REVOLUTIONIZING CANCER THERAPEUTICS: MOLECULAR PATHWAYS AND TECHNIQUES IN CANCER IMMUNOTHERAPY

Bea Co
Grade 12, Garth Webb Secondary School, Halton District School Board (Oakville ON)

ABSTRACT

Contrary to conventional cancer therapies, immunotherapy manipulates an individual’s body cells to fight cancer, enhancing the active and dynamic immune interactions between the tumour and host. Cancer immunotherapy provides evidence of success through a variety of treatment approaches. Utilizing T-cells and antibodies, immunotherapy strategies such as T-cell engaging bodies, checkpoint inhibitors and engineered T-cells have significantly increased the chance of survival for many cancer patients. The combinations of these immunotherapies have also granted greater success in the elimination of tumour cells. Immunotherapy breakthroughs have the potential to have a lasting impact on cancer treatment. This literature review sheds light on the importance in further research for cancer immunotherapy and a glimpse at all of its powerful results.

INTRODUCTION

Cancer immunotherapy began in the 1890s, when Dr. William Coley proved that he could treat advanced cancers with tuberculosis vaccine injections inducing tumour regressions in some patients (Parish, 2003; Hoption Cann et al., 2003). Some significant responses were recorded over the ensuing forty years, but successes were sporadic, difficult to reproduce and were not obtained in a scientifically rigorous fashion (Hoption Cann et al., 2003). Coley’s strategy was further discounted due to the risks associated with the administration of infectious agents to already weakened cancer patients (Mellman et al., 2011).

Thus, the success of immunotherapy seemed unlikely, slowing the development of immunotherapeutic progress in cancer for many decades (Biemar et al., 2013).

The immune system and tumor cells have a dynamic relationship where the immune system is seeking to eliminate tumour cells, whereas tumour cells are seeking to evade the immune system (Yokota, 2000). In cancer, tumour cells use many evasive techniques to avoid the immune system such as T-cell anergy, dendritic cell defects, secretion of immunosuppressive cytokines, resistance to apoptosis, and deficient expression of immunomodulatory molecules (Seliger, 2005). Cancer is treated by reducing or removing tumours as well as avoiding tumour development.
through common methods such as surgery, radiation therapy, chemotherapy, and external drug use (Rees, 2014). However, these methods come along with adverse side effects that critically impact one’s health (Canadian Cancer Society, 2016). Fortunately, these treatments can be replaced with the best natural defense mechanism of all – the immune system.

Cancer immunotherapy encompasses treatments that are meant to strengthen immune responses against tumours so that cancerous cells can be more effectively eliminated (Janeway, 2005). The immune system has not been able to effectively recognize and attack cancer since cancer cells can be altered and grow out of control (American Society of Clinical Oncology, 2016). The body normally kills cancer cells or abnormally growing cells, however, these cells can often evade detection by immune cells when reducing tumour antigen cell surface expression (American Society of Clinical Oncology, 2016). Cancer immunosurveillance is demonstrated when molecules on the surface of cancer cells, known as tumour-associated antigens (TAAs) are detected by the immune system (Remedios, 1980). The identification of tumour markers in TAAs provides targets for attacking the cancer (Remedios, 1980). Innate and adaptive immunity are both critical responses to the immune system (The University of Arizona, 2000). Adaptive immunity confers antigen specific immune responses, while innate immunity refers to nonspecific defence mechanisms (The University of Arizona, 2000). Cancer immunotherapies rely heavily on the adaptive immune response more because it includes a “memory” that makes future responses against a specific antigen more efficient (OpenStax, 2016). Memory T-cells and B-cells are immune cells that remain in the body and retain a memory of a pathogen. If a pathogen is re-encountered, memory T and B-cells can immediately trigger a fast and powerful immune response (OpenStax, 2016). The memory-based advantage associated with cancer immunotherapy significantly helps the long term care of patients as immunologic memory provides the host with long-lived protection from future pathogen encounters (American Society of Clinical Oncology, 2016; Klebanoff et al., 2006).

There are two types of immune cells that are important to the success of these therapies, T-cells and B-cells. T-cells are immune cells that recognize antigen fragments presented at cell surfaces by specialized antibody-like receptors (Janeway, 2005). They work to attack cells carrying certain foreign or abnormal fragments (Janeway, 2005). In addition, B-cells are another type of immune cell that plays a key role in activating T-cells (Janeway, 2005). Each B-cell is programmed to make one specific antibody, and when a B-cell encounters its triggering antigen, it activates and differentiates into plasma cells (Janeway, 2005). The plasma cells then manufacture millions of identical antibody molecules that bind the antigens to mark the cells for destruction (Janeway, 2005). Helper T-cells as well have a critical role in helping B-cells by stimulating nearby B-cells to produce antibodies (Janeway, 2005).

