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Cancer Vaccine Final IQP

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DO CANCER VACCINES REALLY WORK?

An Interactive Qualifying Project Report

Submitted to the Faculty of

WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the requirements for the

Degree of Bachelor of Science

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ABSTRACT

The overall goal of this project was to document and evaluate the technology of cancer vaccines, especially the newer more effective versions, to determine whether cancer vaccines are really worth the recent media hype, to document any problems associated with the technique, and to help prioritize future directions. We performed a review of the research literature and conducted interviews with academic cancer researchers. Based on the research performed for this project, our team’s overall conclusion is that, of the six major categories of cancer vaccines, the tumor infiltrating lymphocyte (TIL) and chimeric antigen receptor (CAR) type vaccines have shown the highest efficacies, with some CAR vaccines producing as high as 90% full cancer remissions in medium-sized (30 patient) studies. We identified several directions for moving the field forward, including using combination vaccines (especially with antibodies for immune checkpoint inhibitors), adjuvants, recall antigens (if the vaccine is delivered into the skin), and identifying and using a patient’s own specific tumor neoantigens. We recommend that funding be continued in the cancer vaccine area of research.
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PROJECT GOALS

The overall goal of this project was to document and evaluate the technology of cancer vaccines, especially the newer more effective versions, to determine whether cancer vaccines are really worth the recent hype, to document problems with the technique, and help prioritize future directions.

The specific objectives were to:

1. Develop a comprehensive assessment of the scientific experiments that led to the development of cancer vaccines, and document the key animal and clinical studies that have been done with them.
2. Characterize what key scientific stakeholders believe are the strengths, weaknesses, reliability, usefulness, and cost of this new technology, and any other concerns.
3. Evaluate all of the obtained evidence and prioritize the remaining problems.
4. Recommend potential solutions to any remaining problems, and prioritize future experiments.
EXECUTIVE SUMMARY

Cancer is a genetic disease caused by mutations in DNA. The mutations can occur in growth factor genes, growth related pathways, or tumor suppressor genes, but regardless of the site of the mutation the outcome is uncontrolled cell growth. Cancer survival statistics have improved in the past several decades due to more accurate and sensitive detection techniques, better drugs to block cell growth by a variety of mechanisms, and more precise surgical tools. But despite these advances and the money provided by the “War on Cancer” initiated by the National Cancer Act of 1971, cancer still remains the second leading cause of death following cardiovascular disease, so better therapies are needed.

The subject of this IQP is a new form of therapy, cancer vaccines, which use the patient’s own immune system to help fight the tumor. Several types of cancer vaccines have been developed, including: 1) injecting peptides present on the surface of the tumor cell into the patient to help the immune system make antibodies and T-cells against the tumor, 2) forming antibodies against tumor-specific proteins outside the body, then injecting them into the patient (passive immunity) to fight the tumor, 3) forming antibodies against immune system inhibitors outside the body, and injecting them into a patient to activate their immune system against the tumor, 4) isolating dendritic cells (DC) from a patient, mixing them with tumor-specific proteins to commit the DC cells to those proteins, then injecting the DC cells back into the patient where they induce antibody and T-cell formation against the tumor, 5) isolating T-cells from a patient’s tumor that have already infiltrated the tumor (tumor infiltrating lymphocytes, TILs), expanding the TILs outside the body, and then injecting them back into the same patient, 6) engineering T-cells to contain chimeric antigen receptors (CARs) that recognize a specific tumor antigen, and injecting the CAR cells back into the patient to lyse the tumor.

Early cancer vaccine attempts did not work well, but the past few years have shown some spectacular successes, including complete cancer remissions in patients with very poor prognosis who had metastatic tumors resistant to all other current treatments. So, the topic of cancer vaccines has become one of the hottest topics in all of cancer research. But the new techniques appear to come with problems, including high costs in the cases of personalized vaccines, the induction of side-effects in some cases, and they do not work well in all patients. The overall goal of this project is to document and evaluate this new technology, to determine whether cancer vaccines are really worth the recent hype, to document problems with the technique, and help prioritize future directions.

The specific objectives of this IQP were to: 1) Develop a comprehensive assessment of the scientific experiments that led to the development of cancer vaccines, and document the key animal and clinical studies that have been done with them. 2) Characterize what key scientific stakeholders believe are the strengths, weaknesses, reliability, usefulness, and cost of this new technology, and any other concerns. 3) Evaluate all of the obtained evidence and prioritize the remaining problems. 4) Recommend potential solutions to any remaining problems, and prioritize future experiments. To accomplish objective-1, we performed a review of the current literature, including reputable academic journal articles, relevant books, scholarly websites, and other pertinent materials. To accomplish objective-2, we conducted a set of interviews with
various academic researchers. The interviewees included individuals working with mitochondrial disease patients, individuals performing IVF or MRT procedures, bioethics experts, and MRT legal experts. The purpose of the interviews was to determine the interviewees full range of opinions on MRT, and to solicit their help gauging the strengths and weaknesses of this new technology. After performing the Literature Review and interviews, the group synthesized all of the information collected to ascertain the strength of the evidence for and against MRT, and created recommendations for approval of MRT in the U.S. and for further research.

Cancer Introduction

Cancer is a disease caused by uncontrolled division of abnormal cells in the body. Cancer is a name given to a collection of related diseases, all sharing this uncontrolled cell division. It can begin almost anywhere in the human body, and there are over one-hundred different variations. The extra cells form growths called tumors. Some cancers form solid tumors, while others, such as leukemia, form diffuse tumors. Malignant tumors spread into, or invade, nearby tissues, or can break off and travel to distant places in the body through the blood or the lymph system to form new tumors. Benign tumors do not spread into, or invade, nearby tissues.

Cancer is the second leading cause of death worldwide, second only to cardiovascular disease. According to the Centers for Disease Control and Prevention, there were approximately 584,881 cancer related fatalities in the U.S. in 2013 (CDC, 2015). Each year, there are approximately 1,597,000 new cancer diagnoses in the U.S. (Cooper and Hausman, 2013). From the prostate gland, to the thyroid glands, the lungs, or the kidney, these malignancies symptomatically grow and strongly affect health. Sometimes the symptoms are not manifested until tumor growth is advanced, making it difficult to diagnose.

Tumor treatments vary widely, depending on the location and type. Benign tumors in a safe area of the body that do not cause organ disruption are sometimes left alone and watched carefully for switching to a cancerous state. If a tumor is cancerous, treatment options include chemotherapy (the use of chemical drugs to block DNA replication or other key cancer processes), radiation (to kill rapidly dividing cells), surgery (to remove the tumor), targeted cancer therapy (drugs that interfere with specific molecules a tumor needs to grow), and biologic therapy (i.e. cancer vaccines, the subject of this IQP).

With respect to cancer vaccines, an important point to note in this section is that the types of proteins present on the surface of a tumor cell can vary for each patient. Cancer is a genetic disease caused by changes to genes that control the way our cells function, especially how they grow and divide. These genetic changes can occur as we age, as random errors inserted during DNA replication, can occur as a result of carcinogens (such as tobacco smoke or radiation, our two best characterized carcinogens), or can be inherited from our parents. Each person’s cancer has a unique combination of genetic changes, and additional changes occur as the tumor grows, so this makes designing a cancer vaccine against a single protein difficult. Recent “personalized medicine” therapies use second-generation DNA sequencing technology to sequence the DNA of a patient’s tumor to identify neo-antigens, new proteins specific to that tumor that might provide a target for the vaccine.
Our review of the literature in this area uncovered the desire of some individuals to design a “universal cancer vaccine” that would be effective against all forms of cancer. However, our research indicated that cancer vaccines are directed against antigens known to be present on one type of tumor (CD19 in B-cell tumors), or (because cancer cells evolve within the patient) against neo-antigens on the surface of a patient’s specific tumor cells. To determine whether a universal vaccine is possible, we interviewed two leading cancer researchers, and both indicated this is not likely given the variability in antigens displayed by different types of tumors.

Immunology Introduction

The immune system is composed of a network of cells, tissues, and organs whose main purpose is to protect the body from disease and infection. Cells in the immune system recognize problems within the body, communicate with other cells, and perform various beneficial functions (NIAID, 2015). One of the main functions of the immune system is to distinguish self from non-self (foreign antigens): to protect the body from invading pathogens and eliminate altered cells (as with cancer) (Shultz and Grieder, 1987).

The immune system is divided into two major subdivisions: innate immunity and adaptive immunity (NIAID, 2015). Innate immunity is the part of the immune system that is ready for immediate maximal response when an infection is first detected. It constitutes the first two lines of a three-line defense system. The first line of defense is non-specific, and begins with physical and chemical surface barriers, such as the skin, sweat, tears, saliva, respiratory tract mucous, stomach acid, and urine. If pathogens are able to get past the first line of the innate defense, the second line of the innate defense is activated which includes the use of immunity defensive cells, defensive proteins, inflammation, and fever. The cells involved in the second line of defense include several types of white blood cells (leukocytes), including natural killer cells (NKs), mast cells, eosinophils, basophils, and the phagocytic cells including macrophages, neutrophils, and dendritic cells (DCs). These cells recognize molecular patterns present on the surface of bacteria and fungi, and act to engulf them (or aid other cells that engulf and kill them).

Adaptive immunity is the third line of defense. If a pathogen survives the nonspecific innate defenses, the body will react with a more advanced response to specifically target the pathogen. The adaptive immune system is known as the antigen-specific immune response. Once contact has been acquired with a pathogen, it activates the B and T-lymphocytes, each boosted by humoral mediators such as cytokine hormones (NCBI, 2001). Once engaged by a foreign antigen (usually one presented by an antigen presenting cell) the B or T-cell matures to commit to that specific antigen. The immune cells that are most importance to our cancer vaccine topic are antigen-presenting cells (including dendritic cells) and T-cells.

Dendritic Cells (DCs): Discovered in 1973 in mice (Steinman and Cohn, 1973), dendritic cells (DCs) are important components of the mammalian immune system. They get their name from their branched appearance at specific stages of their development. DC’s are potent “professional” antigen-presenting cells; their main function is to recognize foreign antigens (usually small epitope domains of proteins) on the surface of invading pathogens (and sometimes cancer cells), process the antigen within the cell, and then present it on its surface to other cells of the immune system (T-cells and B-cells) so they can commit to recognizing that
particular foreign antigen and attack the tumor to reduce its mass (Banchereau and Steinman, 1998; Sallusto and Lanzavecchia, 2002; Trombetta and Mellman, 2005). Half of the 2011 Nobel Prize in Physiology or Medicine went to Ralph M. Steinman "for his discovery of the dendritic cell and its role in adaptive immunity" (The Nobel Prize, 2011). Because of this antigen presentation function, DCs are used in some types of cancer vaccines to induce an immune response against an antigen on the surface of a patient’s tumor cell.

_T-Cells:_ T-cells (also termed T-lymphocytes) are a type of nucleated white blood cell that functions in cellular immunity. They are distinguished from other lymphocytes, such as B-cells and natural killer cells (NK cells), by the presence of a T-cell receptor (TCR) on their cell surface which recognizes presented antigen and commits the cell against it. They are called T-cells because they mature in the thymus (although some T-cells also mature in the tonsils) (Alberts et al., 2002). There are several types of T-cells, each with a different function: helper (CD4+), cytotoxic (CD8+), memory, suppressor, mucosal associated, and gamma delta T-cells. With respect to cancer vaccines, **tumor infiltrating lymphocytes (TILs)** are a type of T-cell found in tumors that help kill it. High levels of TILs in tumors are often associated with a better clinical outcome for the patient (Vanky et al., 1986). TILs isolated from tumors usually include both CD4+ (helper T-cells) and CD8+ (cytotoxic killer T-cells, CTLs). TILs circulate through the bloodstream, recognize the tumor and infiltrate it. The CD4+ cells secrete cytokines to stimulate the immune system, while the CTLs directly lyse the tumor cell.

**Peptide Injection Vaccines**

The simplest type of cancer vaccine involves directly injecting purified proteins into a patient to stimulate the patient’s own immune system to clear the tumor cells. The injected peptide usually represents a protein (or a part of it) present on the surface of the patient’s own tumor cells. The injected proteins are usually previously characterized as present on all cells of a particular type of tumor (for example prostate-specific antigen for prostate cancer). More recently, in a form of personalized medicine, the proteins are identified directly from a patient’s tumor cells by whole exome DNA sequencing to identify neo-antigens. The injected protein is hopefully recognized by the patient’s immune system as foreign. This recognition is by antigen presenting cells (APCs) that present the protein to B-cells (which mature to make antibodies against the peptide) and present it to T-cells (which mature to make cytotoxic T-lymphocytes to home in on the cancer cells containing the peptide). Relatively few success studies of this type exist because the approach appears to be less effective than DC, TIL, and CAR vaccines.

An example experiment in the peptide vaccine category is a 2006 Phase-II clinical study performed on 28 patients with metastatic androgen-independent prostate cancer (Arlen et al., 2006). The patients received a peptide vaccine consisting of a vaccinia virus encoding PSA tumor antigen (specific for the tumor cells), followed by boosters with a fowlpox virus also encoding the PSA antigen. Their data demonstrated a 3.33-fold increase in T-cell response to the peptide (PSA) after 3 months, and the median progression-free survival increased to 6.1 months compared to 3.7 months for the controls. But there were no full remissions, as seen in some of the newer forms of cancer vaccines.

A future trend in the peptide vaccine area is to inject peptide **mixtures**. For example, a 2012 study done in Tubingen, Germany was the first immune vaccine for renal cell carcinoma
(RCC) (Walter et al., 2012). The clinical Phase-I/II study consisted of an injection of 11 different multiple tumor associated peptides (TUMAPs) the authors previously determined were naturally present on the surface of the RCC tumor cells, and most importantly, the peptides were antigenic when injected into humans. The Phase-I data showed that an induction of T-cell responses against multiple TUMAPs correlated best with disease control, and the vaccine appeared to be safe. The Phase-II data confirmed that an immune response against multiple cancer peptides correlates with longer patient survival. But again, there did not appear to be any long-term full cancer remissions.

**Monovalent Antibody Vaccines**

The second type of cancer vaccine involves injecting antibodies previously made against a specific tumor protein. This type of “passive immunity” does not activate the patient’s own immune system to create the antibodies, but instead provides the antibodies by a bio-engineering process. The antibodies combine with the protein on the surface of tumors to create antigen-antibody complexes, which are then recognized and cleared from body by other cells of the immune system, such as macrophages or T-cells. Monovalent antibodies recognize only one type of epitope on a protein (portion of a protein). Most natural antibodies produced by the body in response to an infection are of this type, and both arms of the “Y” shaped antibody molecule recognize the *same* antigen. Anti-tumor monoclonal antibodies (mAbs) represent a major advance in cancer therapy, and in the past decade at least 6 different mAbs have been approved by the FDA for treating cancer, and more are in development (Taylor et al., 2007).

In this approach, the antibodies are made in advance against a protein previously determined to be present on a cancer cell. Examples of this type of vaccine are antibodies made against CD19, CD20, or CD22 on the surface of B-cells for fighting B-cell tumors (such as leukemia), overactive B-cells (autoimmune disorders, transplant rejection), or dysfunctional B-cells. The first antibody approved for cancer treatment in the U.S. was **Rituximab**, a mAb against CD20. It was initially FDA approved in 1997 to treat non-Hodgkin lymphomas resistant to chemotherapy (Maloney et al., 1997). Several studies have used it in clinical trials with various success. For example, one 40 patient study showed a 95% response rate, 55% complete responses, and 40% partial remissions (Czuczman, 1999). Another study of 39 patients at one year showed an overall response rate of 72%, 18% with complete responses, and 77% showing a progression-free patient survival (Hainsworth, 2000). Another study of 33 patients with aggressive non-Hodgkin’s lymphoma showed an overall response rate of 94%, with 61% with a complete response, and 33% with a partial response (Vose et al., 2001). Similar data has been obtained for a mAb against CD22 on B-cells. For example, a study done with 90 patients suffering from refractory acute lymphoblastic leukemia (ALL) (with a very poor prognosis) showed that patients treated with **Inotuzumab** had an overall response rate of 58% (19% complete response, 30% complete response with no platelet recovery, and 9% became stable enough with the antibody treatment to have a bone marrow transplant and showed a complete cancer remission) (Kantarjian et al., 2013).

The monovalent antibody category of cancer vaccine is also represented by **Herceptin** (also known as **Trastuzumab** or Herclon). This antibody was the second mAb approved for cancer treatment in the U.S. and interferes with the HER2/neu receptor over-expressed on some types of cancer cells. Typical clinical trial results in this category are represented by a study of
234 patients with metastatic breast cancer showing that the treatment provided a longer time to disease progression (median 7.4 vs 4.6 months; p<0.001), a higher rate of objective response (50% vs 32%, p<0.001), a longer duration of response (median, 9.1 vs 6.1 months; p<0.001), a lower rate of death at 1 year (22% vs 33%, p=0.008), longer survival (median survival 25.1 vs 20.3 months; p=0.046), and a 20% reduction in the risk of death (Slamon et al., 2001).

Although the treatments in this category sometimes prolonged a patient’s life, full remissions were not common. The reported “complete responses” (full immune involvement) are not the same as “complete cancer remissions”, as have been seen recently with TIL and CAR vaccines (discussed below). A trend seen throughout the entire cancer vaccine field is the design of antibodies against inhibitory receptors present on immune cells (to activate them). An example of this is Siglec CD22, an inhibitory receptor located on B-cells which is activated in leukemia. Our interview with a cancer researcher using antibodies against CD22 indicated he felt this was a good target because it is activated on so very few cell types. So, this allows a highly selective targeting to the tumor, and limited damage to normal cells, and will likely be continued in the future.

**Bivalent Antibody Vaccines**

Bivalent (or bi-specific) antibodies recognize two different epitopes by the same antibody molecule. The best characterized example of this bivalent approach is antibody Blinatumomab, where one antibody arm recognizes CD19 (to attach to B-cells whose numbers are elevated in leukemia), and the other arm recognizes CD3 on the surface of T-cells to bring them into close proximity with the cancer cell to kill it. Researchers sometimes term this antibody a “bi-specific T-cell engager” (BiTE). Studies designed to directly compare the activities of monospecific versus bispecific antibodies have shown the latter have higher potency, improved anti-tumor activity, lower dosing, and lower costs of production compared to other antibody formats (Molhol et al., 2007).

Examples of the spectacular successes with the bi-specific cancer vaccine approach include the treatment of 9 patients with acute lymphoblastic leukemia with Blinatumomab, of which 4 achieved complete remission after their first cycle of treatment, 2 showed a complete remission after the second cycle (Schlegel et al., 2014). And in another study, 189 patients with B-cell acute lymphoblastic leukemia (B-ALL) showed that after two treatments, 81 of the 189 patients (43%) showed complete cancer remission.

In addition to CD19 x CD3 bispecific antibodies, other bispecific antibodies include CD3 (T-cell binder) x glioma marker (Nitta et al., 1990), folate receptor (ovarian cancer cells) x CD3 (Canevari et al., 1995), CD16 x CD30 for Hodgkin’s disease (Hartmann et al., 1997), CD319 x CD28 for B-cell lymphomas (Daniel et al., 1998), CD64 x Fc-Receptor for B-cell lymphomas (Honeychurch et al., 2000), CD30 x CD64 for Hodgkin’s lymphoma (Borchmann et al., 2002).

One problem identified in our Lit Review in this area was with CD19 vaccines for leukemia, where all B-cells are removed from the body, not just cancerous B-cells. Normal B-cells are required for antibody production. To gain more information on this potential problem, we interviewed a researcher using CD19 antibodies to treat leukemia, who indicated that occasionally they see serious opportunistic infections due to the lack of antibody production, but
these are considered a necessary evil compared to the very high death rates that result from leukemia. Our review of the literature also identified combined vaccines as a trend in the field, and our interviews with BiTE bi-specific antibody users indicated they agreed, and liked the idea of using BiTE in combination with a CAR vaccine. Originally, it was not clear for the CD3 portion of BiTE whether it activated the T-cells or simply served to bridge the cancer cell (bound to the CD19 antibody portion) to a T-cell, to bring the two into close enough proximity to allow the T-cell to kill the cancer cell. Our interviews with researchers who use BiTE indicated it is the latter…the bispecific antibody makes physical bridge between a cancer cell and a T-cell simply to bring them close together.

**Immune Checkpoint Vaccines**

One of the most exciting advances in cancer vaccine research in the past decade is the discovery that T-cells that have migrated into a patient’s tumor in the body’s attempt to fight the tumor often become inactivated by the tumor. Proteins present on the tumor surface (such as PD-1 and CTLA-4) engage inhibitory receptors on the surface of the T-cells to inactivate them [reviewed in: Topalian et al., 2012]. To overcome this immune suppression, scientists are researching the use of antibodies against the inhibitory receptors to “block the blocker” and re-activate the T-cells to attack the cancer. The immune system blockers discovered to date include programmed death-1 (PD-1) and cytotoxic T-lymphocyte-associated protein-4 (CTLA-4). This approach provides a new and exciting approach to cancer vaccines that is different than all other approaches.

The most researched anti-PD-1 monoclonal antibody is Nivolumab (marketed as Opdivo). Nivolumab has been approved by the FDA for treating squamous non-small cell lung cancer, and several other studies have investigated its use for treating melanomas (Topalian et al., 2014), Hodgkin’s lymphoma (Ansell et al., 2015), and non-small cell lung cancer (Gettigner et al., 2015; Rizvi et al., 2015; Tanner, 2015). Another well characterized antibody in this category is Ipilimumab, an antibody against CTLA-4. CTLA-4 acts as an "off" switch when bound to ligands CD80 or CD86 on the surface of antigen presenting cells (Walunas et al., 1994). Some scientists have experimented with combination vaccines using both anti-PD-1 and antib-CTLA-4 antibodies. This combination approach appears to be even more successful than using single antibody approaches. For example, one study done with the combination showed that treatment of melanoma patients with a combination of Nivolumab (PD-1 inhibitor) and Ipilimumab (CTLA-4) showed that 80% of the patients had a significant tumor reduction (Wolchok et al. 2013).

Our review of the literature showed that activating the immune system by blocking these checkpoint inhibitors can sometimes lead to autoimmunity (where the immune system is stimulated to react with the body’s own tissues) or inflammation (Melero et al., 2007). And in some cases the treatments led to grade-3 and 4 (serious) side-effects, although most of the side-effects appeared to be relatively mild, transient, and treatable. So these potential problems should be monitored, and the treatment stopped if necessary. Our review of the literature also showed that some scientists obtained promising synergistic effects when they combined checkpoint vaccines with antibodies directed against tumors. We verified this point in an interview with a researcher who used PD-1 antibodies who agreed that combining a PD-1 approach with for example Herceptin antibody for breast cancer would be a good idea.
Dendritic Cell Vaccines

Tumor cells in the body are poor antigen-presenting cells. Tumor cells are derived from normal cells by DNA mutation, so most of the proteins on their surface look like “self” to the immune system, and are ignored allowing the tumor to grow. Only a small portion of the cancer DNA mutations create “neo-antigens” that are unique to the patient’s tumor, and these provide excellent candidates for cancer vaccine designs. Even when tumor cells contain unique neo-antigens on their surface, dendritic cells (DCs) are still required to process the neo-antigen and present it to the immune system to generate active B-cell and T-cell responses against the tumor (Palucka and Banchereau, 2012). Animal experiments (discussed in our Lit Review) have shown that DC cells are a required component of the body’s immune attack against cancer, and are required for activating CD8+ T-cells that infiltrate and attack the tumor. The aim of a DC-type tumor vaccine is to induce DC cells to present tumor antigens on their surface to stimulate the formation of immune cells, especially CD8+ tumor infiltrating lymphocytes (TILs) or cytotoxic T-lymphocytes (CTLs) that recognize, infiltrate, and attack the tumor (Davis et al., 2003; Steinman and Banchereau, 2007; Koski et al., 2008; Schuler, 2010; Ueno et al., 2010). In an *ex vivo* approach, the DC cells are usually isolated from a cancer patient’s peripheral blood mononuclear cells (PBMCs) using various techniques and are cultured to expand their numbers. The DCs are then “pulsed” (mixed) with foreign tumor antigen (either purified antigen or entire tumor cells themselves), and are then injected back into the patient where they hopefully migrate to the lymph nodes to engage B-cells and T-cells to commit them against the tumor antigen. In a less used *in vivo* approach, DC cells in the body are induced to take up tumor-specific antigens, and the antigen-presentation is done naturally to stimulate the T-cells.

The first DC cancer vaccine approved for use in the U.S. by the FDA was **Provenge** (also called **Sipuleucel-T** or APC8015). Provenge is a DC-type vaccine designed for prostate cancer, which is the second leading cause of cancer death in men (after skin cancer) (Ledford, 2015). Provenge consists of autologous (taken from the same patient) peripheral blood mononuclear cells (PBMCs) (that includes DCs) taken from the patient by leukapheresis. The PBMCs are cultured to increase their numbers. They are then mixed for 2 days *in vitro* with a recombinant fusion protein (PA-2024) that contains prostatic acid phosphatase (PAP) (expressed in a majority of prostate adenocarcinomas) (Goldstein, 2002) linked to granulocyte macrophage colony stimulating factor (GM-CSF) (to help activate the immune system). After the DCs are pulsed or primed against PA-2024, the DCs process the antigen within the cell and present it on their surface. The DC cells are perfused back into the same patient over a 30 minute period. Once in the body, the DCs migrate to the lymph nodes and present the tumor antigen to B-cells and T-cells to activate them against the prostate cancer. The results of Provenge are mixed. Early experiments demonstrated that CTLs had been formed against the tumor cells, and that PSA levels dropped, but the survival statistics were relatively unimpressive compared to controls, for example increasing from 4.9 months survival before treatment to 7.9 months after treatment (Beinart et al., 2005). A randomized, double-blind, placebo-controlled phase-III clinical trial of Provenge, testing a total of 225 patients with advanced prostate cancer found an average 33% reduction in death rate over the test period for the patients receiving Provenge versus the placebo (p=0.011), a significant outcome, but the study did not report long term remissions (Higano et al., 2009). Another double-blind, placebo-controlled, multi-center trial on 341 Provenge patients versus 171 placebo patients showed an average 22% reduction of death in the Provenge group,
with survival extending from 21.7 months to 25.8 months (an improvement of only 4.1 months) (Kantoff et al., 2010). Adverse effects included chills, fever, and headache. So, the Provenge Phase-III results were not as dazzling as hoped for, but they did provide a proof-of-principle that cancer vaccines can improve lifespan in a large cohort of patients.

The DC vaccine approach has also been applied to patients with malignant melanoma. Melanoma is a type of skin cancer that forms from pigment-containing melanocytes in the skin (Bajetta et al., 2002). Although it is less common than other types of skin cancers, melanoma is much more deadly if not detected early, causing the vast majority (75%) of skin cancer deaths (Jerant et al., 2000). As with the Provenge findings, most of the early experiments reported on the formation of anti-tumor CTLs that were capable of destroying cancer cells in vitro. A more recent study of 7 patients with stage IV (advanced) melanoma showed that 6 of the 7 patients had sustained T-cell responses (against the tumor), and of those 6 patients, 1 patient showed complete remission, and 2 showed partial remission (Carreno et al., 2013). Strong improvement correlations were observed for patients perfused with DC cells producing the hormone IL-12, screening for DC cells that are functional enough to produce IL-12 (or supplementing the vaccine with IL-12) might help improve DC vaccine outcomes. Very recently, this same team used a personalized medicine approach to identify and vaccinate against “neo-antigens” present only in a patient’s own tumor cells (Carreno et al., 2015), so this approach likely will be further researched in the future.

DC vaccines have also been applied to glioblastomas, the most common and aggressive primary brain tumor for adults (Stupp et al., 2005). Because these tumors have a finger-like shape that protrudes into healthy tissue, it is very difficult to completely remove the tumor by surgery without damaging healthy brain tissue (ABTA, 2014). The median survival time after standard treatment is less than 2 years (Johnson and O’Neill, 2012). An example DC study with this type of cancer on 9 glioblastoma patients showed the median survival time of the DC group was 455 days compared to 257 days for the control group (Yu et al., 2001), but no complete remissions were reported. In another example study, 19 patients were treated with DC cells loaded with glioma-associated antigen (GAA) and supplemented with a synthetic adjuvant poly(I:C). 58% showed a positive immune response against the antigen, 9 patients (47%) had no sign of tumor progression for at least 12 months, 1 patient showed sustained complete cancer remission (Okada et al., 2011).

Some scientists argue that the DC vaccine clinical trials performed so far have produced relatively disappointing results compared to the full remissions seen with the TIL and CAR studies, and that a full potential of DC cancer vaccines has not yet been achieved (Koski et al., 2008). Our review of the literature identified several trends for improving vaccine strategies, including: 1) combining the vaccines with immune stimulatory hormones, like IL-12. 2) Creating combination vaccines composed of DC’s primed to tumor antigens plus antibodies against checkpoint inhibitors CTLA-4. 3) Using a personalized medicine approach to sequence the exomes of a patient’s tumor to identify neo-antigens unique to that patient for priming the vaccine against. Within this area it is especially important to identify which neo-antigens are immune-dominant (of the new neo-antigens synthesized by a tumor and located on the cell surface, which ones are more likely to produce an immune response when used to charge a vaccine, these should be used for the vaccine). This key point of immune-dominance was verified by several of our interviews in this area. 4) Improving the speed at which this DNA sequencing can be done to decrease the time to therapy. 5) Using adjuvants, such as poly-(I:C),
to boost the immune response. This important approach was verified by us in an interview with a researcher who uses poly(I:C) as adjuvant, who verified that the adjuvants should in the future be combined with checkpoint inhibitors. 6) Using pre-injections of a “recall antigen” such as tetanus toxoid Td which has been shown to increase DC migration to lymph nodes to improve the immune response. This important point was validated in an interview we had with a researcher using tetanus toxoid as a recall antigen at the injection site of the cancer vaccine, but he indicated this only works when injecting the cancer vaccine into the skin, not when injecting the DC cells directly into lymph nodes. 7) Identifying biomarkers for which type of patient is most likely to respond to a vaccine. 8) Supplementing the vaccines with other cellular components of the immune system, such as natural killer cells (NKs) which have recently been shown to help other cells present antigens. This latter point is especially interesting, because one of our interviews with an NK researcher indicated they recently discovered that NK’s work in conjunction with DC cells to help the latter present antigens more efficiently to the immune system. So, the use of mixed cell type vaccines might be a field of the future.

**TIL Cancer Vaccines**

T-cell therapy, sometimes referred to as adoptive cell therapy (ACT), has several advantages over any form of vaccine that relies solely on the production of T-cells in vivo, including: 1) Very large numbers of T-cells can now be grown in vitro (up to 10^{11} cells). 2) The T-cells can be selected in vitro for those that recognize tumor antigens. 3) T-cell growth in vitro by-passes any negative regulation those cells might encounter in vivo, for example from PD-1 or CTLA-4. 4) The patient can be treated with traditional chemotherapy to support the tumor therapy prior to the cell infusion. 5) The T-cells can be genetically engineered in vitro to express chimeric antigen receptors (CARs) (discussed below) that directly recognize tumor proteins without relying on MHC-type antigen presenting cells.

The use of TILs to treat cancer was pioneered by Dr. Steven Rosenberg at the National Cancer Institute (for a review, see Rosenberg and Dudley, 2009). In this technique, autologous lymphocytes are isolated from a patient’s tumor and grown to very large numbers in vitro. In some cases, prior to the TIL treatment the patients are given chemotherapy to deplete native lymphocytes that can suppress the function of the perfused TILs. Once lympho-depletion is completed, patients are then infused with their own TILs supplemented by hormones such as interleukin-2 (IL-2). The vast majority of research on TIL cells, and several ongoing clinical trials have been conducted using TILs to treat patients with metastatic melanoma (Dudley et al., 2008; Besser et al., 2010; Rosenberg et al., 2011; Radvanyi et al., 2012; Pilon-Thomas et al., 2012).

One TIL experiment that caught the world’s attention was performed by Steven Rosenberg’s team in 2011 on 93 patients with advanced metastatic melanoma treated with autologous TIL cells and supplemented with IL-2 (Rosenberg et al., 2011). 95% of the patients had ongoing cancer from prior chemotherapy treatments. Their data showed that 20 of the 93 patients (22%) achieved complete cancer remission, and 19 of the 20 regressions remained intact beyond 3 years! The 3 and 5-year survival rates for the 20 remission patients were 100% and 93%, respectively. The same team also developed a new screening approach for identifying newly mutated proteins present in melanomas using whole-exome DNA sequencing of tumor DNA followed by bioinformatics to identify mutations present in the tumor protein-coding
regions not present in normal tissue (Robbins et al., 2013). The method avoided the labor-intensive methods of synthesizing and screening cDNA libraries. And in 2014 they analyzed TIL cells isolated from tumors using deep sequencing techniques to determine which cancer antigens the TIL cells recognized and whether any TILs expressed inhibitory receptors (Gros et al., 2014). Their data indicated that 6 of 6 tumors contained TILs directed against neo-antigens, indicating that neo-antigens likely are the best target for future experiments. And all 6 tumors contained TILs positive for negative immune receptors PD-1, LAG-3, and TIM-3, indicating that the TILs in their in vivo state were functionally impaired by the tumor. Thus, antibody therapy designed against the negative regulators might improve vaccine effectiveness.

