Financial Viability and Technical Evaluation of Dendritic Cell-Carrying "Vaccination Nodes" for Immunotherapy

by

Andrew Song

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ABSTRACT

Cancer immunotherapy attempts to stimulate the immune system to reject and destroy tumor cells. Despite the amount of ongoing intensive research to prevent cancer, tumor cells continue to evade immune responses. Currently, dendritic cell vaccines are in development, in which autologous antigen-loaded dendritic cells are injected back into the patient in order to generate an appropriate immune response. Improving upon this idea, members of the Irvine laboratory are in development of an injectable dendritic cell based formulation that gels in situ around the tumor site. In this way, immune cells (most notably T cells) can be recruited and become activated against specific tumor antigens, and (hopefully) kill tumor cells. Recent studies have shown the potential benefit of incorporation of cytokine interleukin-15 complexed with its soluble receptor interleukin-15Rα, which is discussed.

Economic considerations are also discussed, including topics such as intellectual property, barriers to entry, initial markets and market drivers, and entry into the current supply chain considerations. A business strategy is outlined and evaluated.

Thesis Supervisor: Darrell J. Irvine
Title: Eugene Bell Career Development Associate Professor of Tissue Engineering

ACKNOWLEDGEMENTS

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To my friends who supported me to come here, and to those that I met here along the journey. You have helped shape this experience into something that I will never forget, and moreover, you have helped shape me into a better person. Thank you all.

To my family: Mom, Dad, Steve, and Jen. You have equipped me with everything that I needed to succeed, and so much more. I could not have done this without your unconditional love and support. This thesis is dedicated to you.

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1 Introduction

In 2007, there were an estimated 1.4 million new cases of cancer in the United States alone [1]. Despite the application of increasingly intense chemotherapeutic and radiotherapeutic regimens, overall cancer cure rates have remained essentially unchanged for the past 30 years [2], as shown in the figure below:

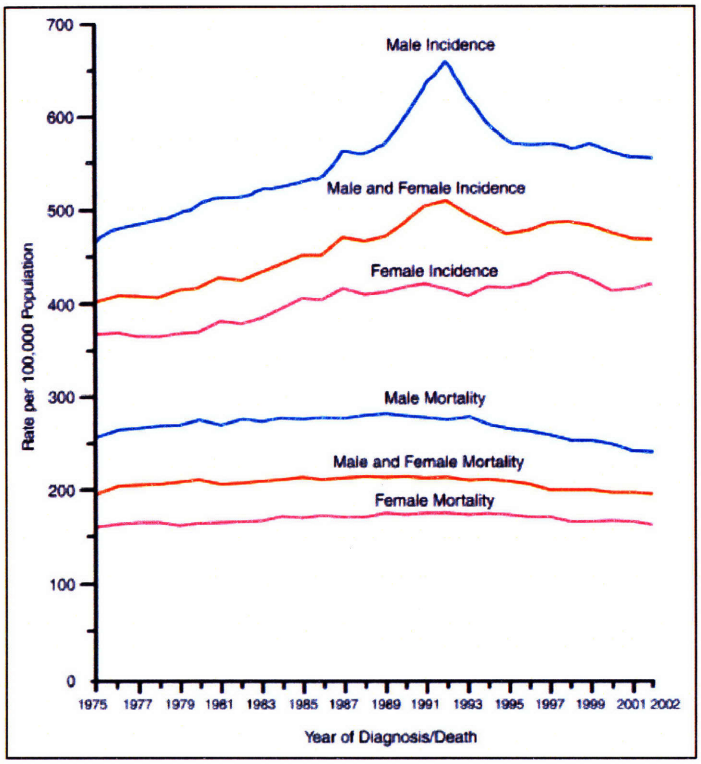

Figure 1: Annual age-adjusted cancer incidence and death rates for all sites, by sex, U.S. 1975 to 2002. Rates are age-adjusted to the 2000 U.S. standard population [2].

1.1 Focusing on Melanoma

Trying to conquer the issue of cancer all at once is quite overwhelming, since different cancers work through different mechanisms. Instead, in order to solve the problem, it is more feasible to try to understand the mechanisms of a particular cancer, and work to solve those problems first. This review will focus on one particular type of cancer: melanoma. Melanoma is a type of skin cancer that develops when melanocytes – the cells responsible for pigment in the skin – become malignant.

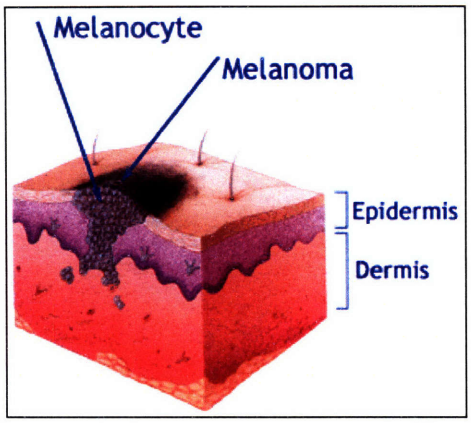

Figure 2: Sample schematic of melanoma in the skin [3]

While melanoma is not as common as other types of skin cancer (such as basal cell carcinoma and squamous cell carcinoma), it is by far the deadliest once it is contracted. To put some numbers to this, melanoma accounts for only 4 percent of skin cancer cases, but causes 79 percent of all skin-cancer related deaths [4]. Each year, an estimated $740 million is spent in the U.S. for treating melanoma [5]. There are five stages of melanoma (0-4), classified by severity.

Stage of Melanoma	Characteristics
0	Involves the epidermis but has not spread deeper into the underlying dermis. Tumor has not invaded the surrounding normal tissue, lymph nodes, or distant sites
1	Tumor thickness is less than 2.0mm. The primary tumor is localized (has not spread to nearby lymph nodes or distant sites), but may affect nearby tissue
2	Tumor is localized, but shows signs of affecting nearby tissue. There are three sub-stages, depending on the thickness and ulceration. Usually up to 4.0mm thick, but may be more
3	Characterized by lymph node metastasis, but no evidence of distant metastasis. May be anywhere from 1.0 to more than 4.0mm thick
4	Associated with metastasis beyond the regional lymph nodes to distant sites in the body, usually to vital organs (lungs, abdominal organs, brains, and bone) or soft tissues. May be any size

Table 1: Characteristics of melanoma by stage [6]

If detected early enough, early stage melanoma is removed surgically with very high success [6]. However, later stage melanoma is where current treatments are not successful. According to the National Cancer Institute, there will be an estimated 62,480 new cases of melanoma in 2008, and 8,420 deaths [7].

1.2 Conventional Treatment of Melanoma

Conventional treatment for melanoma includes surgery, chemotherapy, and radiation therapy. Surgery is the standard treatment for stage 0, 1, and 2 melanoma. As mentioned before, when detected early enough and before metastasis (spreading to other parts of the body), surgery is usually enough to rid the patient of the tumor and essentially "cure" the patient. Surgery is still used in later stages, however because the tumor has begun to spread, it is difficult to remove the entire tumor [7]. As a result, surgery is necessary, but not sufficient. Chemotherapy is used in stages 2, 3, and 4 melanoma, as an adjuvant to surgery. In localized melanoma, chemotherapy is used to further eradicate any parts of the tumor that were not removed from surgery. However, like surgery, chemotherapy has low efficacy in later stage melanoma where the tumor has metastasized, and there is currently no effective systemic chemotherapy treatment [4]. In addition, chemotherapy generally targets fast growing cells, which may not be specific to tumor cells. Harmful side effects may occur as a result of this. Radiation therapy is known to be ineffective in curing melanoma, however it is still used as palliative therapy for stage 4 patients [4].

While incidence rates may vary with time because of changes in detection technology as well as definitions, a fixed measure would be the mortality rate normalized to the population. As shown below, melanoma follows the general trend of cancers that the overall mortality rate has remained relatively unchanged over the past 30 years, and in fact has slowly increased.

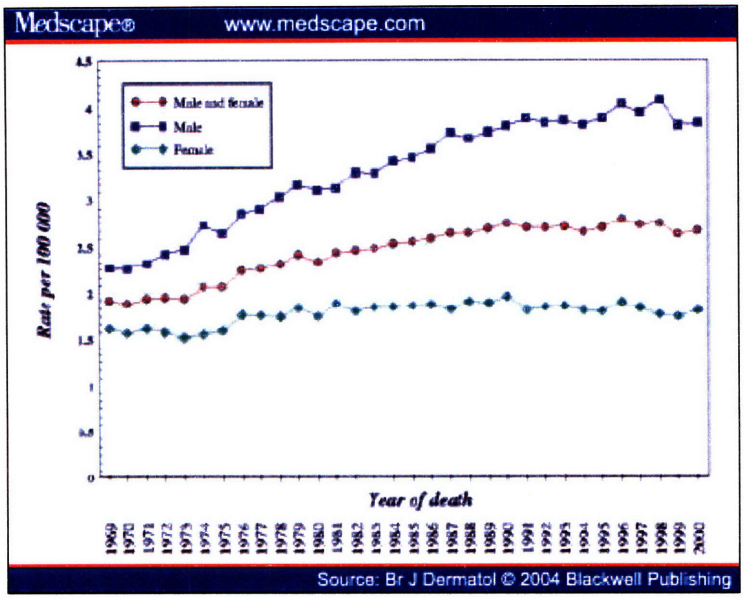

Figure 3: Age-adjusted (2000 U.S. standard population) melanoma mortality rates (per 100 000), total U.S.A., 1969-2000 (data from the SEER Program of the National Cancer Institute) [8].

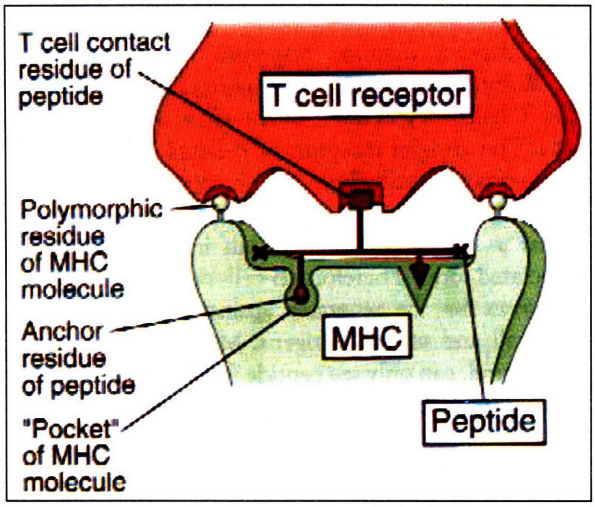

Figure 4: Model of how a T cell receptor recognizes a complex of a peptide antigen displayed by a major histocompatibility complex (MHC) molecule [10]

There are a number of subsets of T lymphocytes, and non-differentiated T lymphocytes are called naïve. However, the type that is thought to be of most importance in tumor regression are called cytolytic T lymphocytes (CTLs or CD8+ T cells) [10]. This is because they kill cells harboring intracellular microbes, and are the chief cells responsible for tumor eradication.

The majority of T lymphocytes do not recognize antigens (i.e. tumor antigens) themselves, and as mentioned before, must have them presented by what is known as an antigen presenting cell. APCs are specialized cells that capture microbial antigens and display them for recognition by T lymphocytes. Examples of APCs are dendritic cells, macrophages, and follicular dendritic cells. Furthermore, naïve T lymphocytes need to see antigens presented by "professional" APCs to initiate an appropriate response against protein antigens. The term "professional" in this sense refers to the ability of these cells to both display antigens for T cells and provide the costimulatory signals needed to activate naïve T cells. The hypothesized method of tumor eradication is that tumor cells are ingested by the professional APCs (usually by phagocytosis or pinocytosis), and the antigens of the tumor cells are processed and displayed by the host APC molecules. At the same time, professional APCs express costimulators that provide "second signals" for the activation of the T cells. This process is known as cross-presentation or cross-priming, because one cell type (the professional APC) presents antigens of another cell

(the tumor cell) and activates (or primes) T lymphocytes specific for the second cell type [10]. A diagram of this is shown in the figure below:

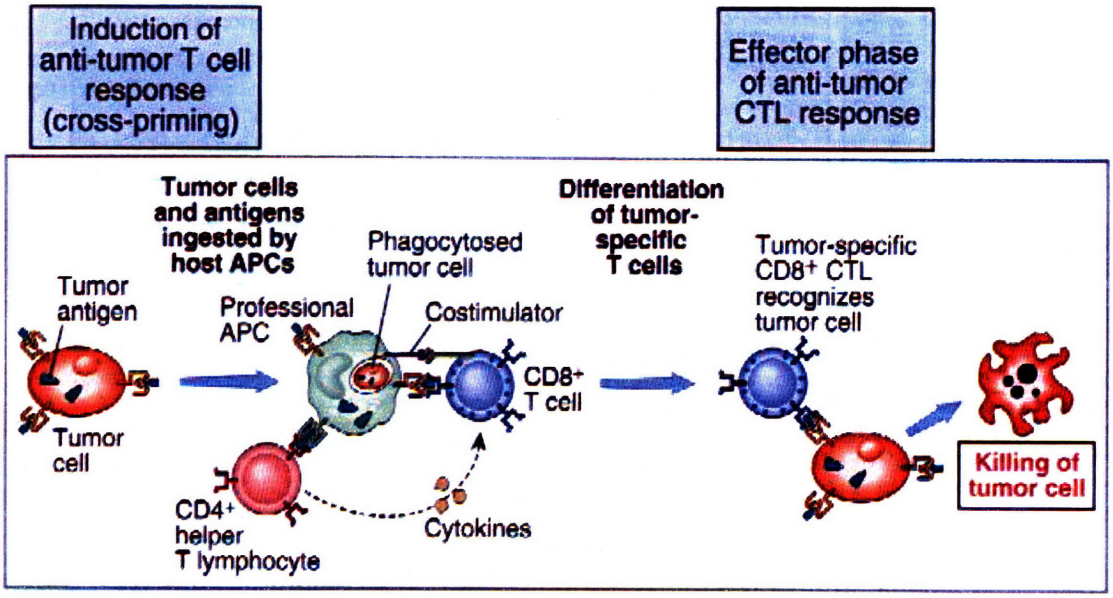

Figure 5: Diagram of tumor cell destruction by CTL [10]

