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Exploring Immunotherapies: Beyond Checkpoint Inhibitors

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Introduction

Immunotherapy is an emerging class of cancer treatment, which stimulates the body's immune system to fight cancer. With the approval of three checkpoint inhibitors (ipilimumab, pembrolizumab and nivolumab) by the Food and Drug Administration (FDA) in the past five years, immunotherapies are seen as exciting treatments able to elicit durable cures. In a previous VirtualScopics whitepaper, *Immunotherapy – Concept Turned Reality* [1], an introduction to the role of the immune system and an overview of checkpoint inhibitors was presented. However, checkpoint inhibitors are only one of the many possible approaches used to harness the antitumor effects of the immune system. This whitepaper will introduce other promising and emerging immunotherapeutic approaches to treat cancer, namely adoptive cell transfer (ACT), anticancer vaccines, and oncolytic viruses.

Evading the Immune System

The immune system identifies and destroys developing tumors thus serving as the primary defense mechanism against cancer [2]. Tumor cells express antigens, substances considered foreign by the body. The immune system identifies and eliminates tumor cells based on the expression of these tumor-specific antigens. Attacking tumor cells requires T-cells, specialized immune cells that move from the blood stream into the tumor. The number of tumor-infiltrating T-cells present in the tumor microenvironment is correlated with better prognosis in various cancer types [3]. Despite what may seem like a straight forward process for tumors to be recognized as abnormal and destroyed by the immune system, tumors develop and spread even when the immune system is otherwise functioning normally. As discussed previously [1, 4], tumors can elude immune surveillance through a number of evasion mechanisms.

Adoptive Cell Transfer (ACT)

ACT is a type of personalized immunotherapy that uses a patient's own T-cells to attack tumors. In one approach, T-cells are harvested from a patient's tumor and grown *in vitro* over the course of several weeks. During this period the tumor-infiltrating T-cells are stimulated with factors (e.g. interleukin-2 and anti-CD3 antibodies) that induce activation and proliferation. The T-cell population expands considerably, with a 1,000-5,000 fold increase in T-cells [5]. The patient is then infused with the highly

activated and tumor-specific T-cells. Prior to infusion, the patient receives chemotherapy to suppress the immune system and augment the antitumor efficacy of the ACT [6]. ACT has shown promise in metastatic melanoma, inducing objective response rates (i.e. the proportion of patients who receive a complete or partial response during treatment) near 50% [5].

Another related form of ACT uses modified T-cells, such as chimeric antigen receptor (CAR)-T cells. The procedure includes apheresis to isolate peripheral blood mononuclear cells from the patient's blood [7]. Once extracted, the T-cells are modified *in vitro* to express specialized proteins, termed CARs, on their surface. The T-cell is therefore genetically re-engineered to recognize a specific antigen found on the tumor. The process of modifying the T-cells is complex and requires gene transfer technologies [7]. Similar to tumor-infiltrating T-cells, the CAR modified-T cells are expanded in culture and reinfused into the patient. With guidance from the engineered receptor, the modified T-cells can preferentially recognize, target, and destroy cancer cells that harbor the CAR-specific antigen. Although genetically modified, CAR T-cells still maintain intrinsic T-cell functionality, including the ability to proliferate, localize to sites of disease, and elicit cytotoxic effects [7].

CAR T-cells have been used in clinical trials to treat both solid and hematologic tumors, with the greatest successes seen in leukemia and lymphoma [8-10]. Recently, a phase I study using CAR T-cells directed at the CD19+ antigen in patients with acute lymphoblastic demonstrated complete remission in 90% of patients [11].

Vaccines

Cancer vaccines can be used as prophylactic measures to protect against cancer causing viruses. For example, vaccinations for hepatitis B and the human papillomavirus are given to reduce the risk of developing liver and cervical cancers, respectively [12]. However, vaccinations can also be used to treat cancer. Several vaccines strategies, including cell-based (tumor or immune), protein/peptide-based, and genetic-based (DNA, RNA and viral) vaccines, have been developed and evaluated [13]. For brevity this whitepaper will introduce two cell-based approaches, tumor cell vaccines and dendritic cell vaccines.