TIL cells have also been used to treat other kinds of cancer besides melanomas, including epithelial and ovarian cancers epithelial cancer (Tran et al., 2014), and ovarian cancer (Robins et al., 2013).

CAR Cancer Vaccines

Chimeric antigen receptors (CARs) (also known as chimeric T-cell receptors or chimeric immune-receptors), are artificially engineered T-cell receptors that provide a specific binding affinity to the T-cell containing it. In the case of cancer immunotherapy, CARs are typically engineered to have a monoclonal antibody-like affinity for a specific tumor antigen, and do not rely on antigen presentation to recognize the antigen. In this approach, an engineered CAR gene is delivered inside the T-cells in vitro using retroviral vectors, the CAR is expressed on the cell surface, and the engineered T-cells are delivered back into the same patient (reviewed in: Pule et al., 2003; Lipowska-Bhalla et al., 012; Curran et al., 2012). The types of CAR structures used have evolved over the years, but the current CAR structure includes a mAb single-chain variable fragment derived from an anti-tumor mAb (which recognizes the tumor), a co-stimulatory domain (like CD28 that activates the T-cells and helps them survive in vivo), a hydrophobic domain that spans the lipid bilayer on the surface of the CAR cell, and one to three cytoplasmic tail domains (like TCR-zeta) that activate the CAR cell once it engages the tumor cell. Thus, structurally, CARs combine the powerful properties of highly specific antigen recognition (the mAb-like portion), with stimulation to increase T-cell survival (CD28), with T-cell signaling activation to kill the cancer cell, all combined in a single engineered receptor molecule (Sadelain et al., 2009). Once the CAR binds its tumor cell target, it sends a signal inside its T-cell to activate it to kill the tumor cell.

The early foundation for the CAR field was laid by Israeli immunologist Zelig Eshhar, but the technique was further refined by other big-name cancer researchers like Steven Rosenberg (National Cancer Institute), Carl June (University of Pennsylvania), and Michel Sadelain (Memorial Sloan-Kettering Cancer Center). Commenting on the technique for transforming the T-cells with foreign DNA, Michel Sadelain stated, “It took me 3 to 4 years to learn to transfer genes [into T-cells] at more than 0.5%. Today we can take a high school student and in an afternoon they know how to take T-cells and blast genes into all of them” (Couzin-Frankel, 2013).

By far, the most successful application of CAR vaccines to date is against CD19 on the surface of B-cells that are elevated in leukemia (reviewed in: Kochenderfer and Rosenberg, 2013). Engineering T-cells to target CD19 has provided some of the most striking successes in
the entire cancer vaccine field. Several labs converged on the CD19 antigen for two good reasons: 1) it is universally expressed on the surface of all leukemic B-cells, and 2) although normal B-cells are also killed by the anti-CD19 treatment, such cells are not absolutely required for patient survival (antibodies can be provided passively, or the patient can undergo a bone marrow transplant after ridding the cancer cells). Early CAR experiments focused on altering and improving the design of the CAR receptor, quickly finding out that without the CD28 co-stimulatory domain, the perfused CAR cells quickly die off.

The breakout year for CAR treatments was 2011. Early studies with CAR cells had shown some success, but in 2011, Carl June’s group at the University of Pennsylvania (one of the founders of the CAR field) published two studies on their design of a CAR against CD19 and its use to treat 3 patients with advanced chronic lymphocytic leukemia (CLL). The first study (Porter et al., 2011), showed that the injected CAR cells expanded at least 1000-fold in the patients, migrated to the bone marrow, continued to produce functional CARs for at least 6 months, each CAR cell destroyed about 1,000 cancer cells, one of the 3 treated patients showed complete remission (33%). In the second paper (Kalos et al., 2011), two of 3 patients (66%) showed complete remission, and they remained in remission two years later in June of 2013 (Couzin-Frankel, 2013). These two 2011 studies, although done on relatively few patients, were later attributed by Carl June as breaking open the CAR funding for his entire research team, and the National Cancer Institute reversed their earlier moratorium on funding CAR research. An interesting follow-up 2013 study (Grupp et al., 2013), showed that of 2 children with relapsed and refractory acute lymphoblastic leukemia (ALL) (normally a very poor prognosis) using CAR cells targeting CD19, 1 of the 2 children (50%) showed a complete remission. This paper observed that the patient that relapsed developed cancer cells no longer expressing CD19, so this evolution problem should be monitored in the future. Also in 2013, a team headed by Drs. Michel Sadelain and Renier Brentjens (Brentjens et al., 2013) showed spectacular success when 5 of 5 patients (100%) with relapsed B-cell acute lymphoblastic leukemia (B-ALL) (normally with a very poor prognosis) showed a rapid tumor eradication, and appear to have no residual disease as assayed by deep sequencing PCR (although one patient eventually relapsed). With respect to side-effects, some patients showed significant cytokine elevations, but those incidences were treatable with steroid therapy. So, the treatment appeared to be generally well tolerated.

Additionally in 2013, a team headed by Dr. Kochenderfer (of Steven Rosenberg’s team at the National Cancer Institute), published their findings of a clinical trial of allogeneic T-cells genetically engineered to express CAR targeting the B-cell antigen CD19 (Kochenderfer et al., 2013). Their study tested 10 patients that had malignancy persisting after initial bone marrow transplants and standard lymphocyte infusions. No new chemotherapy was done to any of the ten patients prior to the CAR T-cell infusions, so the patients were not lymphocyte-depleted at the time of infusions. The data showed that three of the 10 treated patients had regressions of their previously untreatable malignancies. One patient with chronic lymphocytic leukemia (CLL) obtained complete remission after the T-cell infusion, while another CLL patient had tumor lysis syndrome as his leukemia regressed. They discovered that none of the patients developed graft-versus-host disease (GVHD). PCR showed the presence of the CD19-CAR gene in the blood of eight of the ten patients. This study showed that the CAR approach can cause regression of B-cell malignancies previously resistant to standard treatments without causing graft versus host disease.
In one of the most spectacular experiments in all cancer vaccine research, in 2014, Dr. Grupp’s team at the Children’s Hospital of Philadelphia and their main collaborator Dr. Carl June of the University of Pennsylvania (Maude et al., 2014) treated 30 patients (children and adults) with relapsed acute lymphoblastic leukemia (ALL) with CD19-directed CAR cells and 27 of 30 patients (90%) showed complete remission at 6 months! This is quite a success story compared to the relatively weak data obtained in the early cancer vaccine papers. With respect to side-effects, all 30 patients developed cytokine-release syndrome (CRS) (27% with severe CRS), but the problem was effectively treated with anti-interleukin-6 receptor antibody (tocilizumab), and the patients remained in cancer remission.

Although the release of cytokine hormones from the engineered CAR T-cells is normally viewed as desirable, and is one measure of their successful in vivo function, the production of very high levels of cytokines is viewed as a “cytokine storm” which can be fatal. One 2010 paper from Rosenberg’s team reported the death of a patient five days after receiving second-generation CAR T-cells (Morgan et al., 2010). The authors speculated that the large number of perfused T-cells localized in the patient’s lung immediately after the perfusion, triggering cytokine release by recognizing a receptor on the lung cells. So, this study emphasizes the importance of restricting T-cell infusions to those designed against cancer neo-antigens whenever possible, that are specific for the tumor cells whenever possible, to avoid CAR interactions with natural proteins.

Our review of the T-cell literature identified several problems, some of which were followed up in interviews: 1) The best patient survivals appear to occur in patients with the highest TIL load in the tumor, so this underscores the importance of producing large numbers of T-cells for a vaccine. 2) Some of the best cancer remissions occurred by enriching the TILs for a subset of TILs committed against neo-antigens, so perhaps in the future, although this approach is labor intensive, this enrichment approach should be tested further. Our interview with a neo-antigen expert in the TIL area indicated they have found neo-antigens in all tumors they have investigated. So, the neo-antigen approach seems like it would apply to all types of cancer. We also questioned another interviewee about the speed at which this individualized medicine approach takes, as time is critical for patients with advanced cancer. He replied that it currently takes about 10 weeks to make a personalized vaccine, with 6 of those weeks spent on identifying and synthesizing the neo-antigen peptides. Another interviewee pointed out that he has already shortened the time down to 6 weeks overall. So, this critical neoantigen identification process appears to be improving with continuing advances in DNA sequencing and synthetic chemistry. Another interviewee in this area made an important point that a tumor that is too young might contain only a few mutations, which is not sufficient for a targeted therapy. So, the age of the tumor may also be important. The interviewee also noted that it is important for the neoantigen to be present on most of the patient’s tumor cells, not just a few, or they won’t be eliminated. 3) With ovarian cancer, the TILs appeared to be committed to a variety of neo-antigens, not just one type. So, fighting these types of cancer might require broadly expanding all types of TILs directed against a variety of antigens.

Based on the research performed for this project, our team’s overall conclusion is that, of the six major types of cancer vaccines, the tumor infiltrating lymphocyte (TIL) and chimeric antigen receptor (CAR) type vaccines have shown the highest efficacies, with some CAR vaccines producing as high as 90% cancer remissions in medium-sized (30 patient) studies. We identified several ways for moving the field forward, including using using combination vaccines
(especially combined with antibodies for immune checkpoint inhibitors), adjuvants, recall antigens (when injecting the vaccine into the skin), and isolating and using a patient’s own specific tumor neoantigens. We recommend that funding be continued in this interesting area of research.
Cancer is a disease caused by uncontrolled division of abnormal cells in the body. Cancer is a name given to a collection of related diseases, all sharing this uncontrolled cell division. Cancer can begin almost anywhere in the human body, and there are over one-hundred different variations of this anomalous cell progression. The extra cells form growths called tumors. Some cancers form solid tumors, while others, such as leukemia, form diffuse tumors. Malignant tumors spread into, or invade, nearby tissues, or can break off and travel to distant places in the body through the blood or the lymph system to form new tumors. Benign tumors do not spread into, or invade, nearby tissues.

Cancer is the second leading cause of death worldwide, second only to cardiovascular disease. According to the Centers for Disease Control and Prevention, there were approximately 584,881 cancer related fatalities in the U.S. in 2013 (CDC, 2015). Each year, there are approximately 1,597,000 new cancer diagnoses in the U.S. (Cooper and Hausman, 2013, Table 18.1). From the prostate gland, to the thyroid glands, the lungs, or the kidney, these malignancies symptomatically grow and strongly affect our health. Sometimes the symptoms are not manifested until tumor growth is advanced, making it difficult to diagnose.

A tumor, also known as a neoplasm, is defined as an abnormal mass of tissue which may be solid or fluid-filled. A tumor does not necessarily mean cancer; tumors can be benign (not cancerous), pre-malignant (pre-cancerous), or malignant (cancerous). “There are many different types of tumors and a variety of names for them – their names usually reflect their shape and the kind of tissue they appear in. Put simply, a tumor is a kind of lump or swelling, it does not necessarily pose a health threat” (Medical News Today, 2015). Malignant tumors are defined by their ability to metastasize to different parts of the body, which worsens patient prognosis. Any unexpected growth within the body has the potential to disrupt major organ operation, blood flow, or mechanical operation. Medical News Today further states that “according to experts, there is no clear dividing line between cancerous, precancerous and non-cancerous tumors” (Medical News Today, 2015). Benign or non-progressive tumors do not metastasize, but some types of benign tumors indeed have the ability to change and become cancerous. Premalignant tumors are those that are soon to become a malignant growth. Examples of premalignant growths include: leukoplakia, actinic keratosis, and dysplasia of the cervix. Diagnoses can be obtained by excisional biopsies, incisional biopsies, or needle aspiration biopsies.

Tumor Formation

The formation of a tumor requires that a normal cell undergo at least six fundamental steps (Hanahan and Weinberg, 2011): 1) sustaining the ability to proliferate (usually by switching on proliferative signal transduction), 2) evading growth suppressors, including immune evasion, 3) resisting cell death (upregulating anti-apoptotic proteins), 4) enabling
immortality (upregulating telomerase and other factors that allow DNA to continue to replicate), 5) inducing angiogenesis (the tumor needs to expand its blood supply to receive nutrients), and 6) activating metastasis (acquiring the ability to move throughout the body and colonize the new location).

Step-2 mentioned above, involving immune evasion, is an important topic to this IQP project that focuses on immune therapy. Normally the immune system recognizes and removes dividing foreign bodies in our bodies, but tumors represent our own cells and are sometimes recognized as self. So, a key problem for immune vaccines is to identify neoantigens, new proteins (or smaller epitopes) created specifically on the surface of tumor cells that we can design antibodies against to help fight the tumor. In addition, recent evidence indicates that tumors have the ability to suppress our immune system. For example some tumors produce proteins such as PD-1 or CTLA-4 that suppress a T-cell attack. So, second-generation cancer vaccines sometimes include antibodies against PD-1 or CTLA-4 to “block the blockers” to activate the immune system to clear the tumors.

With respect to metastasis, Hanahan and Weinberg state that “as normal cells evolve progressively to a neoplastic state, they acquire a succession of these hallmark capabilities, and that the multistep process of human tumor pathogenesis could be rationalized by the need of incipient cancer cells to acquire the traits that enable them to become tumorigenic and ultimately malignant” (Hanahan and Weinberg, 2011). They also state that once a malignant tumor is established, metastasis occurs in a sequence of discrete steps, known as an invasion-metastasis cascade. Metastasis is the beginning point for cell replication in different locations of the body. “Metastasis begins with local invasion, then intra-vasation by cancer cells into nearby blood and lymphatic vessels, transit of cancer cells through the lymphatic and hematogenous systems, followed by escape of cancer cells from the lumina of such vessels into the parenchyma of distant tissues, the formation of small nodules of cancer cells, and finally the growth of micrometastatic lesions into macroscopic tumors, this last step termed ‘colonization’ (Hanahan and Weinberg, 2011).

**Cancer Causes**

Cancer is a genetic disease caused by changes to genes that control the way our cells function, especially how they grow and divide. These genetic changes can occur as we age as errors inserted during DNA replication, can occur as a result of carcinogens (such as tobacco smoke or radiation, the two best characterized carcinogens), or can be inherited from our parents. Each person’s cancer has a unique combination of genetic changes, and additional changes occur as the tumor grows, so this makes designing an immune vaccine difficult. Recent therapies use second generation DNA sequencing technology to sequence the DNA of a patient’s tumor to identify proteins specific to that tumor to provide a target for the vaccine. Some of the mutations may have nothing to do with causing the cancer, they may merely be the result of the cancer’s increased cell division and a reduced DNA correcting apparatus. But even these mutations could serve as a target for immune therapy if the mutated protein resides on the cell surface.
Tumor Treatments

Tumor treatments vary widely, depending on whether cancer cells are present, the location of the tumor, and the type of tumor. Benign tumors in a safe area of the body that do not cause organ disruption are sometimes left alone and watched carefully for switching to a cancerous state. If a tumor is cancerous, treatments might include chemotherapy (the use of chemical drugs to block DNA replication or other key cancer processes), radiation (to kill rapidly dividing cells), surgery (to remove the tumor), targeted cancer therapy (drugs that interfere with specific molecules a tumor needs to grow), and biologic therapy (i.e. cancer vaccines, the subject of this IQP).

Treatment is sometimes effective when cancer is discovered in its early stages. For example, according to the American Cancer Society, the 5-year survival rate for melanoma when diagnosed early is around 98%, but once it has spread to other parts of the body, the 5-year survival rate drops to about 16% (CDC, 2015). But late stages of cancer are often untreatable, and the physician instead focuses on symptom treatments.

Example Cancers Discussed Later in this Report

The subject of this IQP report is cancer vaccines. Specific types of cancer have been investigated with this type of therapy, so this section of the Lit Review will focus on a few specific types of cancer that we will encounter later in the report.

Prostate Cancer

Among men, prostate cancer is the second leading type of malignancy diagnosed, second only to skin cancer. And with respect to cancer deaths, prostate cancer is second only to lung cancer (Cancer.org, 2015). The American Cancer Society estimates that in 2015, there will be about 220,800 new prostate cancer cases, and about 27,540 deaths. About 1 man in 7 will be diagnosed with prostate cancer during his lifetime. Prostate cancer occurs mainly in older men; about 60% of the cases are diagnosed in men over 65, with an average age of diagnosis around 66. Although prostate cancer can be a serious disease, most men do not die from it. In fact, more than 2.9 million men in the U.S. diagnosed with prostate cancer are still alive today.

Predisposition to prostate cancer can be genetically inherited (as familial-derived prostate cancer, the most prevalent) or it can be spontaneous. According to doctors Jan-Erick Damber and Gunnar Aus, “One obvious possible reason for this is the inheritance of genes that cause prostate cancer, some of which show high penetrance, whereas other genes show polymorphism and low penetrance” (Damber and Aus, 2008). Some genes are thought to correlate with prostate cancer. The first gene locus identified was the hereditary prostate cancer locus-1 (HPC1) (Smith et al., 1996), and that locus contains a strong candidate gene RNASEL (Wiklund et al., 2004). Other genes linked to prostate cancer have also been identified, but they occur at a lower frequency in the population so are thought to be less important: PON1, CAPZB, MSR1, CHEK2, RNASEL, and EPAC2 (reviewed in: Dong, 2006). According to a Swedish study, a high body-
mass index (BMI) strongly correlated with death associated with prostate cancer, but less with its incidence (Andersson et al., 1997). Fat intake did not correlate with increased incidence.

With respect to prostate cancer treatments, if caught in its early stages, it grows so slowly that many physicians take a “wait and see” attitude towards its management. But as is typical of most cancers, it is more difficult to stop once it reaches advanced stages. According to Tsivian et al. (2012), “conventional treatment of prostate cancer is blindfolded; the disease is typically treated on the basis of histological evidence obtained through random sampling of the prostate gland”. The American Cancer Society points out a variety of treatment options, including expectant management, surgery, radiation therapy, cryosurgery, hormone therapy, chemotherapy, vaccine treatment, and bone-directed treatment. These treatment options apply to other types of cancers as well. Precise-targeting approaches and treatment methods are becoming more popular, especially following the advent of precise imaging techniques such as ultrasound and multiparametric MRI. This precise imaging technique allows the use of minimally invasive techniques. Focal therapy is known as “the application of an organ-sparing approach for a disease that has been traditionally treated with radical, whole-gland therapies” (Tsivian et al., 2012). With respect to this IQP, the first cancer vaccine approved for use in the U.S. by the FDA was Provenge (also called Sipuleucel-T or APC8015), a DC-type vaccine designed for prostate cancer.

**Breast Cancer**

On the other side of the gender spectrum, breast cancer is the second most diagnosed cancer in women following skin cancer, and causes the second highest number of deaths after lung cancer (Cancer.org, 2015). In 2015, about 231,840 new cases of invasive breast cancer are expected to be diagnosed in U.S. women, and 60,290 new cases of non-invasive (in situ) breast cancer (Cancer.org, 2015). Breast cancer is also seen in men, but with far less frequency. The formation of a lump within the breast or underarm that persists after a menstrual cycle is often the first symptom of breast cancer. A research study has shown that heightened estrogen hormone levels increases the risk of developing breast cancer (WebMD, 2015). Breast cancer has genetic components. According to WebMD, “a woman whose mother, sister, or daughter has had breast cancer is two to three times more likely to develop the disease, particularly if more than one first-degree relative has been affected (WebMD, 2015). Researchers have identified two genes responsible for some instances of familial breast cancer, BRCA1 and BRCA2, and approximately 7% of breast cancer cases are caused by mutations in those genes (Claus et al., 1996).

Treatments for breast cancer vary depending on the discovery time and stage. Important variables include the size of the host breast, histological grade of the tumor, the age of the patient, the lympho-vascular invasion rate, and other factors. “Choices of adjuvant treatment are based on parameters defined by molecular characterization of breast cancer subtypes, or by approximations to this classification using traditional clinical-pathological features. Clinicians should consider cases within the various distinct sub-populations in order to properly select the most ‘personalized’ adjuvant therapeutic approach. Sensitivity to chemotherapy and/or targeted agents in subtypes of breast cancers are predictable based upon gene pathway alterations and associated gene products” (Curigliano et al., 2012). As with prostate cancer, breast cancer is important to treat before metastasis, to prevent uncontrollable metastatic takeover. Some
management plans include mastectomy or breast-conserving surgery. Radiotherapy and chemotherapy are utilized to clear micro-metastases that can spread throughout the body. Pharmaceuticals can be used to lower estrogen levels, preventing the potential cell division of estrogen-dependent tumor cells (Grimsey, 2003).

**Leukemia**

Leukemia is a cancer of the bone marrow, lymphatic system, or other blood-forming organs which results in the production of increased numbers of immature or abnormal white blood cells. Some forms of leukemia are more common in children, while others predominate in adults. According to the Leukemia Research Foundation, approximately 176,000 new cases are expected this year in the United States, 310,000 Americans are living with leukemia, 731,000 Americans are living with Hodgkin’s or non-Hodgkin’s lymphoma, 88,490 people are living with myeloma, and about 55,000 deaths will result from blood cancer this year (Leukemia Research Foundation, 2015). Directly impacting the blood cells, leukemia originates within the tissues where blood cells form, usually the bone marrow. The outcome is to produce less functional white blood cells to fight infection, or less functional red blood cells to carry oxygen from the lungs to the rest of the body, or fewer platelets to clot the blood. According to the National Cancer Institute, “Leukemia is either acute or chronic. Acute leukemia is a fast-growing cancer that usually gets worse quickly. Chronic leukemia is a slower-growing cancer that gets worse slowly over time. The treatment and prognosis for leukemia depend on the type of blood cell affected and whether the leukemia is acute or chronic” (National Cancer Institute, 2015). The American Cancer Society (2015) classifies leukemia into four types: acute myeloid leukemia, chronic myeloid leukemia, acute lymphocytic leukemia, and chronic lymphocytic leukemia. Myeloid leukemia differs from lymphocytic leukemia with respect to the development location (myeloid cells versus lymphocytic cells).

Leukemia has a wide range of symptoms, including: anemia, swollen lymph nodes, petechiae, or an enlarged liver or spleen (American Cancer Society, 2015). Treatments of all four types of leukemia include chemotherapy (especially for acute leukemia) and/or corticosteroids. Some therapies are applied post-remission to kill any leukemia cells remaining after a primary treatment (National Cancer Institute, 2015). Chronic leukemia is more commonly treated with radiation to treat swollen lymph nodes. Relative to the subject of this IQP, more recent advances that have been extremely effective include combining chemotherapy with monoclonal antibodies against CD19, a protein present on the surface of B-cells to use the patient’s immune system to help rid the cancer cells (National Cancer Institute, 2015).

**Lung Cancer**

Lung cancer is the leading cancer killer in both men and women in the United States, causing more deaths than colorectal, breast, and prostate cancers combined. In 2015, an estimated 158,040 Americans are expected to die from lung cancer, accounting for approximately 27% of all cancer deaths (American Lung Association, 2015). Cancer of the lung is usually categorized in two ways, based upon the growth and spreading: small cell lung cancer (SCLC) or non-small cell lung cancer (NSCLC). According to American Cancer Society, SCLC
is named “for the small size of the cancer cells as seen under a microscope. SCLC often starts in the bronchi near the center of the chest, it tends to grow and spread quickly, and it has almost always spread to distant parts of the body before it is found” (American Cancer Society, 2015). Non-small cell lung cancer is divided into three categories: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. These categories are based upon size as seen in a microscope, shape, and chemical make-up. Both types have similar treatments.

The paramount risk factor for developing lung cancer is smoking. Cells that line the lung are increasingly exposed to carcinogens when inhaling smoke from a cigarette or polluted air. The increased risks are also present for individuals exposed to second hand smoke present from other smokers. Lung cancer is also caused by exposure to radioactive radon gas in the environment.

**Melanoma**

The deadliest form of skin cancer is melanoma. Melanoma is a type of skin cancer that forms from pigment-containing melanocytes in the skin (Bajetta et al., 2002). Although it is less common than other types of skin cancers, melanoma is much more deadly if not detected early, causing the vast majority (75%) of skin cancer deaths (Jerant et al., 2000). According to the skin cancer foundation, melanoma is “the most dangerous form of skin cancer, these cancerous growths develop when unrepaired DNA damage to skin cells (most often caused by ultraviolet radiation from sunshine or tanning beds) triggers mutations (genetic defects) that lead the skin cells to multiply rapidly and form malignant tumors” (Skin Cancer Foundation, 2015). Melanoma is commonly traced back to intense ultra-violet radiation exposure and a genetic predisposition to the disease. As with other cancers, melanoma is treatable if recognized early. But as it moves to other parts of the body, it can be lethal. The Skin Cancer Foundation estimates that in 2015 approximately 135,000 new cases of melanoma in the US will be diagnosed, 73,870 of these will be invasive melanomas, with about 42,670 in males and 31,200 in women (Skin Cancer Foundation, 2015).

The treatments for melanoma range from surgical excisions of the affected and surrounding areas of the skin and chemotherapy. More recently, immunotherapy and immune-checkpoint blockade therapies have been devised, and are the subject of this IQP. In some cases, advanced melanomas have been sent into complete remission using dendritic cell (DC) vaccines, and these will be discussed later in the report.

**Part-1 Conclusion**

Cancer is a genetic disease caused by mutations in growth factor genes, growth related signal transduction pathways, or tumor suppressor genes (making them non-functional). Cancer survival statistics have improved over the past several decades, due to more accurate and sensitive detection techniques, better drugs to block growth by a variety of mechanisms, and more precise surgical tools. But in spite of these advances, cancer still remains the second leading cause of death following cardiovascular disease, so better therapies are needed. The
subject of this IQP is a new form of therapy that uses the patient’s own immune system to help fight the tumor, and this will be discussed in the next several sections.

**Part-1 Bibliography**


Part-2: Introduction to the Immune System

Danielle Healy

This IQP project focuses on recent successes fighting cancer with immune vaccines, so brief introductions to cancer and the immune system are needed. The previous Lit Review section focused on cancer, and this section focuses on the immune system. The immune system is composed of a network of cells, tissues, and organs whose main purpose is to protect the body from disease and infection. Cells in the immune system recognize problems within the body, communicate with other cells, and perform their various beneficial functions (NIAID, 2015).

One of the main functions of the immune system is to distinguish self from non-self (foreign antigens), to protect the body from invading pathogens and eliminate altered cells (as with cancer) (Shultz and Grieder, 1987). The immune system is divided into two major subdivisions: innate immunity and adaptive immunity (NIAID, 2015).

**Innate Immunity**

Innate immunity is that part of the immune system that is ready for immediate maximal response when an infection is detected. It constitutes the first two lines of a three-line defense system. The first line of defense is non-specific, and begins with physical and chemical surface barriers (Science Learning Hub, 2015). The skin is an obvious physical barrier to the entrance of microbes, and is the largest organ of the vertebral body. It also acts as a chemical barrier with its acidic pH, discouraging the growth of organisms via its sweat and oil glands secretions. Tears wash away irritating substances and microbes, and with the help of lysozyme digest the bacterial cell wall to help kill many bacteria. Saliva washes microbes from the teeth and mucous membranes of the mouth. The respiratory tract traps organisms with mucus and uses cilia to sweep away trapped organisms. The stomach uses acid to kill organisms, and the urinary bladder uses urine to wash away microbes from the urethra. The large intestine uses normal bacterial inhabitants to monitor invaders. (NIAID, 2015)

If pathogens are able to get past the first line of defense, the second line of defense is activated which includes the use of defensive cells, defensive proteins, inflammation, and fever. The cells involved in the second line of defense include several types of white blood cells (leukocytes): natural killer cells, mast cells, eosinophils, basophils, and the phagocytic cells including macrophages, neutrophils, and dendritic cells (UIC, 2004). These cells recognize molecular patterns present on the surface of bacteria and fungi, and act to engulf them (or aid other cells that engulf and kill them). Some types of cancer fighting treatments such as chemotherapy or radiotherapy can lower the neutrophil count in the body, leaving the cancer patient more susceptible to bacterial or fungal infections (Cancer.Net, 2012).

**Adaptive Immunity**

Adaptive immunity is the third line of defense. If a pathogen survives the nonspecific innate defenses, the body will react with this more advanced subdivision of immunity to specifically target the pathogen. The adaptive immune system is known as the antigen-specific
immune response. Once contact has been acquired with a pathogen, it activates the B and T-lymphocytes, each boosted by humoral mediators such as cytokine hormones (NCBI, 2001). Once engaged by a foreign antigen (usually one presented by an antigen presenting cell) the B or T-cell matures to commit to that specific antigen.

**B-Lymphocytes (B-cells)**

B-lymphocytes, or B-cells, are produced in the bone marrow (from which they derive their name). Their primary function is to make antibodies against a foreign invader. As shown in **Figure-1**, when a foreign antigen presented by an antigen presenting cell (discussed in a later section) is recognized by a B-cell (diagram left) the B-cell becomes activated and processes the antigen (diagram center). B-cell receptors (BCR), located on the outer surface of the cell, transmit signals inside the B-cell to activate it, causing a clonal expansion and antibody production. With the aid of cytokine hormones (IL-2 and IL-4) and helper T-cells, the B-cell becomes a mature plasma cell which manufactures and secretes antibodies against the foreign antigen (diagram right). Memory cells are also created that have a longer half-life, and respond to the antigen if presented again in a later infection (NIH, 2008).

![Activation of B Cells to Make Antibody](image)

**Figure-1: Diagram of B-Cell Function and Antibody Production.** Shown are the main steps in B-cell function, including the recognition of foreign antigens presented by antigen presenting cells (diagram left), maturation into plasma cells as aided by helper T-cells (diagram center), and antibody production and secretion (diagram right) (NIH, 2008).

The antibodies produced by mature plasma cells, also referred to as immunoglobulin, can be divided into five classes: IgM, IgD, IgG, IgA, and IgE. They function to bind to (coat or opsonize) the specific pathogen to promote phagocytosis. They also promote complement fixation to activate the complement cascade to destroy antibody-bound pathogens (RCN, 2013). IgG is the most abundant type of antibody in the blood, constituting about 80% of all serum antibodies. IgM is the largest antibody, and is secreted by B-cells early in the infection. IgA is
T-Lymphocytes (T-cells)

T-lymphocytes, or T-cells, are white blood cells developed in the thymus gland (from which they derive their name). Their main function is to attack the body’s cells already containing the foreign invaders, or to help other T-cells do so. T-cells contain T-cell-receptors (TCRs) on their surfaces that detect foreign antigens presented by antigen presenting cells (NIH, 2008). Once a pre-T-cell has engaged an antigen presenting cell, the cytokines IL-12 and IFN-gamma help the cells proliferate and differentiate into mature T-cells.