2.2 Tumor Evasive Mechanisms

Unfortunately, tumors evade immune responses through a number of mechanisms. If the immune system is to be effective against malignant tumors, it should in principle kill all tumor cells, which can grow very rapidly. In many cases, the prolific growth simply outstrips immune defenses. Many tumor antigens are weakly immunogenic, perhaps because they only differ slightly from self antigens. Furthermore, emergent tumors also develop mechanisms for evading immune responses. A few of these mechanisms are diagramed below:

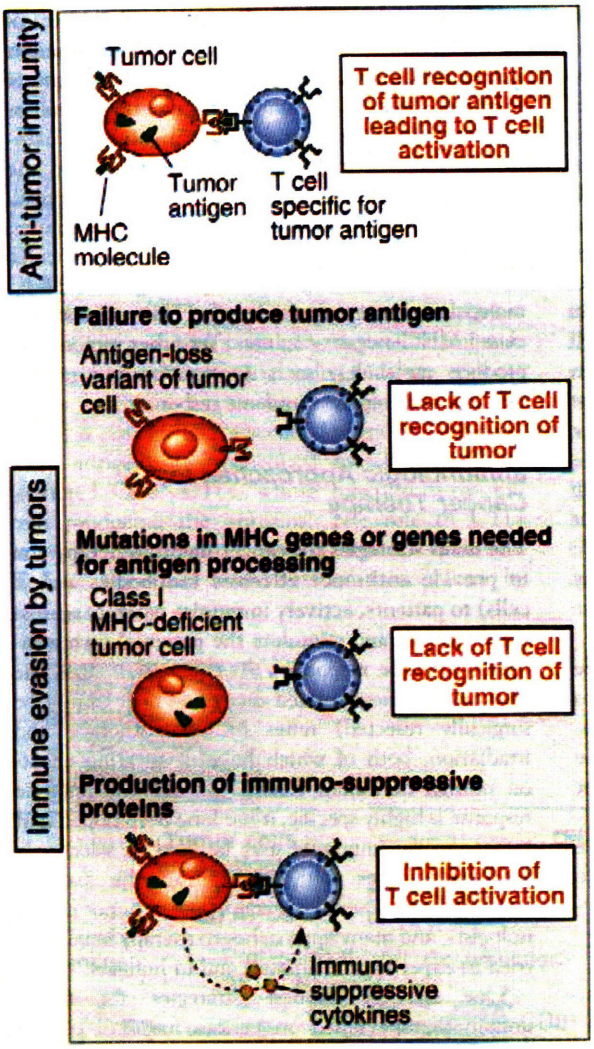

Figure 6: Tumor evasive mechanisms [10]

As shown above, some tumors stop expressing the antigens that are the targets of immune attack (these tumors are called "antigen loss variants"). Other tumors show mutations in the MHC genes or genes necessary for antigen processing, and thus the T cell cannot recognize the tumor cell. Another mechanism of tumor evasion is tumor production of immunosuppressive cytokines (signals), such as transforming growth factor-β, that suppress immune responses [10]. It is therefore unlikely that tumors themselves function as effective professional antigen presenting cells as the necessary step in the process of initiating a de novo a T cell response, priming of naïve T cells [4]. A complete understanding of all tumor evasive mechanisms is currently not available, and this critical

issue must be further pursued in order to maximize the efficiency in the design of tumor eradicating methods.

2.3 Interleukin-2 Immunological Treatment of Cancer

In spite of these tumor evasive mechanisms, there were still indications that immunological manipulations could cause the regression of established, invasive tumors in humans. The first clear indication came from the studies in which humans with metastatic kidney cancer or melanoma were administered a cytokine called interleukin-2 (IL-2) [11]. IL-2 is a cytokine produced by human T-helper lymphocytes, and plays a role in a large number of immune regulatory effects, including the expansion of lymphocytes following activation by a specific antigen. Cancer cells can grow unimpeded in vitro even in high concentrations of IL-2, showing that IL-2 has no direct impact on cancer cells themselves. Thus, the impact that IL-2 brings in destroying cancer cells in vivo is derived from its ability to expand lymphocytes with anti-tumor activity [11]. Combined with other initial studies, high-dose recombinant IL-2 showed regression of even bulky, invasive tumors in selected cancer patients with metastatic melanoma, kidney cancer, and non-Hodgkin's lymphoma in a significant manner [9,11]. Different studies showed similar results, with about 15-20% of patients with metastatic melanoma or kidney cancer achieving a full or partial regression [11-13]. Of those that completely responded, one study showed that with a median follow-up of 7.1 years, 82% of these patients remained in continuous, ongoing, complete regression from three to over twelve years from the onset of treatment [12]. In addition to showing the effectiveness and promise of IL-2, these studies showed that a relatively simple immunological manipulation could have a large impact on tumor regression from a variety of cancers. Furthermore, this spurred intensive efforts to understand, at a molecular level, these complex immunological anti-tumor events [9].

3 Dendritic Cell-based "Vaccination Nodes"

Increasing information highlighted the significance of professional antigen-presenting cells in generating immune responses in humans. Because of this, there was the desire of looking further into antigen presenting cells. Dendritic cells (DCs) are known to be the key antigen presenting cells involved in priming naïve T cells during primary immune responses [14]. Thus the idea of creating a dendritic cell vaccine to combat cancer was proposed. A vaccine is defined as a pharmaceutical product that is a biological medicine, made in, composed of, and/or tested through living systems to elicit an immune response [15]. Generally in this approach, DCs are generated in vitro from a patient's peripheral blood monocytes, activated and loaded (also known as "pulsed") with autologous tumor antigens, and then re-injected back into the patient with the idea to trigger a potent immune response against the target antigens. This approach has been tried in different animal models, and in some cases into clinical studies. While promising animal data has been found using this approach, results from clinical trials have been modest at best [16-18]. A limitation of such dendritic cell vaccines – and many cancer vaccines in general – is that antigen-bearing dendritic cells in lymphoid organs have a limited ability to support the effector phase of the immune response following T cell priming [14]. This is especially troublesome with the concept of dendritic cell vaccines because of the lifespan of activated DCs is known to only be a few days [19]. This means that injection serves as a temporary response, however fails to provide a sustaining solution. Furthermore, in many human cancers and animal models of cancer, activated T cells fail to properly home to the desired site (the tumor site) [14]. As a war analogy, even though the soldiers (T cells) are armed and ready (activated and mobile), they do not know where to go to fight.

Thus a solution developed in the Irvine laboratory was proposed: deliver the DCs in an injectable hydrogel matrix that gels in situ, which could partially or fully encompass the tumor, with the aim of harboring dendritic cells for prolonged periods of time at a defined site and trapping/concentrating factors secreted by DCs to establish an inflammatory milieu in situ. Upon trying this 'vaccination node' approach in mice, it was found that the injected dendritic cells recruited endogenous host DCs and T cells to the site, while

simultaneously a small number of the injected dendritic cells migrated to local lymph nodes. T cells activated by these migrating dendritic cells in the local draining lymph nodes were attracted back to the alginate matrix in response to the local inflammatory milieu established in the gel. In this way, a single injection provided both antigen presenting cells to initiate naïve T cell priming in the native lymph nodes and simultaneously established a microenvironment drawing the activated T cells to the site of injection, supported by host dendritic cells that had infiltrated the gel [14].

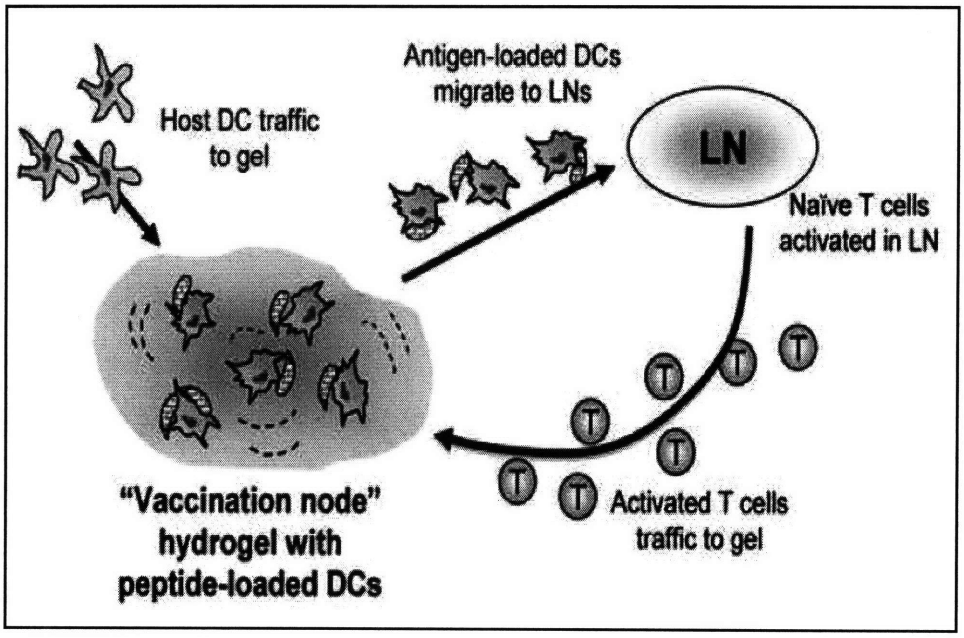

Figure 7: Schematic of dendritic cell and lymphocyte trafficking in response to the vaccination node [14]

Comparing the alginate+DC injection to saline+DC injection in mice shows at least a 125-fold increase in the number of activated T cells at the site. The analysis of this was done by flow cytometry analysis of cells recovered from gels after 7 days in vivo and stained with antibodies against the T cell receptor (TCRβ) or CD19 (a B cell marker) [14]. The results are shown below:

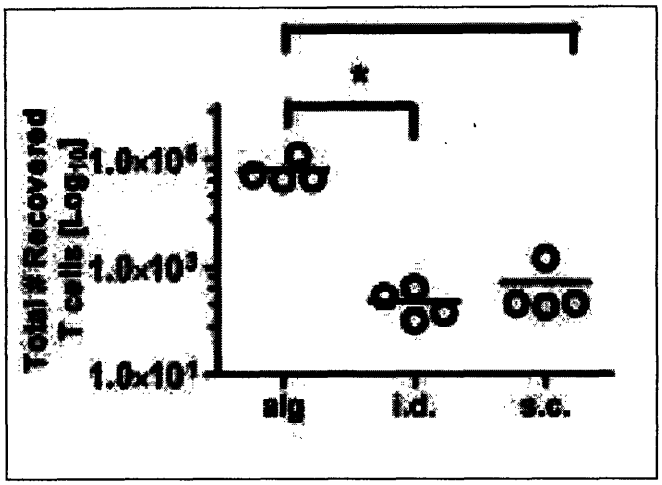

Figure 8: Comparing T cell infiltration in DC-loaded alginate gels with T cell infiltration into intradermal (i.d.) or subcutaneous (s.c.) tissue sites where activated DCs were injected in saline [14]

What are the mechanisms that take place in order for this to happen? Activated dendritic cells are known to secrete cytokines and chemokines that attract both host dendritic cell cells and host T cells. The effect of the matrix is that these signals are initially trapped in the gel and slowly diffuse outward. This provides a gradient of chemokines that directs the activated T cells back to the vaccination node [14]. T cell priming is initiated in the draining lymph nodes, either by the small number of injected dendritic cells that migrate out of the alginate and reach the draining lymph nodes, or by host dendritic cells that infiltrate the alginate and pick up antigen from live or dying injected dendritic cells. Importantly, the presence of activated, antigen-pulsed dendritic cells in the gels conditions the vaccination node to become a site for directed homing/accumulation of activated antigen-specific T cells following their initial priming in the draining lymph nodes. The continuous recruitment of host dendritic cells helps the problem of maintaining a perpetuating response, and the chemokine gradient directing the T cells to a defined site helps the problem of ineffective homing of the T cells. Also, the idea of trying to fully encompass the tumor was proposed. The alginate gel shows to be a good material because it allows some cells (namely dendritic cells and T cells) to penetrate while preventing stromal cells responsible for angiogenesis (new blood vessel growth) from penetrating [20]. This should hinder the tumor growth and spreading, as the tumor's invasive mechanisms are at least partially blocked.

3.1 The Incorporation of Cytokine Interleukin-15 (IL-15)

The immune system is committed to achieving certain objectives, including the rapid generation of different immune responses to invading pathogens, the elimination of autoreactive T cells (to generate tolerance to self) and the maintenance of a specific memory response to these pathogens to protect against future exposure. Such immune responses are normally regulated by cytokines [21]

3.1.1 Background and Motivation

One remarkable characteristic of the immune system is that while it is capable of identifying and reacting to a vast variety of microbes, it does not (normally) react against the individual's own (self) antigens. This unresponsiveness to self antigens is called immunologic tolerance, and is the reason why the immune system usually does not harm normal tissues in its hunt for foreign invaders. Immunologic tolerance is a lack of response to antigens that is induced by exposure of lymphocytes to these antigens [10]. In general, when lymphocytes with receptors for a particular antigen are exposed to this antigen, any of three outcomes is possible. These are shown in the figure below:

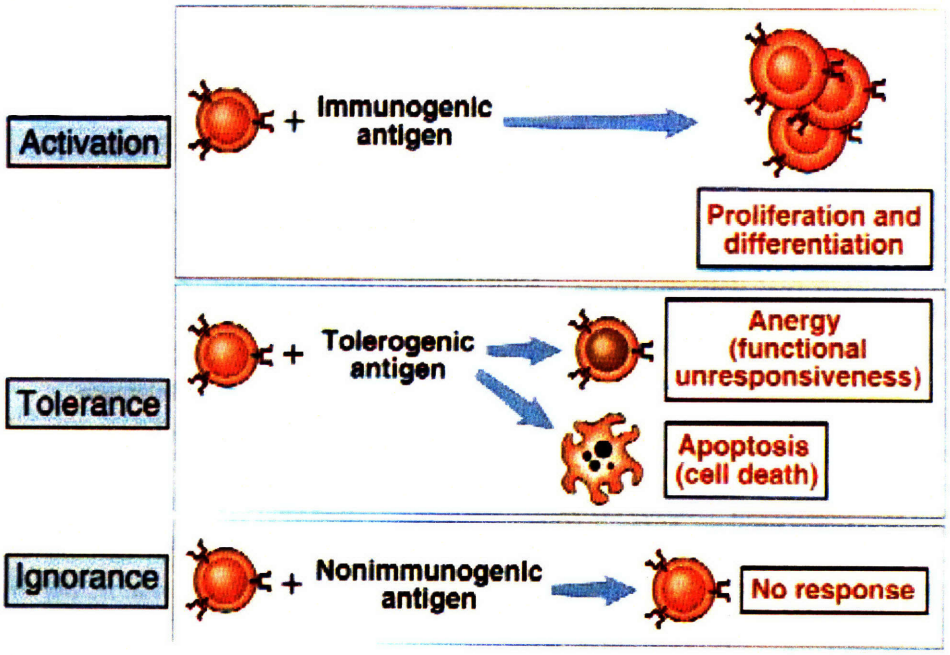

Figure 9: The three possible outcomes when a lymphocyte encounters an antigen [10]

The first case is activation, where the lymphocytes are activated, leading to an immune response. Antigens that elicit such responses are said to be immunogenic. Lymphocytes may also be functionally inactivated (anergy) or killed (apoptosis), resulting in what is known as tolerance. Antigens that induce tolerance are called tolerogenic. Finally, in some situations, the antigen-specific lymphocytes may not react in any way, and this is called ignorance. Antigens of this sort are called nonimmunogenic [10].

