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Recent Patents on Endocrine, Metabolic & Immune Drug Discovery **2013,** *7,* **1-10**

Cancer Therapy Based on a Mechanism of Action for Controlling the Immune System and the Resulting Patent Portfolio

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Abstract: Despite decades of research, current cancer therapies extend survival but often do not actually cure the disease. Immune cell based therapy is described here that can eliminate tumor cells without the toxicity associated with chemotherapy or allogeneic bone marrow transplant (BMT). Allogeneic BMT has beneficial graft vs. tumor (GVT) effects but accompanied by the undesirable graft vs. host disease (GVHD). The mechanism described here relates to generating the Mirror Effect™ that elicits a host vs. tumor (HVT) effect along with a non-toxic host vs. graft (HVG) response. The HVT effect generated by inducing the Mirror Effect™ in a host is effective in cancer therapy, as well as other diseases. The patent strategy, employed for successful patent prosecution related to the elucidation of the Mirror Effect™, is also discussed.

Keywords: Allogeneic cells, cancer therapy, cytokines, HVG, HVT, immune cells, Mirror Effect™.

INTRODUCTION

Typically, an inventor will approach a patent lawyer to protect a new product that he/she desires to commercialize. It is rare when an inventor approaches not only with a product, but with an idea that has as an underlying concept that is fundamental, widely applicable and is capable of producing a multiplicity of products that together can create a new industry. One example of this kind of concept is the US patent that covers the idea of double clicking a user interface element and then interacting with a new user interface element caused by the double clicking [1]. This concept formed the basis for the Internet advertising business, a multi-billion dollar industry.

Our firm was contacted by an inventor who had discovered the mechanism that enables the human immune system to seek out and destroy tumors or infectious diseases, wherever they reside in the body and were also capable of disabling the mechanisms that tumor and viruses use to evade an immune attack. While control of the immune system to treat tumors and infectious diseases has been the dream of scientists and physicians for many years, it has not turned into a reality of successful products in the clinic. This inventor came with a product and data that supported that the product indeed was capable of stimulating the immune system to kill tumors of animals and humans. We recognized that it was not just the product that was important to protect, but also the mechanism the inventor discovered that enabled him to design the first effective immunotherapy product. We also recognized that the knowledge of the mechanism could lead to the development of a wide variety of products aimed to control the immune system for treatment of a wide variety of diseases.

The challenge we faced was to develop a patent portfolio that protected not only the current product, but also the fundamental mechanism and parts and/or steps within the mechanism that enabled the product to work where no others had worked in the past. It was clear that this mechanism was so novel and so fundamental that many new products could be derived from the knowledge of this mechanism and that it had the potential to treat many diseases. We believed that like the "double-click" invention, the mechanism presented to us could create a new industry.

A strong patent portfolio is increasingly viewed as a critical asset for creating and maintaining a successful business, especially a technology based business. Patents are important tools for protecting a company's investment in research. They are also essential for protection against competitors trying to establish a market presence in the same or similar arena and siphoning potential revenue.

Patents can have patent claims directed to a product such as a pharmaceutical or a composition such as an intermediary product useful to produce the pharmaceutical. Patent claims may also be directed to a method of making a pharmaceutical, a method of treating a patient, and a method of making an intermediary product. These are but a few examples. Patent claims to a pharmaceutical or to a composition are often perceived as the most desirable since the infringement of such patent claims is more easily detectable in the marketplace. Method claims can also be very useful in many situations. A successful patent portfolio will contain a variety of such patent claim types.

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A successful patent strategy also includes patent claims of different scopes. Such patent claims should, if possible, also overlap in scope. The scope of a patent claim relates to how broad or specific the patent claim describes the invention.

Here, we describe a patent strategy based on the discovery of a revolutionary biological mechanism related to controlling/directing the immune system. We have employed a comprehensive patent strategy in translating the discovery of this biological mechanism into a patent portfolio that includes a variety of claim types and scopes for composition, pharmaceutical and method claims. These patent claims encompass within their scope, a whole new area in the field of immune cell therapy, especially cancer therapy. We initially focused on the main products and methods resulting from elucidation of this mechanism. Subsequently, we have prosecuted claims that are related to obtaining, generating and using the products as well as claims to phenomenon that are encompassed by the different steps within the elucidated mechanism. This has resulted in a number of patents being granted for products, methods of making such products, methods of treatment, methods of obtaining the specific effects of the treatment. These are all useful for the control/direction of the immune system for disease therapy.

The prevalence of cancer in society, the limitations of the current approaches, the discovery of a novel biological mechanism and the technology resulting from this discovery are discussed below. A discussion related specifically to the patents and patent strategy used is also included.

SCOPE OF CANCER

Over recent decades, the global incidence of cancer has escalated to epidemic proportions, now striking nearly one in two men (44%) and more than one in three women (39%). In 2008, cancer claimed the lives of about 7.6 million people in the world. The World Health Organization estimates that the number of cancer cases will increase to 13.1 million by 2030 because of growth and aging of the population [2].

Despite decades of research and new treatment approaches, reversal in overall mortality rates has been minimal and due largely to a reduction in lung cancer deaths from reduced smoking in men, rather than to advances in treatment. Preventive programs such as smoking cessation programs and early detection methodology have improved the incidence and mortality from cancer but United States national expenditures on cancer continue to rise and could potentially exceed overall medical expenditures combined [3]. The monetary costs for treating cancer are staggering. The cost for cancer treatment in the United States is estimated by the National Institutes of Health (NIH) in 2007 to include $103 billion in direct medical costs. Additionally, indirect costs from loss of wages, taxes, earnings and productivity were estimated at $123 billion in 2007 [4].