There are three main types of T-cells: helper T-lymphocytes, cytotoxic T-lymphocytes (CTLs), and regulatory T-cells (National Multiple Sclerosis Society, 2015). Helper T-lymphocytes are extremely versatile cells, and function in many roles in the adaptive immune system. Four types of helper T-cells have been identified: Th1, Th2, Thh, and Th17. T-cells are formed in a so-called type-1 Th1 response, which also forms B-cells (Figure-2). Th1 cells participate in both cell-mediated immunity and humoral (antibody-mediated) immunity. Th1 cells are created when a pre-Th cell (brown in the diagram) binds an antigen presenting cell (purple). The antigen presenting cell then secretes interleukin 12 (IL-12) as well as IFN-gamma (diagram upper left). These hormones cause pre-Th cells to differentiate into a Th1 cell (green), which further differentiates into both T-cells (lower left) (cellular immunity) and B-cells (lower right) (humoral immunity). If the pre-Th cell matures in the presence of IL-2 and IL-4 hormones (upper right), it matures into a Th2 cell (blue) thus further differentiating into only B-cells (lower right) (humoral immunity). In addition to their main differentiation role, Th1 cells also secrete hormones TNF-beta and IFN-gamma to further support the immune system. The production of cellular immunity is considered the “most potent weapon against intracellular pathogens” (RCN, 2014).
Figure-2: Diagram of Th1 and Th2 Immune Responses. Pre-Th cells (brown) engage antigen presenting cells (purple). In the presence of hormones IL-12 and IFN-gamma (upper left), the pre-Th cell differentiates into a Th1 cell (green) that further differentiates into both T-cells (lower left) (cellular immunity) and B-cells (humoral immunity) (lower right). In the presence of IL-2 and IL-4 (upper right) the Pre-Th cell matures into a Th2 cell (blue), which further differentiates into only into a B-cell (humoral immunity). (RCN, 2014)

Tfh cells, also known as follicular helper T-cells, are CD4+ helper T-cells located in the follicles. When they are exposed to antigen-presenting cells and cytokines which become bound to their TCR, transcription factor Bcl-6 becomes activated, causing the T-cell to form an immunological synapse (connection) with B-cells expressing the antigen fragments in class II histocompatibility molecules that match their TCR. Th2 cells helps B cells by enabling them to develop antibody-secreting plasma cells. (RCN, 2014)

Th17 cells are a subset of CD4+ T-cells located on the boundary of the external and internal environments. When exposed to antigen-presenting cells that bind to their TCR and cytokines, Th17 cells activate a nuclear retinoid receptor which leads to the synthesis and secretion of IL-17, and increased synthesis of the plasma membrane receptor for the interleukin IL-23, causing rapid proliferation. Th17 cells help protect the surfaces against extracellular bacteria, and are a potent effector for autoimmune disorders. (RCN, 2014)

Cytotoxic T-cells, also called cytotoxic T-lymphocytes (CTLs), are a type of T-cell that is programmed to kill host cells infected with foreign pathogens such as virus-infected cells, cells infected with intracellular bacterial or protozoal parasites, allografts, or cancer cells (NIH, 2008; RCN, 2011). This type of T-cell is of strong interest to this IQP project because they directly cause tumor cell lysis and are sometimes used for immunotherapy. CTLs that infiltrate a tumor to help lyse it are called tumor infiltrating lymphocytes (TILs). CTLs have two ways of becoming activated (Figure-3, upper three rows): 1) a CD8+ CTL (orange cell with green receptor, right side) can become activated by a foreign antigen on the surface of, for example, a virus-infected cell (pink cell) while being stimulated by IL-2 hormone secreted from a CD4+ helper T-cell responding to the same virus (orange cell with pink receptor, diagram upper right).
2) A CTL cell can become activated by engaging a foreign antigen presented by a professional antigen presenting cell, such as a dendritic cell (pink cell with dendritic extensions, diagram center). (RCN, 2011)

**Figure-3:** Diagram of the Two Modes of Activation of Cytotoxic T-Cells and the Mechanism for Killing Target Cells. Shown are the two main modes of CTL activation (upper three rows), and mechanism of target cell killing (lowest row). A CD8+ CTL cell can become activated by engaging foreign antigen on the surface of, for example a virus infected cell, while responding to IL-2 secreted from a CD4+ helper T-cell. Alternatively a CD8+ CTL can become activated by engaging a professional antigen presenting cell, such as a dendritic cell. When killing a target cell, the CTL can secrete perforin and granzymes onto the target cell to induce permeability and apoptosis. Or alternatively, a FasL ligand on the surface of the CTL (yellow) can engage fas receptor (blue) on the target cell to activate caspases within the target cell, resulting in apoptosis. (UOH, 1997)

Once a CTL is committed to a particular antigen and is bound to a cell containing that antigen, it has two ways of killing its target cell (Figure-3, lower row). The first mechanism for killing includes the use of perforin and granzymes (blue spheres inside the CD8+ orange cell, lower left diagram). Perforins are cytoplasmic granules discharged by exocytosis once a CTL binds its target. Once a dozen or more granules have inserted themselves into the plasma
membrane of a target cell (such as a tumor cell), they form a pore (orange surface protein in the pink virus infected cell) allowing granzymes to enter (dark pink matter inside the pink virus-infected cell). Granzymes are serine proteases that work inside the cell in two ways: Granzymes-A, once inside the cell, enter the mitochondria to cut a subunit of complex I protein that functions in respiration, and this kills the cell. Granzymes-B cleave caspase precursors to activate them, causing the cancer cell to undergo apoptosis. The second mechanism used by CTLs for killing target cells involves FasL/Fas killing (yellow/blue receptors, lower diagram). All CTLs express the Fas ligand (FasL) (known as the death activator) (yellow surface protein on the CD8+ cell) on their surface. Most targeted cells express a receptor for FasL known as Fas (blue receptor on viral infected cell). When CTLs recognize and bind to their target, FasL is upregulated (more FasL is secreted to the CTL surface) which binds more Fas on the target cell. Activation of the signaling pathway of FasL bound to Fas receptor results in cell death by apoptosis. (RCN, 2011)

When fighting cancer, the adaptive immune response typically responds with a cell-mediated response. Effector helper T-cells help activate naïve CTLs which forms both memory CTLs that continue surveillance of the body and form effector CTLs that lyse the targeted cancer cells (Cancer Research Institute, 2003; Veseley et al., 2011). In addition, the tumor cells can be identified by antibodies against surface proteins to create complement-mediated cytotoxicity (Spurrell and Lockley, 2014).

Regulatory T-cells are important in avoiding immune-mediated pathology and limiting the expansion of the effector T-cell population (for example following an infection). Originally, regulatory T-cells were called suppressor T-cells because they suppress both humoral and cellular immunity. Figure 4 shows the formation of regulatory T-cells within the adaptive immune system. Natural regulatory T-cells (green in the diagram) increase the expression of CD25+ as they migrate from the thymus. Inducible regulatory T-cells (purple) form from naïve CD8+ cells (yellow) in the presence of antigen. These inhibitory T-cells are naturally stimulated by inflammation and disease processes such as autoimmunity and cancer to help inactivate both cellular and humoral immunity. (Shevach and Davidson, 2015)

**Figure-4: Diagram of the Formation of Regulatory T-cells.** Shown in the diagram are three types of T-cells migrating out of the thymus: CD25+ natural regulatory T-cells (green), naïve CD8+ cells (yellow), and naïve CD4+ cells (brown). The CD8+ naïve T-cells can differentiate into CD8+ inducible regulatory T-cells (turquoise). (Shevach and Davidson, 2015)
**Hematopoietic Stem Cells (HSCs)**

Hematopoietic Stem Cells (HSCs) are multipotent progenitor cells responsible for the production of all the cellular components of the blood. They create billions of blood cells within the vertebrate immune system (R&D Systems, 2015). Sources of HSCs include bone marrow, peripheral blood, umbilical cord blood, fetal hematopoietic systems, and embryonic stem/germ cells. HSCs have many characteristics that make them well suited for treating leukemia patients in bone marrow transplants, or making cancer vaccines (Gschweng et al., 2014). HSCs are sometimes identified as having CD34+ on their surface (Figure 5), and the cells they differentiate into are usually fully functional red blood cells, white blood cells, and platelets. Two types of HSCs have been discovered through rate studies: long-term HSCs (top row in the diagram) and short-term HSCs (second row in the diagram). Both types of HSCs are derived from bone marrow, and can renew themselves (circular arrows in the diagram), but short-term HSCs cannot renew themselves for long periods of time. (R&D Systems, 2015)

![Diagram of the Role of Hematopoietic Stem Cells in Hematopoiesis](image)

**Figure-5: Diagram of the Role of Hematopoietic Stem Cells in Hematopoiesis.** Long-lived HSCs (top row, blue) form shorter-lived HSCs (second row, blue), which form Progenitor Cells (third row) that differentiate further into all other blood cells. (Wang & Wagers, 2011)

**Antigen Presenting Cells (APCs)**

Antigen Presenting Cells (APCs) are specialized white blood cells that help fight off foreign invaders by presenting antigens on their surface that are recognized by other components of the immune system (discussed above) to help them commit to that specific antigen during adaptive immunity (Wellness, 2015). When a foreign antigen is first detected by an APC, it is
engulfed by the APC, and proteases inside the cell break it down into smaller peptides. The processed antigen is then transported back to the surface of the APC and bound with either an MHC class I or II molecule. This antigen-MHC complex is then recognized by B-cells and T-cells to help them commit to that particular type of antigen (Kimball’s Biology Pages, 2013).

APCs are classified as two types: professional and non-professional (Figure-6). Professional APCs (diagram upper panel) such as dendritic cells, macrophages, or B-cells, express the foreign antigen via MHC class I molecules on their surface, and are the only type to activate naïve helper T-cells. The interaction between MHC-1-presented antigen and B-cells and T-cells is very strong (upper panel, diagram center). Non-professional APCs (such as fibroblast cells, thymic epithelial cells, thyroid epithelial cells, glial cells, pancreatic beta cells, and vascular endothelial cells) (diagram lower panel) present antigen via MHC class II molecules, in a weaker type interaction to B-cell and T-cell receptors. (Garland Science, 2001)

![Figure-6: Diagram of the Functional Differences Between Professional and Non-Professional Antigen-Presenting Cells.](image)

**Figure-6: Diagram of the Functional Differences Between Professional and Non-Professional Antigen-Presenting Cells.** The upper panel denotes professional APCs with their high affinity interaction with B and T-cells, while the lower panel denotes non-professional APCs and their lower affinity interaction with target cells. (Wellness, 2015)

**Dendritic Cells (DCs)**

DC’s are of special concern to this IQP because they are used as DC vaccines in cancer immune-therapy for some types of cancer. As mentioned above, DCs are a type of professional antigen-presenting cell whose main function is to process foreign antigens and present them on their surface via MHC-1 molecules to B-cells and T-cells to induce them to commit to that antigen (Figure-7). DC’s at some developmental stages grow a branched morphology from which they get their name (dendritic for tree). DC’s are also known as accessory cells of the mammalian immune system. They act as messengers between the innate immunity (recognizing and internalizing foreign antigens) and the adaptive immune system (antigen presentation to B-cells and T-cells). DC’s are present in tissues that are in contact with the external environment,
such as the skin (where they are known as Langerhans cells) and the inner lining of the nose, lungs, stomach and intestines. Immature DCs (also known as veiled cells) are high in endocytic activity and low in T-cell activation potential, and are sometimes found in the bloodstream (RCN, 2013). DCs express pattern recognition receptors (PRRs) on their surface, and are constantly scanning for invading pathogens. When one is detected, DCs engulf the pathogen as part of the innate immune system. DCs also process the antigen and present it via MHC-1 and become activated (NPG, 2015). Once activated, they migrate to the lymph nodes where they interact with T-cells and B-cells to initiate adaptive immunity. Because they migrate, DC’s are distinguished from follicular dendritic cells (FDCs) that do not migrate (discussed below).

Figure 7: Interaction Between Dendritic Cells (DCs) and T-Cells. The interaction between DCs (purple cell) and T-cells (green cell) to activate them involves three signals. The first signal (bright red) is derived from the interaction between the MHC class I molecule (green) on the DC cell which processes and presents foreign antigens to the T-cell receptors (TCR) (dark purple). The second signal (orange) results from the interaction between co-stimulatory molecules, CD80/86 on the DC (green) and CD28 (turquoise) on the T-cell. Signal-3 (dark red) results from the secretion of IL-12 from the DC cell. When these signals work together, they promote a strong T-helper-1 response (Th1) within responding T-cells. (Cambridge University Press, 2002)

In cancer vaccines, a patient’s peripheral blood mononuclear cells (PBMCs) are isolated from his arm, and cultured in vitro. The DC’s are isolated from this expanded PBMC culture, and then mixed in vitro with either tumor cells isolated from the patient or with purified tumor antigen (to expose the DCs to tumor antigens). The DC’s process the tumor antigen and express it via MHC-1 on their surface. The DC’s are then injected in large numbers back into the patient, where they engage B-cells (humoral immunity) and T-cells (cellular immunity) to activate both arms of the immune system against the cancer.

Follicular dendritic cells (FDCs) are different than DCs. FDCs are found in primary and secondary lymph tissues (lymph nodes, spleen) in the B-cell areas of the lymphoid tissue. Although FDCs have a dendritic morphology, they are not DCs. They are not derived from HSCs like DCs, but are of mesenchymal origin. FDCs are a non-migratory population that form a stable connection with follicular B-cell. Opsonized antigens are displayed for long periods of
time without proteolysis or removal by phagocytic cells. FDCs also express complement receptors CR1 (CD35) and CR2 (CD21) (so they trap complement-coated antigens), and the Fc-receptor FcγRIIb (CD32) (so they bind antibody-coated antigens). But FDCs lack MHC-1, so cannot capture non-opsonized antigens. Follicular B-cells must bind FDCs or they undergo apoptosis. FDCs also protect the body from autoimmunity by removing self-reactive B-cells.

Functionally, FDCs enable germinal center responses via antigen, chemokines, and survival factors (Van Nierop and de Groot, 2002) (Figure-8). Although FDCs are normally located in the follicles and stromal cells of secondary lymphoid organs, research has shown that “ectopic FDC formation can also be found in a number of autoimmune diseases and/or chronic inflammatory situations, which indicates development of FDCs is not restricted to secondary lymphoid organs but rather a matter of local conditions that drive a precursor cell type into FDC maturation” (Elsevier Inc., 2015). FDCs are important for the formation of B-cells that produce high-affinity antibodies against pathogens, as they present the foreign antigen on their surface for a long period of time. With respect to autoimmunity, B-cells that are able to bind are able to survive and produce antibodies, but B-cells against self-antigens do not bind FDCs and undergo apoptosis (NPG, 2015).

![Figure-8: Diagram of the Signaling of a Follicular Dendritic Cell with a Target Cell.](image)

Shown are some of the receptors and signaling molecules that participate in follicular dendritic cell (FDC) function. The binding between the dendritic cell (light blue) and target cancer cell (B-cell lymphoma, light purple) is facilitated by the following interactions: ICAM-1 with LFA-1, VCAM-1 with VLA-4, and CD44 with an unknown receptor. FDCs do not present antigens via MHC-1 molecules. (Elsevier Inc., 2015)

Natural Killer Cells (NKs)

Natural killer cells (NKs) are effector lymphocytes of the innate immune system that monitor for cells containing MHC class 1 molecules present on most host cells, and attack cells that lack MHC-1. They also scan for cell stress markers on the cell surface of autologous cells and remove stressed cells (NPG, 2008). NKs constitute about 5-15% of the lymphocytic
circulating population, and are the most aggressive of all white blood cells in the entire immune system. A majority of NKs are located in peripheral blood, lymph nodes, spleen, and bone marrow; however, various chemoattractants have the ability to migrate NKs toward sites of inflammation (ScienceDaily, 2015). What makes NKs of use in the battle against cancer is that they have no immunological memory, so they do not have to recognize a specific antigen before releasing their toxins to destroy cells such as tumor cells, so long as the tumor cell is recognized as “foreign” by the NK (Thornthwaite, 2015).

In humans, two types of receptors regulate the recognition phase of NKs. One is composed of the receptors that are homologous to the C-type lectines, called the NK Regulation Complex (NKRC). The other is composed of Killer Cell Immunoglobulin-like receptors, also known as KIRs, which are shown on the NK cell surface as KIR2 and KIR3. The most predominant control of the NK cell activation comes from the CD94:NKG2. It contains both activating receptors (NKG2A-B) and inhibitor receptors (NKG2C-D) (Figure-9). When MHC1 present on a non-foreign host cell binds with a NK cell receptor (diagram left side), it sends negative signals (veto) to inhibit activation of the NKC receptors to release the ligand (normal healthy host cell). When MHC1 is not dense enough (as for a bacterial cell lacking MHC-1), contains processed antigen (as with an antigen presenting cell containing pathogen), or is sufficiently altered (with some types of cancer) (diagram right side), then the decreased binding of altered MHC1 to the inhibitory receptor activates the NK cell, and it targets and kills the target cell (Vivier et al., 2008).

![Figure 9: Recognition of Host Cells by NKs.](image)

NKs are capable of two types of killing: lysis and apoptosis. Lysis involves the release of cytotoxic molecules (such as Tumor Necrosis Factor, TNF) from NK granule membranes that
cause destruction of the target cell if the inhibitory receptor is not engaged (NPG, 2008). NKs can also kill by apoptosis using perforins and granzymes (as discussed earlier with CTLs). NKs contain small cytotoxic granules in their cytoplasm containing proteins called perforin and proteases called granzymes. When the NK cell releases perforin, it forms cylindrical pores in the target cell that act as an entrance for the granzymes. The granzymes are serine proteases that work inside the cell to 1) enter mitochondria to cleave a subunit of complex I protein that functions in respiration, which kills the cell, and 2) to cleave caspase precursors to activate them, causing the cancer cell to undergo apoptosis.

Unfortunately, some cancer cells have the ability to escape NK activity by encoding proteins that serve as ligands for the inhibitory NK receptors, thus keeping the NKs inactive against them (Vivier et al., 2008). So, scientists are devising new methods for down-regulating these NK-inhibitory tumor ligands (Veseley et al., 2011; Cancer Research UK, 2014).

**Part-2 Bibliography**

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The simplest types of cancer vaccines involve directly injecting purified proteins or antibodies into a patient to stimulate the patient’s own immune system to clear the tumor cells. This section of the Lit Review will focus on this type of cancer vaccine.

**Peptide Injection Experiments**

In the case of peptide vaccines, the injected peptide (or a mixture of peptides) usually represents a protein (or a part of it) present on the surface of the patient’s own tumor cells. This protein is identified directly from the patient’s tumor cells in a form of personalized medicine, or is previously known to be present on all cells of this type of tumor. The injected protein is recognized by the patient’s immune system, including antigen presenting cells (APCs) that present the protein to B-cells (which mature to make antibodies against the peptide) and present it to T-cells (which mature to make cytotoxic killer cells to home in on cells containing the peptide). Relatively few studies of this type exist because the approach has been replaced by DC, TIL, and CAR immune vaccines that appear to be more effective.

In 2006, a Phase-II clinical study was performed on 28 patients with metastatic androgen-independent prostate cancer (Arlen et al., 2006). The patients received a peptide vaccine consisting of a vaccinia virus encoding PSA tumor antigen, followed by boosters with a fowlpox virus also encoding the PSA antigen. The patients also received docetaxel as a type of chemotherapy that in mice had shown activity against androgen-insensitive prostate cancer. The purpose of the study was to determine whether the docetaxel affected the immune vaccine, whether the combination was safe, and whether the combination was effective. Any patient with advancing cancer who had not previously received docetaxel was immediately provided it. An ELISPOT assay was used to monitor immune responses for PSA-specific T-cells. Their data demonstrated a 3.33 fold increase in T-cell response to PSA after 3 months, and an increased immune response against other prostate tumor antigens. Median progression-free survival on docetaxel plus vaccine was 6.1 months, compared to 3.7 months for controls. This study is significant because it is the first clinical trial to demonstrate that docetaxel can be administered safely with immunotherapy, and the combination improves patient survival, but further studies need to be conducted.

In 2007, a team of scientists at the Unit of Immunotherapy of Human Tumors, Istituto Nazionale Tumori Foundation (Milan, Italy) reviewed the published literature related to the use of unique human tumor antigens in cancer vaccines (Parmiani et al., 2007). In this review, the authors highlight the biological and clinical importance of identifying and using unique antigens against tumors, if they are known, to avoid cross immunity with normal patient cells. They also summarize how knowledge of the unique tumor antigens can be applied to both active immune vaccines and adoptive immunotherapy. Because each patient’s tumor mutates DNA individually, personalized DNA sequencing likely will need to be performed to identify “neoantigens” that are
new to the patient’s tumor and not present in his own cells. Tumor specific antigens were first identified fifty years ago in rodents, but their full molecular characterization was limited to a few of obtained in the last 20 years. Human studies on tumor neoantigens were relative sparse until recently.

In 2012, an interesting peptide mixture experiment was performed by Dr. Harpreet Singh-Jasuja’s team at Immatics Biotechnologies (Tubingen, Germany) (Walter et al., 2012). The study represents the first immune vaccine for renal cell carcinoma (RCC). It provides the team’s data from Phase-I and -II clinical trials against RCC. The vaccine (IMA901) consisted of an injection of 11 multiple tumor associated peptides (TUMAPs) that the authors previously determined were naturally present on the surface of RCC’s and were antigenic when injected into humans. In this study, they verified the peptides were present in patient’s own cancer tissue, and treated 96 patients who suffered from advanced stages of renal cell carcinoma. The phase-I part showed that T-cell responses induced against multiple TUMAPs correlated with better disease control, and the vaccine appeared to be safe. The phase-II part confirmed that an immune response to multiple cancer peptides correlates with longer patient survival. Treatment with cyclophosphamide, a DNA alkylating agent that blocks the action of T-cells, confirmed that a T-cell response was necessary for the improved patient survival. They also assayed over 300 serum biomarkers, and found that apolipoprotein A-1 (APOA1) and chemokine ligand-17 (CCL17) best predicted a strong immune response to the IMA901 vaccine and best predicted patient survival. The authors noted that a Phase-III clinical trial with IMA901 is currently ongoing. This peptide injection study directly addressed two of the ongoing problems associated with cancer vaccines: 1) which tumor antigens are best to target, and 2) which biomarkers are best to follow as indicators of a strong vaccine response. Their mixture of IMA901 RCC peptides proved to induce a strong immune response that improved patient outcomes, and they were able to identify two biomarkers as a way to follow vaccine efficacy. It was not clear from the study whether the authors assayed for potential negative effects of the vaccine, so this might be worth following up in an interview.

In 2015, a review article discussed the topic of neoantigens and their importance to tumor therapies (Schumacher and Schreiber, 2015). This study summarized some of the recent technological innovations that have made it possible to dissect the immune response to patient-specific neoantigens that arise as a consequence of tumor-specific mutations. The authors suggest that recognition of such neoantigens is a major factor in the activity of successful clinical immunotherapies. Neoantigen formation can act as biomarkers for tumor formation, and can provide a target for the development of therapeutic approaches to enhance T-cell reactivity against them. Neoantigens will be discussed later in the report.

**Monovalent Antibody Experiments**

Instead of injecting peptides to induce an immune response, some scientists directly inject antibodies previously made against a tumor protein. This type of “passive immunity” does not activate the patient’s own immune system to create the antibodies, but instead provides the antibodies by a bio-engineering process. The antibodies combine with the protein on the surface of tumors to create antigen-antibody complexes, which are then recognized and cleared from body by other cells of the immune system, such as macrophages or T-cells.
Monovalent antibodies recognize only one type of epitope (portion of a protein). Most natural antibodies produced by the body in response to an infection are of this type, and both arms of the “Y” shaped antibody molecule recognize the same antigen. Anti-tumor monoclonal antibodies (mAbs) represent a major advance in cancer therapy. In the past decade, at least 6 different mAbs have been approved by the FDA for treating cancer, and more antibodies are in development (Taylor et al., 2007).

**Antibodies Against CD20 for Leukemia, Lymphoma, and Autoimmune Disorders**

The first antibody approved for cancer treatment in the U.S. was **Rituximab** (also known as Rituxan, MabThera, or Zytux). Rituximab is a monoclonal antibody directed against protein CD20 primarily found on the surface of B-cells. The antibody binds to B-cells, bringing them to the attention of the immune system and their removal. This antibody is used to treat diseases with elevated B-cell numbers (leukemia and lymphoma), overactive B-cells (autoimmune disorders, transplant rejection), or dysfunctional B-cells. The antibody was developed by IDEC Pharmaceuticals (San Diego) as IDEC-C2B8. It was initially FDA approved in 1997 to treat non-Hodgkin lymphomas resistant to chemotherapy based on its observed safety performance (Maloney et al., 1997). A U.S. patent was issued in 1998 and will expire in 2015. The antibody is currently marketed by Biogen Idec (Cambridge, MA) and Genentech (South San Francisco) in the U.S., by Hoffmann–La Roche in Canada and the EU, Chugai Pharmaceuticals in Japan, and AryoGen in Iran. Because the antibody was approved in 1997, several studies have used it in clinical trials.

In 1998, a team of scientists at the Toronto-Sunnybrook Regional Cancer Centre (Ontario, Canada) used Rituximab against CD20 on B-cells to treat patients with low-grade non-Hodgkin’s lymphoma (Berinstein et al., 1998). Their previous phase I and II trials indicated the antibody had minimal toxicity and provided significant therapeutic effects against low grade non-Hodgkin’s lymphoma. Their phase III study included 116 patients with recurrent low-grade lymphoma, who were treated with four infusions of Rituximab. 48% achieved a measurable tumor response, and 6% achieved complete tumor responses. 76% achieved ≥20% reduction in tumor volume. Rituximab antibody was detected in all patients and increased throughout the treatment course. The half-life of the monoclonal antibody increased from 76.3 hours to 205.8 hours by the end of the fourth infusion. It is important to note that a significant correlation was found between the median antibody concentration and the tumor response. The authors concluded that Rituximab is therapeutically effective against B-cell lymphoma, and some patients may even benefit from increased dosing.

A 1999 article reported the first successful clinical trial combining a chimeric anti-CD20 monoclonal antibody (Rituximab) with a standard-dose combination of chemotherapy in the treatment of patients with indolent B-cell lymphoma (Czuczman, 1999). In a 40 patient group, the study found a 95% overall response rate, with 55% complete remission and 40% partial remission. In addition, seven of seven patients with follicular histologies achieving complete remission also showed positive PCR results for clearing of BCL-2 from the blood and marrow, suggesting an eradication of minimal residual disease. The author of this report concluded that based on its single-agent efficacy, excellent toxicity profile, and ability to be successfully
combined with combination chemotherapy; rituximab may be a very beneficial towards treatment of CD20-positive neoplasms.

A 2000 study performed at the Sarah Cannon Cancer Center (Nashville, TN) investigated the ability of rituximab anti-CD20 mAb in 39 previously untreated patients with stage II-IV low-grade non-Hodgkin’s lymphoma (Hainsworth, 2000). The patients received rituximab at 375 mg/m2 by intravenous infusion for four consecutive weeks, and were evaluated for responses at week six. Patients who exhibited stable disease or an objective response at 6 weeks received a repeat four-week course at six-month intervals for a maximum of four treatment cycles. In the 6 week evaluation, 54% of the patients showed objective responses, and 36% had stable disease or minor responses. The response rates were similar in patients with follicular and small lymphocytic lymphoma (52% and 57%, respectively). At 1 year, the overall response rate was 72%, with 18% showing complete responses, and 77% showing a progression-free survival. The treatment appeared to be well tolerated by the patients. It is noted in the evaluation that further follow-up evaluation of the patients will be important.

Another study was done in 2001 by a team of scientists at the University of Nebraska Medical Center (Omaha, NE) who determined the safety and efficacy of the combination of the chimeric anti-CD20 antibody (Rituxan) supplemented with CHOP chemotherapy (a combination of cyclophosphamide, doxorubicin, vincristine, and prednisone) in patients with aggressive non-Hodgkin’s lymphoma (NHL) (Vose et al., 2001). The study consisted of 33 patients with previously untreated advanced aggressive B-cell NHL who received six infusions of Rituxan (375 mg/m2 per dose) on day 1 of each cycle in combination with six doses of CHOP chemotherapy given on day 3 of each cycle. The overall response rate was 94%, with 61% of the patients experiencing a complete response, 33% had a partial response, and two patients were classified has having progressive disease. 18 patients had an International Prognostic Index (IPI) score ≥ 2, for these patients the combination of Rituxan plus CHOP achieved an overall response rate of 89%, and a complete response of 56%. Within the median observation time of 26 months, 29 of 31 responding patients remained in remission during this follow-up period, including 15 of 16 patients with an IPI score ≥ 2. PCR was used to monitor the causing bcl-2 translocation in blood or bone marrow cells, and was positive at baseline in 13 patients, of 11 who did follow-up PCR all 11 had a negative bcl-status after therapy. Only one patient reconverted to bcl-2 positivity, and all patients remain in clinical remission. The most frequent adverse events attributed to the Rituxan antibody were fever and chills, primarily during the first infusion. But the Rituxan did not seem to compromise the ability of patients to tolerate the CHOP chemotherapy, as all patients completed the entire six courses of the combination. This study is the first to demonstrate the safety and efficacy of the Rituxan chimeric anti-CD20 antibody in combination with standard-dose CHOP in the treatment of aggressive B-cell lymphoma.

Another 2001 study was done by a team of scientists in Tours, France who published their findings using CD20 rituximab mAb against follicular non-Hodgkin lymphoma (NHL) (Colombat et al., 2001). The study consisted of 50 patients with follicular CD20+ NHL and a low tumor burden, who were analyzed for clinical and molecular responses. The patients received four weekly infusions of rituximab at a dose of 375 mg/m2. The response rate after 50 days was 73%, with 10 patients (20%) in complete remission, 3 patients in complete remission/unconfirmed, and 23 patients in partial remission. Ten patients had stable disease, and 3 patients had progressing disease. The results indicated that early molecular responses to the antibody can be sustained for up to 12 months, and the response highly correlates with
progression-free survival. A significant association was observed between molecular and clinical responses \( (p < 0.0001) \). A year into the study, 8% of the patients were in complete remission, 39% in partial remission, and 50% had stable remission. Of the pool of patients, 32 were initially informative for polymerase chain reaction (PCR) data on bcl-2-Jh rearrangement. On day fifty, 57% were negative for bcl-2-Jh rearrangement in peripheral blood and 31% were negative in bone marrow. After 12 months, 62% of patients were PCR-negative in peripheral blood. This study provides a very successful use of a monovalent mAb against CD20 for fighting non-Hodgkin lymphoma.

Hainsworth (discussed above) followed up his earlier study with a second one in 2002 (Hainsworth, 2002). Again he analyzed the use of rituximab as a first-line treatment for patients with indolent non-Hodgkin’s lymphoma. The study was a community-based trial on 62 patients with stage II to IV indolent non-Hodgkin’s lymphoma (follicular or small lymphocytic) who had received no previous systemic therapy. All patients received rituximab at 375 mg/m² weekly for four consecutive weeks, and were evaluated for responses at week 6. Patients who had an objective response or stable disease continued treatment every 6 months with repeat four-week courses of rituximab for a total of four treatment courses. The data indicated that at week 6, 47% had objective responses, and 45% had minor responses or stable disease. Following a second rituximab treatment, the major response rate increased from 47% to 65%, and the complete response rate increased from 7% to 27%. The author stated that progression-free survival was not measured in the study, but he stated it will be greater than 24 months. During the investigation, there was no observable toxicity with repeat courses of rituximab. The author concluded by saying that the initial response rate can be improved by using several scheduled maintenance courses of rituximab administered every six months.

**Antibodies Against HER2**

The second monoclonal antibody approved for cancer treatment in the U.S. was Herceptin (also known as Trastuzumab or Herclon). Herceptin is a monoclonal antibody that interferes with the HER2/neu receptor that is over-expressed on some types of cancer cells. It was first developed in the 1990’s by scientists Dr. Axel Ullrich and Dr. H. Michael Shepard at UCLA’s Jonsson Comprehensive Cancer Center, with further refinements by Dr. Dennis Slamon (reviewed in Bange et al., 2001). The antibody is manufactured by Genentech, and it gained FDA approval in September 1998. Herceptin’s main use is to treat HER2-type breast cancers, but it is also used to treat other types of HER2 cancers such as colorectal and pancreatic cancers (Perez et al., 2002). HER2 receptors are embedded in the cell surface membrane, and after binding epidermal growth factor (EGF) ligands, communicate signals to stimulate cell proliferation. Over-expression of HER2 in cancers aids tumor cell division (reviewed in Hudis, 2007).

A 1999 study evaluated the efficacy and safety of a recombinant humanized anti-HER2 monoclonal antibody as a single agent in women with HER2-overexpressing metastatic breast cancer that had progressed after chemotherapy to metastatic disease (Cobleigh et al., 1999). They enrolled 222 women with HER2-overexpressing metastatic breast cancer that had progressed after one or two chemotherapy regimens. The patients received a loading dose of 4 mg/kg antibody intravenously, followed by a 2-mg/kg maintenance dose at weekly intervals. A blinded, independent response evaluation committee identified 8 complete, and 26 partial responses,
providing an overall response rate of 15%. The median response duration was 9.1 months, and the median survival duration was 13 months. Among the most common adverse events were infusion-associated fever and/or chills, observed mostly during the first infusion in about 40% of the patients. Other side-effects commonly observed with chemotherapy were rarely seen, such as alopecia, mucositis, and neutropenia. The authors concluded that recombinant humanized anti-HER2 monoclonal antibody administered as a single agent, produces durable objective responses and is well tolerated by women with HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease.

A 2001 study in the Division of Hematology and Oncology at the UCLA School of Medicine (Los Angeles, CA) evaluated the efficacy and safety of trastuzumab in women with metastatic breast cancer that overexpressed HER2 (Slamon et al., 2001). 234 patients were randomly assigned to receive standard chemotherapy, and in another group 234 patients were assigned to receive standard chemotherapy plus trastuzumab. The results indicated that the addition of trastuzumab antibody to the chemotherapy regime provided a longer time to disease progression (median 7.4 vs 4.6 months; p<0.001), a higher rate of objective response (50% vs 32%, p<0.001), a longer duration of response (median, 9.1 vs 6.1 months; p<0.001), a lower rate of death at 1 year (22% vs 33%, p=0.008), longer survival (median survival 25.1 vs 20.3 months; p=0.046), and a 20% reduction in the risk of death. The most important adverse event observed was cardiac dysfunction, occurring in 27% of the chemotherapy group, 8% of group given partial chemo, 13% of the group given paclitaxel and trastuzumab antibody; and 1% of the group given paclitaxel alone. It was noted that although the cardiotoxicity side effects were sometimes very severe, the symptoms generally improved using standard medical management. It was concluded that trastuzumab antibody increases the clinical benefit of first-line chemotherapy in metastatic breast cancer that overexpresses HER2.