This review will focus on tolerance, and specifically the functional unresponsiveness of cells called anergy, because T cell anergy is often seen in cancer, and there has been promising recent studies on combating this effect. Anergy is the functional inactivation of T lymphocytes that occurs when these cells recognize antigens without adequate levels of the costimulators, or second signals, that are needed for full T cell activation [10]. It is believed that normally, antigen presenting cells in tissues and peripheral lymphoid organs are in a resting state, in which they express little or no second signals, also called "danger signals" (such as B7 proteins). These APCs are constantly processing and displaying the self antigens that are present in the tissues. Without receiving the necessary second signals, the T lymphocytes with receptors for the self antigens are still able to recognize the antigens and receive signals from their antigen receptors (signal 1), but signal 1 without adequate signal 2 may lead to long-lived T cell anergy or deletion [10].

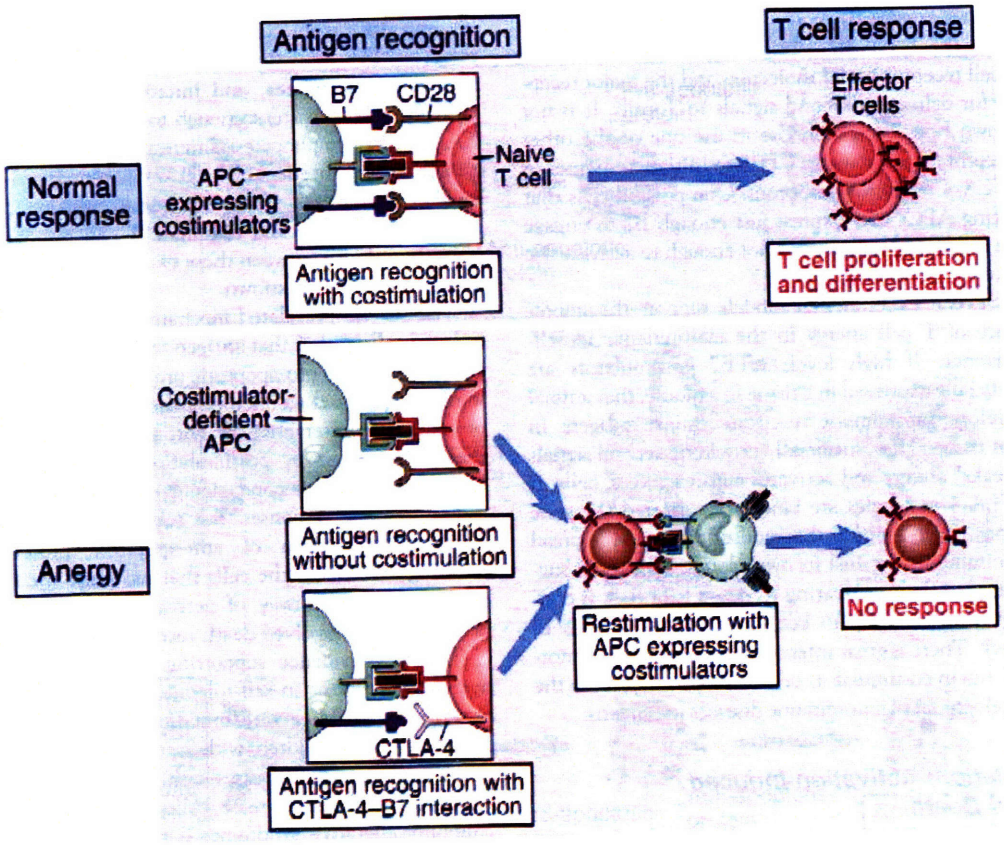

Figure 10: Normal Response and T Cell Anergy [10].

Many cancer vaccine experiments employ a method called adoptive cell therapy (ACT), in which patients are infused with autologous, tumor-specific T cells that can be derived from tumor-infiltrating lymphocytes (TILs) or from peripheral blood lymphocytes engineered to express a tumor-specific T cell receptor [22]. ACT has shown promise and success in select patients with cancer, however most patients still fail to respond despite having increased frequencies of circulating, tumor-specific lymphocytes [23]. This leads to the possible conclusion that it is not necessarily the sheer number of tumor-specific lymphocytes that leads to cancer regression, but instead the ability of immune effector cells to access the tumor and exert their tumoricidal functions there.

Unlike naïve T cells, tolerant CD8+ T cells do not proliferate in response to antigen. Nonresponsiveness is not corrected by the stimulation with activated antigen presenting cells, as shown by Teague et al., and others [24]. However, experimental models have shown that if high levels of B7 costimulators are artificially expressed in a tissue in a

mouse, that animal develops autoimmune reactions (attacks against the individual's own cells and tissues) against antigens in that tissue. Therefore, artificially providing second signals may "break" anergy and activate autoreactive T cells [10]. While generally we want to stay protected from autoimmune effects, it is this same mechanism that may help activate antigen specific antitumor cells and destroy tumor cells.

Since most tumor antigens are also expressed in normal, peripheral tissues, a large fraction of potentially tumor-reactive T cells are deleted in the thymus during development. While some autoreactive T cells evade this deletion, they generally have low affinity T cell receptors, and are unlikely to be effectively triggered by and injure normal tissues – or be effective in tumor therapy. The higher affinity autoreactive CD8+ T cells that evade deletion are potentially harmful and thus are subject to peripheral tolerizing mechanisms, however it is precisely these cells that might be the most effective in tumor therapy [24]. Knowing that anergy of tumor specific T cells takes place in tumors, and that there is a mechanism to "wake" these inactivated T cells, the idea of implementing this into the vaccination node was also proposed.

3.1.2 Comparison of Interleukin-15 to Interleukin-2

Building upon the understanding and relative success of Interleukin-2 treatment for cancer – IL-2 is Food and Drug Administration (FDA) approved for the treatment of renal cell carcinoma and metastatic melanoma [21] – other cytokines have been researched for immunotherapy. One especially promising cytokine was found to be Interleukin-15, which shares many traits with IL-2, but sometimes plays an opposing role during an immune response.

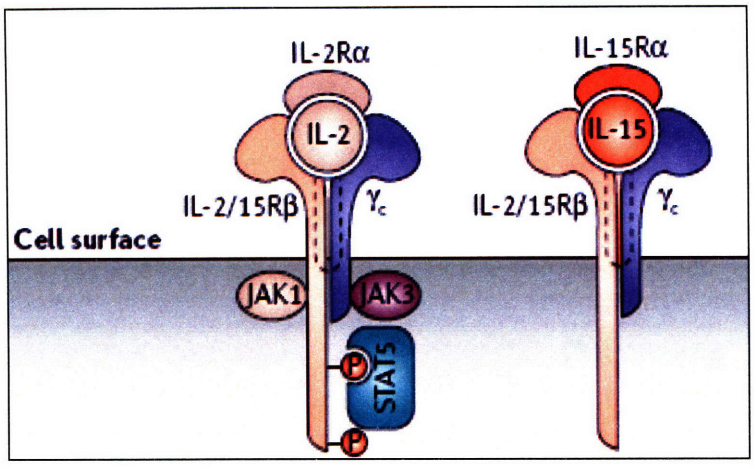

Figure 11: The structure and signaling pathways of IL-2 and IL-15 [21]

As shown in the figure above, the cytokine receptors for IL-2 and IL-15 are heterotrimeric and both contain the receptor subunit γc and also both contain another subunit referred to as IL-2/15Rβ. In addition to these, the high affinity forms of the IL-2 receptor (IL-2R) and IL-15 receptor (IL-15R) contain a third unique receptor: IL-2Rα or IL-15Rα, respectively. Both IL-2 and IL-15 stimulate the proliferation of T cells, induce the generation of CTLs (CD8+ cells), facilitate the proliferation B cells and the synthesis of antibodies by B cells, and induce the generation and persistence of natural killer (NK) cells [21]. Furthermore, both cytokines act as chemoattractants for T cells, and can synergize with IL-12 to facilitate their synthesis of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) [25].

Despite the many overlapping functions, IL-2 and IL-15 also have distinct and often competing roles. IL-2 is responsible for contributing to activation-induced cell death (AICD), which is a process by which fully activated T cells undergo programmed cell death through engagement of cell-surface-expressed death receptors (such as CD95 or tumor necrosis factor receptor). Moreover, IL-2 participates in the maintenance of peripheral CD4+CD25+ regulatory T cells. In these roles, IL-2 is involved in the elimination of self-reactive T cells, which have a role in the pathogenesis of autoimmune diseases. On the other hand, IL-15 is important for the maintenance of long-lasting, high avidity T cell responses to invading pathogens, and it achieves this by supporting the survival of CD8+ memory T cells [21]. IL-15 acts to extend the life and survival of

lymphocytes, both by acting as a growth factor, and by inhibiting IL-2 mediated AICD of CD4 T cells. Thus, IL-15 both increases the proliferation of CD8+ T cells and reduces death by apoptosis (programmed cell death) [26,27].

The antitumor effect of IL-2 is hypothesized to take place because of its ability to expand lymphocyte populations in vivo and to increase the effector functions of these cells, thereby inhibiting tumor growth [21]. However, IL-2 is not optimal for inhibiting tumor growth because in the presence of IL-2, either the cytolytic T lymphocytes generated might recognize the tumor as self and undergo AICD, or the immune response might be subdued by IL-2 dependent regulatory T cells. In contrast, IL-15, with its ability to activate T cells and NK cells, its inhibition of AICD, and its role in the persistence of CD8+ memory T cells, might be a better choice for the treatment of cancer. It is important to note that IL-15 might contribute to autoimmune diseases by this inhibition of self-tolerance mediated by IL-2 induced AICD and by facilitating the maintenance of CD8+ memory T cell survival, including those of self-reactive memory T cells [21]. However, this idea combines well with that of the injectable vaccination node that gels in situ around the tumor, potentially minimizing the systemic activation of self-reacting cells to healthy tissues throughout the body.

3.1.3 Ability of IL-15 to Proliferate Tolerant T cells and "Break" Tolerance

As mentioned before, the importance of the ability of lymphocytes to actually infiltrate tumors is of critical importance. Tolerant T cells are found at tumor sites, thus if these cells lose their tolerance, they may be ideal for effectuating their tumoricidal functions because they are already at the tumor site. Because of the role in facilitating the maintenance of memory CD8+ T cells, it was investigated whether IL-15 could also provide proliferative signals to tolerant T cells. It was found that naïve CD8+ T cells did not proliferate in response to IL-15, even at concentrations as high as 500 ng/ml. In contrast, both tolerant and memory CD8+ T cells proliferated in response to IL-15 at concentrations as low as 50 but not at 5 ng/ml [24]. Since the receptors of IL-2 and IL-15 include common signaling chains and induce similar signaling cascades [28], further

studies were conducted to see if IL-2 could similarly induce proliferation of tolerant T cells. Teague et al. found that at physiologic or low doses of IL-2 ($\leq$ 100 U/ml), neither naïve nor tolerant CD8+ T cells showed detectable levels of the high affinity IL-2 receptor, and thus had no effect on T cell population. High dose IL-2 (1000 U/ml) had a proliferative effect on tolerant T cells similar to that seen when administering 50 ng/ml of IL-15, but IL-15 seemed to be a more efficient proliferative signal, inducing one to two rounds of cell division at doses as low as 10 ng/ml [24]. Furthermore, the study evaluated whether IL-15 mediated proliferation would also restore antigen responsiveness to tolerant CD8+ T cells, and thus "break" tolerance or "rescue" tolerant T cells. This was indeed found to be the case, as after five day culture with 50 ng/ml of IL-15, tolerant T cells formed robust synapses with their target antigen. While high dose IL-2 (1000 U/ml) also restored antigen responsiveness, only a small percentage of the cells that had proliferated responded to antigen, compared to the majority of cells that had been induced to proliferate with IL-15 treatment, and the overall expansion of these responding T cells was blunted [24].

In vivo, tolerant T cells remain unresponsive to antigen despite the ability of the host to make IL-15, thus suggesting that the amount of IL-15 is either limiting in vivo or the tolerizing environment interferes with this proliferative response to IL-15. However, IL-15 may still be critical despite the tolerizing environment by potentially providing survival signals which may promote the persistence of tolerized T cells in vivo [24].