The increasing evidence of a link between obesity and cancer is extremely troubling given the current rise in obesity rates. Poor diet, physical inactivity, and overweight/obesity may account for about 25-30 percent of several of the major cancers in the United States [3].

Despite dramatic advances in our understanding of cancer cell biology, conventional cancer therapy has remained fundamentally unchanged for decades. The current therapies for cancer have such devastating emotional and physical side-effects that many patients view the treatments to be worse than the disease. The three major forms of cancer therapy remain to be surgery, radiation and chemotherapy. Surgery and radiation therapies have reached their logical limits, and chemotherapy remains the current mainstay of cancer management.

Most chemotherapy drugs are broad-spectrum cytotoxic agents. These drugs are designed to inflict greater damage on cancer cells than on normal cells. Nonetheless, all chemotherapy drugs affect normal cells and cause severe side effects. Chemotherapy is an attempt to kill the tumor before the drug kills the patient. While response rates to highly toxic chemotherapy have improved over the last two decades, the modest increase in 5-year survival has come at a severe price in terms of quality of life [5].

The major limitation of all current cancer therapies is the inability to eliminate the last tumor cell. This means that current cancer therapies, for the most part, can only extend survival but rarely can actually cure the disease. While current therapies can often initially eradicate measurable evidence of disease, they generally fail to eliminate all the tumor cells. Therefore, any remaining cells may proliferate and cause a relapse of cancer. In this common scenario, the first sets of remaining cells have resisted chemotherapy/radiation. The offspring of these tumor cells that were not destroyed by the chemotherapy/radiation have a selective advantage, leaving the person with a recurrence of cancer that is often widespread and resistant to chemotherapy/radiation and other techniques.

IMMUNOTHERAPY

Immunotherapy is a new modality for cancer treatment that holds great promise for becoming a curative therapy with minimal toxicity. The human immune system is capable of seeking out and destroying cancer cells wherever they reside in the body. Harnessing the power of the immune system holds the greatest potential for winning the battle against cancer.

The most promising approach for developing curative therapies that eliminate the last tumor cell is through vaccination against the tumor. The immune system, if properly stimulated and educated, is capable of eliminating every last tumor cell. The concept of vaccine immunotherapy against cancer is based on the body's natural defense system, which protects against a variety of diseases. Vaccine immunotherapy has proven to be one of the most effective treatment strategies for prevention of certain infectious diseases. Vaccines using killed or attenuated pathogens have revolutionized public health by preventing the development of many important infectious diseases, including poliomyelitis, small pox, diphtheria, rabies, typhoid, cholera, plague, measles, mumps, hepatitis B, diphtheria toxin and tetanus. Application of these vaccination concepts to cancer could similarly revolutionize cancer treatment.

However, attempts to develop cancer vaccines for treatment of existing tumors have proven to be much more difficult than developing vaccines for prevention of infectious diseases. Attempts to develop immunotherapies such as cancer vaccines, despite many decades of experimental work, have yet to consistently reach their curative potential in human clinical trials.

The one exception to the failure of immunotherapy protocols to produce significant anti-tumor effects in patients is the immune response that occurs in patients that undergo allogeneic bone marrow/stem cell transplant procedures [6, 7]. Allogeneic bone marrow transplantation (BMT) is a proven curative therapy for hematological malignancy and also has shown application for the treatment of solid tumors. BMT is a procedure in which a person receives stem cells (cells from which all blood cells develop) from a genetically similar, but not identical donor. In allogeneic BMT procedures, the transplanted immune system from a normal donor is capable of mediating a powerful anti-tumor mechanism called the graft-vs-tumor (GVT) effect (sometimes referred to as the graft vs. malignancy effect (GVM) or graft vs. leukemia (GVL) effect).

The powerful GVT effect is mediated by T-cells that originate from the donor. The GVT effect is the most powerful and most effective anti-tumor mechanism ever observed in the treatment of human malignancy. However, the same immune cells (primarily T-cells) which mediate GVT also mediate a serious and often lethal side effect known as graft vs. host disease (GVHD). This is due to the fact that the transplanted immune T-cells recognize both normal and tumor cells as foreign and mount attacks against both indiscriminately. Despite the curative effect of BMT, the severe treatment related toxicity has prevented the wide spread application of allogeneic BMT for cancer treatment. The powerful GVT immune response observed in BMT procedures is capable of overcoming tumor immunoavoidance mechanisms, resulting in complete systemic eradication of cancer in many instances. This GVT effect has been shown to be capable of curing patients with large tumor burdens, including patients with tumors unresponsive to chemotherapy, radiation and other forms of immunotherapy.

Therefore, one of the important therapeutic goals has been to develop methods to preserve the beneficial GVT effect while eliminating the detrimental GVHD toxicity [8]. Separation of the GVT/GVHD effect is difficult because the effects are interrelated, both mediated by donor T-cells and both mediated by similar mechanisms [9-12].

IMMUNE CELLS

Products and methods using T-cells of the immune system are currently an exciting and promising area for developing cancer therapies. T-Cells are part of the adaptive immune system of humans. T Cells or T lymphocytes belong to a group of white blood cells known as lymphocytes, and play a central role in cell-mediated immunity. They can be distinguished from other lymphocyte types, such as B cells and natural killer cells (NK cells) by the presence of a special receptor on their cell surface called T cell receptors (TCR). Several different subsets of T cells are known, each with a distinct function.