In 2002, a team of scientists in the Department of Surgical Oncology, The University of Texas M. D. Anderson Cancer Center (Houston, TX) published the results of their study investigating the molecular mechanisms by which Herceptin enhances the antitumor effects of taxol (Lee et al., 2002). They studied the effect of Herceptin treatment on p34Cdc2 kinase activation and apoptosis in taxol-treated human breast carcinoma cell lines MDA-MB-435, SKBr3, MDA-MB-453, and 435.eB (a positive control ErbB2 transfectant of MDA-MB-435). Activation of p34Cdc2 was previously shown to be required for taxol-induced apoptosis, and over-expression of ErbB2 blocks the taxol-induced apoptosis by inhibiting p34Cdc2 activation. Their data indicated the Herceptin antibody treatment down-regulated ErbB2, reduced the inhibitory phosphorylation of Cdc2 on Tyr-15 (improving the molecular requirements for taxol sensitivity), Herceptin plus Taxol treatment led to higher levels of p34Cdc2 kinase activity and apoptosis in vitro, and more effectively inhibited the growth of human tumor xenografts in mice with enhanced in vivo apoptosis. It was concluded that Herceptin treatment of ErbB2-overexpressing cells allows effective p34Cdc2 activation and induction of apoptosis upon taxol treatment.

In 2005, a team of scientists at the Jules Bordet Institute (Brussels, Belgium) studied the use of Trastuzumab, a recombinant monoclonal antibody against HER2, against advanced breast cancers over-expressing HER2 (Piccart-Gebhart et al., 2005). The objective was to investigate efficacy and safety after excision early-stage breast cancer and completion of chemotherapy. This study was an international, multicenter, randomized trial that compared one or two years of trastuzumab given every three weeks with patients with HER2-positive and either node-negative or node-positive breast cancer, who had previously completed locoregional therapy and at least
four cycles of chemotherapy. The total study consisted of 1,694 women with two years of trastuzumab treatment, 1,694 women with one year of trastuzumab, and 1,693 controls. The published findings here pertained only to the one year observation. Recurring breast cancer or death was observed in 347 events (127 events in the trastuzumab group and 220 in the observation group). Severe cardiotoxicity developed in 0.5% of the women who were treated with trastuzumab. Disease-free survival at two years would be about 8.4%. The authors of this investigation concluded that one year of treatment with trastuzumab after adjuvant chemotherapy significantly improves disease-free survival among with HER2-positive breast cancer patients.

In 2007, a team of scientists in the Department of Microbiology at the College of Physicians and Surgeons, Columbia University (New York, NY) published a study to determine whether passive immunotherapy (providing antitumor antibodies against HER-2/neu antigen) can induce active tumor immunity via the opsonic enhancement of immunogenicity of tumor antigen in breast cancer patients (Taylor et al., 2007). The study assessed whether immune sensitization to the HER-2/neu tumor antigen occurs during treatment with the anti-HER-2/neu monoclonal antibody trastuzumab. 27 breast cancer patients were treated with trastuzumab and chemotherapy, then were assayed for the induction HER-2/neu – specific immunity. The assessment was done by obtaining sera and peripheral blood mononuclear cells before and after trastuzumab therapy; these patient cells were compared for the presence of anti-HER-2/neu endogenous antibodies and HER-2/neu – specific CD4 responses (indicating immune activation) by ELISA and enzyme-linked immunospot. In the overall population, anti-HER-2/neu humoral responses significantly increased during therapy (p<0.001). Anti-HER-2/neu antibodies were detectable in 29% of patients before treatment and in 56% of patients during treatment. Interestingly, in ten evaluable individuals, six showed elevated HER-2/neu – specific CD4 T-cell responses during therapy, indicating activation of the patient’s immune system against Her2, not just a passive immune response by the added antibodies. Of the twenty-two individuals treated for metastatic disease, those showing improved clinical responses contained higher levels of HER-2 antibodies. The authors concluded that humoral immune sensitization indeed occurs during treatment with chemotherapy and trastuzumab, but more studies are required to determine whether the newly induced immune responses contribute to the patient outcomes.

**Antibodies Against CD22 for Leukemia**

CD22 (cluster of differentiation-22) is a transmembrane protein located on the surface of mature B-cells (and somewhat on immature B-cells) whose N-terminal immunoglobulin-like domain specifically binds sialic acid residues (reviewed in Macauley et al., 2014). CD22 is a member of the Siglec immunoglobulin superfamily of proteins, and is an inhibitory receptor that once bound to ligand prevents over-activation of the immune system and the development of autoimmune diseases (Otipoby et al., 2001). Because CD22 is located on B-cells, antibodies against it are sometimes used to treat leukemia where B-cells are over-produced.

In 2012, Dr. Hagop Kantarjian and his colleagues at the MD Anderson Cancer Center (Houston, TX) published the results of their Phase 2 clinical trial (Kantarjian et al., 2012). This group designed an antibody against surface protein CD22 highly expressed in patients with acute lymphocytic leukemia (ALL). The antibody was conjugated to the toxin calecheamicin to kill the CD22 cells. They treated 49 ALL leukemia patients, 9 patients (18%) had a complete response, 19 (39%) had a marrow response, 19 (39%) had resistant disease, and only 2 (4%) died. The
most frequent side effects were fever (20 patients), hypotension (12 patients), and liver related toxic effects (12 patients). It would be interesting in an interview with the authors to see how they think their CD22 targeting approach compares with the CD19 approach of killing leukemic B-cells.

One year later in 2013, Dr. Kantarjian published a follow-up study (Kantarjian et al., 2013) using their inotuzumab (a CD22 mAb bound to toxin calicheamicin) shown in their previous study to be active against patients with ALL. For this study, 90 patients with refractory-relapsed ALL received inotuzumab. The first group of 49 patients received a single-dose of inotuzumab i.v. at doses of 1.3 to 1.8 mg/m² every three to four weeks. The second group of 41 patients received inotuzumab weekly at a dose of 0.8 mg/m² on day one, and a dose of 0.5 mg/m² on days 8 and 15, every three to four weeks—based on their earlier observations of higher in vitro efficacy with more frequent exposures. The overall response rate was 58% (19% achieved a complete response, 30% had a complete response with no platelet recover (CRp), and 9% had a bone marrow complete recovery). The response rates were similar between the two groups. The median survival was 6.2 months, 5.0 months with the single-dose, and 7.3 months with the weekly dose schedule. The median remission duration was seven months. Some of the adverse side effects observed were reversible bilirubin elevation, fever, and hypotension. The authors concluded that inotuzumab as a single-agent therapy was highly active, safe, and convenient in patients with refractory-relapsed ALL. A weekly dose schedule proved to be equally effective as a single-dose schedule, and was less toxic.

In 2013, Dr. Dieter Hoelzer of Frankfurt, Germany, was sole author on a review article in Cancer, 119: 2671-2674, entitled “CD22 Monoclonal Antibody Therapies in Relapsed/Refractory Acute Lymphoblastic Leukemia” (Hoelzer, 2013). This review article reminds us that CD22 is present on about 60-90% of B-cell malignancies, but not 100%. So, presumably antibodies against CD22 would not work in 10-40% of ALL patients.

**Part-3 Bibliography**


humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *Journal of Clinical Oncology*, 1999 Sep; 17(9): 2639-2648.


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Bivalent (or bi-specific) antibodies recognize two different epitopes by the same antibody molecule. The best characterized example of this bivalent approach is antibody Blinatumomab, where one antibody arm recognizes CD19 on the surface of leukemic cells, and the other arm recognizes CD3 on the surface of T-cells to activate them.

**Blinatumomab Antibody Against CD19 and CD3**

CD19 (Cluster of Differentiation-19) is a protein found on the surface of B-cells of the earliest recognizable B-lineage, but it is lost on mature plasma B-cells cells. Due to its specificity for early B-cells, its lack of expression in other tissues, and the ability of a human being to live without early B-cells (by passively providing antibodies to the patient), the targeting of CD19 to fight leukemia (where excess immature B-cells are produced) has become one of the biggest success stories in all of cancer vaccine research (Scheuermann and Racila, 1995). Targeting CD19 can be achieved by antibody therapy or by therapy with CAR cells designed against CD19 (discussed later), and it will be interesting in interviews to have the study authors contrast the bi-specific antibody approach with the CAR approach against CD19.

Blinatumomab (also known as AMG103) is a bivalent antibody designed with one arm directed against CD19 (to destroy B-cells whose numbers are elevated in leukemia) and the other arm against CD3 (a T-cell receptor activator) to bind and activate T-cells so they destroy the co-bound B-cell tumor. This antibody is a type of “bi-specific T-cell engager” (BiTE) that specifically binds a tumor cell (in this case a B-cell) while simultaneously binding T-cells. The activated T-cell destroys the co-bound cancer cell. Blinatumomab was created in the early 1990’s by scientists at University Hospital Utrecht (The Netherlands) (Haagen et al., 1992; Bohlen et al., 1993; Haagen et al., 1994; DeGast et al., 1995; reviewed in Weiner and DeGast, 1995). The antibody was further developed by Micromet, Inc. in collaboration with Lonza. Micromet was later purchased by Amgen, which expanded Blinatumomab’s clinical trials. On December 3, 2014, the antibody was approved by the FDA for treating acute lymphoblastic leukemia (ALL) (FDA, 2014).

In 1995, extensive preclinical testing was done testing the effectiveness of bispecific antibody (BsAb) designed against CD3 X CD19 (De Gast et al., 1995). The team, led by Dr. De Gast of the Netherlands Cancer Institute, initially tested antibody effectiveness against a pre-B-cell line, and found that the BsAb induced the killing of malignant B-cells by added resting T-cells that had become activated by the CD3-portion of the bispecific antibody. The study was 14 days long, and showed that BsAb required repeated administration together with IL-2 to achieve a 5-log kill of the B-cells by resting peripheral blood T-cells. The study then expanded into in vivo experiments, and the team tested 3 human patients for safety, intravenously introducing BsAb in 2.5 mg doses into 3 patients with B-cell non-Hodgkin’s lymphoma. The results showed relatively safe toxicity. Next, patients with chronic lymphocytic leukemia (CLL) were treated...
with 0.6 mg doses intravenously, preceded by IL-2. The patients showed improved T-cell activation, and the toxicity remained limited. The team noted that the chemotherapy-resistant CLL patients with a high tumor burden showed no clear clinical effects, so the authors suggested treating patients with a lower tumor load in future experiments. So, these early experiments had modest efficacy.

In 2002, Dr. Patrick A. Baeuerle and his colleagues of Micromet AG (Martinsried, Germany) extended the testing of the in vitro efficacy of their bi-specific single chain antibody directed against CD19 and against CD3 (Dreier et al., 2002). Their data indicated that the in vitro lysis of the tumor cells was mostly mediated by CD8+ T-cells, and that CD19-negative cells (non-leukemic cells) were not harmed by the CD8+ T-cells induced by the antibody vaccine.

In 2003, Dr. Ralf Bargou and his colleagues at the Max Delbruck Center for Molecular Medicine (Berlin, Germany) (also authors on the 2002 paper discussed above) published their study of the bispecific CD3 x CD19 antibody in patients with B-cell chronic lymphocytic leukemia (B-CLL), the most common type of leukemia in adults in western countries (Löffler et al., 2003). B-CLL patients treated with standard chemotherapies often relapse, so there are no current long term treatments. CD19 appears to be a decent target because it is expressed on the surface of the vast majority of B-cell cancers but is not expressed on stem cells or plasma cells. They tested its ability to bind in vitro primary B-cells and autologous T-cells isolated from healthy volunteers and from chronic lymphocytic leukemia (B-CLL) patients, and showed a depletion of lymphoma cells in 22 out of 25 patient cases. In addition to the CD3 activation of the T-cells, no further activation by IL-2 was needed.

In 2005, Dr. Baeuerle’s group (as discussed above for the 2002 study) used video-assisted microscopy to determine that their bi-specific CD19 x CD3 antibody vaccine stimulated the T-cells to switch from a scanning mode into a killing mode (Hoffmann et al., 2005). In the killing mode, each T-cell eliminated multiple tumor cell (CD19 B-cell) targets within a 9 hour time period, and the tumor cell targets were completely eliminated within 24 hours using ratios as low as 1:5 (T-cell to tumor cell). This study nicely shows that their bi-specific antibody can indeed activate T-cells, and each activated T-cell can undergo multiple rounds of target cell lysis.

In 2007, Dr. Molhoj of the University of Connecticut and his colleagues published an article in Molecular Immunology titled “CD19-/CD3-bispecific antibody of the BiTE class is far superior to tandem diabody with respect to redirected tumor cell lysis” (Molhol et al., 2007). In their paper they stated that many kinds of bispecific antibodies recruiting T-cells for cancer therapy have now been developed, but a comparison of antibody structures had not been attempted. They compared the activity of monospecific and bispecific CD19/CD3 antibodies of the single chain format, tandem diabody (Tandab) format, and the quadroma format. Their data indicated that all antibodies had similar cytotoxic activities, but that under identical experimental conditions, the bispecific CD19/CD3 format has far superior activity compared to the monospecific formats. The extraordinary potency of the BiTE class (as represented by MT103) may translate into improved anti-tumor activity, lower dosing and lower costs of production compared to other antibody formats (Molhol et al., 2007).

In 2008, Dr. Ralf Bargou of the University of Wurzburg (Germany) and his team published an article in Science titled “Tumor regression in cancer patients by very low doses of a T-cell-engaging antibody” (Bargou et al., 2008). The article detailed their findings of the ability
of blinatumomab bispecific CD19 x CD3 antibody to engage all of the cytotoxic T-cells in patients and their ability to lyse cancer cells. They reported that doses as low as 0.005 milligrams of antibody per square meter body per day in non-Hodgkin’s lymphoma patients led to an elimination of all target cells in the blood (Bargou et al., 2008). Partial and complete tumor regression was observed at a low dose level of 0.015 milligrams. The team treated seven patients with relatively high dose levels of 0.06 milligrams, and 100% of the patients experienced tumor regression. The team also found that blinatumomab cleared tumor cells from bone marrow and liver.

In 2011, Dr. Gerhard Zugmaier and his colleagues at Micromet (Munich, Germany) (the same large research group discussed above for Drs. Baeuerle and Bargou) published their study in the Journal of Clinical Oncology, entitled “Targeted Therapy with the T-Cell-Engaging Antibody Blinatumomab of Chemotherapy-Refractory Minimal Residual Disease in B-Lineage Acute Lymphoblastic Leukemia Patients Results in High Response Rate and Prolonged Leukemia-Free Survival” (Topp et al., 2011). This paper describes the team’s phase-II human clinical trials for efficacy of their CD19/CD3 bi-specific antibody vaccine for treating acute lymphoblastic leukemia (ALL). 21 patients were treated, and at the end of the study 16 of the 21 showed a successful minimal residual disease (MRD). 12 of the 16 responders had been refractory to previous cancer treatments, so any improvement in their condition is significant. The most frequently observed side-effects were grade-3 and 4 lymphopenia, but were completely reversible. So, this study shows the CD19/CD3 antibody treatment is efficacious and well-tolerated against tumors that would otherwise cause a relapse.

In 2012, the same team discussed above, but here led by Micromet’s collaborators at the University Hospital of Wuerzburg (Germany), published an article in Blood titled “Long-term follow-up of hematologic relapse-free survival in a phase 2 study of blinatumomab in patients with MRD in B-lineage ALL” (Topp et al., 2012). Twenty patients suffering from relapsing acute lymphoblastic leukemia (ALL) were treated in a phase-II clinical trial with the CD19/CD3 bispecific Ab construct, and were observed for about 33 months. Their results showed 61% of the 20 patients had a hematologic relapse-free survival rate. In a subgroup of 9 patients who progressed well enough to also receive an allogeneic hematopoietic stem cell transplant, 65% showed hematologic relapse-free survival (Topp et al., 2012). Of another subgroup of 6 patients, 67% (4) showed ongoing hematologic and molecular ALL remissions. The study concluded that blinatumomab can induce a long-lasting (at least 33 months) complete remission in patients with B-lineage-persistent or recurrent ALL (Topp et al., 2012).

In 2014, Dr. Schlegel of the University of Tubingen (Germany) and his colleagues published a paper in Haematologica titled “Pediatric post-transplant relapsed/refractory B-precursor acute lymphoblastic leukemia shows durable remission by therapy with the T-cell engaging bispecific antibody blinatumomab” (Schlegel et al., 2014). Their study treated 9 post-transplant relapsed pediatric patients with B-precursor acute lymphoblastic leukemia with blinatumomab on a compassionate use basis. The B-cell blast load (as an indicator of residual leukemia) was assessed prior to, during, and after blinatumomab cycle. The 9 patients were administered blinatumomab in a 4-week continuous intravenous infusion at a dosage of 5 or 15 µg/m²/day, with a total of 18 cycles. Of the 9 patients, 4 achieved complete remission after their first cycle of treatment, 2 showed a complete remission after the second cycle, and the remaining 3 patients did not respond to the treatment. After about a year, the patient’s chance of hematologic event-free survival was 30%. The treatment induced some side effects, with 1
patient experiencing grade-3 seizures and 2 patients experiencing grade-3 cytokine release syndrome, but those were eventually treatable. This study shows that even patients with relapsing leukemia can successfully be treated with bispecific antibody treatment.

In 2015, an interesting study was published in *Lancet Oncology*, again by Dr. Topp’s team in Wurzburg, Germany (Topp et al., 2015). The study was entitled “Safety and Activity of Blinatumomab for Adult Patients with Relapsed or Refractory B-Precursor Acute Lymphoblastic Leukaemia: A Multicentre, Single-Arm, Phase-2 Study”. This clinical trial is registered at the website ClinicalTrials.gov (number NCT01466179). The authors treated 189 patients suffering from relapsed or refractory B-Precursor Acute Lymphoblastic Leukaemia (B-ALL) with the Blinatumomab CD19 x CD3 bispecific antibody. After two treatments with the monoclonal antibody, 81 of the 189 B-ALL patients (43%) showed complete cancer remission. In a best practice of open data release, the study listed the negative side-effects of the vaccine. The most frequent adverse events were febrile neutropenia (25%), neutropenia (16%), and anemia (14%). 2% of the patients showed grade-3 cytokine release syndrome. 3 deaths due to sepsis from *E. coli* and *Candida* were thought to result from the treatment, as they are hindering the patient’s ability to produce antibodies, so this topic is worth following up in interviews as to how to minimize these opportunistic infections. Although this vaccine produced several documented side-effects, saving the patient’s life likely is of more direct concern, but it will be important to verify that the side effects were treatable.

**Other Bi-Specific Antibodies**

In addition to therapies against CD19 on B-cells, other bispecific antibodies have been designed against other tumor markers. Examples of non-CD19 bispecific antibodies include: CD3 T-cell activator x anti-glioma marker (Nitta et al., 1990), folate receptor on ovarian cancer cells x CD3 T-cell activator (Canevari et al., 1995), CD16 x CD30 for Hodgkin’s disease (Hartmann et al., 1997), CD319 x CD28 for B-cell lymphomas (Daniel et al., 1998), CD64 x Fc-Receptor for B-cell lymphomas (Honeychurch et al., 2000), CD30 x CD64 for Hodgkin’s lymphoma (Borchmann et al., 2002).

In 1990, a study was done by Dr. Nitta of the Juntendo University School of Medicine (Japan) in which 10 patients with malignant glioma received lymphokine-activated killer (LAK) cells treated in vitro with bispecific antibody against CD3 T-cell activator and anti-glioma marker (Nitta et al., 1990). 10 glioma patients were treated with LAK cells activated by the bispecific antibody, and 10 patients received untreated LAK cells. While 9 patients relapsed (and 8 died within 4 years) in the control group, in the antibody-treated group 0 patients relapsed (in 18 months), 4 showed tumor regression and 4 showed tumor eradication. This study showed that bi-specific antibodies can be effective against gliomas, and warrants further research in the field. It would be interesting to find out if there were any side effects associated with the study.

In 1995, a study was done on patients with ovarian carcinoma who relapsed after chemotherapy. The study was led by Dr. Canevari in Italy using autologous peripheral blood T-lymphocytes that were retargeted in vitro using a bispecific antibody against the folate receptor present on ovarian cancer cells and the CD3 T-cell activator (OC/TR) (Canevari et al., 1995). Before treatment, the patients underwent surgery to reduce the tumor load, and were then given
five daily intraperitoneal infusions of the antibody, a second similar treatment, and IL-2. Of the 19 patients evaluated, 3 showed complete responses (lasting an average of 22 months), 1 showed complete intraperitoneal response with progressive disease in the lymph nodes, 3 showed partial responses, 7 had stable disease, and 5 showed progressive disease. They concluded that immune-therapy of ovarian cancer with bispecific mAb-retargeted T-lymphocytes can result in tumor regression with transient and mild toxicity.

In 1997, Dr. Hartmann of Detmold Germany and his team published an article in Blood detailing their findings treating patients with refractory Hodgkin’s disease with a bispecific monoclonal antibody against natural killer cell activator CD16 and tumor marker CD30 (Hartmann et al., 1997). They treated 15 Hodgkin’s patients where chemotherapy and/or radiation therapy had failed. Their results varied greatly, with 1 patient experiencing complete remission lasting 16 months, one patient experiencing partial remission lasting 3 months, 3 had minor responses, and 1 mixed response. Side effects were rare, and consisted of fever, lymph node pain, and a rash. Despite the varying results, the data are promising but requires more research to determine optimal antibody dose. It would be interesting to pursue in interviews why they believe their results were so varied.

In 2002, Dr. Borchmann of the University Hospital of Cologne (Germany) and his colleagues published an article in Blood titled “Phase 1 trial of the novel bispecific molecule H22Ki-4 in patients with refractory Hodgkin lymphoma” (Borchmann et al., 2002). Their study targeted CD30 rather than CD19 in the immunotherapy of Hodgkin lymphoma (HL), and also targeted immune activator CD64. Ten patients with refractory CD30+ lymphoma were treated with different doses (1.0, 2.5, 5.0, 10.0, and 20.0 mg/m2/d) administered intravenously on days 1, 3, 5, and 7. The main study objectives were to determine the maximum tolerated dose and the dose-limiting toxicities. The observed side effects were transient and mild. The most serious were hypotension (4 of 10), tachycardia (6 of 10), fatigue (10 of 10), and fever (2 patients with grade-I, and 3 patients with grade-II). The administered antibody half-life was about 11.1 hours, resulting in a significant antibody accumulation. The response included 1 complete remission, 3 partial remissions, and 4 with stable disease (Borchmann et al., 2002).

**Bi-Specific Antibody References**


Immune Checkpoint Vaccines

One of the most exciting advances in cancer vaccine research in the past decade is the discovery that T-cells which have migrated into a patient’s tumor in the body’s attempt to fight the tumor often become inactivated by the tumor. Proteins present on the tumor surface (such as PD-1 and CTLA-4) engage inhibitory receptors on the surface of the T-cells to inactivate them (reviewed in: Topalian et al., 2012). To overcome this immune suppression, scientists are researching the use of antibodies against the inhibitory receptors to “block the blocker” and stimulate the T-cells to attack the cancer. The immune system blockers discovered to date include programmed death-1 (PD-1) and cytotoxic T-lymphocyte-associated protein-4 (CTLA-4). This approach provides a new and exciting approach to cancer vaccines that is different than the previously discussed cases.

However, the technique has some problems that must be solved. By stimulating the immune system, some patients develop autoimmunity (the immune system is stimulated to react with the body’s own tissues) or inflammation (Melero et al., 2007). So the topics of autoimmune or inflammatory side-effects are worth asking about in interviews. Some scientists have observed promising synergistic effects when they combine vaccines directed against tumor antigens with vaccines directed against PD-1, so this future topic of mixed vaccines is also worth follow-up questions in interviews.

PD-1 and PD-L1 Vaccines

Programmed death-1 (PD-1) is an inhibitory receptor present on the surface of activated T-cells (Freeman et al., 2000). When the PD-1 receptor is engaged by its ligand (programmed death-ligand-1, PD-L1), signal transduction events decrease T-cell function and limit immune memory. Activation of PD-1 suppresses T-cell migration, T-cell proliferation, secretion of cytotoxic mediators, and restricts tumor cell killing (Herbst et al., 2014). Thus, activation of PD-1 to suppress immunity is often an important reason that growing tumors “avoid” the patient’s immune system (Hirano et al., 2005; Topalian et al., 2012; Sznol and Chen, 2013; Herbst et al., 2014). The purpose of delivering antibodies against PD-1 is to bind and inactivate the PD-1 receptor to allow T-cell functions to remain active in the patient to remove the tumor.

The most researched anti-PD-1 monoclonal antibody is Nivolumab (marketed as Opdivo). It was developed by Ono Pharmaceutical and Medarex (later acquired by Bristol-Myers Squibb), and was approved by the FDA on December 22, 2014, for treating patients with non-surgically treatable metastatic melanoma no longer responsive to chemotherapy. On March 4, 2015, Nivolumab was approved by the FDA for treating squamous non-small cell lung cancer. Several studies have investigated the use of Nivolumab for melanomas (Topalian et al., 2014), Hodgkin’s lymphoma (Ansell et al., 2015), and non-small cell lung cancer (Gettinger et al., 2015; Rizvi et al., 2015; Tanner, 2015).

In one vaccine example, Dr. Suzanne L. Topalian’s team at Johns Hopkins University School of Medicine conducted an ongoing clinical trial using antibody Nivolumab against PD-1 (Topalian et al., 2014). They assayed some of the long-term effects of the vaccine in advanced melanoma patients after removal of the therapy. Their data with 107 patients showed a mean
overall survival of 16.8 months for the treated patients, with 1-year and 2-year survival rates of 62% and 43%, respectively. They concluded that the beneficial effects were durable and persisted after drug discontinuation, and that antibody safety was acceptable (with toxicity rates similar to previous reports). So, the PD-1 vaccine approach appears to have some promise for extending the lifespan of patients with advanced melanomas. It would be interesting to determine whether this research group has tried combining their PD-1 approach with antibodies against tumor proteins.

In another example, Dr. Scott N. Gettinger and his colleagues at the Yale Cancer Center in New Haven (CT) treated patients suffering from advanced non-small cell lung cancer (NSCLC) with an antibody against anti-programmed death-1 (PD-1) (Gettinger et al., 2015). Their previous phase-I trial showed the antibody treatment was generally well tolerated in NSCLC patients. In this phase-II study, they showed in 22 NSCLC patients that the drug produced “durable immune responses and encouraging survival rates”. So, this study shows that stimulating the immune system in a general way by blocking a protein that is suppressing the immune response can be an effective treatment for NSCLC. The authors also report that 14% of the patients showed grade-3 or -4 treatment-related adverse events, so it would be interesting in an interview to determine whether the observed side-effects were fully treatable.

In another recent study published in the April 3 issue of Science, Rizvi et al. (2015) analyzed nonsynonymous mutations (mutations producing amino acid changes, neoantigens) in patients with non-small cell lung cancer patients. The patients were treated with Pembrolizumab, an antibody against PD-1. The data showed that higher neoantigen formation in the tumor (presumably providing new targets for the immune system to attack) was associated with durable clinical benefits and progression-free patient survivals. The anti-PD-1 treatment was found to enhance the formation in vivo of T-cells against the neoantigens. So, this recent data indicates that the formation of an immune response against cancer neoantigens occurs naturally in some patients, is boosted by anti-PD-1 treatment, and correlates with better patient prognosis.

At the May 30, 2015, meeting of the American Society of Clinical Oncology in Chicago, scientists reported their findings of a study performed at Johns Hopkins University treating 600 patients with non-small cell lung cancer, the most common form of lung cancer (Tanner, 2015). They randomly treated the patients either with Nivolumab (Opdivo) (an antibody against PD-1) or with docetaxel chemotherapy. The median survival rate increased from 9 months (for the chemotherapy group) to 12 months (for the immunotherapy group), and the tumors shrank in 12% of the chemotherapy patients versus 20% of the immunotherapy patients. These data may not seem highly significant (they are not complete cures), but any success against highly metastatic tumors that are so common is regarded as a success. Dr. Richard Schilsky, Chief Medical Officer for the American Society of Clinical Oncology, the meeting’s organizer stated “These drugs are among the most promising drugs that have come along in many years” (Tanner, 2015).

In addition to the above mentioned studies, PD-1 antibodies have also been used to treat diffuse large B-cell lymphoma (Armand et al., 2013), refractory solid tumors (Brahmer et al., 2010), and melanoma (Hamid et al., 2013; Robert et al., 2014; Robert et al., 2015).
CTLA-4 Vaccines

Discovered in 1987 (Brunet et al., 1987), **CTLA-4** (cytotoxic T-lymphocyte-associated protein-4) (also known as CD152) is a protein receptor located on the surface of T-cells that functions to down-regulate the immune system. CTLA-4 acts as an "off" switch when bound to ligands CD80 or CD86 on the surface of antigen presenting cells (Walunas et al., 1994). T-cells that migrate to tumors (tumor infiltrating lymphocytes) sometimes become inactivated by the cancer through the CTLA-4 pathway, so scientists are using antibodies against CTLA-4 to activate the T-cells and restore their activity against the tumor (reviewed in: Peggs et al., 2006; Cha et al., 2014). Early studies using anti-CTLA-4 antibodies to fight cancer were done in mice (for example, Chen et al., 1992), and they later progressed to human patients.

In 2010, Dr. Hodi and his colleagues did a study comparing the treatment of metastatic melanoma patients with a combination of **Ipilimumab** CTLA-4 mAb and gp100 (a peptide vaccine) to patients treated only with gp100 (Hodi et al., 2010). Their results were promising, with the median overall survival of patients receiving Ipilimumab + gp100 around 10 months compared to 6.4 months for those receiving only gp100. The side effects observed were severe in only 10-15% of the patients receiving both treatments, and in only 3% of the patients receiving gp100, although most of the side-effects were manageable. 2.1% of the patients died from the study, 7 related to immune adverse events. The team concluded that Ipilimumab, with or without a gp100 peptide vaccine, is a better method of treatment for increasing survival than gp100 alone. It would be interesting to interview Dr. Hodi and to find out how the side-effects were managed, and what they believe caused the deaths in the study.

In 2012, Dr. Weber and his colleagues published an article in the *Journal of Clinical Oncology* titled “Management of immune-related adverse events and kinetics of response with Ipilimumab” (Weber et al., 2012). The article discusses the different kinds of immune-related adverse events associated with Ipilimumab, and the necessary treatments to manage them. The article states that one of the most important aspects of side-effect treatment is early recognition. It would be of interest in an interview to ask Dr. Weber if the side effects were all manageable.

Snyder et al. (2014) used an innovative whole-exome DNA sequencing approach on tumor tissue taken from melanoma patients to identify marker proteins that best correlate with the success of anti-CTLA-4 treatments. A cell’s exome is that portion of the DNA that codes for proteins (exons). Exome sequencing is a technique that sequences all the exon sequences in a genome (the exome). The exons are first isolated (by a targeted capture technique) and are then sequenced using any high throughput DNA sequencing technology (Ng et al., 2009). Human DNA contains about 180,000 exons, constituting only about 1% of the human genome (approximately 30 million base pairs), but mutations in these exons are more likely to have severe consequences than mutations in the remaining 99% (Ng et al., 2009). Snyder et al. obtained tumor tissues from melanoma patients treated with antibodies against CTLA-4 (**Ipilimumab** and Tremelimumab) and performed whole-exome sequencing. They compared the exome sequences from 11 melanoma patients showing a long-term clinical benefit of CTLA-4-blockage to the exome sequences of 14 patients who showed minimal antibody benefit. They then used bioinformatics to determine which neoantigen profiles correlated best with a successful
patient outcome. Assaying for these markers in future patients might help determine which ones could benefit from the CTLA-4 antibody treatments. This study was a nice combination of state-of-the-art cancer vaccine approaches that stimulate the immune system with second-generation sequencing methods for analyzing large amounts of sequence data.

In 2015, a study was done for melanoma patients who progressed after treatment of Ipilimumab (Weber et al., 2015). The study compared treatment of advanced melanoma patients who had not responded to Ipilimumab to treatments with Nivolumab (a PD-1 inhibitor antibody) or standard chemotherapy (ICC). The results were very positive: 38 of 120 (31.7%) of the patients treated with Nivolumab showed positive responses, compared to 5 out of the 47 (10.6%) for patients treated with ICC. The side effects of Nivolumab included increased lipase, increased alanine aminotransferase, anemia, and fatigue. The side effects of ICC patients included neutropenia, thrombocytopenia, and anemia. The study noted grade-3 to 4 serious adverse events in 5% of Nivolumab-treated patients, and in 9% of patients treated with ICC. There were no treatment-related deaths.