The cellular mechanisms that regulate maintenance of tolerance are largely undefined, which makes it difficult to predict how exactly IL-15 (and IL-2 to a lesser degree) rescues tolerant T cells. There are a few hypotheses of why this takes place, such as the activation or silencing of proteins and/or genes that regulate tolerance; dilution of regulatory cellular proteins; and enhancement of functional avidity [24]. In addition, the higher effectiveness of IL-15 may have to do with the unique biology of IL-15 and the IL-15Rα chain, including potential signaling through the IL-15Rα cytoplasmic tail, retention of membrane-bound IL-15 and IL-15Rα, endosomal recycling of IL-15 and differential receptor oligomerization, all of which may alter the quality and kinetics of IL-

15 mediated signals as compared to IL-2 signaling despite sharing the same signaling chains [24]. Further research is necessary in order to better understand the correct mechanism(s) and ultimately maximize the safety and efficacy of treatment.

3.1.4 Toxicity Comparison of IL-2 to IL-15

The issue of toxicity is critically important when considering any therapeutic treatment. In fact, the first clinical phase of getting FDA approval is testing in humans for safety and appropriate dose for therapeutic effect through a process called dose escalation. One significant concept is the therapeutic index, which is a comparison of the amount of an agent that causes a therapeutic effect to the amount that causes toxic effects. Quantitatively, it is the ratio of toxic dose divided by the minimum therapeutic dose. Consequently, a higher therapeutic index is generally desired.

Although FDA approved, two major dose-limiting toxicities associated with IL-2 therapy are pulmonary vascular leak syndrome (VLS) and hypotension [29]. Vascular leak syndrome is a condition in which fluid from the bloodstream escapes into surrounding tissues. While a body can often slowly expel the excess liquid, fluid buildup in critical organs such as the lungs can turn deadly [30]. A study conducted by Rosenberg and colleagues found that treatment with high-dose IL-2 led to a weight gain of more than 5% of total body weight in most patients, stemming from increased fluid extravasation into soft tissues [31]. Often times, patients with VLS are hospitalized in intensive care units and require respiratory and ventilatory support [32]. It has been suggested that the antitumor response to IL-2 might be improved if VLS could be attenuated, so that regimens containing higher doses of IL-2 could be administered [33]. This is important to note, as toxicity may be a limiting factor in treatment efficacy. Side effects of high-dose IL-2 therapy include systemic symptoms such as nausea, vomiting, diarrhea, and malaise. In addition, many of the side effects associated with high-dose IL-2 treatment are similar to those seen in patients with sepsis, including a decrease in peripheral vascular resistance, increase in cardiac index, tachycardia, oliguria, and in some rare cases, even death [31].

Since VLS is a dose-limiting toxicity associated with IL-2 therapy [29], the potency of IL-15 was compared with IL-2 in inducing pulmonary vascular leak in mice by a study conducted by Munger and colleagues. The figure below shows the results of this testing.

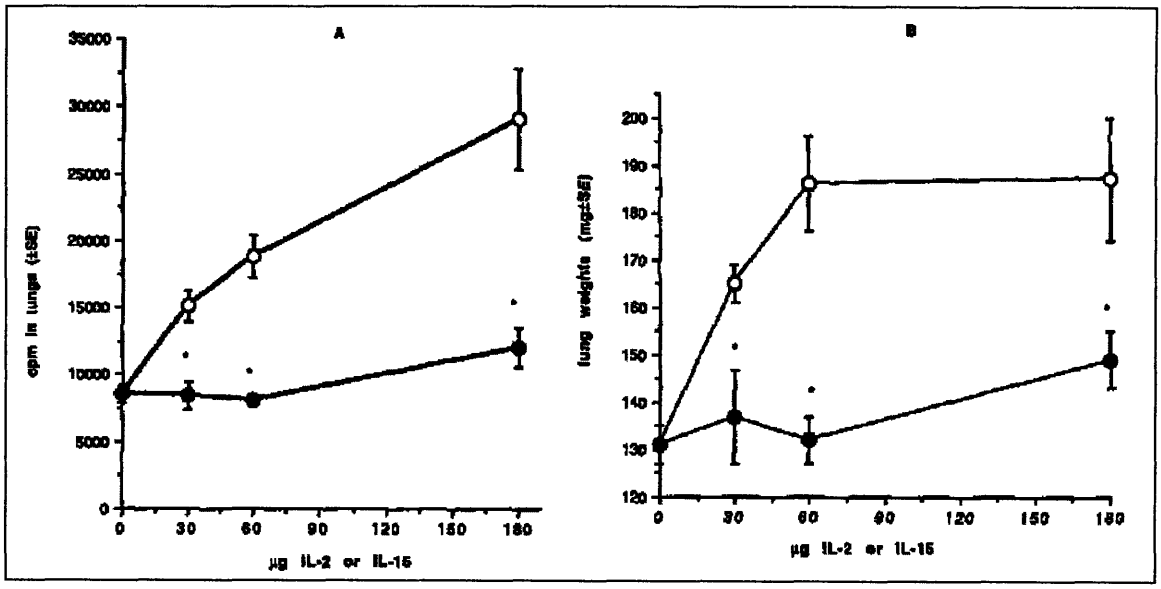

Figure 12: The effect of varying concentrations of IL-2 or IL-15 in inducing VLS in mice. The open circles (○) represent IL-2; the closed circles (●) represent IL-15. For each experiment, n=10 [32]

The figure on the left measures the extravasation (leakage of fluid) of radio-labeled albumin into the lungs (in counts per minute). It shows a dose-dependent increase in the accumulation of radioactivity in the lungs of mice treated with IL-2. The minimal dose of IL-2 required to induce VLS is 30 µg. In contrast to IL-2, IL-15 induces VLS only at the highest dose treated (180 µg). The difference in potency of IL-2 and IL-15 is further evidenced in the figure on the right, which used lung weights as a measure of pulmonary edema (swelling). IL-2 at all doses induced a strong edematous response, whereas IL-15 induced a slight, but significant, edema at the 180 µg dose. Analysis of this data indicates that the VLS induced by 180 µg of IL-15 approached that of IL-2 at 30 µg. Therefore, IL-15 is approximately six times less toxic than IL-2 in this model of VLS. The therapeutic index (defined earlier) was found to be 18 for IL-15 and 6 for IL-2 for their tumor model (MCA-205) [32].

3.1.5 Combining IL-15 with soluble IL-15Rα-Fc

With the significant evidence in favor of implementing Interleukin-15, further evidence indicated the potential in vivo benefits of "complexing" IL-15 by combining it with soluble receptor IL-15Rα-Fc (IL-15Rα).

A study conducted by Stoklasek et al. compared treating mice with phosphate buffered saline (PBS), IL-15 alone (2.5 μg), IL-15Rα alone (15 μg), or a mixture of IL-15 (2.5μg) and IL-15Rα (15μg). After four days of treatment, it was found that the IL-15Rα alone did not significantly alter CD8 T cell proliferation, IL-15 alone showed 8.4% proliferation, and the IL-15/IL-15Rα complex induced a proliferation of 64.3% of the CD8 T cells. Furthermore, the cells responding to the complex treatment underwent about 5 to 7 divisions, resulting in a substantial increase in T cell numbers, whereas the majority of CD8 T cells responding to IL-15 divided only once [34]. Also, the early kinetics of the CD8 T cell proliferation was examined, finding that the maximum effect of a single dose took place about four days after treatment [34].

In addition to looking at the proliferation of CD8 T cells, the study investigated if IL-15 and the IL-15/IL-15Rα complex induced proliferation of B cells, CD4 T cells (helper T cells), NK cells, and NK T cells. Using the same amounts as before, the results indicated that while B cells did not respond to either, the other three types were affected. As seen in the figure below, the administration of 2.5μg of IL-15 alone proliferated the NK and NK T cells very little, and CD4 T cells insignificantly. On the contrary, the IL-15/IL-15Rα complex generated widespread proliferation of NK and NK T cells, and an intermediate level of CD 4 T cells [34].

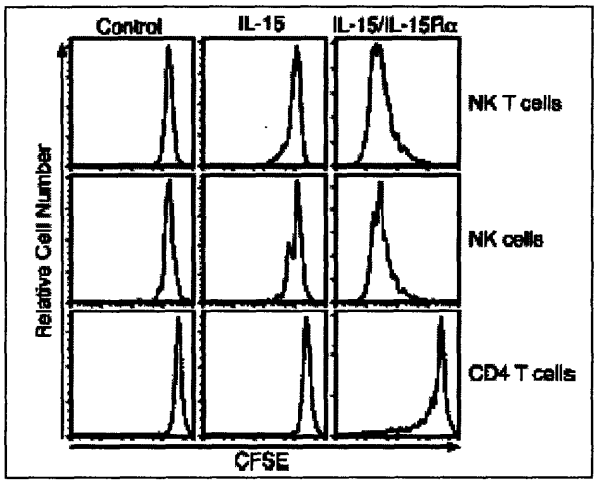

Figure 13: NK T cell, NK cell, and CD4 T cell responsiveness to 2.5 μg IL-15 or 2.5 μg IL-15 complexed with 15μg IL-15Rα [34]

In order to attain an estimate of the level of activity enhancement that complexing IL-15 brings over IL-15 alone, Stoklasek et al. performed titrations of IL-15 and IL-15/IL-15Rα using an adoptive transfer system of CD8 T cells. They found that a dose of 0.1 μg of IL-15 combined with 0.6 μg of IL-15Rα induced a level of proliferation similar to that of 5 μg of IL-15 alone. Therefore, in this experiment, a simple coadministration of IL-15Rα improved IL-15 activity by about 50-fold. They found that the administration of 37.5 μg of IL-15 alone could not achieve the level of proliferation obtained with 0.5 μg of IL-15 complexed with 3 μg IL-15Rα. Similar results were found when examining NK and NK T cell proliferation [34].

CD8 T cell proliferation induced by IL-15 alone plateaued at about 12 μg upon in vivo administration and the addition of more cytokine after this no longer increased cell proliferation. This suggested that IL-15 half-life and/or IL-15Rα availability were limiting in vivo. A short half-life could pose a problem in the development of an effective treatment. It was found that using human IL-15 (hIL-15), the half-life of it alone was about one hour. However, complexing it with IL-15Rα increased the half-life to about 20 hours [34], as shown in the figure below.

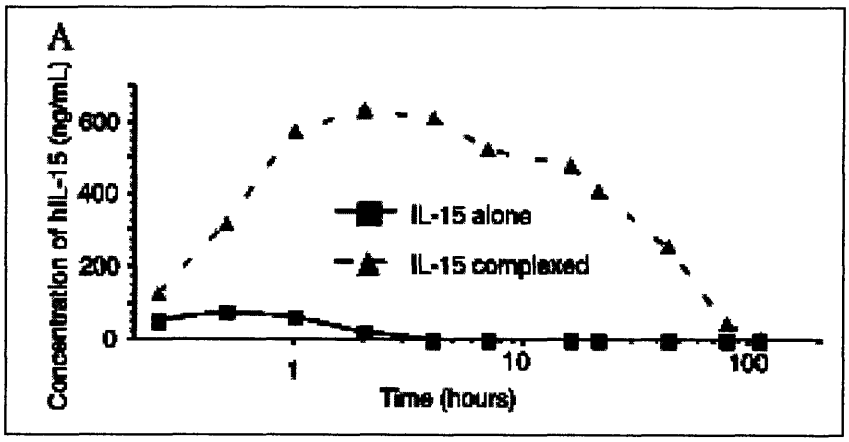

Figure 14: Comparison of lifetimes of IL-15 and complexed IL-15 after injection into mice intraperitoneally [34]

The understanding of exactly how this increases half-life is not complete, but there are several possible mechanisms including: protection of IL-15 from degradation by proteases; inhibition of clearance via receptor binding or other mechanisms; and FcR-mediated binding/recycling of complex [34]. Further research should investigate these hypotheses to ascertain the correct mechanism(s).

In addition to the increase in half-life brought by complexing IL-15, the Irvine laboratory hopes to further increase half-life by immobilizing the IL-15/IL-15Rα complex to the alginate gel vaccination node. Possible immobilization methods involve covalent linking of the IL-15/IL-15Rα complex and/or charge-charge interactions between the alginate and the cytokine/receptor complex. One possible method of accomplishing this is through EDC/NHS chemistry, using the –COOH groups on alginate chains and the $-NH_2$ groups on the proteins.

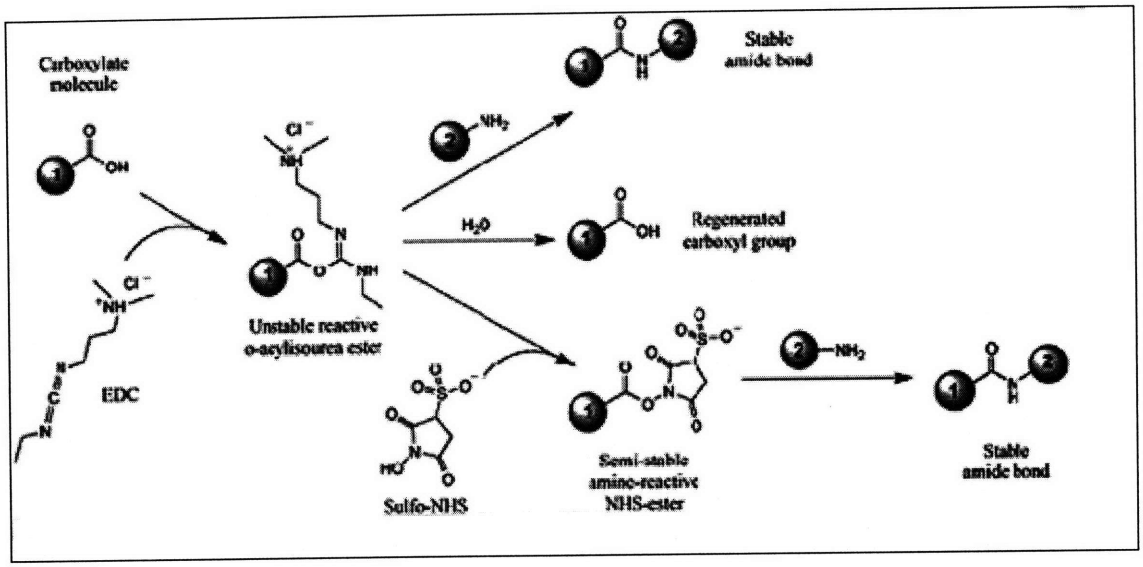

Figure 15: Sample EDC/NHS chemistry [35]

In the figure above, 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) reacts with a carboxyl group on molecule 1, forming an amine-reactive O-acylisourea intermediate. The amine may react with an amine on molecule 2, making a conjugate of the two molecules coupled by a stable amide bond. However, since the intermediate is susceptible to hydrolysis, it is unstable and short-lived in aqueous solution. The addition of *N*-hydroxysulfosuccinimide (Sulfo-NHS) stabilizes the intermediate by converting it to an amine-reactive Sulfo-NHS ester, thereby increasing the efficiency of EDC-mediated coupling reactions. The amine-reactive Sulfo-NHS ester intermediate has sufficient stability to permit two-step crosslinking procedures, which allows the carboxyl groups on one protein to remain unaltered [35].