Cancer immunotherapy dramatically alters the immune-cancer relationship to allow for disease control up to complete remission. Immunotherapy offers several advantages to today’s standard treatments from its unique approach to arming the body’s own immune system with numerous molecular weapons instead of directing a therapy against a single tumour target (National Cancer Institute, 2016). Activated and tumour-specific immune cells can reach areas that a surgeon cannot, and when the immune system is appropriately stimulated, it can target even microscopic diseases and disseminated metastases (Dimberu et al., 2011). Furthermore, immunotherapy avoids the adverse side effects by only attacking the tumour cells, and avoiding the target of healthy cells that chemotherapy and radiation therapy can strike on (Dimberu et al., 2011). Successful trials and experiments have been accomplished with effective results using methods such as T-cell engaging bodies, checkpoint inhibitors and engineered T-cells (Huehls et al., 2014; Alberts et al., 2002; Levine, 2015).

Cancer immunotherapy is an emerging field that is only beginning to show its potential. A revolution of pharmaceutical, academic, and investor interest should ready us for the hope of an exquisitely effective cancer therapy with the adverse side effects present in chemotherapy to be obsolete. This paper encompasses the main methods of cancer immunotherapy treatment and its successful results based on the molecular pathways targeted and techniques used that ultimately triumph cancer immunotherapy’s astounding potential for the future.
CURRENT CANCER IMMUNOTHERAPY METHODS

T-Cell Engaging Bodies

Bispecific T-cell engagers (BiTEs) are molecules that enhance the patient’s immune response to tumours by retargeting T-cells to tumour cells (Huehls et al., 2014). A BiTE is constructed of two single-chain variable fragments (scFv) connected in tandem by a flexible linker. One scFv binds to a T-cell-specific molecule, usually the protein complex CD3, whereas the second scFv binds to a tumour-associated antigen (Huehls et al., 2014). These T-cell engaging molecules stimulate the immune system by bringing the tumour cell in close proximity to T-cells activated through their cell membrane receptors (Huehls et al., 2014). T-cells expand rapidly upon activation producing durable cytotoxic responses with the potential to generate immunologic memory by continually executing the immune response and increasing the likelihood of the eradication in cancer cells (Huehls et al., 2014). T-cells have been found to attack tumours from the outside and as well as infiltrate into the tumour. These features make T-cells optimal therapeutic effectors for cancer as specificity allows a BiTE to bridge a T-cell with a tumour cell, stimulating T-cell activation, tumour cell lysis and cytokine production (Huehls et al., 2014). The efficiency and exceptional potency of BiTEs for therapeutics have been demonstrated multiple times in both animal models and humans (Huehls et al., 2014). In animals, mouse models have shown that microgram doses of this agent are able to promote tumour regression of colorectal and ovarian cancer (Huehls et al., 2014). In humans, BiTEs have shown complete and partial responses in a phase I trial of the BiTE blinatumomab at small doses in non-Hodgkin B-cell lymphoma patients (Huehls et al., 2014).

Checkpoint Inhibitors

T-cell activation is tightly regulated in order to maintain self-tolerance for minimal collateral damage in peripheral tissues. With checkpoint inhibitors, the tight regulation system that dampens down the immune response is impaired, removing immune-checkpoint blockades for a stronger physiological immune response (Alberts et al., 2002). This is exploited as a method of cancer treatment that intensifies the activation of T-cells (Alberts et al., 2002). T-cell activation begins when antigens are presented during the engagement of the T-cell receptor (TCR) and peptide from the major histocompatibility complex to the T-cell (Alberts et al., 2002). Afterwards, costimulation occurs when T-cells receive signals usually from B7 proteins such as CD80 and CD86 (Pardoll, 2012).