In 2015, Dr. Lussier and her colleagues did a study of PD-1 blockade as a therapeutic strategy against metastatic osteosarcoma, the most common bone cancer in children and adolescents (Lussier et al., 2015). Osteosarcoma is sometimes curable by chemotherapy and surgical resection, but patients with metastatic osteosarcoma are sometimes refractory to treatment. Dr. Lussier and her colleagues researched the role of PD-1 in limiting the effectiveness of cytotoxic T lymphocytes (CTLs). The study showed that by blocking PD-1/PD-L1 interactions, osteosarcoma tumor burden and overall survival was increased. She later followed up this study with a combination PD-1 + CTLA-4 study, discussed below.

Combination Vaccines (PD-1 + CTLA-4)

Some scientists have experimented with combination vaccines using both anti-PD-1 and antib-CTLA-4 antibodies. This combination approach appears to be even more successful than using single antibody approaches.

In 2013, Dr. Wolchok and his colleagues published an article in The New England Journal of Medicine titled “Nivolumab plus ipilimumab in advanced melanoma” (Wolchok et al. 2013). The article detailed their investigation of treatment of melanoma patients with a combination of Nivolumab (PD-1 inhibitor) and Ipilimumab (CTLA-4). The combination treatment was rationalized because of their “distinct immunologic mechanisms of action and supportive preclinical data” (Wolchok et al. 2013). 53% of the patients treated with the combination therapy reached the objective response rate with a tumor reduction of 80% or more, compared to only 20% of individually or sequentially-treated patients. Grade-3 and 4 side-effects occurred in 53% of the combined antibody patients, and in 18% of the sequentially treated patients. However, the study noted that the side-effects of the concurrently treated patients were qualitatively similar to monotherapy side-effects, and were generally reversible.

In 2013, a study was done in mice by Dr. Duraiswamy and his colleagues which concluded the dual treatment was superior to individual treatments. In this study, mice treated with antibodies against both PD-1 and CTLA-4 rejected various types of tumors injected into the
mice (Duraiswamy et al., 2013). When the two drugs were combined, 100% of the CT26 tumors, and 75% of the ID8-VEGF tumors were rejected from the mice. Their evidence further supports combined PD-1 CTLA-4 therapy.

In 2013, Dr. Weber and his colleagues did an interesting study of sequential therapy with patients resistant to treatment with Ipilimumab + Nivolumab (Weber et al., 2013). The study took patients who were resistant to one drug and treated them with the other. They found that in most cases the second drug was well received and that after administration of the second drug, the first was also received.

In 2015, Dr. Lussier of Arizona State University (mentioned above in the study of osteosarcoma) and her colleagues conducted a study on the combined treatment of metastatic osteosarcoma with CTLA-4 + PD-L1 antibodies (Lussier et al., 2015). Their results indicated that a combination blockade prevented tumor immune escape and led to complete control of metastatic osteosarcoma in a mouse model. The mice were also resistant to any further tumor cell injections.

So, the combination PD-1 + CTLA-4 treatment appears to be more effective than either antibody alone, and is a promising future method of therapy.

Section Conclusions and Problems

Overall, the use of antibodies against tumor antigens has proven to be a successful approach in cancer therapies, and have provided complete cancer remissions in some experiments. The use of antibodies against CD19 on B-cells has been quite promising, and this antigen appears to be an especially good target for removing early stage B-cells in leukemia. Other successes have been achieved with difficult to treat cancers such as gliomas and ovarian cancers. Especially promising are the use of bi-specific antibodies that combine in one antibody molecule a reactivity against a tumor antigen (like CD19) with a reactivity against an immune activator (like CD3 on T-cells). This bi-specific approach uses the antibody molecule itself to bring the tumor cell into close proximity with the T-cell that will kill it. Of equal promise is the use of antibodies against immune checkpoint inhibitors, such as PD-1 and CTLA-4, which remove the immune suppression provided by the tumor. Recent experiments have also shown that tumors as they grow and divide often produce new DNA mutations, and if those mutations occur in exon portions of the genome, they encode new neo-antigens that act as excellent targets for the antibody therapies. Strong patient antibody responses to neo-antigens correlate strongly with better patient prognosis. Some scientists have observed promising synergistic effects when they combine vaccines directed against tumor antigens with vaccines directed against PD-1, so this future topic of mixed vaccines is also worth follow-up questions in interviews.

However, the antibody approaches appear to have some problems that should be followed up in interviews. In some cases, grade-3 and 4 (serious) side-effects appear to be caused by the vaccine, although most of the side-effects appear to be relatively mild, transient, and are treatable. Also, when stimulating the immune system by PD-1 or CTLA-4 antibodies, some patients developed autoimmunity (the immune system is stimulated to react with the body’s own
tissues) or inflammation. So, for labs doing immune stimulation experiments the topics of potential autoimmune or inflammatory side-effects should be asked about.

References for PD-1 and CTLA-4 Antibodies


Dendritic Cells (DCs)

Discovered in 1973 in mice (Steinman and Cohn, 1973), dendritic cells (DCs) are important components of the mammalian immune system. They get their name from their branched appearance at specific stages of their development. DC’s are potent “professional” antigen-presenting cells; their main function is to recognize foreign antigens (usually small epitope domains of proteins) on the surface of invading pathogens (and sometimes cancer cells), process the antigen within the cell, and then present it on its surface to other cells of the immune system (T-cells and B-cells) so they can commit to recognizing that particular foreign antigen and attack the tumor to reduce its mass (Banchereau and Steinman, 1998; Sallusto and Lanzavecchia, 2002; Trombetta and Mellman, 2005). Half of the 2011 Nobel Prize in Physiology or Medicine went to Ralph M. Steinman "for his discovery of the dendritic cell and its role in adaptive immunity" (The Nobel Prize, 2011). Because of this antigen presentation function, DCs are used in some types of cancer vaccines to induce an immune response against an antigen on the surface of a patient’s tumor cell.

DCs are present in tissues that contact the external environment, such as skin (in that location they are called the Langerhans cell), and the inner lining of the nose, lungs, stomach and intestines. DCs can also be found in an immature state in the blood. Once activated by a foreign antigen, they migrate to the lymph nodes where they interact with the T-cells and B-cells to initiate the adaptive immune response against the foreign antigen. As discussed in the Immunology section of this IQP, the migration of DCs to the lymph nodes to engage T-cells and B-cells distinguishes them from the non-migrating follicular dendritic cells.

Dendritic Cells and Cancer Vaccines

With respect to cancer, tumor cells themselves in the body are poor antigen-presenting cells. Tumor cells are derived from normal cells by DNA mutation. So, most of the proteins on the surface of cancer cells look like “self” to the immune system, and are ignored allowing the tumor to grow. Only a small portion of the cancer DNA mutations create “neo-antigens” that are unique to the patient’s tumor, and these provide excellent candidates for cancer vaccine designs.

Even when tumor cells contain unique neo-antigens on their surface, DC cells are still required to process the neo-antigen and present it to the immune system to generate active B-cell and T-cell responses against the tumor (Palucka and Banchereau, 2012). Animal experiments (discussed below) have shown that DC cells are a required component of the body’s immune attack against cancer, and are required for activating CD8+ T-cells that infiltrate and attack the tumor.
The aim of the DC-type tumor vaccine is to induce DC cells to present tumor antigens on the cell surface to stimulate the formation of immune cells, especially CD8+ tumor infiltrating lymphocytes (TILs) or cytotoxic T-lymphocytes (CTLs) that recognize, infiltrate, and attack the tumor (Davis et al., 2003; Steinman and Banchereau, 2007; Koski et al., 2008; Schuler, 2010; Ueno et al., 2010). In an ex vivo approach, the DC cells are usually isolated from a cancer patient’s peripheral blood mononuclear cells (PBMCs) using a variety of techniques, cultured to expand their numbers, and are then “pulsed” (mixed) with foreign tumor antigen (either purified antigen or entire tumor cells themselves). The treated DCs (that have processed the foreign antigen) are then injected back into the patient where they hopefully migrate to the lymph nodes to engage B-cells and T-cells to commit them against the tumor antigen. In a less used in vivo approach, DC cells in the body are induced to take up tumor-specific antigens, and the antigen-presentation is done naturally to stimulate the T-cells.

References for Dendritic Cells


DC Vaccine Animal Experiments

Animal experiments have been crucial for the current human patient clinical successes of DC cancer vaccines. In some cases, animal experiments have provided the use of controlled experiments not possible in humans, while in other cases they provided initial tests of new DC therapies prior to moving into humans. A few key experiments done on mice with DC vaccines are discussed below as examples.

In 2004, a team of scientists in the Department of Molecular Preventive Medicine in the School of Medicine at the University of Tokyo, experimented with DC precursor cells containing a surface protein “signature” of CD11-pos, B220-neg, F4-neg, CCR1-pos, CCR5-pos (Zhang et al., 2004). In previous studies, the team showed the DC precursor cells rapidly mobilized into the circulation in mice infected with a test bacterium (Propionibacterium) and were recruited into inflammatory tissue by hormone MIP-1-alpha binding CCR1 and CCR5 receptors on the DC surface. In this study, the team showed that mice genetically engineered to lack CCR1 or CCR5 recruited less DC cells into the circulation than WT mice. They also showed that injecting MIP-1-alpha into the mice mobilized the DC cells, and that DC cells pulsed with B16 tumor lysates produced a strong anti-tumor response in vitro and in vivo. So, this study shows that hormone MIP-1-alpha and its receptors are important in the DC response, and this hormone is worth asking about in DC vaccine interviews.

In 2006, a team in the Lymphocyte Biology Section of the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, NIH (Bethesda, Maryland) showed that the interaction of CD8+ T-cells and CD4+ helper T-cells with DC cells is important in the vaccine response (Castellino et al., 2006). They also showed that the immunization process upregulates CCR5 receptor on naïve T-cells allowing their attraction to the DC and helper T-cell sites in the lymph nodes. So, this paper also shows the importance of CCR5 type signaling in the DC vaccine process.

In 2008, a team of scientists at the Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China) (containing some of the same team members from the 2004 study above) identified a different type of precursor DC cell in mice with the following surface signature: CD11c-pos, B220-neg, CCR5-neg, CCR1-neg, CCR6-pos (He et al., 2008). These precursor DC cells differentiated into mature DC cells when treated with GM-CSF, IL-4, and TNF-alpha. And hormone MIP-3-alpha treatment of the mice mobilized this subset of DC cells. They showed that a combined treatment of MIP-1-alpha and MIP-3-alpha elicited strong anti-tumor responses, suggesting that a combination hormone strategy may improve vaccine effectiveness.

Modern animal experiments allow scientists to delete specific types of receptors on cells to determine whether those receptors and cells are required for a particular biological response. In the case of the immune system trying to suppress the growth of cancer cells, scientists showed...
using mice that type I interferons (IFN-alpha and IFN-beta) are required to initiate the anti-tumor response (Diamond et al., 2011). Using mice engineered to have DC cells lacking interferon type-1 receptors, they also showed that a DC cell response to the interferons is required for producing activated CD8+ T-cells against the tumor.

This same result of the importance of type-I interferons in the anti-cancer response was shown in mice independently by another lab in the same year (Fuertes et al., 2011). So, these animal experiments show that DC cells are a critical part of the body’s attack on cancer, and underscore the importance of using them in immunotherapy treatments.

Over the last 15 years, multiple studies have been performed on the relationship between natural killer (NK) cells and DCs. The results hypothesized that NKs play a role in DC maturation by killing immature DCs, leaving fully activated mature DCs. This was shown in vitro, but until recently, studies were lacking in vivo. In a recent study of this relationship, researchers from the Department of Experimental Medicine at the University of Palermo, Italy, showed this DC cell lysis was indeed performed by NK cells in vivo in mice (Morandi et al., 2012). The team reported a substantial decrease in CD11c+ immature DC cells in lymph nodes inoculated with YAC-1 cells, a MHC-negative cell line used to activate the NKs. The NK killing of immature DCs was dependent on perforin, as perforin-knockout mice failed to diminish the DC numbers. The NK killing was also shown to increase the effectiveness of a cancer vaccine, increasing the CTL expansion against the tumor when the NKs were activated by the YAC injection. Although the types of NK cells responsible for the immature DC killing and the sites of elimination are still unclear, the data show that activating NK cells may help improve the effectiveness of a cancer vaccine by helping mature DCs, leading to a strong activation of CTLs. A clearer picture of this relationship will hopefully be the subject of future experiments.

In a 2014 study, conducted at the Campbell Family Institute for Breast Cancer Research, Princess Margaret Cancer Center, University Health Network (Toronto, Canada), a team created a transgenic mouse model for testing the effectiveness of various cancer vaccine strategies (Dissanayake et al., 2014). They engineered mice to express a target antigen on the surface of pancreatic islet cells, and measured the ability of different immune vaccine protocols to induce a response against the target antigen (as measured by the induction of diabetes). They found that a DC vaccine using DC cells primed against the target antigen was far more effective than injecting the target antigen itself, with or without an adjuvant. And in further important findings, the best vaccine allowed the primed DC cells to mature prior to infusion and occurred when the DC cells were primed against more than one epitope.

In June of 2015, an interesting study was done by a team of scientists in the Department of Medicine, Weill Cornell Medical College (New York, NY) in mice, identifying a new pathway in DC cells that inhibits their function (Cubillos-Ruiz et al., 2015). Previous studies had shown that endoplasmic reticulum (ER) stress response factor XBP1 directly promotes tumor cell growth, but it was not known whether XBP1 affected the DC cells that had migrated to a tumor. In a mouse model of ovarian cancer, the team showed that XBP1 becomes activated within the DC cell in response to reactive oxygen species (ROS) and lipid peroxidation, limiting their ability to support anti-tumor T-cells. And in an experiment that could only be done in animals, not humans yet, they showed that deleting XBP1, or inhibiting it by siRNA, restores DC function and induces a type-1 anti-tumor response. This study offers a new unique approach for
restoring the function of DC cells by blocking an inhibitory pathway, and perhaps could be combined with the use of tumor-specific antigens as a vaccine.

Although some scientists argue that cancer vaccines as a whole have yet to produce high efficacy levels in clinical trials, using animal models, much knowledge is being gained about improving vaccine effectiveness and for determining exactly which immune cells are involved. Scientists have learned much about DCs and their immunological role in the body. From the groundwork laid with animal studies, hopefully the ongoing clinical trials will be informed by their findings to improve the results in human patients.

**References on Animal DC Vaccines**


DC Cancer Vaccine for Prostate Cancer: Provenge

The first cancer vaccine approved for use in the U.S. by the FDA was Provenge (also called Sipuleucel-T or APC8015). Provenge is a DC-type vaccine designed for prostate cancer. In the U.S., prostate cancer is the second leading cause of cancer death in men (after skin cancer). According to the National Cancer Institute (2015), an analysis of the data from 2008-2012 prostate cancer cases showed approximately 137.9 new cases (and 21.4 deaths) per year per 100,000 men. About 14.0% of all men will be diagnosed with it during their lifetime, and there are approximately 2,795,592 men living with it in the U.S.

Provenge was developed in the early 1990’s by two scientists: Edgar Engleman, an immunologist at Stanford University (who had been studying cancer vaccines for lymphomas), and Samuel Strober. In 1992, those two scientists founded Dendreon Corporation (Seattle, WA). In spite of Engleman’s early success in animals with lymphomas, they chose to pursue prostate cancer, in part because more people have it, and also because men can live without a prostate, so if anything went wrong with the vaccine and it destroyed healthy prostate tissue it would not be fatal (Ledford, 2015).

Provenge consists of autologous (taken from the same patient) peripheral blood mononuclear cells (PBMCs) (a cell mixture that includes DCs) taken from the patient by leukapheresis. The PBMCs are cultured to increase their numbers, and are then mixed for 2 days in vitro with a recombinant fusion protein (PA-2024). PA-2024 contains prostatic acid phosphatase (PAP) (expressed in a majority of prostate adenocarcinomas) (Goldstein, 2002) linked to granulocyte macrophage colony stimulating factor (GM-CSF) (to help activate the immune system). After the DCs are pulsed or primed against PA-2024, the DCs process the antigen within the cell and present it on their surface. The DC cells are perfused back into the same patient over a 30 minute period. Once in the body, the DCs migrate to the lymph nodes and present the tumor antigen to B-cells and T-cells to activate them against the prostate cancer.

In 2010, Provenge made history by being the first cancer vaccine approved by the FDA for use in the U.S. (Ledford, 2015). Dendreon Corporation was the first company to show that complex cell treatments prepared fresh from each patient could be made reactive against an antigen on the surface of prostate cancer cells, could be expanded in vitro, and could be placed back in the same patient without rejection. Provenge is usually touted as the main early success story of DC-type cancer vaccines.

Provenge has already undergone several clinical trials in human patients. Small et al. (2000) performed a combined Phase-I and -II clinical trial of Provenge on a small number of patients with hormone-refractory (androgen resistant) prostate cancer. The main point of the study was safety, not efficacy, and the patients appeared to tolerate the treatment well. The most common side-effect observed was fever, occurring in about 14.7% of the patients. All of the patients developed an immune response against the PA-2024 recombinant stimulatory protein mixed with the DCs. Three patients showed a 50% decline in PSA levels (an indicator of prostate cancer lowering).

In 2000, scientists at the Mayo Clinic (Rochester, MI) performed a phase-I test of Provenge on 13 patients with progressive hormone-refractory metastatic prostate carcinoma.
The purpose of the phase-I test was safety, and the treatment appeared to be well tolerated. The patients experienced only mild grade-1 and -2 side effects, such as fever, chills, myalgia, pain, and fatigue. Only 1 of the 13 patients developed grade-3 fatigue. Circulating levels of PSA dropped in 3 patients, an indicator of cancer lowering. T-cells drawn from patient blood after the treatments, but not before the treatments, could be stimulated in vitro with components of the DC antigen mix (GM-CSF and PAP), indicating the patient’s had overcome their previous self-tolerance of those key proteins, and had now created T-cells committed against those tumor antigens.

In 2004, the same Mayo Clinic group performed a phase-II test of Provenge on 21 patients suffering from androgen-independent prostate carcinoma (Burch et al., 2004). Most of the side-effects were grade-1 and -2, with only 4 of the 21 patients showing grade-3 or 4 side-effects. 2 of the 21 patients showed a 25-50% drop in PSA levels, and one patient dropped PSA to undetectable levels and resolved his cancer. It is not clear in this study why only 2 of 21 patients lowered their PSA levels, so perhaps this indicates the Provenge vaccine should be combined with other vaccines to stimulate the immune system.

In 2005, scientists at the MD Anderson Cancer Center (Houston, TX) performed a phase-II test of Provenge on 18 patients with non-metastatic prostate cancer who had not responded to previous treatments (Beinart et al., 2005). 13 of the 18 patients slowed the rate of increase of their serum PSA levels, with a median increase in doubling time of 62% (4.9 months before treatment, 7.9 months after treatment, p=0.09). So, in this experiment a higher percentage of patients responded to the vaccine, and it appeared to be well tolerated.

In 2009, scientists at the Seattle Cancer Care Alliance published the results of a Provenge phase-III clinical trial (Higano et al., 2009). The trial was a randomized, double-blind, placebo-controlled phase-III clinical trial of Provenge, testing a total of 225 patients suffering from advanced prostate cancer. The patients were treated with Provenge (147 patients) or placebo (78 patients). They found an average 33% reduction in death for the patients receiving Provenge versus the placebo (p=0.011), a significant outcome. The beneficial effects of Provenge remained strong even after correcting for baseline prognostic factors, post-study chemotherapy, and non-prostate cancer deaths. The most common Provenge-induced side-effects were chills, pyrexia (fever), headache, asthenia (weakness), dyspnea (labored breathing), vomiting, and tremor, all of which were only grade-1 or -2, with short durations of only 1-2 days. So, in this experiment the vaccine caused only modest toxicity while showing statistically significant clinical improvements for the patients.

In 2010, scientists at the Dana Farber Cancer Institute (Boston, MA) performed a different phase-III test on 512 patients with metastatic castration-resistant (androgen-independent) prostate cancer (Kantoff et al., 2010). In a double-blind, placebo-controlled, multi-center trial, Provenge was given to 341 patients (administered intravenously every 2 weeks for a total of 3 infusions), and a placebo was given to 171 patients. The Provenge patients saw an average 22% reduction of death, with survival extending from 21.7 months to 25.8 months (an improvement of 4.1 months). Adverse effects included chills, fever, and headache. Overall, the phase-III results were not as dazzling as hoped for, but did provide a proof-of-principle that cancer vaccines can improve lifespan in a large cohort of patients.
In 2011, scientists in the Laboratory for Tumor and Transplantation Immunology, University Hospital of Cologne (Cologne, Germany) performed a systematic review of the literature on 17 DC clinical trials for prostate cancer to determine whether there is a significant link between the immune responses induced by the vaccine and improved clinical outcomes (Draube et al., 2011). Overall, a clinical benefit (stable disease) was observed in 54% of the prostate cancer patients receiving the vaccine that significantly correlated with a T-cell immune response against the tumor. High DC doses also significantly correlated with clinical benefit.

But in spite of Provenge’s U.S. approval and measurable (but minor) efficacy, Dendreon Corporation (Seattle, WA) (the innovative biotech company who developed Provenge) financially collapsed (Ledford, 2015). The company was strained by the long 18-year wait from its initial foundation in 1992 to the 2010 approval by the FDA. Although the procedure was approved by Medicare in 2011, confusion over reimbursement by private insurance companies left many U.S. doctors reluctant to use the expensive procedure (about $73,000 per treatment), so revenues came in far below the company’s initial estimates. And at the same time, the vaccine results were not as dazzling as they hoped for. As discussed above, one phase-III study showed the vaccine only extended survival time by a median of 4.1 months (Kantoff et al., 2010).

On February 23, 2015, the rights to the Provenge vaccine and the assets of the bankrupt Dendreon were purchased by Valeant Pharmaceuticals (Laval, Canada). Valeant hopes to continue to develop and use Provenge.

References for Provenge Vaccine


DC Vaccines for Melanoma

The DC vaccine approach has also been applied to patients with malignant melanoma. Melanoma is a type of skin cancer that forms from pigment-containing melanocytes in the skin (Bajetta et al., 2002). Although it is less common than other types of skin cancers, melanoma is much more deadly if not detected early, causing the vast majority (75%) of skin cancer deaths (Jerant et al., 2000). In 2012, melanoma occurred in about 232,000 people worldwide, resulting in 55,000 deaths (World Cancer Report, 2014). Its incidence is strongly related to exposure to ultraviolet (UV) light (Wang et al., 2001; Kanavy and Gerstenblith, 2011) relative to the amount of skin pigmentation to protect from UV exposure (Jost, 2003), so it is especially common in Caucasians living in sunny climates. If found early, the typical treatment is surgical removal of the tumor. If completely removed, the chance of survival is high. But if the melanoma recurs or spreads deep, other treatments are used including chemotherapy or radiation therapy.

One of the earliest attempts to treat melanoma patients with DC cells was in 1998 by a group of scientists at the University of Zurich Medical School (Switzerland) (Nestle et al., 1998). In a clinical pilot study, these scientists treated 16 patients with advanced melanoma. The patient DC cells were pulsed with either tumor lysate from the same patient, or were pulsed with a mixture of peptides known to be recognized from animal studies against melanoma. The vaccinations appeared to be well tolerated, and did not generate any visible autoimmune responses. 11 of the 16 patients showed immune responses against the vaccine and the recruitment of CTLs to a challenge site. Regression of metastases was observed in 5 of the 16 patients.

In 2000, Dr. Andreas Mackensen and his group published their findings in an International Journal of Cancer article entitled “Phase I study in melanoma patients of a vaccine with peptide-pulsed dendritic cells generated in vitro from CD34+ hematopoietic progenitor cells” (Mackensen et al., 2000). They vaccinated 14 patients with peptide-pulsed DC cells generated in vitro from hematopoietic progenitor cells. Each patient received at least 4 vaccinations, and was monitored before each vaccination and 4 weeks after the final vaccination. Of the 14 patients, 4 showed strong immunological responses. 2 of the 14 showed anti-tumor responses, including regression of metastasis. 1 patient developed vitiligo, but that skin discoloration was minor and treatable. This early study appears to show a modest vaccine success, but did provide proof-of-principle that DC vaccines can cause remissions in some patients.

In 2001, a group of scientists from the Rockefeller University published their findings on the immune effects of subcutaneous injections of progenitor-derived DC vaccines (Banchereau et al., 2001). 18 patients were vaccinated with melanoma peptide-pulsed DCs four times. The peptides used for the pulsing were derived from 4 different melanoma specific antigens. The vaccine seemed to be well tolerated, except 2 patients showed progressive vitiligo (skin
16 of the 18 patients showed enhancement of CD8+ T-cell immunity. The scientists further analyzed 12 of these patients and published their results in 2004 (Paciesy et al., 2004). According to their study, 9 out of 12 vaccinated patients with metastatic melanoma expanded their cytolytic CD8+ T-cell precursors. After single re-stimulation by melanoma peptide-pulsed DC cells, these precursors differentiated into cytotoxic T-lymphocytes (CTLs) that were able to kill melanoma cells. So, 9 of 12 patients developed CTLs capable of destroying melanoma cells. 3 of the patients who did not show melanoma-specific CTLs had early disease progression.

In 2005, a team of scientists in the Division of Hematology-Oncology, Department of Medicine, Massachusetts General Hospital (Boston, MA) treated 12 patients suffering from advanced melanoma with DC cells pulsed with tumor-specific peptide G280-9V. The patients were treated every 3 weeks with 6 doses of cells, in an escalating dose regime. 67% of the patients showed CD8+ cells reactive against peptide G280, and 9 of 9 patients tested had T-cells that were able to lyse tumor cells in vitro. 3 of the 9 patients tested showed stable disease, and 2 showed partially stable disease. This study shows that peptide G280 appears to be a good candidate for pulsing DC cells against melanoma.

In 2006, Dr. Salcedo’s group at the IDM Research Laboratory (Paris, France) published their finding on the immune responses to a DC vaccine loaded with allogeneic (genetically unrelated) tumor cell lysates (Salcedo et al., 2006). Instead of using tumor cells from the same patient, they made a tumor cell bank from a M17 tumor cell line that is known to express high level tumor-specific antigens, and used that cell line as the source of tumor lysates. DC vaccines pulsed with allogeneic tumor lysates appeared to be more reproducible and had a better control of quality compared with autologous tumor lysate-pulsed vaccines. 15 patients were included in this study, and 9 of them received all four doses of vaccine. 11 of the 15 showed enhancement of immune responses against targeted antigens. 2 out of 9 evaluable patients showed clinical responses and received more vaccines. Of these two patients, one showed complete cancer regression, the other one showed disease stabilization. One patient had no evidence of disease after resection surgery. Because some scientists had debated whether it is best to use mature DCs (Adema et al., 2005; Nestle et al., 2005), Dr. Salcedo’s team compared DCs matured ex vivo and non-matured DCs in another study, and suggested that there is no difference in the immune response rates. They believe that other ways of DC maturation, however, could result in improvement of vaccine efficacy.

In 2009, a team of scientists in the Department of Oncology at Herlev Hospital (Herlev, Denmark) performed a data review of 38 published articles using DC vaccines to treat a total of 626 patients with malignant melanoma (Engell-Noerregaard et al., 2009). Their data showed that improved clinical responses appeared to correlate with the use of peptide antigens to pulse the DC cells (p=0.03), the use of adjuvant (p=0.002), and the induction of antigen-specific T-cells (p=0.0004). No specific route of vaccination administration was superior to another.

In 2011, a group of scientists in the Department of Medical Oncology at the Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre (Nijmegen, The Netherlands) published their paper entitled “Cell Vaccine–Induced Antigen-Specific T Cells in Advanced Melanoma Patients” (Lesterhuis et al., 2011). 43 patients were vaccinated with peptide-loaded DCs via intradermal or intranodal injection. In 7 out of 24 patients, no DC redistribution was found at all after intranodal vaccination, whereas in all of the intradermally vaccinated patients at least a small percentage of injected DCs migrated to other lymph nodes.
Both intranodal and intradermal vaccinations induced melanoma-specific T-cells, but intradermal vaccinations more often induced functional T-cells that could recognize tumor cells. This study indicates that intradermal injection may be superior to intranodal injection for inducing immunological responses.

In 2013, Dr. Beatriz Carreno and her team at Washington University School of Medicine (St. Louis, MO) published their findings of the clinical outcomes of 7 patients with stage IV (advanced) melanoma (Carreno et al., 2013). The patients were vaccinated with DC cells pulsed against cancer antigen gp100. Six of the 7 patients showed sustained T-cell responses (against the tumor); of those 6 patients, 1 patient showed complete remission, and 2 showed partial remission. Strong improvement correlations were observed for patients perfused with DC cells producing the hormone IL-12, and for patients with strong T-cell responses to the vaccine. So, this paper indicates that screening for DC cells that are functional enough to produce IL-12 (or supplementing the vaccine with IL-12) might help improve vaccine outcome. This IL-12 conclusion was also discussed by the same team in their 2013 review article of DC vaccines published in Oncoimmunology (Linette and Carreno, 2013). They indicate that although the early DC-based cancer vaccines produced disappointing results in clinical trials, the newer data shows the efficacy can be improved if the patients (or DC vaccine cells) produce the hormone interleukin-12 (IL-12).

Very recently, Carreno’s group also published a paper in the May 15 issue of Science, entitled “A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells” (Carreno et al., 2015). In this paper, the authors provided a landmark proof-of-principle demonstration that a DC vaccine could be designed against “neo-antigens” present only in a patient’s own tumor cells (not naturally occurring). In an interview with The Scientist on April 2, 2015, senior author Dr. Carreno stated that the problem with making a normal vaccine against shared antigens (those that are over-expressed in tumor cells but also present in normal cells) is the “immune response is not so strong” (Azvolinsky, 2015). The team used new sequencing technologies to identify neo-antigens present only in the patient’s tumor cells, not in normal cells. The team performed exome sequencing (sequenced the protein encoding exon portion of the genome) on surgical tumor samples taken from 3 patients with stage-III melanoma. They then used bioinformatics and prediction algorithms to identify missense mutations present in the tumors but not in healthy tissue. They restricted the list of neo-antigens to those with an affinity for HLA-type HLA-A*02:0, a type of antigen-presenting receptor present in 45% of Northern Europeans that are more prone to melanomas. They then selected about 7 neo-antigens per patient, synthesized them chemically, mixed the cocktail with the patient’s DC cells, and injected the primed DC cells back into the respective patients (Carreno et al., 2015). The purpose of this initial study was not to assay vaccine efficacy but to assay the immune responses for proof-of-principle the neo-antigen approach works. After 3 rounds of vaccines over 20 weeks, the team extracted mononuclear cells from the patient’s blood and assayed them in vitro for T-cell responses. The data showed that T-cell responses were generated against some, but not all 7 of the neoantigens, present in the vaccine, indicating the neo-antigens are not alike in their ability to induce an immune response. The results also showed that the vaccine expanded the patient’s own immune response against the neo-antigens, likely because TILs had been primed against them already in vivo. This study proves that new rapid DNA sequencing technologies can be used to identify neo-antigens present in the patient’s tumor, and that priming the DC’s against them may make the DC vaccines more effective by minimizing immune responses against normal shared antigens. When asked their comments on this landmark
study, Dr. Nina Bhardwaj, Director of Immunotherapy at the Tisch Cancer Institute at Mount Sinai Hospital (New York City) (not involved with the work) stated, “The work is an early proof of principle that immunization against these neo-antigens results in patient immune responses” (Azvolinsky, 2015). And Jeffrey Weber, a tumor immunologist at the Moffitt Cancer Center in Tampa (Florida) (also not involved in the study) stated that, “Scientifically and immunologically, this was a tour de force as the first example of a personalized vaccine strategy” (Azvolinsky, 2015).

With respect to the problem of neo-antigens producing different levels of immune responses, 2015 study co-author Dr. Elaine Mardis, Co-Director of the Genome Institute at Washington University said that “We need to improve [our] immunogenicity-predicting algorithm by training [it] on real data obtained from patient responses” (Azvolinsky, 2015). So, this seems to be an important future direction to focus on: using actual patient immune responses against specific tumor neoantigens to help formulate guidelines for making more accurate predictions of which tumor neo-antigens are likely to be more immunogenic, and thus are the best candidates for designing the vaccine against. With respect to the immune responses being relatively low, lead author Dr. Carreno reminded us that the purpose was to establish proof of principle, stating, “There were activated T-cells in the blood after the vaccination that were not there before vaccination - these were not high in number, but they were there” (Azvolinsky, 2015). So, perhaps a future direction for the use of neo-antigens would be to use a combination vaccine against neo-antigens combined with antibodies against immune blockers CTLA-4 or PD-1, as discussed in the previous vaccine section. One important future direction will be the speed of the overall approach. The types of neo-antigens created in a patient’s tumor varies from patient to patient, so the neo-antigens must be assayed for every new patient (unless that type of cancer has an excellent marker protein shared by all patients). Jeffrey Weber (mentioned above) reminded us that the time it takes to produce individualized vaccines is critical, “melanoma patients do not have multiple months to wait on vaccine production”. So, future research likely will need to improve the speed at which we identify neoantigens from each patient, and improve the speed at which the patient’s extracted DC cells can be amplified in vitro.