Finally, since the IL-15/IL-15Rα complex was found to significantly proliferate CD8 T cells and NK cells – two populations known to play an important role in tumor surveillance by directly killing malignant cells [36] – experiments were conducted to compare the ability of IL-15 verses IL-15/IL-15Rα complex in tumor immunity. Stoklasek et al. approached this by injecting 1 x 10^5 melanoma cells (B16 F1) intravenously (a protocol that leads to the establishment of tumors in the lung and liver) on day 0 and treated mice with either PBS, IL-15 (2.5μg), or IL-15/IL-15Rα (2.5μg/15μg) intraperitonally on day 1 and day 10. From two separate experiments, the study found

that 9 out of 10 mice treated with PBS or IL-15 were tumor positive and exhibited a similar tumor burden between groups: multiple tumors that were greater than 5 mm in diameter. In striking contrast, only 1 out of 10 of the IL-15/IL-15Rα complex treated mice was tumor positive, and moreover that one case displayed only a single 2 mm lung tumor [34].

The conclusions that can be drawn from Stoklasek et al. show the potential of IL-15/IL-15Rα to be a potent prophylactic (preventative) vaccine, as injection essentially prevented tumor growth from occurring in the melanoma model used. However, what implications that this has to established, highly vascularized tumors is unspecified. In another study conducted by Epardaud et al., the effect of IL-15/IL-15Rα complex on solid tumors was studied in two different tumor models: transplanted melanoma cells (similar to the work of Stoklasek), and another where tumors arose spontaneously in the endocrine pancreas of transgenic mice [37]. For the first case, mice were injected subcutaneously with 5 x 10^5 B16 melanoma cells and given 10-14 days for it to mature. Thereafter, they were given one intravenous injection of IL-15/IL-15Rα complex per day for two days, and evaluated three days later. The schematic and results are shown below:

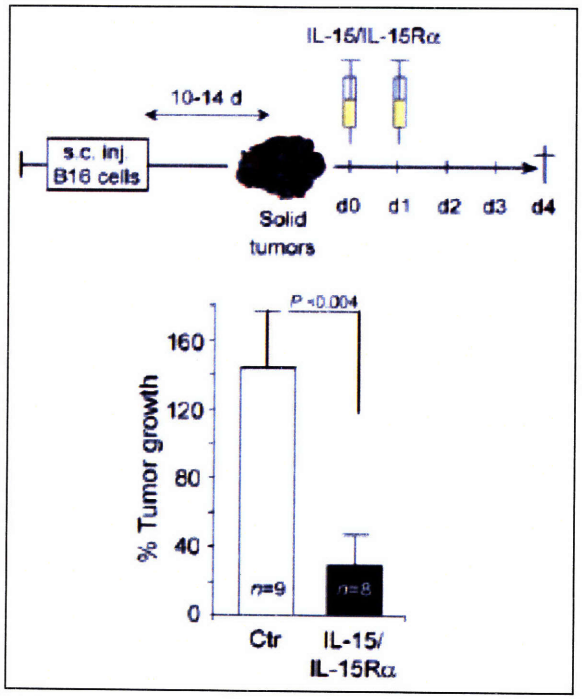

Figure 16: Schematic of IL-15/IL-15Rα treatment (2μg/12μg in 300uL PBS) for B16 melanoma mice and resulting tumor growth [37]

The average tumor growth in the control group (n=9) was 140%, while the IL-15/IL-15Rα treated mice (n=8) averaged a tumor growth of 30% over the same course of time.

These positive results were promising, however melanoma is known to be a very immunogenic cancer, and a more daunting accomplishment would be to see if this treatment proved effective in spontaneous, solid tumors in vital organs. Epardaud and colleagues chose the RIP1-Tag2 transgenic mouse model in which the SV40 T antigen (Tag) is expressed under the control of the rat insulin promoter (RIP), causing oncogenic transformation of the majority of pancreatic β cells [38]. Tumor growth in this model follows a trend, where the first 3-7 weeks is characterized by hyperplasia, the abnormal increase in the number of cells and consequent enlargement. At about 7 to 8 weeks, there is a distinct transformation as new blood vessels form along with alterations in the microvasculature (angiogenic switch). Finally, by 10 weeks, solid tumors will have developed in 100% of mice [37]. After 10 or 11 weeks, mice were injected with IL-15 alone or IL-15/IL-15Rα once per day for two days, and results were analyzed three days later.

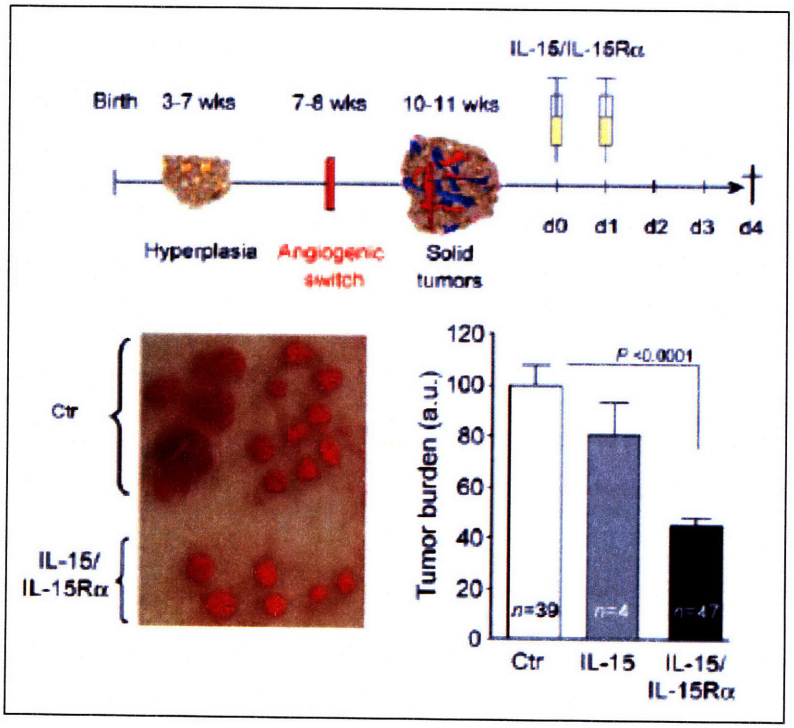

Figure 17: Schematic and results of control (300uL PBS) and IL-15 (2μg in 300uL PBS) compared to IL-15/IL-15Rα (2μg/12μg in 300 mL PBS) in the RIP1-Tag2 transgenic mice model [37]

As clearly seen in the bottom left of Figure 17, complexed IL-15 led to a swift and considerable reduction in the size of tumors. To quantify this, the tumor burden was measured by excising the solid tumors and measuring their diameters (d), separating them into four categories: A, $d < 1mm$; B, $1 \leq d \leq 2$ mm; C, $d = 2mm$; and D, $d > 2mm$. Pancreatic tumor burden was calculated as (A x 1) + (B x 2) + (C x 3) + (D x 4) [37]. Using this quantification, the mice who received complexed IL-15 injections saw a reduction in tumor burden of over 50% compared to the mice that were injected with only PBS. In addition, no observable toxicity or autoimmune effects on normal tissues was found in mice treated with IL-15 alone or IL-15/IL-15Rα complex [37]. This indicates that the systemic administration of IL-15/IL-15Rα complex not only significantly hinders growth in both B16 and spontaneous solid tumors, but does so without detectable autoimmune or toxic consequences.

Reduction in tumor size, however, does not necessarily translate to longer survival time, and so a test of long-term survival was performed on RIP1-Tag2 mice. Five of these mice were administered 13 injections of IL-15/IL-15Rα complex, and survival rates were compared to 10 untreated mice. The results are shown below:

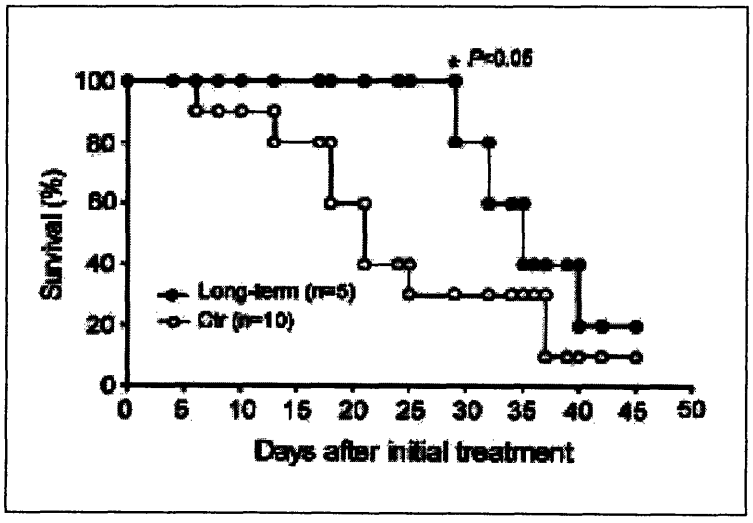

Figure 18: Comparison of long-term survival rates of RIP1-Tag2 mice treated with IL-15/IL-15Rα verses control [37]

The prolongation of survival was greatly increased with treatment of complexed IL-15, showing that treatment appears to be well tolerated and effective. Although, it is noteworthy to point out that while median survival increased, the rate dropped relatively quickly after a certain time period.

While tumor cell destruction has been found in vivo with the addition of IL-15/IL-15Rα, as alluded to before, it is not necessarily due to the presence of circulating lymphocytes – even if tumor-specific. It was reasoned that there are two mechanisms, not necessarily mutually exclusive, that could account for the tumor regression. The first mechanism is that CD8+ T cells within secondary lymphoid tissues or blood would undergo expansion and activation upon exposure to IL-15/IL-15Rα complex, traffic via to blood to tumors, infiltrate the tumor parenchyma, and finally kill malignant cells. The second mechanism is that T cells that are already resident in the tumor would expand in the tumor itself upon signaling by IL-15/IL-15Rα complexes and then destroy neighboring tumor cells [37]. It has been found that solid tumors may become impenetrable by circulating leukocytes after the angiogenic switch because of alterations in the local vasculature that mitigate leukocyte adhesion [39], thus Epardaud and colleagues tested and found that tumor-resident CD8+ T cells rapidly proliferated in response to systemically delivered IL-15/IL-15Rα complexes [37]. Furthermore, they established that advanced solid tumors in RIP1-Tag2 mice are not readily accessible to any circulating leukocytes, and also that systemic treatment of IL-15/IL-15Rα complex did not markedly increase leukocyte infiltration of solid tumors [37]. Therefore, the above results indicate that the tumor-resident CD8+ T cells have a major role in the destruction of advanced solid tumors in vivo with the injection of IL-15/IL-15Rα. Deeper investigation revealed that within 48 hours of treatment, the apoptotic tumor cells were often in close proximity to, or even in direct contact with, tumor-resident CD8+ T cells, suggesting that the lymphocytes themselves were lysing the malignant tumor cells [37]. Thus, despite efforts of the tumor to suppress an immune response, systemic injection of IL-15/IL-15Rα complex rapidly proliferated resident T cells and "broke" the tolerance, activating the killing potential of these T cells against their tumor cell neighbors. Moreover, these results come at a relatively low

dosage, and importantly, without the addition of chemotherapeutic agents, vaccination, adoptive cell transfer, or other cytokines.

As seen by the results, the full therapeutic effect of IL-15 may not be optimal without complexing it with IL-15Rα. The simple addition of the receptor shows improvement in the efficacy of IL-15 potency in many different aspects. With the addition of IL-15/IL-15Rα, IL-15 effectiveness in increased by: increasing half-life by about a factor of 20; increasing IL-15 affinity for IL-15Rβ/γC [40]; and providing a platform for transpresentation [34]. Additionally, complexed IL-15 has shown effectiveness in prophylactically preventing tumor growth, as well as reducing established tumor growth in different cancers in mice. While these results show great promise and excitement, further studies should be conducted to understand the underlying mechanisms, perhaps look into reversing the vascular barriers provided by the angiogenic tumors, and minimize toxicity and autoimmunity.

3.2 Application to Melanoma Treatment

Because dendritic-cell based vaccines are still for the most part considered experimental, widespread use is currently not employed. This is one reason that makes melanoma a good candidate for DC vaccine technology, because conventional treatment so far has not proven to be effective, and there is a clear need for better treatment. This review will concentrate on later stage melanoma for a few reasons. First, early stage melanoma is considered very treatable [6] and the population will be hesitant to try something new to replace something that already works well. Second, the death rate of later stage melanoma is so devastating – even with current treatments – that patients would more likely be more willing to try an alternative solution. Surgery is unlikely to cure metastatic melanoma, but long-term survival may be improved through resection of metastatic tumors. As mentioned before, metastatic melanoma is relatively unresponsive to systemic chemotherapy, and is considered to be very unresponsive to radiation therapy [4]. Finally, melanoma is a highly immunogenic cancer, with the body's own immune system launching strong immune responses against the disease [4]. All of these factors

make melanoma an ideal starting platform, where the probability of success would be the greatest.

In the grand scheme of things, what the technology really does is it allows injectable drugs or biological materials to be delivered as a formation that gels in situ. This serves to dramatically improve an existing drug/biological material delivery method rather than try to re-invent the wheel.

