system to attack the tumor. However, these proteins can also be found on normal organs and side effects are similar to what is often observed with autoimmune-like illnesses. A number of checkpoint inhibitors have now received rapid approval from the US FDA for cancer, including ipilimumab, pembrolizumab and nivolumab [4–6]. These and other immune checkpoint therapies represent one of the most promising approaches in cancer treatment today. In addition, another area of great interest in the immuno-oncology space is the utilization of adoptive transfer of T cells expressing chimeric antigen receptor (CAR). CAR-modified T cells can be engineered to target virtually any tumor-associated antigen [7,8]. Ongoing clinical trials have demonstrated encouraging results especially in the hematologic malignancies.

As our knowledge of the immune system has continued to develop, an emerging area for novel immunotherapy is occurring through the discovery and characterization of neoantigens in cancers. A neoantigen is defined as a newly discovered antigen that is expressed on tumor cells. Neoantigens occur as somatic mutations and may develop through exposure to viruses and

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carcinogen exposure that is introduced into the host. Alterations in the tumor DNA occur during the development and progression of cancer. Since these antigens are considered foreign to the immune system, the host can potentially mount immune responses to these neoantigens [9,10]. High-throughput sequencing of a number of tumor specimens has provided the ability to map out somatic mutations that occur in a variety of human cancers. Thousands of mutations found during screening provide the basis for multiple potential targets that could be used for immunotherapies. Computer modeling and simulation algorithms have been created that can identify these neoantigens and calculate their ability to bind to MHC-I and MHC-II peptides, thus providing a potential antigenic source for cancer vaccine strategies.

The use of these evolving platforms has provided the basis for the use of neoantigens in next generation cancer vaccine strategies. For many years, cancer vaccines have represented an exciting area of potential therapy for patients with cancer. There have been several prevention vaccines that are FDA approved targeting viruses such as human papillomavirus, to reduce the risk of developing cancer. Therapeutic vaccines have also been tested for patients with existing cancer. In the past, a number of therapeutic cancer vaccines did not meet the primary end points of their respective clinical trials. However, in 2010, the first anticancer vaccine approved by the FDA, Sipuleucel-T for the treatment of asymptomatic metastatic castrate-resistant prostate cancer. This autologous vaccine demonstrated a statistically meaningful 4.1-month improvement in median survival in the active arm with respect to the placebo arm (25.8 vs 21.7 months) [11].

Recently, an HLA-A_02:01-restricted neoantigen-based dendritic cell vaccine was tested for safety, tolerability and immunologic responses in patients with metastatic melanoma [12]. Post vaccination patients were able to mount T-cell reactivity against predicted neoantigens. Furthermore, vaccination both stimulated the proliferation of T cells and induced T-cell immunity to several neoantigens. Treatments led to the establishment of CD8 neoantigen-specific T cells and memory T cells and T-cell receptor (TCR) repertoire of T cells was broadened upon treatment [12]. These results demonstrated that treatment produced neoantigen specific cytotoxic T-cell reactivity and there was a selection toward the specific clones [12].

Another novel melanoma vaccine employed as many as 20 predicted neoantigens as targets to elicit antitumor immune responses. Most patients' vaccinated, developed immune responses targeted $CD4^+$ T cells (60%), while less than 20% of patients developed $CD8^+$ T-cell responses to the vaccine [13]. It was interesting to note that the T-cell responses were directed against mutations in known tumor antigens. At a 25-month follow-up post initial vaccination, four of six patients were disease free. Furthermore, the two patients with disease recurrence, when treated with an anti-PD-1 antibody experienced both a complete regression of tumor as well as an expanded immune response to additional neoantigen epitopes [13]. Further studies are being performed with this vaccine strategy both as a monotherapy approach as well as in combination with checkpoint inhibitor antibodies.

Most neoantigen approaches rely on computational next generation sequencing (NGS) and computational analysis that select epitopes to be used for vaccine therapy. However, these approaches rely on algorithms to predict immune response to the correct epitopes that are chosen for vaccination. A biologic approach is currently being pursued to identify immunogenic functional neoantigens. Ensituximab is one of several monoclonal antibodies raised against an allogeneic colorectal cancer vaccine that had previously been tested in human clinical trials in the USA [14]. Surgical samples from dozens of patients with various stages of colorectal cancer including metastatic foci were obtained as a source for antigen screening. Surface membrane from pooled allogeneic tumor was fractionated and various molecular weight components were tested for immunogenic reactivity which was the basis of the initial vaccine. Results revealed safety of the vaccine, but clinical benefit that directly correlated with the ability of the patients' immune system to mount IgG responses against the vaccine [14]. Based on the results of these early studies, the original vaccine was used as the source of antigenic material to produce monoclonal antibodies that specifically reacted with colorectal cancer and could kill tumor via antibody-dependent, cell-mediated cytotoxicity. Several functional antibodies were identified and were used to identify the neoantigens in colorectal cancer. The lead antibody, Ensituximab was found to target a neoantigen with sequence homology to *MUC5AC*, but functionally distinct [15]. Ensituximab has been used in clinical

trials for patients with metastatic colorectal cancer previously treated with standard chemotherapy agents. Selection criteria for patient participation were based on an immunohistochemistry (IHC) assay demonstrating the presence of the neoantigen on the patient's tumor [16].

Conclusion

In recent years, through scientific discovery and advances, immunotherapy has become a central focus for the treatment of cancer. Cancer vaccines, adoptive T-cell and check point therapies have led to several exciting therapies that are now FDA approved for patient treatment. The number of ongoing clinical trials is now in the hundreds to test new targets as well as combination approaches. Neoantigen discovery has supplemented the armamentarium of novel immune therapies which can harness a patient's immune system to attack cancer.

As these immune therapies are being explored, laboratory based science is driving our understanding of how to combine traditional treatments, in other words, traditional chemotherapy, radiation therapy, along with immune-mediated approaches.

Financial & competing interests disclosure

PM Arlen is an employee of Precision Biologics, Inc. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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