References for DC Treatment of Melanoma


**DC Treatment of Glioblastomas**

Glioblastoma (GBM) is the most common and aggressive primary brain tumor for adults (Stupp et al., 2005). About 23% of primary brain tumors are GBMs in the US (IRSA, 2015). GBMs arise from star shaped glial cells that form supportive tissues in the brain. GBMs usually contain multiple cell types. Some traditional therapies may kill parts of the GBM, but have little effect on other cells of the tumor. So, GBM treatments usually have several steps, usually tumor resection (if possible) followed by radiotherapy or chemotherapy. Because GBMs have a finger-like shape that protrudes into healthy tissue, it is very difficult to completely remove the tumor by surgery without damaging healthy brain tissue (ABTA, 2014). The median survival time after standard treatment is less than 2 years (Johnson and O’Neill, 2012).

In 2001, Dr. Yu’s group at the Maxine Dunitz Neurosurgical Institute, Cedars-Sinai Medical Center (Los Angeles, CA) published their findings on treating GBM with DC vaccines (Yu et al., 2001). They vaccinated 9 patients with DCs loaded with peptides isolated from the...
surface of tumor cells. DCs were amplified from the patients’ blood stem cells \textit{ex vivo}. No serious side effects were seen. 4 out of 7 evaluated patients showed sustained CTL responses against the tumor. 4 patients that had signs of tumor progression underwent reoperation. Strong cytotoxic and T-cell infiltrations were observed near the tumors in 2 of the 4 relapsing patients after re-perfusions. The median survival time of the study group was 455 days, which was considerably longer than the control group (257 days). This study demonstrates that DC vaccinations can positively correlate with longer patient survival.

One study showed that the time required to prepare peptide-pulsed DC vaccines is limited by the time needed to extract antigens from the patient’s tumor cells (Yu et al., 2004). The extraction is also more difficult if the patient received radiotherapy. To avoid these limitations, Dr. Yu’s team chose to load DCs with tumor lysates isolated from the same patient. 14 patients were included in this study. The vaccines appeared to be well-tolerated, and no evidence of autoimmune disease was seen. 6 of 10 evaluated patients showed robust T-cell responses against the tumors. 4 out of 9 patients showed significant expansion of antigen-specific T-cells against tumor associated antigens such as mege-1 or gp100. 6 patients underwent another round of vaccination. Of those 6 patients, significant T-cell infiltration of the tumors was detected in 3 patients. This study demonstrates that DC vaccines pulsed with autologous tumor lysate appears to be a safe, feasible way to induce strong cytotoxic immune responses against brain tumors, and that antigens mege-1 and gp100 appear to be excellent candidate antigens.

In 2005, Dr Liau’s group in the Division of Neurosurgery at the Brain Research Institute of the David Geffen School of Medicine, UCLA (Los Angeles, CA) published their findings on the ability of DC vaccines to induce immune and clinical responses in GBM patients (Liau, et al., 2005). They had previously shown that DC vaccines can induce antitumor responses against GBM’s in animal experiments. 12 patients with grade-4 GBM’s were vaccinated with tumor peptide-pulsed DCs, and the patients were followed for 5 years. 1 out of 12 patients had clinical response, as documented by MRI. 6 had measurable systemic antitumor CTL responses, but the immune responses did not translate into clinical responses or prolong survival. Of 8 patients that received second vaccines, 4 showed increased tumor infiltration by cytotoxic cells. The secretion of TGF-β2 into the blood often correlates with GBM tumor progression (Derynck et al., 2001), and these results showed that T-cell infiltration increased with lower TGF-β2 expression.

Another paper from UCLA in the Department of Neurosurgery, Brain Research Institute (Los Angeles, CA) attempted to identify which GBM patients were most likely to respond to a DC vaccine (Prins et al., 2011). In this study, 23 patients with grade-4 GBM were vaccinated with tumor lysate-pulsed DCs accompanied by adjuvant. The vaccines appeared to be well tolerated, with a median survival time of 31.4 months. They determined that patients whose tumors showed a mesenchymal-like gene expression had longer overall survival and higher CD3+ and CD8+ T-cell infiltrations into the tumor. Thus, mesenchymal gene expression profiles may help the patient eliminate the tumor.

In 2011, Dr. Okada’s group at the Hillman Cancer Center (Pittsburgh, PA) published their findings on the clinical and immune responses induced by alpha-type-1 polarized DC cells (αDC1) loaded with glioma-associated antigen (GAA) supplemented with synthetic adjuvant polyinosinic-polycytidyllic acid [poly(I:C)] stabilized by lysine and carboxy-methylcellulose-poly-ICLC (Okada et al., 2011). αDC1 cells can produce high levels of IL-12 which can strongly boost the type-1 immune response (cellular and humoral immunity). Their previous
experiments with poly-(I:C) stabilized with poly-ICLC showed it can enhance the efficacy of glioma antigen-targeting vaccinations in animal experiments (Zhu et al., 2007). This was the first study to treat recurrent human malignant gliomas with the combination of αDC1 cells and adjuvant. The vaccines were well-tolerated. Of 19 evaluable patients, 58% had positive immune responses against glioma associated antigen. 9 patients had no sign of tumor progression for at least 12 months. And 1 patient showed sustained complete remission. Thus, replacing standard mature DC cells with αDC1 cells might improve vaccine response and efficacy.

Some scientists argue that cancer stem cells (cells with stem cell characteristics found within the tumor) survive chemotherapy and are responsible for tumor survival post-chemo treatments (Yao et al., 2011). A group of scientists in the Department of Neurosurgery at Wuhan University (China) investigated whether pulsing DC cells with glioma stem cell lysate increases the anti-tumor effects of the T-cells (Ji et al., 2013). The DCs were obtained from murine bone marrow cells, and glioma stem cells were cultured from the glioma cell line U251. Previous studies showed that heat-treating the cultured tumor cells for 3 hours at 44°C up-regulates heat-shock protein expression which increases the immunogenicity of the cell lysate. So, the team heat-shocked their cultured stem cells prior to making a lysate by repeated freeze/thawing. The lysate was then used to pulse their DC cells. The data showed that the stem cell antigen-pulsed DCs can significantly stimulate the formation of tumor-specific T-cells that kill glioma cells more effectively than DCs in the control group. This study shows that reacting the DC cells against cancer stem cells, a key component of glioma tumors, has the potential to improve DC-based vaccines.

In 2015, a group of scientists from Duke University published a paper entitled “Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients” (Mitchell et al., 2015). The team randomized GBM patients to pre-conditioning with non-pulsed DCs or to tetanus toxoid Td (to generally boost the immune system). They then vaccinated mice or patients with cytomegalovirus phosphoprotein-65 (pp65) RNA-pulsed DCs. The data suggested that patients in the Td + DC group had considerably greater DC migration to the vascular draining lymph nodes (VDLN) (where presumably they would induce a strong immune response) and a longer progression-free and overall survival compared with patients treated only with non-pulsed DCs. To fully understand the mechanism for improved patient survival, the team performed similar experiments on mice. The Td pre-conditioned group had a threefold increase of DCs within inguinal lymph nodes, whereas no increase of DC migration occurred in the control group. Injection of CD4+ Helper T-cell-dependent protein antigens could also induce DC migration, and therefore the authors indicated that this could be a generalizable event that would apply to human patients. Thus, a recall response against tetanus toxoid appears to improve DC migration to a lymph node location required for a strong immune induction, and should be considered for future DC vaccines.

**References for DC Treatment of Glioblastoma**


**Improving DC Vaccine Strategies**

Some scientists argue that the early DC vaccine clinical trials produced disappointing results, and that a full potential of DC cancer vaccines has not yet been achieved (Koski et al., 2008). Our Lit Review identified several potential areas for improvements:

1. **Stimulatory Hormones**: Include full knowledge of the immune system in the vaccine, and especially include the use of stimulatory hormones such as IL-12, and knowledge of how DC cells engage other cells of the immune system (Topalian et al., 2011).

2. **Combination Vaccines**: Create combination vaccines composed of DC’s primed to tumor antigens plus antibodies against immune blockers such as PD-1 and CTLA-4 to stimulate the immune system. Recent vaccine success stories have taught us the importance of the tumor environment suppressing the immune response, so it is important to overcome this suppression by stimulating the immune system (Topalian et al., 2011).

3. **Cellular and Humoral Immunity**: The vaccine should not only stimulate CD8+ T-cell responses (cellular immunity), but also B-cell responses (humoral immunity) to make full use of the immune system.

4. **Personalized Medicine**: The tumor antigens used to prime the DC cells should be determined for each patient using rapid DNA sequencing technologies and bioinformatics to identify mutations specific for that patient. Neo-antigens produced in the patient’s tumor by gene mutation should be assayed and targeted to make a personalized therapy (Rajasagi et al., 2014).

5. The tumor disease stage best suited for immunotherapy (the stage at which it is best eliminated) should be identified and targeted in the patient if possible.

6. **Speed**: The speed of the entire process should be improved, especially when treating tumors with rapidly poor prognosis, such as advanced stage melanomas.
7. **DC Developmental Stages**: Investigations of how DCs generate immune responses, and which DC developmental stage can bring the best results should be investigated. A key questions is whether DCs should be matured before injection. Most DCs are isolated as CD14+ monocytes from the patient’s peripheral blood, but those DCs are immature. Is the use of immature DC’s best? Some scientists have shown that expanding peripheral cells with hormone Flt3L is better than isolating CD14+ cells. Other scientists have shown that isolating alpha-DC1 cells works better than regular DCs. (Andrews et al., 2008)

8. **Tumor Heterogeneity**: Some cancers are very heterogeneous, containing many types of cells. The presence of cancer stem cells is especially important as they are thought to seed tumor growth following chemotherapy (Pollack et al., 2002). So, designing the vaccines against tumor neo-antigens and against cancer stem cell markers might provide a strong vaccine.

9. **Adjuvants**: Synthetic polymers poly-(I:C) and poly-IC-LC are adjuvants that can effectively improve immune responses. So, adding these adjuvants to a DC vaccine might result in a stronger immune response (Ammi et al., 2015). Other adjuvants such as T-cell stimulator anti-CD173 or CpG also have the potential to improve DC vaccine responses (Cheever, 2008).

10. **Pre-Injections**: Tetanus toxoid Td pre-injection has been shown to increase DC migration to lymph nodes, which improves the immune response. Patients pre-treated with Td also have a longer median survival time and longer progression-free survival (Mitchell et al., 2015).

11. **Patient Selection**: Some patients seem to be better suited for cancer vaccines than others. Identifying markers for those patients more likely to have immune response to DC-based vaccines would enable those patients to be treated first while trying other treatment for those patients likely not to be helped.

12. **Tumor Evasion**: Some tumors are not eliminated by cancer vaccines, so the process of tumor evasion should be further investigated. In resistant cases, perhaps activating other arms of the immune system, such as natural killer cells (NKs) would help. One study has shown that adding protein NKG2D activates NK cells against cancer (Deng et al., 2015).

**References for DC Vaccine Strategies**


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Introduction to TIL Cells

**T-cells** (also termed T-lymphocytes) are a type of nucleated white blood cell that functions in cellular immunity, a key arm of the immune system. T-cells are distinguished from other lymphocytes, such as B-cells and natural killer cells (NK cells), by the presence of a T-cell receptor (TCR) on their cell surface. They are called T-cells because they mature in the thymus (although some T-cells also mature in the tonsils) (Alberts et al., 2002). There are several types of T-cells, each with a different function: helper (CD4+), cytotoxic (CD8+), memory, suppressor, mucosal associated, and gamma delta T-cells.

With respect to cancer vaccines, **tumor infiltrating lymphocytes (TILs)** are a type of T-cell found in tumors that help kill it. High levels of TILs in tumors are often associated with a better clinical outcome for the patient (Vanky et al., 1986). TILs isolated from tumors usually include both CD4+ (helper T-cells) and CD8+ (cytotoxic killer T-cells, CTLs). TILs circulate through the bloodstream, recognize the tumor and infiltrate it. The CD4+ cells secrete cytokines to stimulate the immune system, while the CTLs directly lyse the tumor cell. Naïve T-cells have an amazing capacity for reacting with pathogens (or tumor antigens), by differentiating into effector T-cells that recognize other cells containing that pathogen or antigen, massively expanding themselves by cell division, and migrating throughout the body to clear the infection (or tumor) with minimal damage to surrounding tissue (Zhang and Bevan, 2011).

T-cell therapy, sometimes referred to as adoptive cell therapy (ACT), has several advantages over those forms of immunotherapy that rely on the production of T-cells *in vivo* (Rosenberg and Restifo, 2015):

1. Very large numbers of T-cells can now be grown *in vitro* (up to $10^{11}$ cells).
2. The T-cells can be selected *in vitro* for those that recognize tumor antigens.
3. T-cell growth *in vitro* by-passes any negative regulation those cells might encounter *in vivo*.
4. The patient can be treated with traditional chemotherapy to support the tumor therapy prior to the cell infusion.
5. The engineered T-cell receptors do not rely on MHC-type antigen presentation but recognize the antigen directly.
6. The T-cells can be genetically engineered *in vitro* to express T-cell receptors (TCRs) or chimeric antigen receptors (CARs) that specifically recognize tumor proteins. CAR cells “merge the exquisite targeting specificities of monoclonal antibodies (engineered T-cell receptors given mAb-binding sites against tumor antigens) with the potent cytotoxicity, expansion potential, and long-term persistence of cytotoxic T-cells” (Lee et al., 2012).
References to Introduction to TIL Cells


T-Cell Therapy for Melanoma

The use of TILs to treat cancer was pioneered by Dr. Steven Rosenberg at the National Cancer Institute (for a review, see Rosenberg and Dudley, 2009). In this technique, autologous lymphocytes are isolated from a patient’s tumor and grown to very large numbers in vitro. In some cases, prior to the TIL treatment the patients are given chemotherapy to deplete native lymphocytes that can suppress the function of the perfused TILs. Once lympho-depletion is completed, patients are then infused with their own TILs supplemented by hormones such as interleukin-2 (IL-2).

The vast majority of research on TIL cells, and several ongoing clinical trials have been conducted using TILs to treat patients with metastatic melanoma. In some cases, the teams have observed 50% tumor reduction in about half the patients (Dudley et al., 2008; Besser et al., 2010; Radvanyi et al., 2012; Pilon-Thomas et al., 2012), and in one study, 22% of the melanoma patients even experienced complete cancer remission with no detectable tumor 3 years after the treatment (Rosenberg et al., 2011).

In 1994, Steven Rosenberg’s team designed an experiment to correlate the characteristics of TIL cells versus the patient responses (Schwartzentruber et al., 1994). They analyzed 41 patients with metastatic melanoma receiving TIL cells plus IL-2 hormone. 9 of the 41 patients (22%) achieved complete or partial cancer remissions. Parameters such as patient age, sex, sites of disease, or prior therapies appeared to be similar in both the responders and non-responders. However, the responders appeared to receive 33% more TILs, showed higher lysis abilities in vitro (30% versus 15%), and contained TILs that produced GM-CSF following in vitro stimulation with tumor cells. These data indicate that functional TILs (such as those secreting GM-CSF hormone and those with high lysis abilities) correlate with patient improvements.
In 2002, a research group at the Fred Hutchinson Cancer Research Center (Seattle, WA) performed an adoptive T-cell therapy phase-I clinical trial on 10 patients with metastatic melanoma (Yee et al., 2002). They isolated CD8+ T-cells reactive against tumor antigens MART1 and gp100 from each patient, expanded the T-cells \textit{ex vivo}, and then perfused the expanded T-cells back into the same patient. The purpose of the phase-I experiment was to evaluate the safety, \textit{in vivo} persistence and efficacy of the treatment. 43 cell infusions were performed on the 10 patients. No serious toxicity was observed, the T-cells persisted \textit{in vivo} at least 21 months (using IL-2), and the T-cells localized to the tumor and helped eliminate antigen-positive tumor cells.

In 2002, Mark E. Dudley and his group of researchers from Rosenberg’s team performed an adoptive transfer of TIL cells to 13 patients with metastatic melanoma (Dudley et al., 2002). The TILs were selected to target overexpressed self-derived differentiation antigens. The patients all received pre-treatment with cyclophosphamide and fludarabine chemotherapies for 7 days. The results indicated that of the 13 patients, 6 exhibited significant regression of metastatic melanoma, and 5 exhibited the onset of anti-melanocyte immunity. These results are a success to some of the team’s previous experiments where the patients did not show any responses to treatment, nor did they show any persistence of the TILs. The results from this test show that the destruction of metastatic tumors can be achieved by treatment with autologous T-cell transfer and high-dose IL-2 therapy.

In 2006, Steven Rosenberg’s group performed TIL therapy on 15 patients with metastatic melanoma with TILs that had been genetically engineered to express a retroviral encoded T-cell receptor (Morgan et al., 2006). Their data showed that the infused TILs resulted in a durable engraftment, with 10% presence in the peripheral blood lymphocytes positive for the TILs two months after injection. 2 of the 15 patients showed high levels of circulating TILs one year after injection, and observable tumor regression. This study provides evidence that TILs can be genetically engineered to improve effectiveness.

In 2008, Mark E. Dudley (of the Rosenberg team) and colleagues performed a study to evaluate the safety and efficacy of two TIL therapies accompanied by increased-intensity myeloablative lympho-depleting chemotherapy and radiation regimens (Dudley et al., 2008). The patients were given a pre-treatment with cyclophosphamide and fludarabine accompanied by either 2 or 12 Gy of total-body irradiation. This was followed by the TIL therapy. The results showed that 49% of the TIL patients had an objective response accompanied by just the chemotherapy, adding 2 Gy of irradiation increased the response to 52%, and increasing the radiation to 12 Gy raised the response rate to 72%. The conclusion of this experiment was that treating metastatic melanoma patients with TILs accompanied by strong doses of radiation can provide positive tumor decreases in up to 72% of the patients.

In 2010, Dr. Besser and his team at the Ella Institute of Melanoma, Sheba Medical Center (Tel-Hashomer, Israel) performed a study to test the efficacy and toxicity of TIL cells following lympho-depleting chemotherapy in 20 metastatic melanoma patients that were refractive to previous treatments (Besser et al., 2010). The results indicated that 50% of the patients achieved an objective clinical response, comprising 2 complete remissions, and 8 partial remissions. They concluded that refractory melanoma can show tumor regression in 50% of patients treated with chemotherapy followed by TIL cells, with manageable toxicity.
In 2011, the Rosenberg team studied 93 patients with metastatic melanoma treated with autologous TIL cells (isolated from each patient’s tumor and expanded in vitro) and IL-2 (Rosenberg et al., 2011). The patients were pre-treated with chemotherapy or radiation regimes to improve their prognosis. 95% of the patients had ongoing cancer from prior chemotherapy treatments. Their data provide a great success story, showing that 20 of the 93 patients (22%) achieved complete tumor regression, and 19 of the 20 regressions remained beyond 3 years! The 3 and 5-year survival rates for the 20 remission patients were 100% and 93%, respectively. The likelihood of achieving a complete remission was similar regardless of prior therapies, but the success correlated with longer TIL cell telomeres (to allow more population doublings in vivo), a higher number of TILs infused, and the persistence of the TILs in the circulation at one month.

In 2012, Dr. Radvanyi and a group of researchers in the Department of Melanoma Medical Oncology, at the University of Texas MD Anderson Cancer Center (Houston, TX) investigated the use of TIL cells in 31 patients with metastatic melanoma (Radvanyi et al., 2012). Each patient was treated with chemotherapy followed by his or her expanded TILs, and two cycles of high-dose interleukin (IL)-2 therapy. 15 of the 31 (48.3%) patients in the study had an objective clinical response. Of those 15, two patients had a complete response. They concluded that immunotherapy with expanded autologous TIL cells can achieve durable clinical responses in patients with metastatic melanoma, and that CD8+ T-cells in the TILs are important for the response.

In 2012, a team led by Dr. Shari A. Pilon-Thomas at the Donald A. Adam Comprehensive Melanoma Research Center in the H. Lee Moffitt Cancer Center and Research Institute (Tampa, FL) performed a clinical trial on 19 patients with metastatic melanoma treated with a combination therapy of non-myeloablative chemotherapy plus TIL cells and IL-2 (Pilon-Thomas et al., 2012). Of the 19 patients enrolled, 13 of them successfully completed treatment. Out of these 13 patients, 2 had complete responses, and 3 had partial responses. In addition, 4 patients had stable disease. This study concluded that adoptive therapy with infiltrating lymphocytes is possible and can be effective, but is labor intensive.

In 2013, Steven Rosenberg’s group performed TIL therapy on 69 patients with metastatic melanoma (Dudley et al., 2013). 34 patients received TILs not selected for any marker protein, and 35 patients received TILs enriched for CD8+ cells. 12 patients receiving the unselected TILs responded to the therapy, while only 7 responded to the CD8+-enriched TILs. This data indicated that the significant amount of time and cost associated with enriching for CD8+ cells is likely not worth the effort.

In 2013, Rosenberg’s team developed a new screening approach to identify newly mutated proteins present in melanomas (Robbins et al., 2013). The method used whole-exome DNA sequencing of tumor DNA followed by bioinformatics to identify mutations present in the tumor protein-coding regions not present in normal tissue. The method avoided the labor-intensive methods of synthesizing and screening cDNA libraries. They synthesized the neo-antigens synthetically, and tested their recognition by patient TILs. They also extended the approach to identify melanoma neo-antigens that when targeted by TILs have a higher probability of causing a full tumor regression. These neo-antigens were present in about 40% of long-term survival melanoma patients (5-years after TIL therapy). The team identified 3 specific TIL cell lines with
high capacity for therapy. The authors speculate that their technique can be applied to any type of cancer.

In 2014, Rosenberg’s group analyzed the phenotypic traits of TIL cells isolated from 6 melanoma tumors (Gros et al., 2014). They used deep sequencing techniques to determine which cancer antigens the TIL cells recognized and whether any TILs expressed inhibitory receptors. Their data indicated that all 6 tumors contained TILs positive for mutated neo-antigens, indicating that neo-antigens might be the best target for future experiments. And all 6 tumors contained TILs positive for negative immune receptors PD-1, LAG-3, and TIM-3, indicating that the TILs in their in vivo state were functionally impaired by the tumor. Thus, antibody therapy designed against the negative regulators might improve vaccine effectiveness.

References for T-Cell Therapy of Melanoma


**Examples of Fighting Other Cancers with TIL Therapy**

Besides melanoma, TIL cells have been used to treat other kinds of cancer, including epithelial and ovarian cancers (discussed below), and two ongoing clinical trials will investigate a variety of other cancers (Clinical Trial NCT01174121; Clinical Trial NCT01585428). Trial NCT01174121 aims to determine whether TILs can shrink tumors resulting from tumors of the digestive tract, urothelial cells, breast, and ovarian/endometrial cancers. In addition to testing the
effectiveness of the treatment, the trial will also focus on TIL safety. The ongoing trial has an estimated enrollment of 260 patients, and is expected to be completed in December of 2019. Eligible participants will first undergo surgery to remove tumors and TILs will be extracted from the tumors. Patients may also be subjected to leukapheresis if additional white blood cells are required. The patients will return to the clinic for additional tests to record progress and potential side-effects.

Trial NCT01585428 will focus on the use of TILs to shrink HPV-related cervical and non-cervical cancers. Similar to the other ongoing trial, white blood cells will be extracted from the patient’s tumors, will be grown in vitro, and will be then given back to the patients. The estimated enrollment for this trial is 73, and is expected to end in December of 2017. After receiving the treatment, the patients will return for physical exams and tests to review progress and side-effects. The exams will occur every 1-3 months for the first year, and then every 6 months to 1 year thereafter.

Steven Rosenberg’s team that originally pioneered the use of TIL’s for treating melanoma has also used the TIL approach on patients with epithelial cancer (Tran et al., 2014). Prior to the study, it was not clear whether T-cells isolated from patients with metastatic cholangiocarcinoma are directed against mutated neo-antigens in the tumor. So, the team performed whole exome sequencing (discussed previously) on TILs isolated from epithelial tumors, and showed the TILs specifically reacted against tumor erbb2-interacting protein (erbb2ip). When treated with a TIL cell population where 25% were specific for erbb2ip, the patient showed a decrease in lesions and disease stabilization. After a relapse, the patient was retreated with a TIL population where 95% were against erbb2ip, and showed a tumor regression.

TIL cells have also been used to fight ovarian cancer. In 2013, Dr. Jason H. Bielas’ team at the Fred Hutchinson Cancer Research Center (Seattle, WA) published a study in Science Translational Medicine, entitled “Digital Genomic Quantification of Tumor-Infiltrating Lymphocytes” (Robins et al., 2013). The team designed a new digital DNA-based assay (termed QuanTILfy) to count TIL cells and assess their clonality (percent activity against various antigens) in tissue samples (including tumors). They applied the approach to metastatic ovarian tumor tissue, and demonstrated an association between higher patient TIL counts and improved patient survival, which is consistent with previous findings that those parameters are meaningful patient prognostic factors. They also found a diverse TIL repertoire for all their tumors, with no apparent strong clonal expansions against a specific type of antigen for the ovarian tumors, so this makes designing a specific type of TIL (or CAR) against ovarian cancer more difficult, and may necessitate the need for a more complex vaccine.

References for Other Cancers and TIL Cells

Clinical Trial NCT01174121. A Phase II Study Using Short-Term Cultured, CD8+-Enriched Autologous Tumor-infiltrating Lymphocytes Following a Lymphocyte Depleting Regimen in Metastatic Digestive Tract Cancers. ClinicalTrials.gov.
Clinical Trial NCT01585428. A Phase II Study of Lymphodepletion Followed by Autologous Tumor-Infiltrating Lymphocytes and High-Dose Adesleukin for Human Papillomavirus-Associated Cancers. ClinicalTrials.gov.


Part-7 Summary, Problems and Questions

With respect to TIL cell treatments, most of the studies performed to date are with melanoma cancer, performed by Steven Rosenberg’s team at the National Cancer Institute who pioneered the TIL approach. In interviews, it would be interesting to determine why the team chose that particular cancer for so many of the early experiments. Was the cancer chosen because of its high fatality rate after metastasis and lack of response to traditional treatments?

Some of the melanoma TIL clinical trials resulted in patients with complete cancer remissions. The best patient survivals appear to occur in patients with the highest TIL load in the tumor, so does this emphasize the importance of growing large numbers of the TILs prior to infusion? And some of the best remissions occurred by enriching the TILs for those cells committed to the tumor-specific neo-antigens, so should this type of enrichment be done routinely, or just for resistant cases?

In a few studies, TILs have also been used to treat epithelial and ovarian cancers. But in ovarian cancer, the TILs appear to be committed to a variety of neo-antigens, not just one type. So, will these ovarian cases require the enrichment and expansion of all TIL clones to be effective, or will expanding only one type of TIL be sufficient for remission?

Besides the published TIL experiments with melanoma, ovarian, and epithelial cancers, it is not yet known whether the TIL therapy works with other types of cancer, so the ongoing clinical trials testing other cancers will be important.
Chimeric antigen receptors (CARs) (also known as chimeric T-cell receptors or chimeric immune-receptors), are artificially engineered T-cell receptors (TCRs) that provide a specific binding affinity to the T-cell containing it. In the case of cancer immunotherapy, CARs are typically engineered to have a monoclonal antibody-like affinity for a specific tumor antigen. An engineered CAR gene is delivered inside the T-cells in vitro using retroviral vectors, and then the engineered T-cells are delivered back into the same patient (reviewed in: Pule et al., 2003; Lipowska-Bhalla et al., 2012; Curran et al., 2012). Genetically altered T-cells were first developed in the 1980s by Prof. Zelig Eshhar and his colleagues at the Weizmann Institute of Science in Rehovot, Israel. By 1989, they had created the first functional CAR T-cells (reviewed in: Urba and Longo, 2011).

The current CAR structure (Figure-1, right side) often fuses together: 1) a monoclonal antibody single-chain variable fragment (scFv) derived from an anti-tumor mAb (which recognizes the tumor) (orange in the diagram), 2) a co-stimulatory domain (like CD28 that activates the T-cells and helps them survive in vivo) (white in the diagram), 3) a hydrophobic domain that spans the lipid bilayer on the surface of the CAR cell (purple in the diagram), and 4) one to three cytoplasmic tail domains (like TCR-zeta) that activate the CAR cell once it engages the tumor cell (blue, yellow, and green in the diagram).

Figure-1: Diagram of the Typical Structure of a CAR Cell. Shown are first-generation, second-generation and third-generation CAR cells, panels left to right, respectively. The T-cell is shown in the lower half of each panel, and the cancer cell the upper half (green). Gray denotes the cancer antigen targeted by the engineered T-cell receptor in the CAR cell. Orange represents the monoclonal antibody variable fragment that recognizes the tumor antigen, purple represents the hydrophobic transmembrane domain. Blue, orange, and green denote cytoplasmic tail activation domains that signal the T-cell to activate when the receptor is engaged by the tumor cell. White denotes a co-stimulatory domain, such as CD28, that binds ligands present on antigen presenting cells to help the CAR cells divide and survive in vivo. Figure is from Brower, 2015.
Thus, structurally, CARs combine the powerful properties of highly specific antigen recognition and T-cell activation in a single fusion receptor molecule (Sadelain et al., 2009). The first generation CARs (diagram left panel) used CD3-zeta with no co-stimulatory domain, but these molecules failed to enable T-cell proliferation and survival in vivo. So, newer designs (diagram middle and right panels) often use a CD28 co-stimulator domain (white in the diagram) plus the CD3-zeta for the cytoplasmic domain (Sadelain et al., 2009). The engineered CAR gene is preceded by a signal sequence to target the receptor to the cell surface. When this CAR is in place on the cell membrane, once it binds its tumor cell target, it sends a signal inside the T-cell to activate it to kill the tumor cell.

References for CAR Introduction


Early CAR Animal Studies

The early foundation for the CAR field was laid by Israeli immunologist Zelig Eshhar who began the CAR work in mice while on sabbatical in Palo Alto (CA). He later returned to the Weizmann Institute of Science (Rehovot, Israel) and continued his studies (reviewed in Couzin-Frankel, 2013; Eshhar, 2014). Dr. Eshhar’s goal was to determine whether T-cells could be reprogrammed to recognize and latch onto an antigen they would normally ignore (like a cancer antigen). He eventually realized that the best way to do this was to insert foreign DNA into the T-cells encoding an engineered receptor that specifically recognizes the antigen. His early work frequently failed because most T-cells do not readily accept foreign DNA, but in the late 1980’s he learned to transform a type of immortalized T-cell that more easily accepts foreign DNA. Other big-name cancer researchers like Steven Rosenberg (National Cancer Institute), Carl June (University of Pennsylvania), and Michel Sadelain (Memorial Sloan-Kettering Cancer Center) picked up on this early work, and continued improving the technique and adapting it to
their patients. Commenting on the technique for transforming the T-cells with foreign DNA, Michel Sadelain stated, “It took me 3 to 4 years to learn to transfer genes [into T-cells] at more than 0.5%. Today we can take a high school student and in an afternoon they know how to take T-cells and blast genes into all of them” (Couzin-Frankel, 2013).

The early pre-clinical CAR studies in mice focused on determining the optimum CAR structure and the best methods for activating T-cell signaling (Bendle et al., 2009). They focused on designing high-affinity receptors that could overcome the immune tolerance induced by the cancer environment, improving the efficiency of the gene transfer into the T-cells using lentiviral vectors, and improving the methods for expanding the T-cells in vitro by using co-activation receptors or domains and by adding various hormones (reviewed in Kalos and June, 2013).

With respect to activation, the early mouse experiments used CARs containing only one cytoplasmic tail activating domain, usually TCR-zeta. But the use of a single activation domain resulted in low levels of T-cell activation and low homing to the bone marrow to produce more T-cells. More recently, second and third-generation CARs containing multiple activation domains (TCR-zeta plus CD28 and/or CD137) show enhanced activation signals, better T-cell proliferation, higher levels of cytokine production, and stronger effector T-cell function (tumor killing).

In addition, the use of a “co-stimulatory signal” receptor also placed on the CAR cell was an important advance that enabled the CAR cells to divide and survive long-term in the patient. Steven Rosenberg’s group and Michel Sadelain’s group use CD28, while Carl June’s team uses 4-1BB (CD137). CD28 (Cluster of Differentiation 28) is a receptor protein expressed on the surface of T-cells that binds ligands CD80 or CD86 present on antigen presenting cells engaged by the T-cells. Binding of APC ligand to T-cell CD28 produces co-stimulatory signals required for T-cell activation and survival. The CAR cells can contain CD28 receptor as a separate co-stimulatory molecule, or can merge the co-stimulatory domains directly into the T-cell receptor, in the latter case the viral treatment is with only one gene.