4 Technological Barriers

4.1 Barriers That Have Been Overcome

Before thinking about what barriers there are to overcome, it is important to note what barriers have already been overcome. As mentioned earlier, the introduction of a vaccination node addresses two major shortcomings of cancer vaccines: the limited ability to support the effector phase of the immune system following T cell priming; and the failure of activated T cells to properly home to tumors to carry out their effector functions [14]. The need to identify a material that works with cell delivery extremely effectively has been tackled. The alginate gel allows DC and T cells to migrate through, while blocking other stromal cells from permeating the gel. In this way, further angiogenesis of the tumor is difficult to take place, as part or all of it is surrounded by the alginate gel. The gel needs no vascularization and remains in situ, shows promise of great T cell recruitment, and is non-inflammatory and non-toxic [20].

The idea of implementing IL-15/IL-15Rα complex shows the potential proliferating T cells in vivo, as well as side-stepping some of the tumor evasive mechanisms to "break" tolerance in inactivated T cells that are located at or near the tumor site [37]. By complexing the IL-15, the short half-life of IL-15 was found to increase by a factor of 20 [34], and ideas to immobilize the cytokine complex serve to further increase half-life. Not only has complexed IL-15 shown to be a potentially potent prophylactic treatment against tumor growth, it has also shown significant inhibition of tumor growth in already established tumors in mice models. Finally, it has also shown to prolong survival compared to untreated mice [37].

4.2 Barriers Left to Overcome

The technical challenges that remain mostly stem from the remaining immuno-evasive mechanisms that tumors currently utilize. These mechanisms are the limiting factors in the development of successful cancer vaccines, and research of understanding and combating these mechanisms is currently being extensively performed. For the

vaccination node technology, a technical barrier to overcome is the destruction of tumors with products of perpendicular diameters in the order of $50mm^2$ in mice. In 2003, Overwijk et al. showed curing of tumors of this magnitude using a combination of adoptive transfer of tumor-specific T cells, T cell stimulation through an antigen-specific vaccination with an altered peptide ligand, and the coadministration of a T cell growth factor and activation factor [41]. This is considered the "gold standard" of the field, and progress at least equal to this should be generated to show the value of the new technology.

5 Business Strategy

If the technological barriers can be overcome, then a business strategy should be proposed which describes where value is generated, how to extract value, and at what costs this comes. There were two options that were considered: starting a manufacturing facility to produce the entire vaccination node; or starting a manufacturing facility that only produces the alginate microspheres/alginate gel, and trying to partner with an existing dendritic cell vaccine manufacturer. There were numerous factors to consider, and the idea of each had its own set of pros and cons.

For the idea of manufacturing the entire vaccine, the most prominent benefit would be the idea of total control. Each aspect would be designed to the liking of the founder(s), including price, and profit – if obtained – would not have to be split between so many different forces. However, manufacturing of the entire vaccine would entail starting at the beginning of the learning curve for the technical know-how of the field. Significant start-up costs would have to be raised. Even if these hurdles could be overcome, existing DC vaccine manufacturing companies would already have established production lines, economies of scale, perhaps established relationships in the supply chain, as well as overall experience. Furthermore, in order to be independently sufficient, a DC manufacturer would have to have an intellectual property portfolio containing patents on any antigens, adjuvants, methods of obtaining these, and delivery mechanisms. Obtaining each piece without collaboration with other companies would take an extremely significant investment of money and time. Also, testing for the safety and efficacy of the vaccine would have to be proven through the Food and Drug Administration (FDA), and the necessary clinical trials are usually on the order of hundreds of millions of dollars [42]. Even if sufficient capital could be raised for this entirety, the time spent researching and patenting supplementary technologies would also be a great investment to be considered.

The other option was manufacturing only the alginate microspheres/alginate gel and working to partner with other established companies. This option shows much less costs

in manufacturing, since it is only the production of one aspect of the vaccine. Under this schematic, the company can focus on the core competency, working to maximize the efficiency of this one aspect and specializing in it. The time and capital which would be required to establish a full patent portfolio is saved. Moreover, the FDA costs can be shifted to the partner who has the funds to push the technology through the expensive clinical trials.

6 Intellectual Property

In order for a technology to become viably commercialized, it cannot infringe on any existing patents (without a prior agreement), and thus, a patent search was conducted to assess the intellectual property landscape surrounding the vaccination node technology, broken down into different sections. Some of the listed potential intellectual property conflicts are still patent applications, which may or may not be approved, but should still be considered and watched.

6.1 Patents Related to Dendritic Cells

Looking at the intellectual property landscape, there are many patents that seem to be relevant regarding dendritic cells. This includes the methods of obtaining and growing the dendritic cells, as well as methods for activating them and their uses. A short list of possible conflicting patents about dendritic cells is given below:

Patent Title	U.S. Patent Number	Date of Filing
Methods and compositions for obtaining mature dendritic cells	6,274,378	1/27/1998
Rapid one-step generation of antigen loaded dendritic cell vaccine from precursors	App No: 10/537,682	12/3/2003
Activated DCs and methods for their activation	6,017,527	12/12/1996
Preloaded dendritic cell vaccines for treating cancer	App No: 11/202,319	8/10/2005
Therapeutic method and composition utilizing antigen-antibody complexation and presentation by DCs	6,689,355	5/11/2001
Intratumoral delivery of dendritic cells	App No: 10/251,148	9/20/2002

Table 2: A list of patents regarding methods for obtaining and loading dendritic cells

The first four patents (or patent applications) on the list refer to different methods of obtaining mature dendritic cells. U.S. 6,274,378 describes a two step method of generation of mature dendritic cells, first by culturing T cell depleted mononuclear cells in medium supplemented with cytokines granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-4 to produce immature dendritic cells, then differentiating by exposure to DC maturation factor such as monocyte conditioned medium. U.S. Application Number 10/537,682 describes a one-step method of producing antigen loaded APCs from monocytes ex vivo using an activator (such as TNF-α) in combination with a growth factor such as GM-CSF. U.S. 6,017,527 describes a method of transfecting DCs and activating them using a CD40 binding protein. Finally, U.S. Application Number 11/202,319 specifies methods for obtaining a large volume of dendritic cells differentiated from human stem cells, and furthermore goes on to describe pulsing these with tumor antigen for potential use in treating cancer. These four are only a few of many which seem similar. Thus while it may be possible to obtain a highly specific patent on the topic, the overall situation is crowded and may be a source of future problems for a start-up.

The fifth patent on the list, U.S. 6,689,355 illustrates a method for exploiting dendritic cells to present antigen to a patient by combining ex vivo an antigen and APC binding agent specific for the antigen, followed by administering into the patient suffering from a disease associated with the antigen. The patent seems a little vague, however it does specifically indicate that the antigen claimed is prostate specific antigen. Thus while for initial purposes this patent will not be a hindrance, it should be noted in case of future expansion into prostate cancer.

The last application on this list, 10/251,148 is noteworthy because it describes administration of dendritic cells either directly into the tumor and/or into its surrounding tissue. It also describes that autologous DCs are harvested and grown using cytokines GM-CSF and IL-4 before being replenished into the patient. However, the patent only claims certain types of tumors – namely brain, breast, gastrointestinal, and respiratory

tumors or tissues surrounding them. Furthermore, the patent specifically states the dendritic cells are unprimed, which is not the proposed strategy for the vaccination nodes.

6.2 Patents Related to Alginate Gels and Microspheres

The intellectual property surrounding the idea of self-gelling alginate microspheres is much less crowded, with a fewer number of titles that seem to be relevant. These are listed below:

Patent Title	U.S. Patent Number	Date of Filing
Self-gelling alginate systems and uses thereof	App No: 11/248,984	10/12/2005
Hydrogels and water soluble polymeric carriers for drug delivery	7,186,413	5/27/2003
Polymers containing polysaccharides such as alginates or modified alginates	6,642,363	5/3/1999
Immunostimulatory Microsphere	5,008,116	11/14/1988
Medical uses of in situ formed gels	5,958,443	12/13/1996

Table 3: A list of patents regarding the idea of self-gelling alginate microspheres for immunotherapy

The first item on the list, U.S. Application Number 11/248,984 might be the most difficult issue to circumvent. The patent specifies the method of formulation of self-gelling alginate by combining a dispersion of insoluble alginate/gelling ion particles with solution containing soluble alginate. It also mentions that this dispersion may be into the body of an individual. While the methods of gel formation may be conflicting, there is no mention of dendritic cells in the patent. Approval of this patent should be closely monitored.

The next item, U.S. 7,186,413 also shows potential conflict. The patent describes a water-soluble polymer/drug compound which is bonded by an in vivo degradable

covalent bond. The patent only mentions different drugs, which may or may not apply to the biological compounds that the vaccination node technology looks to employ. The technicalities should be closely scrutinized if full commercialization is to be pursued.

The third listed, U.S. 6,642,363 claims a modified alginate, which is composed of at least one alginate chain section to which is covalently bonded to at least one cell attachment peptide or RGD peptide which promotes cell adhesion and growth. The range of molecular weights for the alginate chain section specified is wide, as is the list of potential biologically active molecule(s) bonded to the alginate side chain. While there is no specific mention of dendritic cells or cytokines, this patent should be appropriately examined in deciding whether to partner/license or pursue an alternative path.

The immunostimulatory microsphere patent, U.S. 5,008,116 describes a macroporous microsphere comprising a particle and antigenic component, with the purpose to be an improved carrier or adjuvant to induce a therapeutic response to a weak antigen. This patent is from 1988, so even if it is found to be conflicting, the patent expires in late 2008. However, this patent is very broad, and nowhere in the patent is there specific talk about alginate, employing any type of dendritic cell, or incorporating any cytokines.

Finally, U.S. 5,958,443 claims a long list of potential drugs to attach to a composition that is capable of gelling in situ. However, the composition is claimed to compromise at least one ionic polysaccharide and at least one film forming agent, the latter which does not really apply. Furthermore, the patent only claims the in situ gels for topical, protective layer purposes, and should not be a future conflict.

6.3 Intellectual Property Summary

Upon examination of the intellectual property landscape, a few insights can be made. The dendritic cell aspect – methods for obtaining and activating – of the technology seems to have a lot of contenders with a number of different but similar mechanisms and methods. Thus the potential to obtain a highly specific, similar method of expansion and activation seems plausible, however on the other hand, there are many opportunities to

partner in order to save research investment and time. On the alginate gel side, the option to partner or license with a company that owns one or more of these patents should be considered, as there seem to be many similarities. However, mention of dendritic cells, IL-15/cytokines, melanoma alone or in combination is not found in any of these seemingly conflicting patents, and the incorporation of any or all of these factors may or may not prove to be conflicting. Furthermore, the ingenuity of the vaccination node is in combining the dendritic cell vaccine with the gelling microspheres for a synergistic effect. It is the combining of these two factors that makes the effects better, and is what intellectual property should be protected.

7 The Supply Chain

From the above analysis, the route of greatest probability of success points toward being a microsphere manufacturer, as there seems to be fewer barriers to entry, and because the true value stems from the fusion of existing technologies rather than the individual technologies themselves. The aim is to leverage the industry know-how of existing dendritic cell vaccine manufacturers, and combine it with the self-gelling alginate microspheres in order to create a final vaccination product. This product will then be distributed to hospitals and doctors, and makes its way to the patient. An illustration of the supply chain is shown below:

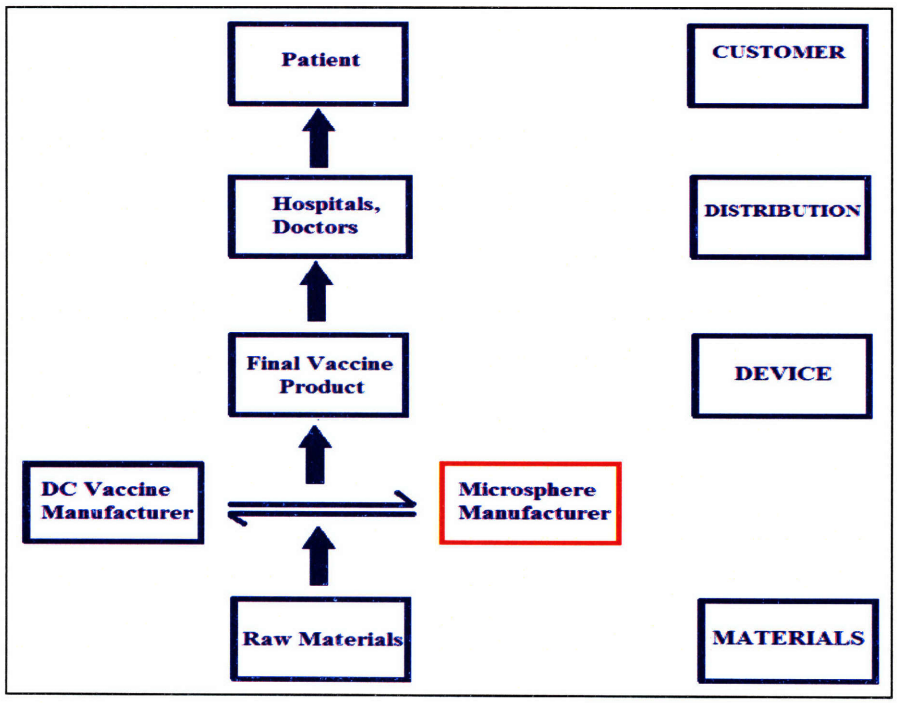

Figure 19: Illustration of supply chain and where implementation of microspheres would be into this supply chain

Potential companies to partner with include companies like Dendreon, Argos Therapeutics, Genzyme Corporation, Northwest Biotherapeutics, and IDM Biotech. A few companies, like Dendreon, specialize primarily in dendritic cell technology, and would be the ideal candidates.

7.1 Typical DC Vaccine Manufacturing Chain

Since the technology of DC vaccines in general is still relatively in its infancy, the supply chain is somewhat vertical, unlike the disaggregated horizontal supply chains of other mature industries. An example of a typical manufacturing chain is shown below, as demonstrated by Argos Therapeutics.