T Helper cells (Th cells) are a subset of T-cells that assist other white blood cells in immunologic processes, including maturation of B cells into plasma cells and memory B cells, and activation of cytotoxic T cells and macrophages, among other functions. These T helper cells are also known as $CD4^+$ T cells because they express the CD4 protein on their surface. Helper T cells become activated when they are presented with peptide antigens by MHC class II molecules that are expressed on the surface of Antigen Presenting Cells (APCs). Once activated, they divide rapidly and secrete small proteins called cytokines that regulate or assist in the active immune response.

Memory T cells are another subset of antigen-specific T cells that persist long-term after an infection has resolved. They quickly expand to large numbers of effector T cells upon re-exposure to their cognate antigen, thus providing the immune system with "memory" against past infections. Memory cells may be either $CD4^+$ or $CD8^+$.

TH1/TH2 RESPONSE

Immune responses are generally described by two polarized responses, the T-helper type 1 (Th1) and the T-helper type 2 (Th2). A Th1 response mediates cellular immunity and is critical for immune-mediated tumor eradication; a Th2 response mediates humoral immunity, the type of immunity that can protect against some infectious diseases, but is the "wrong" or inappropriate response to a tumor. Tumor-mediated deviation of T-helper cell differentiation to Th2 is a tumor strategy for immunoavoidance and tumor survival. In fact, an ineffective Th2 response can be detected in most patients with advanced cancer and metastatic disease. This explains why simply boosting the immune response in cancer patient's fails, as this only serves to enhance a resident Th2 response that has already failed to protect against tumor formation and is incapable of eradicating cancer.

Th1 and Th2 responses are counter-regulatory, increased Th1 responses down regulate Th2 responses and vice versa [13]. Therefore, a successful cancer therapy would avoid enhancing an existing "wrong" Th2 response, and would create, *de novo*, an excessive Th1 "right" response to the tumor sufficient to downregulate the Th2 response. The Th1 response must be created against an overwhelming tumor mediated resident Th2 response and natural mechanisms that suppress autoimmune destruction of normal tissues.

DEVELOPING THE MIRROR EFFECT™

The Mirror Effect™ addresses the problem of separating the beneficial GVT effect from the detrimental GVH effect of BMT procedures [14]. The solution is to develop a T-cell infusion that will mirror the mechanisms mediated by the transplanted donor immune system to instead be mediated by the host immune system. This concept is called the Mirror Effect™ and it is designed to provide the same anti-tumor effect that has been proven to be curative in allogeneic BMT procedures without the lethal toxicity.

In the Mirror Effect™, the direction of the intimately related GVT/GVHD effects originating in the graft are reversed or "mirrored" so that they instead originate from the host mediating host vs. tumor (HVT) effects coupled to a

non-toxic host vs. graft (HVG) rejection effect. To generate the Mirror Effect™, T-cells from a normal donor are infused into a patient and instead of these foreign cells mediating the GVT effect, these cells instead stimulate the patient's own immune system to attack the tumor (HVT effect) and create a highly inflammatory environment which disables tumor immunoavoidance mechanisms.

Since the HVT effect requires coupling to a HVG rejection, the patient in these cases is not preconditioned by an immunosuppressive chemotherapy regimen and therefore, has an intact immune system to mount a response to reject the normal donor T-cells. Further, since rejection of the graft (HVG) is a desired outcome, it is preferred that the donor cells be mismatched to the recipient. This is an important improvement to allogeneic BMT procedures where only 1 of 3 patients has a related donor suitable for the procedure.

The Mirror Effect™ thus involves infusion of intentionally mismatched normal donor cells into a cancer patient that has not received any chemotherapy pre-conditioning so that the infused allogeneic donor cells are rejected by the host (HVG). The HVG effect is the "mirror" of the GVHD effect, but is not toxic. The HVG rejection provides an adjuvant effect to initiate a host-mediated tumor destruction response (HVT), which is the "mirror" of the GVT effect. Thus, the "mirror" effect is capable of preserving the beneficial GVT effect of allogeneic BMT while eliminating the devastating GVHD toxicity.

TYPE I CYTOKINE STORM

The reason that the GVT effect is such a powerful immune mechanism for curing cancers is that the interaction between the host and donor creates the release of an array of inflammatory cytokines that signal the body of an imminent danger. These danger signals shut down the ability of the tumor to avoid an immune attack and enable immune-mediated killing of tumors disseminated throughout the body. The Mirror Effect™ creates these same danger signals in the context of a rejection response to a foreign cell infusion (HVG) rather than as an attack against normal tissues (GVH). Thus, the Mirror Effect™ has the potential to cause a proven curative anti-tumor effect of BMT without the extremely toxic side-effects. This represents a new concept in the treatment of cancer.

Tumors actively produce Type 2 cytokines as a strategy to avoid immune elimination [15]. Therefore, switching the cytokine environment in cancer patients from Type 2 domination to Type 1 is an essential requirement for eliciting anti-tumor immunity [16]. GVHD causes a "cytokine storm" of endogenous Type 1 cytokines that serve to adjuvant an anti-tumor immune response by suppressing Type 2 immunity, promoting development of protecting Type 1 immunity, as well as down regulating a variety of other tumor immunoavoidance mechanisms [17-19]. A HVG response also can cause a "cytokine storm" of endogenous Type I cytokines and the effects engendered by the cytokine storm can also be seen in a HVG response [20, 21].