An example of an early mouse experiment was in 2003 by Dr. Eshhar’s group who tested a CAR treatment (termed by their lab as a T-body approach) against erbB2 on the surface of human prostate cancer cells xenografted into immunocompromised mice (Pinthus et al., 2003). The study was titled “Immuno-gene therapy of established prostate tumors using chimeric receptorredirected human lymphocytes”. The CAR therapy was supplemented by systemic administration of IL-2, and the results slowed tumor growth in all mice, prolonged mouse survival, and provided complete tumor remission in several mice.

Clinical trials with the newer CAR’s show some complete remissions in patients with B-cell leukemias, and are also being used for lymphoma, ovarian cancer, and neuroblastoma (Lipowska-Bhalla et al., 2012).

**CAR Vaccines and Melanoma**

One of the earliest human clinical trials using genetically engineered T-cells was by the Rosenberg team at the National Cancer Institute, the same team that pioneered the use of TIL
cells to treat melanoma. In 2006, they expanded their earlier TIL approach by transforming the T-cells cells with a retrovirus encoding a specific T-cell receptor, so the cells presented the receptor on their surface (Morgan et al., 2006). They treated 15 patients with metastatic melanoma. The CAR cells survived in the peripheral blood for at least 2 months, and survived for at least one year in two patients who showed significant tumor regression. So, this study with first-generation CAR cells showed some success.

**CAR Vaccines and Ovarian Cancer**

In another early clinical trial using first-generation CAR cells, Steven Rosenberg’s team also treated patients with ovarian cancer using CAR cells engineered against the α-folate receptor, a normal cell antigen upregulated in ovarian cancer (Kershaw et al., 2006). In their study entitled “A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer”, they treated 14 patients with the CAR cells, but none of the patients showed tumor reduction. PCR analysis showed that the engineered T-cells were present in the circulation within the first 2 days, but they quickly declined to undetectable levels only one month after treatment. So, this early experiment did not show patient improvement, but showed the importance of seeking new ways for improving CAR cell survival in the body, including using dual activation domains to stimulate T-cell replication and activation.

**CAR Vaccines and Renal Cell Carcinoma**

Another early attempt to treat cancer using CAR cells was in 2006 by a team of scientists at Erasmus University Medical Center (Rotterdam) (Lamers et al., 2006). The team treated 3 patients with metastatic clear cell renal cell carcinoma (RCC) with T-cells engineered to express a T-cell receptor against G250, a carboxy-anhydrase-IX (CAIX) epitope, frequently over-expressed in RCC. Although initially the T-cell treatment appeared to be well tolerated, after 4-5 infusions, each patient developed liver toxicity, the treatment had to be discontinued, and the patients continued to show progressive disease. The authors speculated that the liver toxicity likely resulted from antibodies against G250 in liver bile duct epithelial cells. So, this study was not a success, and reminds us of the importance of selecting cancer-specific antigens not present in normal tissues.

**CARs and CD20 Lymphoma**

A pilot clinical trial done in the Clinical Research Division of the Fred Hutchinson Cancer Research Center (Seattle, WA) showed that CAR cells have the potential to cause remissions in lymphoma patients (Till et al., 2008). Four patients participated, and three received lymphoma-specific CAR cells after pre-treatment with cyclophosphamide lympho-depletion. The treatment was relatively well tolerated. Two patients became progression-free, with no measurable disease at 12 and 24 months. The third patient initially had a measurable remission, but relapsed at 12 months. The trial demonstrated that modification of T-cells with a CAR receptor was well tolerated, and was associated with antitumor activity.
CARs and CD19 Leukemia

By far, the most successful application of CAR vaccines to date is against CD19 on the surface of leukemic B-cells (reviewed in: Kochenderfer and Rosenberg, 2013). Engineering T-cells to target CD19 has provided some of the most striking successes in the entire cancer vaccine field. Several labs converged on the CD19 antigen for good reasons: 1) it is universally expressed on the surface of all leukemic B-cells, and 2) although normal B-cells are also killed by the anti-CD19 treatment, such cells are not absolutely required for patient survival (antibodies can be provided passively, or the patient can undergo a bone marrow transplant after ridding the cancer cells). So CD19 is an excellent target.

One example of an early experiment was performed in 2009 by Carl June’s group at the University of Pennsylvania, one of the founders of the CAR field (Milone et al., 2009). In this study the team addressed one of the early problems with the CAR cells which was their lack of long term persistence in the body. They hypothesized this resulted from a lack of T-cell activation, so they designed a new generation CAR gene containing 2-3 activation domains (CD28 and/or CD137, and TCR-zeta). A lentiviral vector was used to introduce the CAR gene into human T-cells isolated from a humanized mouse containing lymphoblastic leukemia cells. More than 85% of the treated T-cells expressed CD19 TCR on their surface indicating the lentiviral treatment worked efficiently to deliver the CAR gene, and the cells survived at least 6 months. They identified a previously unknown antigen-independent effect for CAR cells containing the CD137 tail, and recommended using this activation domain in the future.

The first successful clinical treatment of leukemia with CD19 CAR cells was in 2010 by Steve Rosenberg’s team in their study entitled “Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19” (Kochenderfer et al., 2010). The team first pre-treated one patient with advanced follicular lymphoma with preparative chemotherapy to destroy the patient’s own bone marrow cells, then perfused patient with CAR cells containing a CD28 co-stimulatory signal. Following the CAR treatment, the results indicated that all B-cell precursors were eliminated from the bone marrow, and peripheral B-cells were absent at least 39 weeks, despite the healthy rebound of other blood cell components. Consistent with the eradication of B-cells, the serum levels of antibodies also decreased to very low levels. Importantly, the patient’s B-cell lymphoma underwent a dramatic regression, providing a proof-of principle that the CAR approach can work, even if the normal B-cells are also eliminated. The results of this paper were controversial, and competitor Carl June later claimed the success could have resulted from the use of chemotherapy used to make room for new cells, not from the CAR cells (Couzin-Frankel, 2013).

The breakout year for CAR treatments was 2011. Early studies with CAR cells had shown some success, but in 2011, Carl June’s group at the University of Pennsylvania (one of the founders of the CAR field) published two studies on their design of a CAR against CD19 and its use to treat 3 patients with advanced chronic lymphocytic leukemia (CLL). The first study entitled, “Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia” (Porter et al., 2011), described the team’s construction of CAR cells engineered to express a T-cell receptor that recognizes CD19 on the surface of B-cells and leukemia cells. The CAR cells also
contained a CD137 (4-1BB) co-stimulatory signal, and a TCR-zeta cytoplasmic tail to activate the T-cells once bound to ligand. The data showed that the CAR cells expanded at least 1000-fold, migrated to the bone marrow, and continued to produce functional CARs for at least 6 months, well beyond the trivial survival of earlier CAR cells. Each CAR cell was calculated to destroy about 1,000 cancer cells. One CLL patient showed complete remission. The second 2011 paper entitled, “T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia” (Kalos et al., 2011), expanded the earlier approach to 3 CLL patients. Two of the 3 patients showed complete remission. Normal B-cells expressing CD19 on their surface were also destroyed in this process, resulting in grade-3 and 4 B-cell aplasia (although in theory this could be treated with a traditional bone marrow transplant later on). These two 2011 studies were later attributed by Carl June as breaking open the funding for his research team. He fielded 5,000 requests from patients and their families for the therapy, and 800 media outlets world-wide covered the story (Couzin-Frankel, 2013). After these two articles were published, the National Cancer Institute reversed their earlier moratorium on funding CAR research, and funded Dr. June’s lab with nearly $500,000 a year for 4 years to create engineered T-cells for patients. The two patients in remission from his CAR treatment were still in complete remission in June of 2013 (Couzin-Frankel, 2013).

Also in 2011, a group of researchers, headed by Dr. Renier Brentjens and Isabelle Rivière, part of Dr. Sadelian’s team in the Department of Medicine of the Memorial Sloan-Kettering Cancer Center (New York, NY) published their findings on 10 patients with chemotherapy-refractory chronic lymphocytic leukemia (CLL) or relapsed B-cell acute lymphoblastic leukemia (ALL) treated with autologous CAR cells (Brentjens et al., 2011). The data showed that eight of the nine CAR-treated patients tolerated T-cell infusions well. Three of four evaluable patients with CLL who received prior conditioning with cyclophosphamide showed a significant reduction. They concluded that genetically engineering T-cell with CAR was a promising approach, and is more likely to show clinical benefit with prior chemotherapy conditioning and low tumor burden.

In 2013, Dr. Stephan A. Grupp’s team at the Children’s Hospital of Philadelphia, and Dr. Carl H. June’s team of the University of Pennsylvania published an article in the New England Journal of Medicine (Grupp et al., 2013). The team treated two children with relapsed and refractory acute lymphoblastic leukemia (ALL) (normally a very poor prognosis) using CAR cells targeting CD19 to reduce the proliferation of leukemic B-cells in the blood. The data showed that the CAR cells expanded to levels at least 1,000 times higher than the levels injected, and were found in the bone marrow and cerebrospinal fluid. Of the 2 children treated, complete remission was observed in one patient, while the other patient showed initial remission, but then had a relapse with his B-cells no longer expressing CD19 (the CAR cells would not kill them). The patients developed grade-3 and -4 adverse events, including a cytokine release syndrome, but those events were fully treatable with cytokine blockade antibodies. The authors concluded that so-long as the target antigen CD19 remains expressed on the tumor cells, the CAR therapy is capable of providing a complete remission. But the emergence of tumor cells during treatment that no longer express the target antigen can be a problem, and this study shows the need to perhaps target more than one antigen in the tumor.

Also in 2013, a team of scientists at Memorial Sloan-Kettering Cancer Center (New York, NY) headed by headed by Drs. Michel Sadelain and Renier Brentjens, published an article in Science Translational Medicine (Brentjens et al., 2013). The team treated 5 patients with
relapsed B-cell acute lymphoblastic leukemia (B-ALL) that normally has a very poor prognosis. They treated the patients with autologous T-cells transformed with a gene encoding a CD-19-specific second-generation dual-signaling (containing both a CD28 co-stimulatory signal, and CD3-zeta cytoplasmic signaling tail) chimeric antigen receptor (CAR) (termed 19-28z). Amazingly, all 5 patients showed a rapid tumor eradication, and appear to have no residual disease as assayed by deep sequencing PCR, although one patient eventually relapsed. With respect to side-effects, some patients showed significant cytokine elevations, but those incidences were treatable with steroid therapy. So, the treatment appeared to be generally well tolerated.

Additionally, in 2013, a team headed by Dr. Kochenderfer (of Steven Rosenberg’s team at the National Cancer Institute), published their findings of a clinical trial of allogeneic T-cells genetically engineered to express CAR targeting the B-cell antigen CD19 (Kochenderfer et al., 2013). Their study tested 10 patients that had malignancy persisting after initial bone marrow transplants and standard lymphocyte infusions. No new chemotherapy was done to any of the ten patients prior to the CAR T-cell infusions, so the patients were not lymphocyte-depleted at the time of infusions. The data showed that three of the 10 treated patients had regressions of their previously untreated malignancies. One patient with chronic lymphocytic leukemia (CLL) obtained complete remission after the T-cell infusion, while another CLL patient had tumor lysis syndrome as his leukemia regressed. They discovered that none of the patients developed graft-versus-host disease (GVHD). PCR showed the presence of the CD19-CAR gene in the blood of eight of the ten patients. This study showed that the CAR approach can cause regression of B-cell malignancies previously resistant to standard treatments without causing graft versus host disease.

In 2014, this same team from the Memorial Sloan-Kettering team discussed above published their continuation study on 16 patients with refractory B-ALL treated with the 19-28z CAR cells (Davila et al., 2014). They found that 88% of the patients responded well enough to the therapy to transition to receive a “routine” allogenic stem cell transplant. They also defined the diagnostic criteria for predicting which patients will develop a severe cytokine release syndrome (sCRS) that sometimes occurred within the first 21 days of the cell perfusion. When this occurred, the patient was treated with corticosteroids and IL-6-receptor antibodies. The data showed that serum C-reactive protein (CRP) can serve as a reliable marker for the severity of the CRS, which might help predict which patients will need corticosteroid interventions to lower the cytokines. It was not clear from this study whether the severe cytokine release syndrome is restricted to B-ALL type leukemia, or is predicted to occur with other cancers.

In 2014, Dr. Grupp’s team at the Children’s Hospital of Philadelphia and their main collaborator Dr. Carl June of the University of Pennsylvania, also published a paper in the New England Journal of Medicine, entitled “Chimeric Antigen Receptor T-Cells for Sustained Remissions in Leukemia” (Maude et al., 2014). The team treated 30 patients (children and adults) with relapsed acute lymphoblastic leukemia (ALL) with CAR cells transduced with a lentiviral vector encoding a CD19-directed chimeric antigen receptor CTL019. Their data showed that complete remission was achieved for 27 of the 30 patients (90% remission), with 67% event-free at 6 months! This is quite a success story compared to the relatively weak data obtained in the early cancer vaccine papers. The infused cells proliferated in vivo, and were detectable in blood, bone marrow, and cerebrospinal fluid. With respect to side-effects, all 30 patients developed cytokine-release syndrome (CRS), 27% with severe CRS, but the problem
was effectively treated with anti-interleukin-6 receptor antibody (tocilizumab), and the patients remained in cancer remission.

In 2014, Dr. Maude’s team at the Children’s hospital of Philadelphia and Perelman School of Medicine, University of Pennsylvania, published a paper entitled “Managing cytokine release syndrome associated with novel T-cell-engaging therapies” (Maude et al., 2014). They described a cytokine release syndrome (CRS) that correlates with both toxicity and efficacy in patients receiving CAR therapies. They demonstrated that T-cell activation by the cell treatment can produce abnormal macrophage activation. But they also discussed an effective way to manage the CRS, the IL-6 blockade by tocilizumab was effective at reversing CRS without inhibiting the vaccine efficacy.

In 2015, scientists at the National Cancer Institute published their findings of a phase-I trial for children and young adults with refractory B-cell cancers treated with CD19-CAR cells (Lee et al., 2015) (registered as clinical trial NCT01593696). 21 patients were treated with autologous CD19-CAR cells engineered by the team’s 11-day manufacturing process. The authors included the most recent vaccine designs into their study: the CAR molecule used both TCR-zeta and CD28 signaling domains to improve cell activation. The patients also received fludarabine and cyclophosphamide chemotherapy treatments prior to CAR infusion. They also tested different CAR doses. The maximum tolerable dose was determined to be $1 \times 10^6$ cells per kg, because all the side-effects were reversible at that dose. The most severe side-effects were a grade-4 cytokine release syndrome observed in 3 of the 21 patients (14%). The authors concluded from their phase-I data that at $1 \times 10^6$ cells per kg, the vaccine is safe, and likely will proceed to a phase-III trial.

Although the release of cytokine hormones from the engineered CAR cells is normally viewed as desirable and is one measure of their successful in vivo function, the production of very high levels of cytokines is viewed as a “cytokine storm” which can be fatal. One 2010 paper from Rosenberg’s team reported the death of a patient five days after receiving second-generation CAR T-cells (Morgan et al., 2010). The patient had colon cancer that had metastasized to the lungs and liver, and was treated with a CAR containing the monoclonal antibody Trastuzumab (Herceptin) against ERBB2 (HER-2/neu, CD28). The patient received cyclophosphamide chemotherapy followed by $10^{10}$ (100-fold higher than the optimum dose described above) T-cells intravenously, and within 15 minutes experienced respiratory distress with pulmonary infiltrate. Despite intensive medical intervention, the patient died 5 days later. Consistent with a cytokine storm, the patient’s serum samples showed high levels of cytokines gamma-interferon, granulocyte macrophage-colony stimulating factor, tumor necrosis factor-alpha, interleukin-6, and interleukin-10. The authors speculated that the large number of perfused T-cells localized in the patient’s lung immediately after the perfusion, triggering cytokine release by recognizing low levels of ERBB2 on lung cells (Morgan et al., 2010). So, this study emphasizes the importance of restricting T-cell infusions against neo-antigens that are specific for the tumor cells whenever possible. The T-cell perfusions are not cleared from the body as fast as antibody infusions, so their experiments should be monitored closely.
Neuroblastoma and GD2 CAR Cells

In 2011, a team headed by Dr. Louis at the Center for Cell and Gene Therapy, Baylor College of Medicine, Texas Children's Hospital (Houston, TX) published their long-term clinical consequences of CAR T-cell infusion for neuroblastoma patients. The paper was titled “Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma” (Louis et al., 2011). The team generated CAR T-cells against the GD2 antigen expressed by neuroblastoma tumor cells. Three of 11 patients with active disease achieved complete remission and showed the persistence of CAR T-cells. They concluded that the CAR cells designed against GD2 can induce complete tumor remissions in some patients with active neuroblastoma.

References for CAR Cells and Cancer


Strategies for Improving T-Cell Vaccines

Overall, with respect to CAR vaccines, as stated in a 2013 review of the topic, “although some scientists are urging caution, it is hard not to be swept up in this moment. Despite the small numbers of patients tested so far, many oncologists believe that [what these studies show], and what others are replicating is unprecedented. No cell therapy has proliferated in the body as well, endured so well, and slain cancer quite like this therapy (Couzin-Frankel, 2013).

But no therapy is perfect, and one of the purposes of this IQP is to identify potential problems of cancer vaccines and discuss future directions. Although researchers are newly energized with recent success stories, some scientists argue the development of a totally effective T-cell vaccine remains elusive, and there is room for improvement. We have identified the following areas for improvement, discussion in interviews, and future directions:

- **More Patients:** Almost everyone agrees that more patients need to be tested, and we need to follow their success long-term. In the case of CAR vaccines, because those treatments are so recent, only a handful of patients have been followed for more than a year.

- **Which Cancers?** Can the treatments work on solid tumors as well as they work for leukemia?

- **Which Antigens?** According to Carl June, one of the founders of the CAR field, “The major challenge currently facing the [cancer vaccine] field is to increase the specificity of the engineered T-cells for tumors, because targeting shared antigens leads to off-tumor toxicities, as observed in recent trials” (Kalos and June, 2013). This means we need to identify which tumor antigens are best to prime T-cells against to increase their ability to control a tumor. Recent studies indicate that T-cell activity directed against newly formed **neo-antigens** (natural proteins altered by DNA mutations in the exon portions of DNA, and present on the tumor surface) represent a major predictor of which TIL or CAR cells will be effective (Schumacher and Schreiber, 2015). Because the neo-antigens were not present during the patient’s own fetal development (when his immune system was forming), the neo-antigens...
are not viewed as “self”, and this increases the likelihood of their being recognized as “foreign” by TILs. A recent study with patients with non-small cell lung cancer showed that a high amino acid substitution rate in tumors (to create neo-antigens) was associated with durable clinical benefits and progression-free patient survival (Rizvi et al., 2015). So, we need to improve our methods for rapidly identifying neo-antigens to avoid immune attack on healthy tissue, and we need to develop algorithms to help predict which neo-antigens will actually be antigenic in vivo (Lee et al., 2012; Curran et al., 2012; Lipowska-Bhalla et al., 2012; Robbins et al., 2013; Rajasagi et al., 2014; Delamarre et al., 2015). As an example, in the Carreno et al. (2015) study of melanoma patients, their DNA sequencing identified several hundred mutations in exons in the tumor DNA, but only a small fraction of those were presented by MHC-1 and -2, and of the 7 used for vaccination only 3 turned out to actually be antigenic! So, we need to continue to better improve our bioinformatics computer algorithms based on more data obtained from real patient responses.

- **What Correlates with the Best Protection?** We need to more clearly define the “correlates of protection” for specific cancers (Appay et al., 2008). Do the patients that show the longest survival have the highest number of TIL and CAR cells in vivo? Do they have CAR cells that target multiple tumor antigens, or one tumor antigen?

- **Loss of Tumor Antigens.** In some cases, a tumor may no longer express a neo-antigen that was expressed earlier, so any immune vaccine would no longer be effective against that patient’s tumor (Delamarre et al., 2015). Perhaps more experiments need to be performed to understand this loss of antigenicity, and the vaccines should be designed against multiple antigens if possible.

- **Personalized DNA Sequencing:** The exact type of neo-antigen present in a patient’s tumor varies from patient to patient. So, we need to apply the new technologies of rapid DNA sequencing to identify the mutations present in each tumor, and design a personal vaccine against this specific neo-antigen. But this type of personalized medicine increases the expense of the vaccine, so who pays for this? Also, one famous patient Emily Whitehead (6 years old in 2013) a survivor of end-stage leukemia, treated in Carl June’s lab with CAR cells, developed an immune overdrive following her treatment (Couzin-Frankel, 2013). Near death in the hospital’s intensive care unit, the doctors realized she was over-producing IL-6, and managed to save her life by giving her an arthritis drug that disables IL-6. When her DNA was sequenced, the doctors realized that Emily had a mutation that causes a hyperactive immune response. So, perhaps these mutations should be screened for prior to T-cell therapies, to know in advance whether to expect an adverse side-effect from the vaccine.

- **Speed of T-Cell Amplification:** Some types of cancer develop faster than others. For example, malignant melanoma if diagnosed in stage-IV can kill a patient within weeks, while prostate cancer usually develops over several years. So, when treating patients with rapidly growing tumors, speed is critical, and we need to develop methods for accelerating the production of T-cells. Carl June of the University of Philadelphia says that one major
engineering challenge [for the cancer vaccine field] is the development of automated cell culture systems (Kalos and June, 2013), so perhaps this would help accelerate the process.

- **Overcoming Immune Suppression**: Recent studies have shown that the tumor environment suppresses the immune system to allow the tumor to survive, and recent successes have been achieved blocking this suppression using antibodies against PD-1 or CTLA-4 (Topalian et al., 2011; Curran et al., 2012; Sharma and Allison, 2015). Steven Rosenberg’s lab showed that TILs isolated from patient melanoma tumors (i.e. those that successfully targeted the tumor) were PD-1 positive (suppressed) (Gros et al., 2014). So, perhaps combination vaccines comprised of T-cells against neo-antigens plus PD-1 antibody should be tested.

- **Patient Pre-Treatments**: Recent clinical trials have demonstrated that chemotherapy treatment of leukemia patients prior to T-cell transfer helps reduce the number of B-cells in the patient, allowing the engrafted T-cells to expand. This pre-treatment appears to improve the effectiveness of the vaccine (Curran et al., 2012). Would this pre-treatment be effective for all kinds of tumors?

**References for Strategies for Improving CAR Vaccines**


METHODS

To accomplish objective-1, we performed a review of the current research literature, including reputable academic journal articles, relevant books, scholarly websites, newspaper articles, and other pertinent materials.

To accomplish objective-2, we conducted a set of interviews with various academic researchers who have performed cancer vaccine research on animals or in human clinical trials, or scientists who have used traditional cancer fighting technology, to determine their range of opinions on the strengths and weaknesses of this new technology and whether other techniques could accomplish the same goal of eliminating cancer.

Who: The stakeholders included academic experts on cancer vaccines and on traditional cancer therapies, and general health experts on cancer. These experts helped answer questions resulting from our Lit Review search, and helped us prioritize any remaining problems. Some of the stakeholders were initially identified by referral from the project advisor, David Adams, while other interviewees were identified from the published literature as authors on key scientific papers, or by referral from the initial interviewees.

Where and When: Once contact had been made with a potential interviewee (see below), a time and place was set up for the interview to be performed at the interviewee’s workplace. Whenever possible, interviews were conducted in person, although most were conducted by email, phone, or Skype.

How: Initially, an interviewee was contacted by email and/or phone. If no response was received, we used follow-up emails or phone calls. We developed our interview questions (see a list of questions in the Appendix) based on our review of the literature, and tailored the questions to the interviewee’s expertise. Based on the interviewee’s initial responses to our first questions, we designed follow-up questions to best obtain information from that person. We informed the interviewee about the purpose of our project, and asked for permission to quote them (see interview preamble in the Appendix). When necessary, we explained how we would protect their confidentiality, by giving them the right to review any quotations used in the final published report, explaining that the interview is voluntary, and explaining that they may stop the interview at any time or refuse to answer any question. After the interview, we asked for permission to follow-up with them at a later date if needed to fill in any gaps in the information, and sometimes asked their guidance for other potential stakeholders we might interview. With respect to the total number of interviews needed for our project, we stopped interviewing additional subjects when sufficient information had been obtained representing all sides of the cancer vaccine story, and when all unclear points had been clarified.

To accomplish objectives-3 and 4, the group synthesized all of the information collected in the literature research, interviews, and follow-up interviews, to ascertain the strength of the evidence on cancer vaccines and to create recommendations for further research.
Cancer

Anthony Kassas

This section of the IQP project focused on a general introduction to cancer. One problem identified in the review of the literature in this area was the need for a universal cancer vaccine that could treat all types of cancers. Most cancer vaccines target either a specific antigen on the surface of a tumor cell known in advance, or target a mixture of antigens present during the cell “charging” stage. But these antigens vary depending on the type of tumor. To gain insight into whether it is feasible given our current approaches to cancer vaccines to make a universal vaccine, we interviewed world-famous cancer researcher Dr. Robert A. Weinberg of the Whitehead Institute for Biomedical Research (Cambridge, MA). We identified Dr. Weinberg as a corresponding author on a 2011 paper in *Cell*, Mar 4; 144(5): 646-674, entitled “Hallmarks of cancer: The next generation”. When asked whether he thought it would be feasible to design a universal cancer vaccine that would target all types of tumor cells at this time, he stated “Thanks for your interest. I must say that at present it seems unlikely that we will have a universal anti-cancer vaccine, given the variability in the antigens that are displayed by different types of tumors. One should never say “never”, but to my mind, it seems unlikely that a universal vaccine will be developed”. So, Dr. Weinberg indicates that based on our current knowledge of tumors it is not likely a universal vaccine will be developed. So, we should celebrate our recent successes with targeted therapies.

This same important question of the possibility of a universal cancer vaccine was asked of Dr. Giuseppe Curigliano of the Viale Romagna, 43 20133 (Milano, Italia). Dr. Curigliano was one of the authors of a 2012 paper published in *Expert Opinion on Investigational Drugs*, February 2012, Vol. 21 (2): 191-204, entitled "Targeting the Subtypes of Breast Cancer: Rethinking Investigational Drugs." When asked about universal cancer vaccine design, he replied “A universal vaccine should be driven by a specific target expression. You can develop a peptide vaccine to enhance immune response against a specific antigen potentially expressed in multiple tumors but, in any case, you need the expression of the antigen that should be targeted by your adaptive immune response”. So, Dr. Curigliano indicates that it might be feasible to design a cancer vaccine against multiple tumors, if those tumors all expressed the same protein targeted by the vaccine. But this is not the same as a truly universal cancer vaccine that would target. Perhaps an example would be a vaccine targeting CD19 on the surface of B-cells that might be effective against any type of B-cell tumor, but that vaccine would not work on non-B-cell tumors, so would not truly be universal.

Another problem identified in the cancer Lit Review section was the application of new second-generation sequencing technologies to identify a specific mutated surface target protein for the cancer vaccine to be designed against. Questions arose concerning personalized medicine, and whether this sequencing approach can be fast enough to help a patient with advanced cancer, whose prognosis is limited. To obtain more information on the subject, we interviewed Dr. Jin-Tang Dong of 1365-C Clifton Road NE (Atlanta, GA). Dr. Dong is the author of a 2006 paper published in the *Journal of Cellular Biochemistry*, 2006 Feb 15; 97(3):
433-447, entitled “Prevalent mutations in prostate cancer.” In that study, the team used new generation sequencing methods to identify mutations common in human prostate cancers. When asked, based on his experience with second generation sequencing methods, whether he thought the new technologies were fast enough to provide a protein target information for a patient with advanced cancer so the vaccine could be delivered fast enough, he stated that “sequencing can be done fast enough nowadays to apply to a specific patient.” However, he did not mention specific lengths of time required for this type of analysis.

Peptide Injections and Monovalent Antibody Vaccines  Muhammad Siddiq

This section of the project focused on cancer vaccines composed of peptides injected in vivo to induce an immune response, or cancer vaccines using monovalent antibodies (designed against one target). One problem encountered in this section was it was unclear what constitutes a successful versus an unsuccessful cancer vaccine design. To gain more insight, we interviewed Dr. James C. Paulson, President and CEO (Acting), and the Departments of Cell and Molecular Biology, Immunology and Microbial Science, and Physiological Chemistry, Scripps Research Institute, MB-202, 10550 N. Torrey Pines Road (La Jolla, CA). Dr. Paulson was senior author on a 2014 paper in *Nature Reviews Immunology*, 2014 Oct; 14(10): 653-666, entitled “Siglec-mediated regulation of immune cell function in disease”. The Siglec immunoglobulin superfamily of proteins are complex. Some members like CD22 are inhibitory receptors that once they are bound to their ligand prevent over-activation of the immune system and prevent the development of autoimmune diseases. Because CD22 is located on B-cells, antibodies against it are sometimes used to treat leukemia where B-cells are overproduced. When asked his opinion about the basis for the effectiveness of his Siglec CD22 vaccine and whether early interventions are important, he stated “I am happy to hear that our publication is of interest to you. We believe that Siglecs have an advantage as targets for some types of leukemia and lymphoma because they are expressed on very few cell types. This allows highly selective targeting of chemotherapeutic agents. Many siglecs are also endocytic receptors that take the antibody or nanoparticle into the cell once it binds. As for the timing of the cancer treatment, earlier is always better than trying to treat a late stage of the disease. But otherwise, I think that siglec-targeted therapies do not have an advantage over others based on the treatment timing”. So, Dr. Paulson indicates that siglecs in general are excellent targets for some forms of leukemia and lymphoma because they are expressed almost exclusively on those cells, so there would be little damage to normal tissue during the treatments. It’s also interesting that many siglecs are also endocytic receptors, so items bound to them are internalized in the cell by endocytosis in vesicles, and can be used for drug delivery (although this is not the subject of our project). Dr. Paulson also indicates that in cancer therapies, earlier treatments are always better, whether targeting siglecs or any other cancer target.

In some interviews, our questions led the interviewee to point us to other published studies or to examples of scientific meeting where these types of topics are debated. An example of this was our interview with Dr. Dieter Hoelzer, MD, PhD, of the Onkologikum, Frankfurt am Museumsufer, Schaubstrasse 16, D-60596 (Frankfurt, Germany). Dr. Hoelzer was sole author on a 2013 review article in *Cancer*, 119: 2671-2674, entitled “CD22 Monoclonal Antibody Therapies in Relapsed, Refractory Acute Lymphoblastic Leukemia”. When asked how his CD22-targeting approach to B-cell tumors compares to the widely known CD-19 approach for
B-cell tumors, he responded “The information about CD22 and CD19 you can see in recently published papers, e.g. Kantarjian et al, *Lancet Oncology*, 2012, but the main source for answering your questions are the recent abstracts of the American Society of Hematology”.

Continuing to gain information on the possibility of combination vaccines, we contacted Professor Philippe Colombat, the Hepator-Gastro-Onco-Enterlogie Professor at CHRU de Tours, Hospital Trousseau, Avenue de la Republique, Chamray-les Tours, 37044 Tours Cedex 9, France. Dr. Colombat was author of a paper entitled “Rituximab (anti-CD20 monoclonal antibody) as single first-line therapy for patients with follicular lymphoma with a low tumor burden: clinical and molecular evaluation.” In this study, the team used CD20 rituximab mAb against follicular non-Hodgkins Lymphoma (NHL) in 50 patients. The patients received four weekly infusions of rituximab at a dose of 375 mg/m2. Their data showed that molecular responses to the therapy could be sustained for up to 12 months, and the response highly correlated with progression-free survival (p<0.0001). Professor Colombat was asked whether rituximab therapy might provide stronger results if used as a combination treatment with other types of antibodies or cells, and he responded, “In fact, at this time we know that six patients have been cured with this approach. I think that future will be the combined association with other targeted therapies with no late secondary effects for these patients who need no chemotherapy.” So, rituximab has been effective in treating some patients with NHL, and combination vaccines appear to be on the horizon.

**Bivalent Antibodies and Immune Checkpoint Inhibitors**  
*Isaac Vrooman*

This area of the IQP focused on two types of cancer vaccines: 1) bivalent antibodies (directed against two different targets simultaneously), and 2) antibodies against proteins PD-1 and CTLA-4 (the antibodies block PD-1 and CTLA-4 which are inhibitors of the immune system; blocking the blocker stimulates the immune system to fight the tumor).

One problem identified in this section of the project is the targeting of the vaccine not just to the cancer cells but to normal cells in the body. A good example of this is provided by the use of Blinatumomab, a monoclonal antibody directed against CD-19 protein on the surface of B-cells. Removing B-cells from the body is a desired outcome when treating various types of B-cell tumors, such as leukemia, especially when treating acute lymphoblastic leukemia (ALL) with its 90% death rate. But in doing so, normal B-cells that produce antibodies are also removed from the body. These B-cells help to fight infections, so the treated patients sometimes die from opportunistic infections from routine organisms such as *E. coli* or yeast.