CTLA-4 (Cytotoxic T-lymphocyte Associated Protein 4) and PD-1 (Programmed Cell Death Protein 1) are both inhibitory receptors that suppress and down regulate the T-cell effector function (Bristol-Myers Squibb Canada, 2016). CTLA-4 primarily counteracts the activity of the T-cell co-stimulatory receptor CD28 that is essential to amplify TCR signaling when activating T-cells (Bristol-Myers Squibb Canada, 2016). CTLA-4 down-modulates CD4+, harming T-cell activity since it enhances regulatory T-cells that are responsible for immunosuppressive activity (Bristol-Myers Squibb Canada, 2016). The major role of the inhibitory receptor PD-1 also limits the activity of T-cells in peripheral tissues during an inflammatory response to infection (Bristol-Myers Squibb Canada, 2016) (Fig 1). Removing CTLA-4 and PD-1 interactions with their receptors using an inhibitor, strengthens T-cell activation and downstream effector responses (Hodi et al., 2010). In 2012, Health Canada approved the antibody ipilimumab, which targets the blockade molecule CTLA-4 (Hodi et al., 2010). Patients with terminal melanoma showed an outstanding 20% response rate when treated with ipilimumab (Awad et al., 2015). In a phase 3 study of ipilimumab, the overall survival was 10 months among patients taking the therapy as compared to the previous expectancy of 6.4 months (Awad et al., 2015). Additionally, 18% of the ipilimumab-treated patients survived beyond two years, a remarkable improvement from the 5% with previous medicinal treatments (Brower, 2016). The impressive survival benefits stay consistent with drugs that block PD-1 action. Disruption of PD-1 pathway signaling represents a major breakthrough in treatment of tumour types like melanoma, lung cancer, bladder cancer, and Hodgkin’s lymphoma (Brower, 2016). Compared to chemotherapy, which has a response rate less than 10% with a one year survival rate of 30%, Gettinger et al. showed that PD-1...
inhibition had a response rate of 17% and an 18% three year survival rate (Brower, 2016). Over half of the patients in the trial had received more than three lines of systematic therapy beforehand, therefore providing exceeding results from such a heavily pre-medicated group (Brower, 2016). These studies show that immunotherapy can be a significantly more effective form of cancer treatment than traditional chemotherapy.

**Engineered T-Cells**

Engineered T-cells with synthetic receptors known as chimeric antigen receptors (CARs) have also been proven effective in eliminating tumour cells (Wolchok et al., 2014). The process of engineered T-cells begins by separating white blood cells from the blood, extracting T-cells, and returning red cells and platelets to the patient (Jarvis, 2014). A viral vector inserts into T-cells with genes carrying the instructions for a CAR that tethers the antibody to promote co-stimulatory signals to divide the cell. A bioreactor is used to increase the amount of T-cells with the help of magnetic beads coated with two antibodies, anti-CD3 and anti-CD28, that signal the T-cells to proliferate (Levine, 2015). The patient's blood is then infused back with the reprogrammed T-cells that will destroy cancer cells expressing the antigen targeted by the CAR (Levine, 2015). The customization of T-cells available to the patient's specific form of cancer is a unique benefit of engineered T-cells. The modifications in T-cells promote convenience and efficiency in the particular treatment exploited.

The reduction of tumour growth is incredibly effective through CARs, but despite promising clinical results, there are critical concerns for engineered T-cells as their excessive activity can make them difficult to control (Wu et al., 2015). This can result in adverse side effects, including life threatening inflammatory risks for severe toxicity from their potent immune activity (Wu et al., 2015). However, a study by Lin and Onuffer has found a way to control the activity by developing a CAR that is switched on only in the presence of both of its cognate antigen and a small-molecule drug (Bird, 2015). Previously with conventional CARs, the cognate antigen is usually activated upon binding from the TCR and co-stimulatory molecules (Bird, 2015). Although, for better regulation of this activity, the new design has different constructs in which the extracellular antigen-binding and intracellular signaling components were split in the presence of a small molecule referred to as a rapalog (Bird, 2015). The on-switch CAR exemplifies a simple and effective strategy, diminishing unwanted side effects and allowing precise control over the amount, timing and location of CAR T-cell activities.

**Combination of Immunotherapy Treatment**

Preclinical models have proven the importance of a complex integrated immune response in eliminating tumours (Vanneman et al., 2011). Combinations of immunotherapies are anticipated to be far more effective than monotherapies (Vanneman et al., 2011). Various integrations of cancer immunotherapy treatments suggest intriguing potential in what lies ahead for therapeutic synergy with complementary modes of action targeting cancer cells (Pardoll et al., 2012). Preclinical models have validated dramatic synergy between tumour vaccines and inhibition of most of the immune checkpoints (Pardoll et al., 2012). Anti-CTLA-4 therapy strongly enhances the amplitude of vaccine-induced anti-tumour responses in many poorly immunogenic tumour models, as does anti-PD1 therapy (Pardoll et al., 2012). BiTEs may also be significantly more effective in combination with other therapies. In particular, their association with immune checkpoint inhibitors, such as CTLA-4 and PD-1 inhibitors, allows for greater T-cell activation (Hodi et al., 2010). For melanoma patients, a staggering 65% of patients treated with a combination of blocking both CTLA-4 and PD-1 had their tumours stop growing (Hodi et al., 2010). These results prove to be very promising, showing the great potency achievable by combinatorial strategies.