ACTIVATED ALLOGENEIC T-CELLS

Critical to eliciting the Mirror Effect™ effect in a patient is the use of activated allogeneic T-cells that produce interferon-gamma and express high density CD40L on the cell surface. To assure that allogeneic T-cells from healthy donors express these properties when infused in a patient, they must be first differentiated *ex vivo* to develop Th1-like properties and then be activated just prior to being administered to a patient that has not been immunosuppressed. Activation of T-cells occurs by engagement of cell surface proteins, i.e. CD3 and CD28, on the T-cells. Binding of the CD3 protein and costimulation of the CD28 protein on the $CD4^+$ T-cells is required for initiating an effective T-cell mediated immune response. In particular, cross-linking of the CD3 and CD28 surface molecules is critical to activating the $CD4^+$ T-cells [22, 23]. Although activation of T-cells during proliferation is well known in the art, the methods of generating T-cells that exhibit enhanced activation properties are unique and have been patented [24] and result in T-cell compositions with unique characteristics that have also been patented.

When the activated allogeneic T-cells are administered to a non-immunosuppressed patient, the patient's immune system is able to mount an attack against the donor allogeneic T-cells in a milieu that also induces a Type I cytokine storm by the patient's immune system. The effect of the Type I cytokine storm is multifactorial. The Type I cytokine storm enables the patient's immune system to develop a de novo Th1 response against the tumors or tumor antigens. The Type I cytokine storm diminishes the Th2 cells and promotes the development of a Th1 environment. Furthermore, it also overcomes the immunoavoidance mechanisms promoted by the tumors.

Allogeneic cell therapy is dependent on the patient having a robust immune system because the desired Mirror Effect™ is based on the rejection response elicited by the patient's immune system against the administered donor T-cells. The donor T-cells are not HLA-matched to the patient and it is preferable that the HLA-mismatch is maximized between donor T-cells and the patient. However, due to the powerful immune stimulatory effect of mis-matched alloantigens, even highly immunosuppressed patients are capable of mounting a rejection response. This feature makes the Mirror Effect™ widely applicable to even heavily pretreated advanced cancer patients.

The novel activated T-cells developed at Immunovative Therapies, Ltd. (Immunovative) have one or more agents, i.e. anti-CD3 and anti-CD28 antibodies, bound to the T-cell surface proteins and these bound agents are in turn, cross-linked by the use of a cross-linking agent. The cross-linking agent can be attached to a support [25, 26]. Figure **1** schematically illustrates the activation of the T-cells used at Immunovative. In the Immunovative activation method, as shown in Fig. (**1**), a support bead is coated with a cross-linking agent that cross-links one or more agents that can bind to T-cell surface proteins. T-Cells activated according to the Immunovative method exhibit enhanced activation characteristics that are different than T-cells activated by prior art methods Fig. (**2**) and elicit desired therapeutic immune responses in patients. For example, one of the enhanced activation characteristics of the allogeneic T-cells include eliciting a "type I cytokine storm" by the patient's immune system in response to administration of the activated allogeneic T-cells.

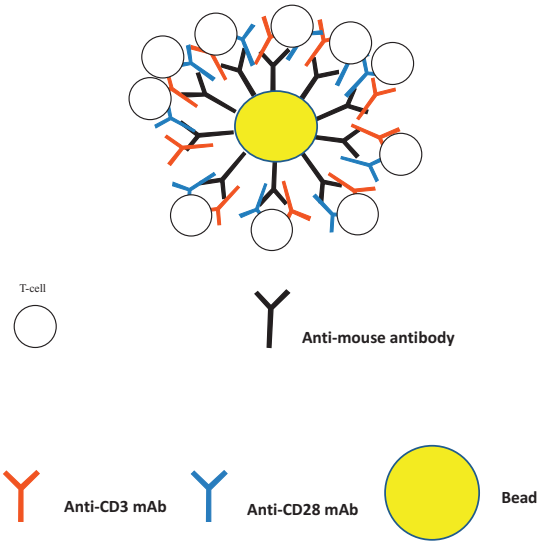

Fig. (1). Activation of T-cells.

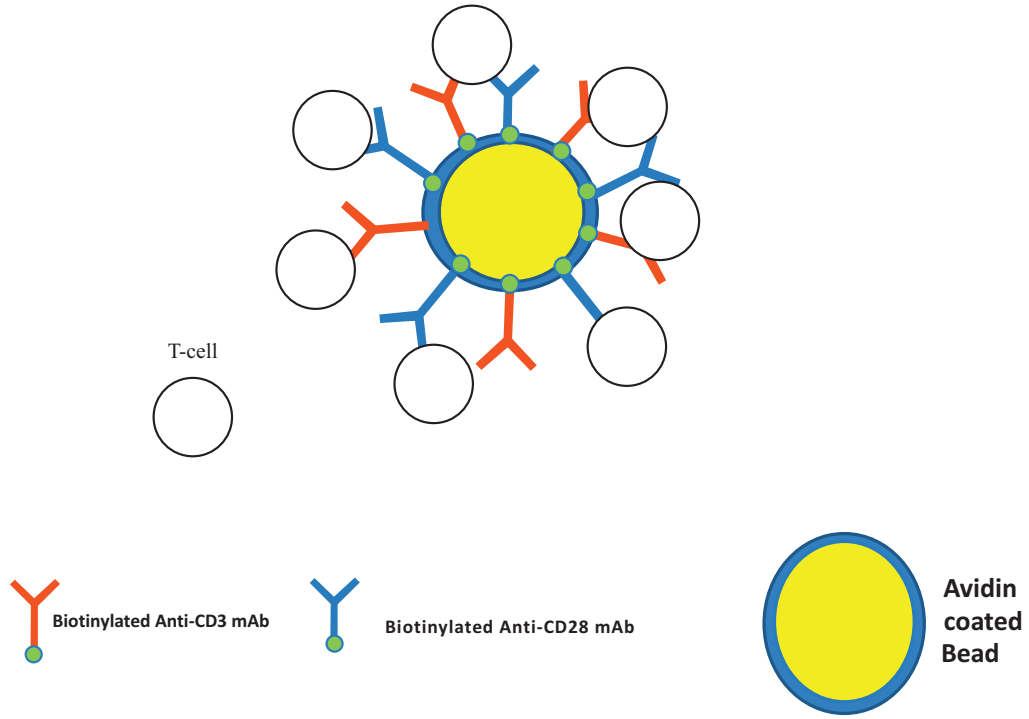

Fig. (2). Old method.