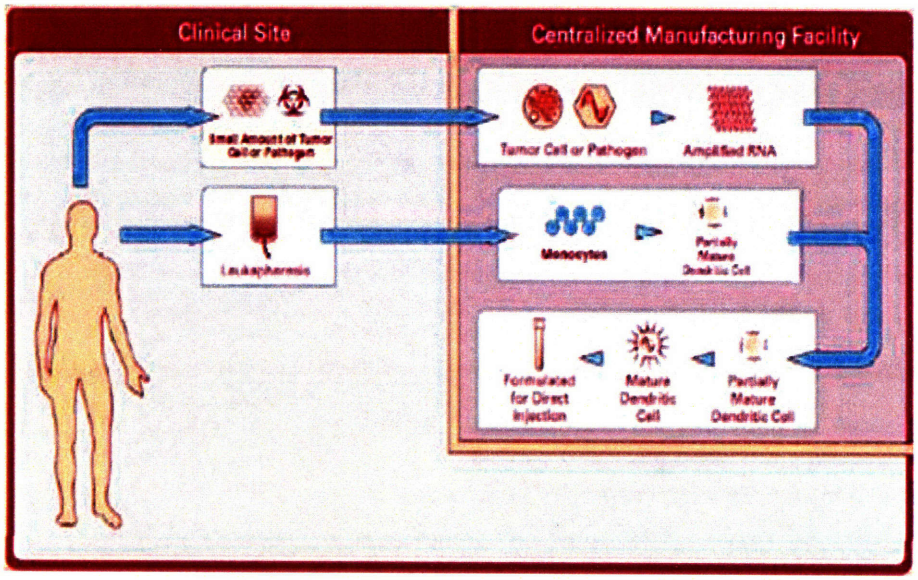

Figure 20: Sample dendritic cell vaccine manufacturing procedure, as shown from the Argos Therapeutics website [43].

As shown, the tumor cell or pathogen is individually derived from the patient, but a known tumor antigen could also be manufactured and purchased from a big pharmaceutical company. Leukapheresis is also performed on the patient; this process extracts blood monocytes which are then derived into dendritic cells through known cytokines. Next the immature dendritic cells are exposed, or "pulsed," with the tumor antigen. Here they phagocytose the pathogens and are ready to display the signal to lymphocytes in the body. They are now called mature dendritic cells.

7.2 Microsphere Production Description

It is at this step where the implementation of alginate microspheres would take place. Developed in parallel to the DC maturation process, alginate microspheres are synthesized via a water-in-oil emulsion of alginate in organic solvent. Span 80 and

Tween 80 are added to isooctane under magnetic stirring, and homogenized for 2 minutes. Then SLG20 (1% alginate, 99% PBS by weight) is added dropwise and homogenized for an additional 3 minutes. Next 5% wt/vol $CaCl_2$ is added dropwise (to crosslink the alginate), and homogenized for 4 more minutes. The resulting solution is transferred to another tube, and the mixture is centrifuged at low temperature. The isooctane supernatant is discarded, and the particle pellet is washed with more isooctane. After another centrifugation, the particles were re-suspended in deionized, distilled water and washed three times. The particles are re-suspended in deionized, distilled water for the final volume and stored at 4 degrees C until used. This completes the alginate microsphere synthesis. In another container, the alginate microspheres are suspended in deionized water [14]. These are stored at around 4 degrees Celsius and delivered to the clinical site. The mature, pulsed dendritic cells are loaded in an alginate solution (SLM20 - 0.01g/mL alginate in PBS) just before injection. Shown below is a picture of the formed microspheres and diagram of the final mixing procedure

.

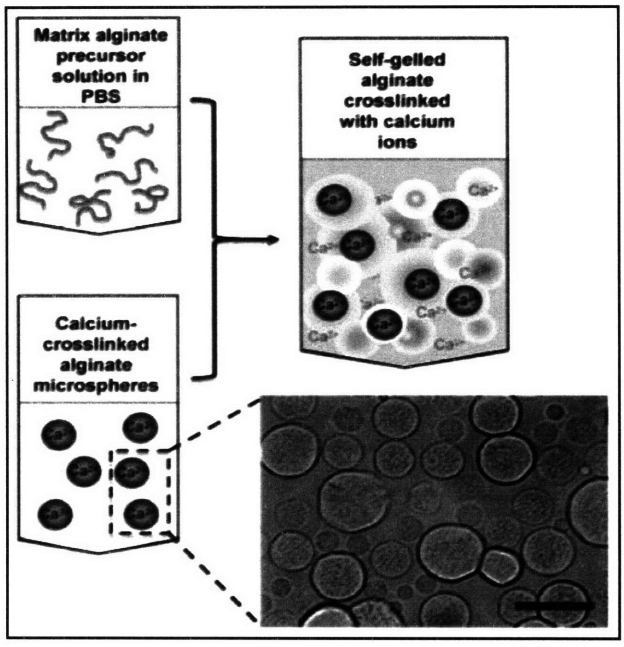

Figure 21: Schematic of the final mixing procedure and actual picture of the calcium crosslinked alginate microspheres [14]

8 Entry into Supply Chain Considerations

Entry into the supply chain of one of these dendritic cell vaccine manufacturers would seemingly come with ease. As stated before, the manufacturing of the actual dendritic cell vaccine is a largely vertical process, and the implementation of the microspheres would come at the very end of that process. For the dendritic cell vaccine manufacturer, the only change would be that they would be mixing their final, mature dendritic cells into a different solution before storage or shipping it off. Higher up the chain, the clinician administering the vaccine would simply mix the dendritic cell-loaded solution with the microspheres by pipetting them together, draw in the mixture into the syringe, and then inject it. The technical know-how necessary to perform this is minimal, and there is little added hassle. The patient would see no difference in getting the injection, except for (hopefully) more effective results. The fact that only a few, minor procedural changes take place is a large benefit of employing the technology. In many fields, including the immunotherapy field, minor changes are much easier to implement than large, paradigm shifts.

8.1 Economic Considerations – Cost Model

In order to access the feasibility of the technology, economic considerations had to be thought out. Even if a technology can show superiority, it will not realistically be implemented if the economics of it come up unprofitable. Because the technology proposed to implement is still in the research phase, many estimates and assumptions had to be made. However, the calculations conducted were to simply show the ballpark estimates, and as will be shown later, ended up being a minor additional cost. The cost model below does not take into account the added costs of including cytokine IL-15 or the IL-15/IL-15Rα complex.

For the amount of microspheres to produce during the first year, this review estimated that out of the prevalence of 700,000 cases of melanoma in the U.S [1], there would be an initial adoption rate of 0.25%. This was thought to be a justifiable and reasonable estimate since the majority of the population would probably be hesitant to try this new

approach (or any new approach for that matter). While the details are not quite clear, many current vaccines employ multiple injections, and an assumption was made that because of the gel technology, the vaccination node vaccines would need less injections than current therapeutic vaccines. Thus an average of 6 injections per patient was chosen, which gave a total that rounded to 10,000 units. One unit is defined as the quantity of microspheres necessary for one injection. The estimated costs stemming from machines, variable materials, and other costs are shown in the chart below.

Machine	**Price ($) of 1**	**# Needed**	**Cost ($)**
UltraTurrax T25 Homogenizer	1500	18	27000
Mechanical Stirrer	150	6	900
Centrifuge machine 1	1500	6	9000
Centrifuge machine 2	8000	6	48000
VWR Analog Vortex mixer	250	4	1000
Refrigerator	5000	2	10000
Zeiss Axiovert 200 microscope	200000	1	200000
Machine Cost Subtotal:			**295900**
Variable Materials	**Price per L or g**	**L or g needed**	**Cost ($)**
Isooctane	20	6000	120000
Acetone	10	5000	50000
Span 80	71.3	150	10695
Tween 80	56	50	2800
Pronova SLG20	408	400	163200
CaCl2	0.01	0.0125	0.000125
Deionized, distilled water	1.25	400	500
PBS	40	53	2120
Pronova SLM20	408	130	53040
Variable Materials Cost Subtotal:			**402355**
Other Costs	**Price per unit**	**# Needed**	**Cost ($)**
Lab Space	$30/sqft/year	1000 sqft	30000
Beakers,containers,etc	$5/batch	10000	50000
Labor	$40000/pp/year	4	160000
Other Costs Subtotal:			**240000**
TOTAL COST FOR 1 YEAR ($)			**938255**
ADDED COST FOR 1 VACCINE ($)			**93.83**

Table 4: Preliminary cost model in determining the added cost of microspheres for one vaccine. The cost model was based on one year's worth of production that translated to 10,000 units

The additional cost for implementing microspheres was found to be $93.83 per vaccine injection, which translates to $562.98 per patient treated. The average cost to treat a patient with metastatic melanoma is $59,440 [44]. Thus, with these calculations, the additional cost to implement these microspheres is adding less than 1% of the current cost of treatment. This shows much promise in the economic perspective, since the additional cost is relatively low compared to the current treatment cost. Furthermore, the average cost of microsphere making may go down with economies of scale, learning curves, and other changes that may be executed during the subsequent years of production. Setting of an actual price would be a future step, however even if the final vaccine product only added an additional $563 per patient (the exact cost to make it), the addition of the microsphere manufacturing step would break-even after the first year, and in subsequent years profit close to the amount spent on fixed costs the first year. Obviously, there will be a mark-up in price since the vaccination node adds value, and the exact mark-up and price setting would be a task decided with negotiations with the partnering company.

8.2 The Candidate Customers

The initial candidate customers would be the sufferers of later stage melanoma, specifically stage III and stage IV patients. As published in the Journal of the American Medical Association, each year roughly $740 million dollars is spent on melanoma treatment [5]. As stated before, one reason that melanoma is an ideal candidate for cancer vaccines because of its high immunogenicity. By improving the immune system's mechanism of recognizing cancer cells, cancer vaccines aim to eradicate tumors and prevent cancer recurrence [4]. Another reason is the urgency of improved treatment for late stage melanoma. The 5-year survival rate for patients with stage III melanoma is 50%, and only 13% for patients with stage IV melanoma, with a median survival time of 6-9 months [4]. This statistic is shown below in this graph showing survival of melanoma patients by stage with time

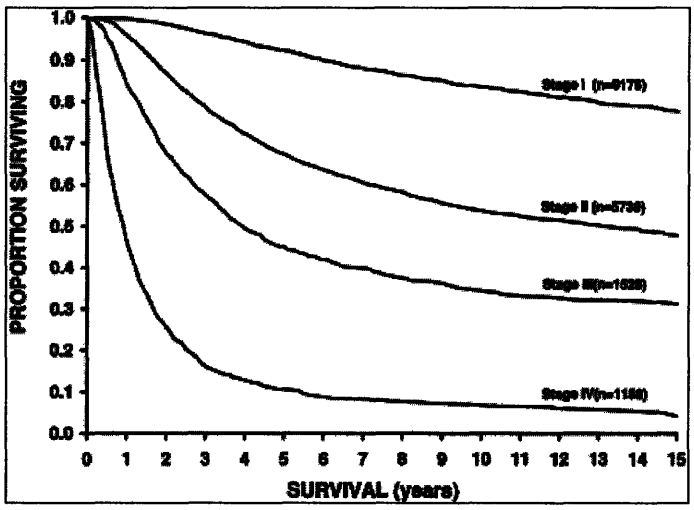

Figure 22: Proportion surviving of melanoma patients by year. The different lines show different stages of melanoma [1]

9 Market Drivers Discussion

9.1 Urgency of Need but Strict FDA Guidelines

Despite other barriers, there are many factors that drive the market. First and foremost, the incidence and low survival rates of melanoma point to the pressing need of an effective treatment. The relatively unchanged low survival rates imply that, as of today, there is no effective treatment for cancer [45]. Because melanoma is known to be both immunogenic and fatal, many vaccines as well as other types of immunotherapeutics are being developed. Melanoma vaccines have been researched for multiple decades, however to date, there is not a single FDA approved melanoma vaccine. This may be looked at as discouraging, since the FDA standards appear to be nearly insurmountable in this field. It may also be looked upon as an opportunity, in that the first vaccine to get approval will not face any competition in its field. Recently, research in novel treatments has accelerated, and in the melanoma vaccine industry alone there are a number of ongoing phase III clinical trials from companies like Genta (Genasense), Progenics Pharmaceuticals (GMK), and Vical (Allovectin-7). Recently, Antigenics' vaccine Oncophage was denied FDA approval in the US, but obtained Russia's equivalent approval [46]. Similarly, Avax's Mvax vaccine is approved for use in Australia and New Zealand, and is currently undergoing phase III clinical trials in the US [45]. Since the use of vaccines would mostly be used as an adjunct therapy, in theory it can be given to each and every patient, provided they work well. Even an incremental benefit provided by a cancer vaccine encourages the adjunct therapy, expanding the cancer therapeutic market. The current basis of cancer vaccines is therapeutic and they act by stimulating the body's own defense system. Vaccines are able to elicit immune responses without having any major side-effects [45]. The vaccination node, like other vaccines, is designed to stimulate the immune system to launch an immune response against a specific target (the tumor cells).

9.2 Specific Targeting and Safety Profile of Novel Immunotherapeutics

As seen in the case of treatment with interleukin-2, side effects from cytokine therapy are numerous and often serious. In some cases, patients have to terminate treatment early because of problems with side effects [31]. Safety is always an important consideration. As more research is conducted, novel immunotherapies are being discovered that allow higher, more effective doses, with greater specificity and lower toxicity. An example of this is seen in a study conducted by Munger and colleagues, who found that IL-15 has a therapeutic index three times greater than that of IL-2. Furthermore, the proposed vaccination node is delivered with the intent of surrounding the tumor, which adds to the safety and efficacy. In this way, the T cells that have broken tolerance near the tumor site and correspondingly attack self (tumor) cells are mostly trapped near the cancer cell neighbors and away from the majority of healthy tissue. Because of this high safety profile, it will be easier for companies to convince physicians to try these therapies in a greater number of patients. In addition, because of the high toxicities displayed by chemotherapy and other therapies like high-dose IL-2, patients need to be hospitalized during and after treatment [4]. The trend of immunotherapeutic melanoma treatments is towards administration on an outpatient basis. This eliminates the need for extra hospitalization time, cutting down both the costs and inconveniences that such actions bring.