Tumor cell vaccines can be prepared using patient-derived tumor cells [13]. From the tumor cells, antigens specific to the tumor are isolated and combined with an adjuvant. Adjuvants are substances that when combined with antigens enhance the body's immune response to the antigen [14]. This cocktail is then injected into the patient from which the tumor fragments were isolated and an immune response is triggered via dendritic and T-cell interactions.

Another type of vaccine uses dendritic cells. Dendritic cells are specialized immune cells that engulf antigens. The antigen is processed by the dendritic cells and presented to naïve T cells, resulting in the priming and activation of the T-cells against cancer-specific antigens [4]. For vaccine creation, dendritic cells are removed from the blood and manipulated *in vitro*. Specifically, dendritic cells are given tumor-associated antigens and treated with adjuvants [13]. The antigen-loaded dendritic cells are then returned to the blood stream to prime and activate T-cells.

Dendritic cell vaccines have been tested in various indications including prostate cancer, melanoma, renal cell carcinoma, and glioma [13]. In 2010, sipuleucel-T (marketed as Provenge™) was approved by the FDA for the treatment of metastatic castrate-resistant prostate cancer [15]. The sipuleucel-T vaccine consists of unfractionated peripheral blood mononuclear cells (which include dendritic cells) that have been incubated with a fusion protein. The fusion protein links the cytokine, granulocyte-macrophage colony stimulating factor (GM-CSF), to the prostate cancer antigen, prostatic acid phosphatase (PAP). In the randomized phase III trial, patients receiving the vaccine demonstrated a significantly increased survival benefit of 4.1 months compared to unvaccinated patients [16]. Sipuleucel-T is currently the only therapeutic vaccine to receive regulatory approval in the United States.

Oncolytic Viruses

Oncolytic viruses are a class of viruses that selectively infect and kill cancer cells without harming normal tissues. Oncolytic viruses have dual mechanisms of action to induce anti-tumor responses [12]. The first is direct lysis of the cancer cell by the virus. The second is the indirect activation of tumor-specific T-cells resulting from the cell lysis. Specifically, the antigens released from the dying cancer cells are released, engulfed by dendritic cells and presented to T-cells to induce cytotoxic effector T cells [4].

Oncolytic viruses can be grouped into two general classes. The first are those viruses that naturally favor tumor cells as their host. These include viruses such as autonomous parvoviruses, myxoma virus, Newcastle disease virus, reovirus, and Seneca valley virus [17]. The second class includes viruses that are also genetically modified for use in vaccines (e.g. measles virus and poliovirus), as well as those viruses that are reprogrammed to specifically target cancer cells (e.g. adenovirus and herpes simplex virus).

In the last few years, there has been a spike in the number of viruses that have been genetically modified for use as oncolytic viruses. For example, talimogene laherparepvec (T-VEC) is a genetically altered oncolytic virus, based on a modified herpes simplex virus type 1, that has been used to treat late stage melanoma [18]. The T-VEC includes several gene modifications allowing the oncolytic virus to selectively replicate within tumors and produce GM-CSF to enhance systemic antitumor immune responses [18].

In the randomized phase III OPTiM trial, which evaluated T-VEC in late stage melanoma against GM-CSF, the durable response rate was significantly higher in the T-VEC group (16.3%) compared to the GM-CSF group (2.1%). The objective response rate was also significantly higher in T-VEC patients (26.4%) compared to GM-CSF participants (10.8%) [18]. The OPTiM study was the first phase III trial showing clinical efficacy of an oncolytic virus. In 2015, the FDA approved T-VEC, as the first-in-class oncolytic immunotherapy for treatment of advanced melanoma.

Summary

The potential promise of immunotherapy to serve as a treatment strategy against cancer has long been postulated. However, recent successes in clinical trials using ACT, vaccines, oncolytic viruses, and check-point inhibitors, are positioning immunotherapies to become a key pillar of cancer treatment. Future research will undoubtedly identify new ways to harness the power of the immune system to fight cancer.

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