In order to stimulate the HVT effect, the allogeneic cells require active production of Type I cytokines upon administration to a patient and the upregulation of effector molecules such as CD40L. The cells can be administered intradermally as part of protocols designed to immunomodulate an existing Th2 immune response to a dominant Th1 immune response. The intradermal injection of the activated allogeneic cells causes the development of Th1 alloantigen-specific immunity. In this manner, patients become immune to the allogeneic cells. The allogeneic cells can also be delivered intratumorally into a tumor that has been destroyed by a process that releases internal contents of the tumors to the microenvironment, such as cryoablation, radiofrequency ablation or electroporation methods. The combination of intratumoral allogeneic cells with necrotic tumor creates an *in situ* anti-cancer vaccine which educates the immune system to systemically identify and eliminate tumors wherever they reside. The allogeneic cells can also be delivered intravenously to cause the extravasation of host immune cells from circulation to sites of inflammation, including tumors. The intravenous infusion also activates elements of both innate and adaptive immunity to mediate anti-tumor effects and create inflammatory environments to disable tumor immunoavoidance mechanisms.

Protocols can be designed to take advantage of these different properties of the activated allogeneic cells that combine intradermal, intratumoral and intravenous administrations to achieve the desired anti-tumor effect.

PRODUCTS AND USES OF ACTIVATED ALLOGENEIC T-CELLS

The therapeutic products resulting from development of the Mirror Effect™ concept and the activated allogeneic cells are T-Stim™, an intermediate product that is produced *ex vivo* and stored long-term in liquid nitrogen; AlloStim™, which is activated T-Stim™ formulated for infusion; CryoStim™, which is the combination of cryoablation (or other ablation methods) and intratumoral AlloStim™ to create an *in situ* vaccine; and AlloVax™, which is the combination of enriched chaperone proteins derived from a tumor sample combined with AlloStim™ to create an anti-tumor vaccine. Table **1** lists the patents that form the basis of these specific products.

The majority of the discussion herein will focus on two products T-Stim™ and AlloStim™. T-Stim™ and AlloStim™ are products of two sequential production processes. In the first production process, blood from normal donors is collected and manipulated to form T-Stim™ in an *ex vivo* culture process that occurs in a bioreactor taking up to 9 days or longer [25]. The T-Stim™ production process involves: (1) purifying precursor immune cells from the blood (from leukapheresis or buffy coat source material) of normal screened donors; (2) culturing the precursors in the presence of custom monoclonal antibodies (mAbs) conjugated to a biodegradable tissue-like matrix simulating a lymph node (the mAbs provide maturation signals to the precursor cells and cause them to expand and acquire the beneficial immunomodulatory and stimulatory characteristics of T-Stim™ cells); (3) aliquoting the resulting cultured T-Stim™ cells into single dosage forms; and (4) freezing the aliquoted T-Stim™ cells in liquid nitrogen for long-term inventory storage. One blood donor can produce enough T-Stim™ to treat up to 100 patients. T-Stim™ cells have the following phenotypic identity: $CD4^+$, $CD45RO^+$, $CD40L^{lo}$, $CD62L^{hi}$, $CD25^+$ which produce > 1500pg/10^6 cells/4h interferon-gamma and < 50pg/10^6 cells/4h of IL-4 (Th1 cells). The manufactured T-Stim™ is aliquoted into individual dosages and stored in liquid nitrogen. The frozen product can be distributed through hospital pharmacies.

In order to produce the Mirror Effect™ in a patient, the T-Stim™ cells require activation prior to infusion into the patient. The second production process involves converting T-Stim™ to AlloStim™. T-Stim™ cells are activated with monoclonal antibody-coated particles that may or may not be removed prior to infusion. When T-Stim™ cells are activated and formulated for infusion the product is called AlloStim™. The product AlloStim™ and method for producing AlloStim™ have been developed at Immunovative [27]. Specifically, AlloStim™ represents activation of the allogeneic T-Stim™ cells prior to infusion converting them to the following phenotypic identity: $CD4^+$, $CD45RO^+$, $CD40L^{lo}$, $CD62L^{hi}$, $CD25^+$ and actively producing inflammatory cytokines such as interferon-gamma, tumor necrosis factor-alpha and GM-CSF. The administration of AlloStim™ into a patient produces the Mirror Effect™ in the patient.

Table 1. Related Patents and Patent Publications [24-27], [30-46].