**DISCUSSION**

**Ethical Implications of Access to New Therapies and Costs**

Immunotherapy has proven to be a stronger approach to fighting cancer cells when compared to traditional chemotherapy. Chemotherapy is a popular method for cancer treatment using chemical based drugs to treat cancer (National Center for Biotechnology Information, 2016).
Information, 2016). It is an effective approach that targets cancer cells, but also attacks healthy, fast dividing cells, causing unpleasant side effects for the patient (National Center for Biotechnology Information, 2016). To begin with, a very short ten-minute drip using immunotherapy can effectively treat leukemia, while current chemotherapy treatments for leukemia can last for over a year (MIT Technology Review, 2016). Chemotherapy is endured over a longer period of time since treatment is given in cycles for the cancer cells to be attacked at their most vulnerable times, and to give normal cells time to recover from the damage (Chemocare, 2016). Treatment sessions can last up to four hours and more time may be needed for treating side effects. Not only does chemotherapy concern time, but also evokes permanent damage attacking healthy cells formed in the bone marrow, digestive tract, reproductive system, and hair follicles, while immunotherapy conveniently avoids all of these chronic side effects (Themeli et al., 2013). Furthermore, the hospital bills for leukemia patients can reach up to two million dollars, which is four times the cost of immunotherapy (MIT Technology Review, 2016).

Others misleadingly advocate cancer immunotherapy to be corrupt because of the controversial high financial costs of treatments that have been constantly disputed. Cost is a remarkable obstacle against immunotherapy. The price of one treatment is half a million dollars (MIT Technology Review, 2016). It is only available to those are more affluent, and not an option for the majority of the population. This is incredibly unfortunate; the help is available yet the accessibility is not. It is understandable that there is a high asking price relative to the expenses of health care and the lasting value of the treatment. It is inevitable for the treatment to be costly when immense efforts are continuously put into research involving highly educated professionals, high quality equipment, and complex analyses of data. However, the government needs to consider making it more widely available as the treatment has been shown to work successfully for various types of cancers. Cancer immunotherapy may have high initial costs, but it provides powerful, and long lasting results. The likely improvements in mass production of immunotherapy treatment will also be able to decrease the costs. The rising possibility of T-cell mass production continues with optimizing growth of T-cells in the lab, using electricity or pressure to introduce genetic material into cells, and the continued success of therapies in mouse cancer models (Topalian et al., 2011).

CONCLUSION
The remarkable effects seen in T-cell engaging bodies, checkpoint inhibitors and engineered T-cells are only the beginning of cancer immunotherapy. There are still numerous more immunotherapeutic methods and findings to be discovered to advance the future of cancer immunotherapy. In the past, immunotherapy was not respected as a field because of the lack of demonstrated clinical benefits and adverse effects. Today, some still believe immunotherapy to be ineffective. Although there was initial skepticism towards this field because of the many failed attempts in the earlier decades, there is still a need to start paying attention to the powerful results seen in current clinical trials rather than stay put with the past mindset of immunotherapy. A paradigm shift has already shown clear clinical benefits, therefore, it is counterintuitive to stick to the standards developed a generation ago. Proper research funding for cancer immunotherapy is needed, especially with its impressive impacts. It has the potential to replace traditional treatments with new molecular pathways and techniques that have been proven to be more effective. With cancer immunotherapy comes the beginning of revolutionizing cancer therapeutics, leading to an incredible future in the fight against cancer.

ABBREVIATIONS

<table>
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<tr>
<th>Acronym</th>
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<tbody>
<tr>
<td>TAA</td>
<td>Tumour-Associated Antigen</td>
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<tr>
<td>BiTES</td>
<td>Bispecific T-cell engagers</td>
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<td>CD3</td>
<td>Cluster of differentiation 3</td>
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<td>scFv</td>
<td>Single-chain variable fragments</td>
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<td>TCR</td>
<td>T-Cell Receptor</td>
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<td>CTLA-4</td>
<td>Cytotoxic T-lymphocyte Associated Protein 4</td>
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<td>Programmed Cell Death Protein 1</td>
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<td>CAR</td>
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REFERENCES


7. Rees, R.C. Tumor Immunology and Immunotherapy; Oxford University Press, 2014.