9.3 Growing Melanoma Patient Population

Another market driver is the fact that ageing baby boomers increase the potential patient population, as the majority of new cases are still diagnosed in people older than 50 years of age [4]. The baby boomer generation – generally thought of those born between 1946 and the early 1960's – had different information regarding the potential harm of ultraviolet rays and are now increasingly becoming victims of melanoma. Furthermore, despite educational efforts and awareness programs, melanoma is the most common form of cancer for young adults aged 25-29 and the second most common form of cancer in women aged 30-34 years (second to breast cancer) [4]. Therefore, simply having a more sophisticated knowledge of melanoma does not necessarily translate to fewer cases.

10 Competition

10.1 Conventional Treatment

Currently, the traditional methods of cancer treatment (chemotherapy, radiation therapy, and surgery) are not considered true competitors, for a few different reasons. Chemotherapy has been shown to be relatively ineffective in treating later stage melanoma. Radiation therapy is known to be even less effective, though may still be used to treat pain in the patient. Surgery will still take place, as it has shown to have some correlation to higher long-term survival, however it does nothing to treat the underlying cause. Furthermore, surgery leaves the patient susceptible to recurrence [4]. The implementation of the technology might be best after surgery as an adjuvant, to patients who are at high risk of recurrence. In this way, some of the tumor is removed, and the vaccination node can focus more on killing the remaining problem. Most of the products in the clinical pipeline for melanoma are intended as adjuvant therapies, many times in conjunction with other drugs. The largest potential for adjuvant therapy implementation may lie in patients with stage 3 melanoma, where the elevated risk of recurrence is a major driver in exploring the clinical benefits of adjuvant therapy [4]. Thus initially, these other techniques of cancer treatment may still be necessary, although ideally the vaccination node treatment will be able to stand alone as a sufficient therapy, as potentially demonstrated using complexed IL-15 by Epardaud et al. [37].

10.2 Other Types of Cancer Vaccines

While there is no FDA approved melanoma vaccine currently on the market, there are many other technologies competing to solve the same problem, and progress and success of these should be monitored. With increasing information concerning the importance of professional antigen-presenting cells in generating immune responses, many attempts have and are being made to use these cells in cancer vaccines [9]. Vaccines based on cancer cells and genetic identification of cancer antigens are currently being pursued. A short description of a few of these attempts – namely tumor cell based vaccines, peptide-based vaccines, DNA vaccines, and viral vector vaccines – are discussed below.

10.2.1 Tumor Cell Based Vaccines

Tumor cell based vaccines, whether autologous or allogenic, are designed with the benefit in mind that many different specific tumor antigens are able to be presented through a number of different mechanisms. The two major proposed pathways of host immune cell activation are either by direct migration of the tumor cells to the draining lymph nodes, or by the uptake of apoptotic or necrotic tumor cells by host DC's [47]. While presumably they are providing the appropriate antigenic stimulus to the host immune system, they actually may not necessarily stimulate a potent enough signal, moreover they may induce tolerance in the immune system [47]. A notable polyvalent, antigen-rich whole cell vaccine called Canvaxin (by CancerVax Corp) has been derived from three melanoma cell lines to contain over 20 immunogenic melanoma tumor antigens, yet showed only mediocre results in clinical trials and minimal benefit in most patients [48]. However, methods to improve this idea have been tried in some experiments with and achieved selective success (such as adding dinitrophenol to improve immunogenicity), therefore progress should be monitored [49].

10.2.2 Peptide Based Vaccines

Peptide-based vaccines have stemmed from the identification of genes encoding cancer antigens. Because of their relative simplicity, peptide-based vaccines are potentially advantageous because of their ease of production under good manufacturing practices, which translates to lower production costs. However, peptide vaccines that target one specific tumor antigen may not be effective due to the fact that most, if not all, melanoma tumors are heterogeneous in their antigenic profile [47]. One possible solution to this that has been tested is administering multiple injections with multi-peptide vaccines, but while a high level of specific T cell responses were noted, there was very little evidence of actual tumor reduction [50]. Thus while immunologic responses have been achieved using peptide-based vaccines, no study of the kind has shown these responses to be directly correlated to regression of an established tumor [47]. At least until this relationship is manifest, competition from this class of vaccine should not be major.

10.2.3 DNA Vaccines

DNA vaccines are composed of a gene encoding a specific cancer antigen that is manipulated from a foreign agent to form a strong eukaryotic or viral promoter. The promoter is next integrated into a plasmid vector (carrier). The vaccine can be delivered as is (called "naked"), or attached to an adjuvant, liposome, or bacterial vector. Injection into patient cells lets the antigen be transcribed, translated, and expressed so that antigen-presenting cells can display the antigen on their surface and generate the preferred immune response [51]. A great advantage is that if successful, DNA vaccines would be easy to produce at a relatively low cost, and show high versatility in engineering possibilities if targeting or co-stimulatory genes are integrated in the vaccine [52]. While DNA vaccines have been shown to induce long-lasting immunity against infectious agents and protection from tumor growth in several animal models, results from many human clinical trials have proven to be disappointing and ineffective [47]. Prolonged survival was found as a surrogate result from one clinical trial [53], showing that while there is potential to vaccinate with DNA, much research still needs to be performed before this treatment becomes actual.

10.2.4 Viral Vector Vaccines

Viral vector vaccines utilize attenuated viruses (i.e. adenovirus, vaccinia, and poxvirus) as carriers of foreign DNA encoding an antigen (the tumor antigen) in order to express the antigen intracellularly. Generally, viruses are attenuated by deleting genes encoding one or more metabolic factors from wild type pathogens. Once the patient is infected with the virus, the patient's immune system should respond to the detection of a foreign pathogen, the antigen carried by the vector is expressed, and the patient is protected from infection [54]. Viral vector vaccines have the potential because they can be made highly immunogenic and highly specific to different host cell components [51]. A drawback of viral vectors is that several different viral vectors are known to induce a large antiviral neutralizing antibody response to the first and subsequent vaccinations. This reduces the ability of the virus to immunize, and severely limits the effectiveness of the approach [4]. Furthermore, clinical studies have so far shown to be unsuccessful, testing several different vectors encoding different melanoma-associated antigens and genes [55].

11 Additional Considerations

11.1 Assessment of Success

There are a number of factors that may benefit or add risk to the feasibility of implementing this technology. First, the current standard of measuring success in a treatment is measured in terms of reductions in morbidity or mortality [56]. Early vaccines set a high standard because they were cost-saving, but health interventions do not have to save money to be cost-effective [57]. Groups are pushing toward using other measures such as quality-adjusted life years (QALYs) saved. This is because the metric of dollars per life year saved does not give credit for averting pain, suffering, or disability attributable to disease [56]. As stated before, treatments like high intensity chemotherapy in some cases may show to extend life, but this comes with other side effects, and that extended life may be lived in extreme pain. While the assessment of the "quality of life" is very subjective, it may better reflect how the effects of treatment are progressing. If the addition of microspheres can show to improve the quality of life, even a minor way, the cost-effectiveness of adding the microspheres could be shown to be better than in the traditional, strictly economic sense.

11.2 Improving Chances for FDA Approval

Besides the technical barriers, there are other industry challenges that also need to be accounted for. As demonstrated currently with the lack of melanoma vaccines on the market, high standards in the regulatory approval process extend the time to market, which restrains revenue potential. Because there is no melanoma vaccine currently on the market, the FDA will most likely set the standard high for the first vaccine to make it to the market, so that subsequent vaccines will have to surmount equally high regulations. The lack of conclusive evidence that supports the efficacy of vaccines will justifiably hinder the approval of the first melanoma vaccine. Unfortunately, part of this is most likely because the patients who try the melanoma vaccines in clinical trials usually have undergone – and failed – conventional treatments (chemotherapy, radiation therapy, and surgery). Many of these other treatments severely weaken the immune system, and subsequently, efficiencies of melanoma vaccines have been disappointingly low. While

ideally trials would be conducted with patients who have yet to try other, more invasive treatments, patients understandably expect a certain standard of treatment first. To risk conventional treatment – although known to be somewhat inefficient – for an unproven vaccine trial, is a risk that many patients do not want to take. Along the lines of the heavy regulations, the failure of a clinical trial still involves millions of dollars (and up to tens or hundreds of millions depending on the phase of the trial), which can easily bankrupt a smaller research company. The investment spent on a failed clinical trial is completely lost. Thus there is a large risk involved, especially for a market that has yet to see the first approved product. To better increase the chance of approval, a company should set out to find a clear understanding of exactly what is required for FDA approval, including what endpoints to test for, what to look for and measure. For the case of melanoma therapy, the FDA requires survival and response rate data as clinical endpoints for immunotherapeutics [4]. The first phase of clinical trials has a primary focus on safety, during which usually a small study population consisting of healthy volunteers is involved in dose escalation (starting with a very low dose), in order to identify the maximally tolerated dose (MTD) [58]. The phase one program should ask a limited number of focused questions, because its purpose is to justify exposure to larger study populations for effectiveness in stage two. In early stage development of cancer vaccines however, the identification of a pharmacologically effective dose or optimal biologic dose might be more logical, since biologically actives doses may occur well before the MTD and furthermore it may not be feasible to achieve the MTD [59]. Phase two trials continues to evaluate safety, but the primary objective is to determine whether the regimen has biologic activity that is likely to translate into patient benefit, through an exploration of things like appropriate dose, schedule, and route of administration. In tumor vaccine studies, clinical endpoints include tumor shrinkage (an indicator of benefit, not necessarily a direct measure of patient benefit), reduction in tumor marker levels or delay in time to tumor progression [58]. Understandably, the FDA sets strict guidelines in order to standardize what constitutes effective treatment, and until (if ever) these guidelines change, companies should make sure to show the results for what the FDA considers meaningful. Appending to this, the importance of a well-designed clinical trial should be noted. In addition to a longer clinical trial being more costly, there is generally

a shortage of volunteering clinical trial candidates. Thus the clinical design should focus on obtaining the maximum amount of useful data possible, from the least number of patients, in the shortest amount of time. Because larger companies have more resources and likely industry know-how, smaller companies may opt to form strategic alliances and partner with these large companies.

11.3 Treatment Combinations

A challenge that must be overcome is finding the optimal treatment combination(s) and doses for prolonging median survival in metastatic patients. This criterion is obviously of critical importance to patients, and is also the main criterion of what the FDA looks at as success in clinical trials. As immunotherapies become more common as adjuvant therapies to chemotherapy, surgery, etc., certain combinations may prove to be more effective and safe than others. Different combinations should be tested and optimized. The statistics of late stage melanoma are extremely devastating, and so perhaps research should also be conducted in earlier stage melanoma in order to assess the preventative capabilities. Along these lines, better detection methods, education, and technology may also help reveal more treatable melanoma cases. Since late-stage metastatic melanoma makes up only a small portion of all melanoma cases, working to prolong survival in earlier stage melanoma would open more of a market, and potentially help more people.

11.4 Recent Tumor Reduction Basis of Approval

In research in mice models, the vaccination node showed a 125-fold increase in activated, antigen-specific T cells brought to the tumor. Also, the gel surrounding the tumor prevents further angiogenesis of the tumor. These factors hint at the possibility of great tumor shrinking/killing abilities. Very recently, Genentech's cancer drug, Avastin, was approved by the FDA. The drug is a recombinant humanized antibody to vascular endothelial growth factor (VEGF), and is basically designed to inhibit VEGF, which is a protein that plays a critical role in tumor angiogenesis [60]. While the FDA traditionally approved drugs for late-stage cancer if the drug prolonged or improved the patient's quality of life, Avastin did neither [61]. The basis of approval was Avastin's tumor-

shrinking capabilities [61], which may make it easier for the vaccination node to be implemented if it can show similar tumor shrinking capabilities.

11.5 Prophylactic Vaccines

Another consideration is that while our technology is aimed at implementation for therapeutic vaccines, there is much research being performed on prophylactic vaccines [45]. These vaccines have the objective of preventing a disease to begin with, rather than treating an existing problem. While success in prophylactic cancer vaccines is likely far adrift, a factor to note is that the successful invention of a prophylactic melanoma vaccine would eliminate the future market for the therapeutic treatment.

12 Conclusion

Vaccination nodes have shown promise in the research stage, overcoming a number of limitations found in other vaccination strategies for cancer. The addition of IL-15/IL-15Rα shows great promise in appending to the tumor fighting abilities of the immune system. If the vaccination node technology can show decent safety and effectiveness in humans, then its implementation into the market should not be difficult. The technology is not necessarily aiming to replace conventional treatments, but rather make significant improvements to ones that are in development. Also, if the tumor antigens are being derived from the individual patient, then some form of surgery would most likely still be necessary.

While late stage melanoma patients are the initial intended target for these vaccines, the vaccination nodes would ideally be used for treatment for earlier stage melanoma and in other types of cancers as well. The short-term goal is to conquer the problem for melanoma patients, while always keeping the bigger picture in mind. If melanoma can be treated, then this would open the door to more extensive research in other areas, and possibly extending the effective treatment to more people who suffer from other cancers.

Despite the large number of market barriers to entry, the urgency of effective cancer treatments is a pressing need, and there are many factors pushing for the pursuit of this. Upon an estimated cost model, the added cost of implementation would be slight, relative to current treatments. The manufacturing of alginate microspheres and alginate solution is relatively inexpensive, especially when considering today's high mark-ups on drugs, thus the added cost should not be significant. Supplying the microspheres and solution to the vaccine manufacturer is an easy step to implement in the supply chain, so the success of the vaccination node technology would be contingent on FDA approval of the vaccine. To ensure a higher probability of FDA approval, there should be a clear understanding of what the FDA requires, and clinical tests should be set up accordingly. There is a need to explore options with respect to which dendritic cell vaccine manufacturer(s) to potentially partner with. Commercialization of the technology and all these other factors

are contingent on success in the research stage, which is currently being pursued. Current studies are aimed at optimizing immunization and achieving a better understanding of the mechanisms that tumors employ to evade tumor destruction. Once a better understanding is achieved, efforts will be focused on dealing with these evasive mechanisms or circumventing them. The addition of complexed IL-15/IL-15Rα has shown many benefits, however further research is needed in order to maximize efficiency. Thus this research is the critical step, and efforts now should be primarily focused on this.

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