Group I
US Patent No. 7435592* [25]
US Patent No. 7943180 [26]
US Patent No. 8273377 [30]
US Patent Pub. No. 20110040259 [31]
US Patent Pub. No. 20110206707 [32]
US Patent Pub. No. 20110206708 [33]
Group II
US Patent No. 7592431 [34]
US Patent No. 7678572* [24]
US Patent No. 8012750 [35]
US Patent No. 7956164 [36]
US Patent No. 8071374 [37]
US Patent No. 8313944 [38]
Group III
US Patent No. 7402431* [27]
US Patent No. 8076135 [39]
US Patent Pub. No. 20110033435 [40]
US Patent Pub. No. 20110028912 [41]
Second generation patent and publications
US Patent No. 7972594 [42]
US Patent Pub. No. 20110229502 [43]
US Patent Pub. No. 20060115487 [44]
US Patent Pub. No. 20120128656 [45]
US Patent Pub. No. 20100086561 [46]
US Patent Pub. No. 20110142887 [47]
US Patent Pub. No. 20110250173 [48]
US Patent Pub. No. 20120045423 [49]

AlloStim™ is formulated in syringes and is packaged and shipped to points of care. AlloStim™ remains stable in formulation at 2-8°C for at least 72h, enabling the drug to be delivered by overnight courier from a central formulation facility to points of care in most of the world.

AlloStim™ can be used alone therapeutically or in conjunction with other methods or agents. Cryoablation of the tumor or other techniques that cause necrotic death and release of tumor antigens can be used in conjunction with the use of AlloStim™. Necrosis of tumors results in release of tumor antigens at the site of the ablation. After cryoablation, AlloStim™ is administered at the site of necrosis. This method causes the released tumor antigens to be processed

by APC, such as dendritic cells, in a highly inflammatory environment that causes the dendritic cells to mature and express IL-12. IL-12 is a key cytokine for mediating and directing a Th1 anti-tumor immune response. Further, IL-12 can be monitored as a marker which indicates successful activation of the immune response. IL-12 expression also correlates to increased survival in advanced cancer patients treated with AlloStim™.

CryoStim™ relates to the use of AlloStim™ in conjunction with cryoablation in a patient with a solid tumor. CryoStim™ is a novel *in situ* therapeutic cancer vaccine for patients with disseminated metastatic tumors. CryoStim™ combines AlloStim™ with cryosurgical ablation of a single tumor, stimulating a tumor-specific immune response that eliminates tumors wherever they are located throughout the body.

Patients are first infused with a dose of AlloStim™. The rejection of the allogeneic mis-matched AlloStim™ cells results in the patient being immune to the drug. Immune patients then undergo a procedure to freeze an accessible tumor. This causes the tumor to die by a process of necrosis, a pathological mechanism that results in an immune response to cleanup and repair the damaged tissue. Additional AlloStim™ is injected into the necrotic tumor lesion resulting in a memory recall immune response to reject the allogeneic cells. The combination of these two powerful immune responses results in the education of the immune system to perceive the dead tumor tissue as a danger.

AlloStim™ is also critical to the development of AlloVax™ that is a customized personal vaccine product in which the disease antigens of a patient are administered along with AlloStim™. AlloVax™ is an individualized anticancer vaccine that is designed to educate the immune system during the period of cancer remission to prevent the tumor from recurring. Chaperone proteins isolated from cancer cells have been shown to carry hidden tumor antigens that can be used to educate the immune system to identify and kill the cancer cells from which they were derived [28].

The AlloVax™ treatment protocol involves first removing a sample of the cancer cells from the patient prior to chemotherapy/radiation therapy. After the patient is in remission, the patient receives intradermal injections of AlloStim™ which are rejected by the patient immune system making the patient immune to the AlloStim™ cells. The cancer sample obtained prior to chemotherapy/radiation treatment is then processed in the laboratory to isolate the chaperone proteins containing the unique tumor antigens. The chaperone protein sample is then combined with AlloStim™ and injected intradermally. In this setting, AlloStim™ acts as an adjuvant for the chaperone protein vaccine formulation. This causes an immune response to reject the AlloStim™ and an immune response against the unique tumor antigens carried by the chaperone proteins. The combination of these immune responses serves to educate the immune system that the tumor antigens are a danger to the body. In this manner, if there is a recurrence of the tumor, the immune system is primed to destroy the tumor without any further treatment in order to keep the patient in remission.

AlloStim™ can also be used against opportunistic infections caused by pathogens such as *Aspergillus* that are prevalent in patients after BMT. An effective immunotherapy against opportunistic infections in the post-engraftment BMT setting first stimulates innate immunity and then induces a fungus-specific (or other pathogen-specific) Th1 adaptive immune response against a background of immunosuppressive drugs, as well as existing and imprinted Th2 skewed immunity to the offending pathogen. An additional challenge is the need to elicit Th1 anti-fungal (or other pathogen-specific) immunity in this background without concurrent stimulation of Th1-mediated GVHD. The infusion of HLA-mismatched allogeneic cells (AlloStim™) into an immunocompromised host elicits a strong host allorecognition response capable of triggering a cascade of events that are beneficial in treating these opportunistic infections mediated in part by activation of innate immunity.

TUMOR IMMUNOAVOIDANCE

Tumors have numerous active mechanisms to suppress host Th1 immunity, including altering the function of antigen presenting cells (APCs), fostering dysfunctional T-cell co-signaling, generating an immune-subversive Th2 cytokine milieu, downregulating HLA molecules, and recruiting tolerogenic dendritic cells (DCs) and myeloid suppressor cells. Many of the mechanisms that impede anti-tumor immunity result in the development of T regulatory (Treg) cells. These Treg cells are the dominant immune escape mechanism in early tumor progression. In addition, most human tumor cells produce high levels of transforming growth factor (TGF)-β that can directly convert naïve T cells to Treg cells. TGF-β also suppresses the transcription of genes encoding multiple key proteins of $CD8^+$ cytotoxic T-cells (CTL), such as the cytolytic molecules perforin and granzymes. Tumor-mediated immunoavoidance shifts and promotes the immune system to an ineffective Th2 response which renders it tolerant, permitting tumors to grow unimpeded by the surveillance mechanisms.

Immune tolerance of disease antigens and/or disabling tumor immunoavoidance mechanisms in a patient can be suppressed by activated allogeneic cells [29]. These cells can stimulate immunity against the disease antigens, in particular Th1 immunity. Administration of disease antigens and allogeneic, activated Th1 cells as adjuvant can lead to suppression of Treg cell activity. The activated, allogeneic Th1 cells are effector/memory Th1 (emTh-1) cells. A mixture of disease related antigens and chaperone proteins isolated from diseased tissue, such as Chaperone Rich Cell Lysate (CRCL) are also effective in this context. This is the composition called AlloVax™.

The suppression of immune tolerance includes impairing the suppressive activity of Treg cells by inhibiting the conversion of naïve T-cells ($CD4^+CD25^-FoxP3^-$) to Treg cells ($CD4^+CD25^+FoxP3^+$), referred to herein as iTreg. The suppressive activity of both naturally occurring Treg (nTreg) cells and iTreg cells can be reduced or eliminated by the activated allogeneic Th-1 cells (AlloStim™). In addition, the immunosuppressive activity of Treg is inhibited while simultaneously promoting the function of conventional effector T lymphocytes in the patient. The inhibitory activity on Treg is

dependent on IFN-γ, which in preferred compositions is produced by the AlloStim™ cells, which in turn increase the production of IFN-γ from host innate and adaptive immune cells.

CYTOLYTIC T-HELPER CELLS

Activation of the $CD4^+$ cells results in T-cells with unique characteristics. Cytolytic activity is generally carried out by natural killer (NK) cells and/or $CD8^+$ cytotoxic T-lymphocytes (CTL). T-helper cells do not directly exhibit cytolytic activity but produce cytokines and other factors that then elicit other cells, such as NK cells and CTLs to destroy the diseased cells. The T-Stim™ cells are unique in that they exhibit both Th1 helper cell activity and cytolytic cell activity, expressing the cytolytic molecules granzyme B and perforin.

The activated $CD4^+$ T-cells (AlloStim™) can directly inactivate the diseased cells as well as produce cytokines to stimulate other cells, such as NK cells, to carry out their cytolytic activity. The cytolytic activity of these AlloStim™ cells is mediated through granzyme B and perforin resulting in the killing of the tumor or infected cells.

DETECTION OF IL-12

IL-12 is a heterodimer composed of a p35 and a p40 subunit. It is produced primarily by Antigen Presenting Cells (APC). IL-12 can also be produced by monocytes and macrophages, dendritic cells and B-cells. IL-12 exerts immunomodulatory effects on T-cells and natural killer cells. Endogenous IL-12 is known to be involved in generating optimal Th1 responses and can play an important role in cell-mediated immunity against intracellular pathogens. Generally, IL-12 is not found in cancer patients because tumors can inhibit expression of IL-12.

IL-12 has been the subject of intense investigation because it modulates important components of the immune system and has been demonstrated to have dramatic anti-tumor effects in the laboratory and in animal studies. IL-12 has been implicated, for example, in inhibiting growth of human lung adenocarcinoma and acute myeloid leukemia. However, the use of exogenous IL-12 in a therapeutic regimen has been limited by high toxicity in humans.

Administration of activated AlloStim™ cells can lead to the production of detectable levels of endogenous IL-12 in the patient's plasma, without any significant toxicity. T-cells are not capable of producing IL-12, therefore the T-cell composition administered to the patient elicits the production of IL-12 by the patient's own APC. Administration of the activated, allogeneic T-cells can overcome the inhibition promoted by a tumor and create an environment sufficient to induce expression of IL-12 for extended periods of time, for example, several months or even a year. Furthermore, the presence of endogenous IL-12 in plasma does not lead to significant toxicity in the patient as does the administration of exogenous IL-12 as a medicant.

BENEFITS OF ALLOGENEIC CELL THERAPY

Allogeneic cell therapy with living immune cells in the form of activated T-cells can be used to treat a wide range of diseases. One of the most important benefits of the use of AlloStim™ is the lack of severe side effects in the patient. Therapies using AlloStim™ are extremely well-tolerated by patients. Generally, the patient exhibits flu-like symptoms. These symptoms occur as a result of the patient's immune system reacting against the administered donor allogeneic T-cells.

PATENT STRATEGY

The focus of the patent strategy has been to translate the elucidation of a fundamental mechanism such as the Mirror Effect™ into marketable assets that can potentially define a whole industry. These assets include patents as well as products. To carry out this patent strategy, we used a multi-pronged, overlapping claim strategy that lends itself to protecting a potential industry, instead of a single product.

The overall claim strategy was to procure claims focused on different phenomenon encompassed within and related to the Mirror Effect™. These include, for example, eliciting HVT and HVG, generation of Type I cytokines, cross-linking of CD3 and CD28 surface moleucles, methods of performing allogeneic cell therapy, stimulating immune systems, treating diseased patients, methods of generating T-cells, methods of formulating T-cells, methods of making devices for generating T-cells, methods of storing and transporting T-cells, etc. By focusing not only on the whole mechanism, but the many parts of the mechanism, we hope to protect a large segment of this new field.

The first generation of patents includes 3 groups or families of patents (Group I, II and III) [24-27, 30-46]. Group I relates to T-Stim™, Group III relates to AlloStim™ and Group II relates to devices for preparing T-Stim™ and AlloStim™. Appendix A lists the specific patents and patent publications that belong to each of the groups. Each group has a parent application with disclosure that provides support for all of the claims in the subsequent applications of the group. The parent applications of each group are indicated with an asterisk. The claims in the patents or patent applications of each group include composition claims, method claims and use (treatment) claims.

The disclosures in the parent applications relate to the fundamental discovery of the Mirror Effect™ and how the Mirror Effect™ can be harnessed into development of products that control and/or modulate the immune system. The patents obtained also include methods of using the products, making the products, devices used in making the products and methods of making the devices.

In obtaining patent protection in all of the above-stated areas, an initial strategy of narrow, overlapping claims was utilized. Once the narrower claims were successfully prosecuted, then broader, overlapping claims have been prosecuted. This was advantageous because we were assured of some patent protection that was beneficial in procuring

further investments. Furthermore, since the examiner was educated and familiar with the technology and the prior art, he was less apprehensive about allowing broader claims.

Initially, for example, the claims included language that the T-cells were activated by the use of anti-CD3 and anti-CD28 monoclonal antibodies. The latter claims, however, used language that the CD3 and CD38 cell surface molecules need to be cross-linked but do not limit how or what enables this cross-linking to occur. In other words, there is no limitation on how the cross-linking is performed. Of course, all of these claims (broad and narrow) relied on support in the original disclosures of the parent applications of Groups I, II and III patents.

Timing and cost were also relevant issues in determining a patent strategy. In order to be mindful of the costs related to pursuing patents, the parent applications were the sole pending applications in the United States until the Examiner indicated that there were allowable claims. It did not seem prudent to file multiple concurrently pending applications when there had been no indication of allowability over the prior art. This is particularly true for a start-up business that does not initially have extensive monetary resources. While we were confident regarding the ground-breaking nature of the mechanism elucidated, we were cautious with our expectations. However, great care and time was spent on ensuring that the disclosure of each parent application was thorough in order to provide support for future claims.

Initially, the claims related to offensive patent strategy, i.e., claims to products and methods that would be marketable. These claims generally relate to the main product or methods resulting from practicing the mechanism. The claim language generally included, for example, "A composition of T-cells …" and describes the composition contents. Additional divisional and continuation applications were filed once the Examiner had indicated that the parent applications had allowable claims.

Claims that were more defensive in nature have been pursued in the later applications. First, the defensive claims generally protect the steps or parts within an elucidated mechanism. Second, the defensive strategy discourages competitors from entering the field. Third, patents with claims that are broader and defensive in nature can also be prime candidates for sub-licensing. Sub-licensing can provide revenue, a key concern for any company. Sub-licensing can also allow other companies into the arena that provide supportive or complementary roles. They may provide products or processes that enhance the patented products or processes. They may also provide products or process that are different from but yet function in the same manner as the patented products or processes.

The claims in the patents for AlloStim™ demonstrate the advantages of using a defensive strategy. In the technology discussed herein, the mechanism for developing the Mirror Effect™ requires that a patient needs a source of alloantigens, needs components to signal dendritic cells (DC) to undergo maturation and needs a source of inflammatory cytokines. Claims have been obtained to T-cells that enable all of these functions. In order to solidify the advantages of being the first to discover this important mechanism, claims have also been designed to a medicament having the properties of: a DC maturation molecule, an alloantigen and an inflammatory cytokine. Currently, AlloStim™ is the product of choice to provide all of these components. However, we are pursuing the broader claims to prevent other companies from entering the field by developing technology that can carry out these functions without the use of AlloStim™ . Alternatively, if such technology is developed and is desirable, it is strategic to hold patents with the above-mentioned broader claims so that the company with the new technology must sub-license in order to legally market its products.

The initial groups of patents have been followed by a second generation of patents and patent applications. The second generation of patents includes additional methods and compositions that can be used in conjunction with the methods and compositions of the claims in Groups I, II and III. They include novel uses of T-Stim™ and Allo-Stim™. Patent applications have been crafted that relate to scaling up for production and also to methods for conducting a business using these novel products. As further study on these products has continued, additional patent applications have been filed that include elucidation of details regarding the mechanism, the product and the processes.

CURRENT & FUTURE DEVELOPMENTS

Current treatment methods do not have the fine specificity to identify and eliminate microscopic tumor cells wherever they reside in the body. Only the human immune system has this feature. Thus, the ability to harness the power of the immune system to eliminate cancer has the potential to be curative. The compositions and methods involved in the Mirror Effect™ technology represent a break-through in our ability to both direct immune responses against tumors and dysregulate the ability of tumors to avoid the immune response.

The conception of the Mirror Effect™ and the discovery of a mechanism to engender the Mirror Effect™ have the potential to spawn a whole industry. The patent strategy used must translate these revolutionary discoveries into profitable, sustainable and marketable assets. We have and still continue to build a patent portfolio that captures the depth, breadth as well as the subtle features of harnessing the Mirror Effect™ to control and modulate the immune system in powerful ways to tackle some of the most complex diseases presently known. Thus the patent portfolio in Appendix A represents not only protection of a new product and method, but has broad implications for creating a new industry based on knowledge of controlling the immune system. This knowledge can lead to an eventual possible cure for currently incurable diseases. This makes the patent portfolio especially notable as it is a paradigm shift ushering in a new era of immune-based medicine.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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All human trials were conducted in accordance with international standards for Good Clinical Practices which includes obtaining informed consent. In addition, all animal studies were conducted according to the Guide for the Care and use of Laboratory Animals as published by the National Academy of Sciences as stated in our publications.

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