To obtain more information on this issue, we interviewed Dr. Nicola Gökbuget who is GMALL Chair of the University Hospital Frankfurt at Goethe University (Frankfurt, Germany). Dr. Gökbuget was second author on a 2015 paper published in *Lancet Oncology*, 16(1): 57-66, entitled “Safety and Activity of Blinatumomab for Adult Patients with Relapsed or Refractory B-Precursor Acute Lymphoblastic Leukaemia: A Multicentre, Single-Arm, Phase-2 Study”. In this phase-2 clinical study, the team treated 189 patients with relapsed or refractory B-Precursor Acute Lymphoblastic Leukaemia (ALL) with Blinatumomab, a monoclonal antibody targeting CD19, an antigen consistently expressed in B-lineage acute lymphoblastic leukemia cells. After two vaccine treatments, 81 of the 189 (43%) showed complete cancer remission, compared to a
standard ALL life expectancy of only 10%. The most frequent adverse events observed were febrile neutropenia (25%), neutropenia (16%), and anemia (14%). 2% showed a grade-3 cytokine release syndrome, and 3 deaths resulted from sepsis from *E. coli* and *Candida*, likely as opportunistic infections occurring once the B-cells had been eliminated by the CD-19 vaccine. When asked about some of the adverse events seen with their CD-19-targeting cancer vaccine, Dr. Gökbuget stated “Acute lymphoblastic leukemia [ALL] in relapse is a life threatening disease. Survival is less than 10%. The standard therapy is intensive chemotherapy which is associated with adverse events in more than 90% of the patients; nearly all patients experience cytopenias. There is also a considerable mortality e.g. due to [opportunistic] infections. Compared to this standard, and given the poor overall outcome [of ALL patients], the adverse event profile of blinatumomab is really acceptable”. So, in this interview the author verifies that indeed some side-effects are seen with their Blinatumomab antibody treatment, including occasional death by opportunistic infection, but the side-effects also routinely seen during all treatments for acute leukemias, and are negligible considering the usual death rate of 90%. So, their 43% complete remission success rate is a strong improvement for acute leukemia treatments.

Patients with chronic lymphocytic leukemia often relapse after traditional chemotherapy treatments, and currently there are no long-term cures. Patients with chronic lymphocytic leukemia face horrific odds with a less than 10% survival rate. In recent years, research has begun testing bi-specific antibody vaccines. One of the best characterized bi-specific treatments targets CD19 (expressed on the surface of most B-cell cancers) and CD3 (expressed on T-cells). To gain more information on the CD19/CD3 approach, we interviewed Dr. Ralf Bargou of the Max Delbruck Center for Molecular Medicine, Robert-Rossle-Str. 10, 13125 (Berlin, Germany). Dr. Bargou and his colleagues published an article in 2003 in *Leukaemia* entitled “Efficient Elimination of Chronic Lymphocytic Leukaemia B-Cells by Autologous T-Cells with a Bi-Specific Anti-CD-19 / Anti-CD3 Single Chain Antibody Construct”. In their article, the team discussed their compound’s ability to bind in vitro primary B-cells and autologous T-cells isolated from healthy volunteers and from chronic lymphocytic leukemia (B-CLL) patients. Their results showed a depletion of lymphoma cells in 22 out of 25 patient cases. In our interview with Dr. Bargou, we asked him to compare his team’s BiTE bi-specific antibody approach with the CAR approach against CD19. In response, Dr. Bargou stated that “he hoped to bring his bi-specific antibody tests to clinical trials very soon”. He also answered “How does the BiTE approach [his team’s bi-specific drug] compare to CARs? The future will show. Both are very similar approaches to utilize T-cells for cancer therapy and show similar clinical results. Maybe in the future one will test a new target antigen in the first step with the BiTE approach, because it is safer. And then in the second step by CARs. One might also speculate on combination approaches utilizing different immunotherapies and different target antigens.” So in this interview Dr. Bargou indicated that his bi-specific antibody approach works well enough to enter clinical trials, and that it would be interesting to use BiTE in combination with a CAR vaccine, either targeting the same antigens or different antigens.

To obtain more information, we interviewed Dr. Patrick Baeuerle of Amgen Research in Munich, Germany. Dr. Baeuerle was a senior author on a 2002 paper in the *International Journal of Cancer* entitled “Extremely Potent, Rapid, and Costimulation-Independent Cytotoxic T-Cell Response Against Lymphoma Cells Catalyzed by a Single-Chain Bispecific Antibody”. In their research, the authors tested the efficacy in vitro of the same vaccine that was tested by Dr. Bargou and his colleagues. Dr. Baeuerle and his team found that the lysis of the tumor cells
in vitro was mediated by CD8+ T-cells, and that CD19-negative cells (non-B-cells) were not harmed by the CD8+ T-cells induced by the antibody vaccine. In our interview with Dr. Baeuerle, we asked him if thought the bi-specific antibody approach was as specific in vivo as in vitro. In his response, Dr. Baeuerle said “The bivalent antibody approach is not a vaccination! Any CD8+ T-cells, regardless of T-cell receptor specificity, will be physically bound for lysis. There may be a vaccination effect once tumor tissue is destroyed by redirected polyclonal T-cells, but we never explored this in detail.” So, this response indicates this team does not term their passive delivery of a bispecific antibody into a patient as a “vaccine”, because it does not by itself induce an adaptive immune response against the tumor. Instead, the antibody physically connects a tumor cell to any T-cell (regardless of its commitment) to activate the T-cell to lyse the tumor. The T-cell does not undergo a response to produce a T-cell receptor against the tumor cell, it is simply physically bridged to the tumor cell. This physical bridging is an important concept with bi-specific antibodies.

The physical behavior of blinatumomab was further confirmed by our next interviewee, Dr. Matthias Klinger of BiTE Immunology at Amgen Research in Munich, Germany. Dr. Klinger was the corresponding author on a recent 2015 paper in Molecular Immunology entitled: “Clinical overview of anti-CD19 BiTE® and ex vivo data from anti-CD33 BiTE® as examples for retargeting T-cells in hematologic malignancies”. The authors describe their clinical programs to treat B-precurso acute lymphoblastic leukemia (ALL) and B-cell non-Hodgkin lymphoma (NHL). For the ALL patients, they found a relapse-free survival (RFS) rate of 60% with sustained negative minimal residual disease (MRD) at 31 months. The remissions were seen in both pediatric and adult patients, and have allowed the patients to progress to successful allogeneic hematopoietic stem cell transplantation (HSCT). The antibody also resulted in durable responses in low-grade B-cell NHL. Blinatumomab recently gained approval in the United States by the U.S. Food and Drug Administration for treatment of Philadelphia chromosome-negative B-precurser relapsed/refractory acute lymphoblastic leukemia. The authors also describe their AMG 330, an investigational anti-CD33 BiTE® antibody construct. This antibody has not been used in patients yet, but has worked against cells taken ex vivo from patients with acute myeloid leukemia (AML). In the interview with Dr. Klinger, we asked to clarify his lab’s hypothesis on the CD3 targeting. We also asked if the bispecific antibody physically brings together activated T-cells with the target B-cell to facilitate the killing, or if CD3 engagement also helps activate the T-cells. In response, Dr. Klinger wrote “Blinatumomab bridges T-cells to B-cells by simultaneously binding to CD3 and CD19, thereby activating T-cells and leading to redirected lysis of bound B cells.” So, Dr. Klinger validated that the bi-specific antibody Blinatumomab directed against CD3 on T-cells and CD19 on B-cells makes a “bridge” that physically holds the two cells together, thus activating the T-cells to kill the B-cell (which in this case is a leukemia cell).

With respect to PD-1 inhibitors such as Nivolumab, one interesting idea we quickly settled on was combining checkpoint inhibitor antibodies (like anti-PD-1, to activat the immune system) with antibodies targeting specific tumor antigens. This approach might be more successful than using a singular approach. To obtain more information on this, we chose to speak with Dr. Suzanne Topalian of the Department of Surgery in Johns Hopkins University School of Medicine (Baltimore, Maryland). Dr. Topalian was the first author on a 2014 study published in the Journal of Clinical Oncology, entitled “Survival, Durable Tumor Remission, and Long-Term Safety in Patients with Advanced Melanoma Receiving Nivolumab”. This article was a continuation of the author’s ongoing clinical trials with Nivolumab, an antibody against
programmed cell death-1 (PD-1), but here they assayed some of the long-term effects after removing the antibody. The purpose of Nivolumab is to bind and inactivate PD-1 to allow T-cell functions to remain active. The authors of this paper analyzed 107 patients with advanced melanoma. Their data showed a mean overall survival of 16.8 months for the treated patients, with 1-year and 2-year survival rates of 62% and 43%, respectively. They concluded that the effects were durable and even persisted after drug discontinuation, and safety was acceptable (toxicity rates similar to previous reports). In our interview with Dr. Topalian, we asked if she and her team had tried combining the PD-1 approach with antibodies directed against tumor proteins and if the surface proteins specific for melanomas were identified yet. In her response, Dr. Topalian stated that “Combination therapies based on anti-PD-1 or anti-PD-L1 are now moving into clinical testing. This includes combinations with tumor-specific antibodies. One example is anti-PD-1 plus Herceptin in breast cancer. For melanoma, it has been difficult to find antibodies that are truly melanoma-specific and do not target other tissues.” This was very useful information. First we determined that indeed our idea of combination approaches have appealed to other scientists, and some labs are starting to combine the PD-1 approach with other immune responses against tumor antigens (she gave the example of breast cancer and PD-1 and Herceptin). And we also learned that as we expected, tumor-specific antigens for melanoma have not yet been identified.

**DC Vaccines: Animal Experiments and Provenge**  
*Derek Brinkman*

With continued research in the field of dendritic cells (DCs) and their immunological properties, their relationships with other immune system components are consistently being discovered by researchers that help shed light on the pivotal role DC’s play in antitumor immunity. Important connections and discoveries continue to be made within the DC vaccine subfield that are opening doors for future treatment possibilities. However, one of the challenges our team recognized while researching DC cancer therapies is what defines the effectiveness of these treatments in tumor regression. Relationships with DCs with natural killer cells (NKs) was recently identified, but how this interaction could benefit cancer vaccines was unclear. And all the interactions occurring in between various cells of the immune system often fail to eliminate the tumor, so the role of checkpoint inhibitors becomes very important. Even if all other processes succeed to create T-cells specific for tumor antigens, these cells can become inhibited by immune checkpoint proteins in the tumor cells engaging receptors on the T-cells. This discovery has helped our team understand just how easily one inactivation step can lead to ultimate vaccine failure.

A problem that we encountered in the DC section is those vaccines do not seem to have the same spectacular successes as the TIL and CAR vaccines. Provenge was the first DC vaccine approved by the FDA, but its effectiveness is somewhat controversial. The interactions DC’s produce within the immune system have proven to be important, but after decades of investigation, the question remains whether scientists believe in the viability of DC-based cancer treatments when used alone.

To assist our team with this query, we contacted Dr. David Raulet who is a Co-Chair in the Department of Molecular and Cell Biology at the University of California Berkeley. Dr. Raulet was the senior author on a 2015 paper published in *Science*, 348: 136-139, entitled: “A
Shed NKG2D Ligand That Promotes Natural Killer Cell Activation and Tumor Rejection.” In this publication, the team discusses the traditional method of immune-surveillance used by natural killer cells (NKs) to identify and eliminate cancer cells. The paper was aimed at investigating a model in which tumors are able to evade detection by shedding a membrane protein that links to NK receptor NKG2D to inactivate the NKs. Raulet’s study demonstrated that in murine subjects, a shed form of MULT1 actually enables the NK cells to create an immune response when bound with NKG2D, which promotes tumor rejection in both subcutaneous and peritoneal tumor types. Based on this successful finding, our team inquired about the future of combinations of NK and DC vaccines, most importantly whether DC vaccines would be used jointly or if the NK vaccines would become the new “gold standard.” To this, Dr. Raulet speculated that, “ultimately most immunotherapies will be used in combinations. Whether DC therapies are the way to go will require more experimentation. It is too soon to have a strong opinion about that. DCs do seem to be important for activating NK cells, so it is a reasonable approach to consider.” Here Dr. Raulet states his belief that vaccine combinations are a very likely option for future experimentation. He also states that while not enough is known about DC vaccine methods to make concrete determinations, he feels it is a promising avenue to explore.

To supplement the above information on NK and DC cells, our team turned to Dr. Guido Ferlazzo for his input on the subject. Dr. Ferlazzo works both as a Professor in the Human Pathology Department and researcher in the Immunology and Biotherapy Lab at the University of Messina (Messina, Italy). In addition to over 80 other scientific publications, Ferlazzo was the corresponding author on a 2012 paper in PLoS ONE, 7(6): e39170, entitled: “Dendritic Cell Editing by Activated Natural Killer Cells Results in a More Protective Cancer-Specific Immune Response.” In this study, the team demonstrated for the first time the cause-effect relationship when DCs are activated in vivo and NKs subsequently kill off immature DCs to create a more effective immune response. The team achieved this result by injecting YAC cells into mice, which lack the MHC-1 component so they activate NK cells. The study showed that an NK cell-mediated killing of DCs resulted in higher cytotoxic T lymphocyte (CTL) expansion against the invading tumor cells. To understand the magnitude to which this NK-DC-CTL relationship could be utilized, we contacted Dr. Ferlazzo to ask him whether this YAC cell injection method that activated NKs to kill immature DCs could be used to treat all forms of cancer or only specific subsets. Ferlazzo responded stating that, “the single limitation I can see is a poor immunogenicity of the tumor. The one described in the paper is an experimental model with an immune-dominant epitope expressed by cancer cells employed for vaccination. What we show is that the interaction between activated NK cells and DCs can improve the T-cell response, most likely because of a better antigen presentation secondary to DC editing by NK cells. So, in general there would be no limitation for the use of this approach on tumor types as long as the tumor cells present some antigenicity.” So, here our team learned that as long as the tumor is immunogenic (it can provoke an immune response) Ferlazzo believes that the NK activation approach would work for any type of tumor. By jointly using NKs and DCs, not only are the strongest DC’s surviving to maturity, but the NKs are also performing intricate edits to the DCs to promote more effective T-cell responses.

Often times, even when using a combined vaccination effort, an immune response can ultimately fail due to the activation of checkpoint inhibitors by the tumor. Understanding what causes these inhibitors to nullify immune functions and how to eliminate the block led us to Dr. Laurie Glimcher’s lab in New York. Dr. Glimcher manages a team of researchers in the
Department of Medicine at Weill Cornell Medical College (New York, NY). She is also the corresponding author on a 2015 paper in *Cell*, entitled “ER Stress Sensor XBP1 Controls Anti-Tumor Immunity by Disrupting Dendritic Cell Homeostasis.” Previous studies identified that the endoplasmic reticulum (ER) stress response factor XBP1 directly promotes tumor growth. Glimcher and her team discovered that it does so by becoming active in DCs that migrate towards the tumor thus inhibiting the T-cell support that DCs typically provide. The team used a mouse model of ovarian cancer to demonstrate this inhibitory effect, and showed that by deleting the stress factor, it allows the DCs to function again to induce an anti-tumor response. So, by blocking this XBP1 inhibitory pathway, the team showed that DCs could regain their function to attack the cancerous cells. When asked about the possibility of vaccinating with tumor-specific antigens to improve treatment efficiency, first author of the study Dr. Juan Cubillos-Ruiz responded. In his reply he stated that, “We have observed that these DC are capable of engulfing tumor-derived proteins in vivo. The problem is that the DCs are unable to properly process and present the antigenic peptides to T-cells in the tumor microenvironment. We have never tested your idea because it has been difficult to identify ovarian cancer-specific antigens that can be used as an effective “vaccine.”” He goes on to add that if they performed vaccinations with immune-dominant tumor antigens, that these could “prime and elicit tumor-specific T cells (both effector and memory), which can migrate to tumor sites.” From this interview, our team learned that the use of cancer-specific antigens for vaccination is not currently an option with ovarian cancer because none have been identified yet. However, Dr. Cubillos-Ruiz believes that in some cases, this treatment can be effective if immunodominant antigens are used. This paper and Cubillos-Ruiz’s insight has demonstrated that it is possible to eliminate or nullify checkpoint inhibitors, but more research is required to direct the immune activation against immunodominant tumor-specific antigens.

Based on the knowledge our team gained from the publications and interviews, we can draw conclusions about what future research in this subfield will likely focus on. Clearly the trend is to use combination vaccines. This is demonstrated in the studies performed by Raulet and Ferlazzo on the key interactions between NKs and DCs. From the literature review, we can say that type-I interferons are necessary to mount an effective anti-tumor response, and DC involvement is necessary to process tumor antigens to produced activated CD8+ T cells. So, although the subtopic of this section is DC vaccines, their effectiveness would not be possible without other relevant immunity components. Another topic of importance is that of immune process editing. At all times, the immune system is evolving to counteract the expanding cancerous cells. The relevance of this adaptation factor is shown in Dr. Ferlazzo’s study where NKs modify DCs to promote the most mature cells and enhance the overall effectiveness of the immune response. In other cases, checkpoint inhibitors evolve to inhibit DC functions that, if not restored, can cause the entire anti-tumor response to fail. If researchers can study the constant evolution of both tumors and the evolving immune responses, it will hopefully lead to more effective eradication of cancerous cells before severe damage is done.

**DC Vaccines for Melanomas and Glioblastomas**  
Zhizhen Wu

In the review of literature in this section we found that pre-conditioning patients with recall antigen tetanus/diphtheria (Td) toxoid could improve DC vaccine effectiveness. It was shown in a clinical trial that pre-conditioning the vaccine site with Td can stimulate migration of
DCs to the lymph nodes (where they function to present antigens to T-cells), and the DC vaccine significantly prolonged patient survival. This finding introduces a question whether this pre-conditioning approach would work with other recall antigens and with other types of cancer. To obtain more information about this question, we interviewed Dr. John H. Sampson, the Chief of Neurosurgery Division of the Department of Surgery of Duke University Medical Center. Dr. Sampson was the senior author on a paper published in a 2015 issue of Nature (519: 336-369), entitled “Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients”. In this blinded clinical trial, the team randomized 12 newly diagnosed glioblastoma patients and pre-conditioned them with Td or un-pulsed DCs. Then all patients were vaccinated with DCs pulsed with Cytomegalovirus phosphoprotein 65(pp65) RNA. The successful accumulation of injected DCs in the lymph nodes (where they can process antigen and present it to T-cells) and overall patient survival was higher for the patients pre-conditioned with Td. When asked about the potential use of other recall antigens’, Dr. Kristen Batich, another co-author of the same paper, stated, “In a pre-clinical setting in mice, we have tried two other protein antigen formulations, the pedvax hib vaccine and the prevnar-13 strep pneumo formulation. We found that these other pre-conditioning vaccines worked well for facilitating the migration of our DC vaccine.” Thus, in this interview the author verified that they have indeed tested two other recall antigens in mice, but they have not yet tested those antigens’ ability to stimulate anti-tumor responses or improve survival in humans. So Td pre-conditioning remains the most effective approach for now.

In our review of the literature, we also identified a problem that while some studies showed the stimulation of anti-tumor immune-responses, the patients actually show the same level of improvement as with traditional therapies. To obtain more information of this issue, we interviewed four scientists. We first interviewed Dr. Philip W. Kantoff, who is the Vice Chair and Chief of the Division of Solid Tumor Oncology, Department of Medical Oncology, Dana-Farber Cancer Institute, the Jerome and Nancy Kohlberg Professor of Medicine, Harvard Medical School. He was the first author on a 2010 paper published in the New England Journal of Medicine, 363(5): 411-422, entitled “Sipuleucel-T Immunotherapy for Castration-Resistant Prostate Cancer”. In this study, the team vaccinated 341 advanced prostate cancer patients with the Provenge (Sipuleucel-T) vaccine and 171 patients with placebo. The risk of death of the sipuleucel-T group was reduced by 22% compared with the placebo group. The median survival was prolonged by 4.1 months. When asked about potential ways to improve the vaccine efficacy, Dr. Kantoff stated, “The most promising way would be to add a checkpoint inhibitor.” So in this interview the author pointed out that using antibodies against PD-1 or CTLA-3 to block the receptor that leads to an inhibition of T-cell responses by the cancer environment has high potential for improving the vaccine. This approach should be further tested for its ability to improve the vaccine efficacy.

We then interview Dr. Evelien L.J. Smits of the Center for Oncological Research, University of Antwerp. Dr. Smits was the corresponding author of a 2015 paper published in Pharmacology & Therapeutics, 146, 120-131, entitled “Poly (I:C) as cancer vaccine adjuvant: Knocking on the door of medical breakthroughs”. In this paper the author summarized studies using poly (I:C) as a cancer adjuvant. According to their study, poly (I:C) has the potential to improve immunotherapy. In the discussion section of the paper, the authors stated “Hence, additional selective blocking of inhibitory molecules might be able to improve the adjuvant effect of poly (I:C)/poly-ICLC in cancer vaccination strategies”. When asked whether they have tested this “blocking of inhibitory molecules” approach, Dr. Smits stated “We are currently
testing pl:C in combination with immune checkpoint inhibitors and in combination with hypoxia-inducible factor inhibition. The tests are conducted in vitro, so we have no human or mouse data yet”. In this interview, the author pointed out that pl:C application in combination with checkpoint inhibitors is a promising way to further improve cancer vaccine effectiveness.

We also interviewed Dr. I. Jolanda M. de Vries from the Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre. Dr. de Vries was corresponding author on a 2011 paper published in Clinical Cancer Research, 17(17): 5725-5735, entitled "Route of Administration Modulates the Induction of Dendritic Cell Vaccine–Induced Antigen–Specific T-Cells in Advanced Melanoma Patients". In this study, the team vaccinated 43 patients with peptide-loaded DCs via intradermal or intranodal delivery. The results showed that both intranodal and intradermal vaccinations can stimulate T-cell responses against tumors, but intradermal vaccinations tend to induce more functional T-cells than intranodal. When interviewed about other strategies for improving cancer vaccines, Dr. Kalijn Bol of the same college as Dr. de Vries responded, stating, “One can think of ways to improve the maturation of the DC’s (e.g. with TLR ligands) or the loading of antigens on DCs. Further, we started using naturally occurring DCs instead of monocyte-derived DC’s, and believe they are more potent.” Thus, in this interview the author stressed that the choice of DCs, the method of DC maturation, and how they are loaded with antigens all play crucial parts in improving DC-based cancer vaccines.

We then interviewed Dr. Hideho Okada, currently of the University of California San Francisco, Department of Neurological Surgery, 505 Parnassus Ave, M-779 San Francisco, CA. Dr. Okada was corresponding author on a 2013 paper published in the Journal of Clinical Oncology, 29(3): 330-336, entitled “Induction of CD8+ T-Cell Responses Against Novel Glioma-Associated Antigen Peptides and Clinical Activity by Vaccinations With α-Type 1 Polarized Dendritic Cells and Polyinosinic-Polycytidylic Acid Stabilized by Lysine and Carboxymethylcellulose in Patients With Recurrent Malignant Glioma”. In this paper, the team vaccinated 19 patients with alpha-type-1 polarized DC’s loaded with glioma-associated antigens complemented with poly (I:C) adjuvant. Nine of the 19 patients showed 12 months of progression-free status, and 1 patient showed sustained complete remission. When asked whether strategies like pre-conditioning the vaccine site with Td could help improve the vaccine efficacy, Dr. Okada stated “We inject DCs directly into lymph nodes, but not in the skin. So, I do not think we need the priming in the skin”. He then mentioned they have come up with several strategies for improving the vaccine, but he is unable to provide more details due to his busy schedule. Thus, in this interview the author stressed that Td pre-vaccine may not be very helpful if the DCs are injected into the lymph nodes, not the skin. For those experiments that inject DCs into the skin, Td pre-conditioning is still a potential way to help improve vaccine efficacy.

**TIL Vaccines**

Eric Williams

This section of the IQP focused on the use of tumor-infiltrating lymphocytes (TILs) as cancer vaccines. In this approach, TILs that have migrated to a patient’s tumor site are isolated, amplified ex vivo, and injected back into the same patient. One problem identified in this section was the need for the TILs to be directed against novel neo-antigens created on the surface of the patient’s tumor as it evolves. Identifying these neoantigens requires new second-generation
sequencing approaches. One lab using this technology is Dr. Paul F. Robbins of the Surgery Branch, National Cancer Institute, National Institutes of Health (Bethesda, MD). Dr. Robbins and his team developed a new screening method that uses bioinformatics and whole-exome sequence data from tumor cells to determine if there is a connection between the ability of TIL to mediate durable tumor regressions and their ability to recognize potent neo-antigens. Dr. Robbins was first author on a 2013 paper in Nature Medicine, 19(6): 747-752, entitled “Mining Exomic Sequencing Data to Identify Mutated Antigens Recognized by Adoptively Transferred Tumor-Reactive T-cells”. This paper detailed their new method and successful results. The first step of the process was to mine whole-exome sequence data (protein encoding areas of the DNA) to identify mutated proteins expressed in patient tumors. They then synthesized and evaluated specific mutated T-cell epitopes for recognition by TILs from the patient. These were chosen using a major histocompatibility complex-binding algorithm for recognition by TILs. To learn more about this method, we interviewed Dr. Robbins, and asked whether he had attempted to use this method for identifying mutated antigens in tumors other than melanomas. He responded that they have done so “in colon, breast, lung and ovarian cancers”. In addition, he said that “it appears that all or nearly all tumors possess immunogenic mutations”. So, this means that the whole-exome sequencing approach developed by Dr. Robbins and his colleagues could be applied to other types of tumors, and that their data indicates it is likely that all types of tumors create neo-antigens that could serve as targets for therapy.

Several studies done in both animals and humans show that T-cells active against neo-antigens mediate the tumor regressions in most cancer vaccines. The problem with this approach is the long time it takes to sequence a patient’s exome (exon portion of the genome) to identify the neoantigens. Most indications are it takes about 10 weeks, which is a long time if the patient has advanced cancer. To ask about the speed of the new neo-antigen methods, we interviewed Dr. Robert D. Schreiber who is director of the Center for Human Immunology and Immunotherapy Programs at Washington University of Medicine (St. Louis, MO). Dr. Schreiber was second author on a 2015 paper published in Science, 348(6230): 69-74, entitled “Neoantigens in Cancer Immunotherapy”. This paper detailed some of the new technologies used to identify patient-specific neoantigens. We asked Dr. Schreiber how he thinks the speed of identifying the patient neoantigens and getting them back into the patient could be improved, and he responded that of the 10 weeks from tumor biopsy to vaccination, 6 of those weeks are used to identify and produce the synthetic long peptides. He estimated that this process could be reduced to 3 weeks with newer sequencing and synthesis technologies. We also asked what types of cancers will best be treated by neoantigens, and he said that the most susceptible tumors would be those with mutation rates of ~100 mutations per tumor or greater.

CAR Vaccines Zhuohao Ling

In some cases, researchers isolating T-cells from tumors found them to be committed against a broad spectrum of antigens, not one type. This implies that cancer vaccines directed against only one antigen might not work here. To learn more about this broad-type response, we interviewed Dr. Jason H. Bielas, who is a researcher of the Human Biology Division at Fred Hutchinson Cancer Research Center (Seattle, Washington). Dr. Bielas was senior author (last author) on a 2013 paper in Science Translational Medicine, 5, 214ra169, entitled “Digital Genomic Quantification of Tumor-Infiltrating Lymphocytes”. The authors designed a new
digital DNA-based assay (termed QuanTILfy) to count TILs and assess T-cell clonality in tissue samples, including tumors. They applied the approach to metastatic ovarian tumor tissue, and demonstrated an association between higher TIL counts and improved patient survival, which is consistent with previous findings that the patient’s immune response is a meaningful and independent prognostic factor. Surprisingly they found a diverse TIL repertoire for all their tumors, with no apparent clonal expansions. When asked about whether the broad TIL response and lack of clonal expansion of a specific type of TIL against a specific tumor antigen is specific to the ovarian cancer tissues, or is likely to apply to other types of cancer, Dr. Bielas stated “The assay isn’t as powerful for identifying clonal expansions as NGS-based methods, nor have we looked at other tumor types”. So, perhaps future experiments could analyze other types of tumors for this “broad-type” TIL response.

This speed question for identifying neoantigens was also asked of Dr. James N. Kochenderfer who is a physician-scientist of Center for Cancer Research at National Cancer Institute (Bethesda, Maryland). Dr. Kochenderfer was the second author on a 2015 paper in Lancet, 385(9967): 517-528, entitled “T-Cells Expressing CD19 Chimeric Antigen Receptors for Acute Lymphoblastic Leukaemia in Children and Young Adults: A Phase-I Dose Escalation Trial”. Researchers treated 21 patients with refractory B-cell malignancies with CD19-CAR cells. The autologous cells were engineered in an 11-day manufacturing process to express an anti-CD19 single chain antibody variable fragment. They determined the maximum tolerated dose to be 1 x 10E6 CAR cells per kg weight. Any toxicities observed were reversible. Three of the 21 (14%) showed grade-4 cytokine release syndrome, 9 (43%) showed fever, and 8 (38%) showed neutropenia. The purpose of this phase-1 was not efficacy, but safety. When asked if his current 11-day manufacturing process is fast enough to treat advanced cases and if there are any efforts being to decrease the time for this therapy, Dr. Kochenderfer replied “we can make the cells in 6 days now. Eleven days is fast enough to accommodate most patients’. So, in this interview the author confirms that the 11-day manufacturing process is fast enough to treat most patients with CD19-type cancer. And more importantly, the process has been improved to 6 days, which might help with other types of cancer.

With respect to how universal the neoantigen approach might be, we interviewed Dr. James C. Yang who is a senior investigator at Center for Cancer Research at National Cancer Institute (Bethesda, Maryland). Dr. Yang was senior author on a 2014 paper in Science, 344: 641-645, entitled “Cancer Immunotherapy Based on Mutation-Specific CD4+ T-Cells in a Patient with Epithelial Cancer”. These researchers used a whole-exome sequencing strategy to show that TIL cells isolated from a patient with metastatic cholangiocarcinoma contain CD4+ helper cells that recognize a neoantigen mutation in Erbb2-interacting protein (ERBB2IP) expressed by the cancer cells. They performed a therapy using TILs, of which 25% were active against this mutation, and achieved a stabilization of the cancer progression, while a second treatment with 95% TILs against the mutated antigen provided tumor regression. Their data showed that TIL cells designed against a mutated neoantigen on the tumor cells can help mediate tumor regression. When asked whether the approach of identifying mutated antigens targeted by the TILs could be a universal protocol for treating almost any form of cancer, Dr. Yang stated “the immune recognition of a mutated "neoantigen" should be universal, i.e. not specific for the type of cancer it is expressed by, although more highly mutated cancers are more likely to have more target antigens. Having a recognized mutated antigen may be an important, but not sufficient component for inducing the immune tumor rejection. Some cancers may have too few mutations, such that none get processed by the proteasome correctly or do not bind well to any of
the patient's HLA alleles. Others may get out to the cell surface as an epitope properly mounted on an HLA molecule, but the T-cell response may prove to be weak. Additionally, the mutation may only be on a portion of the tumor cells, not all of them as would likely be the case for a true "driver" mutation (such random and functionally unimportant mutations are called bystander mutations and T-cells against them would only treat part of the tumor). And lastly, there may be some powerful immunosuppressive mechanisms in the tumor microenvironment that would dampen even a good immune response against a good driver mutation (these factors could be somewhat tumor type specific--e.g., a tumor type that frequently secretes an immunosuppressive cytokine or expresses the ligand for a T-cell inhibitory receptor). Having considered all these things, it still remains a possibility that any human tumor could be rejected by an immune response against a mutated antigen, especially if combined with other agents such as a checkpoint blocking antibody (e.g., anti-PD1). And the more mutations a tumor has, the more likely that one will be an important/ubiquitous driver mutation that also provokes a potent T-cell response. That is probably why some tumors seem clinically "more immunogenic" than others (e.g., a melanoma seems immunogenic because it often has many mutations generated by UV irradiation). Still the case we described had only 26 point mutations (not hundreds like the typical melanoma) and was successful, so sometimes you just get lucky.” Therefore, in the interview Dr. Yang explained that the age of the tumor is important, as older tumors have a higher number of neoantigen targets. And it is important for the neoantigen to be present on most of the patient’s tumor cells, not just a few if that neoantigen is to be used as a target.
CONCLUSIONS / RECOMMENDATIONS

Based on the research performed for this project, our team has made several conclusions and recommendations.

1. **For the cancer area in general**, no single antigen has been identified that represents all cancers, and cancers evolve by mutating more genes to create neoantigens, so it is not likely that a universal cancer vaccine will be developed in the near future for curing all forms of cancer. Although, as one of our interviewees stated “You should never say never”.

2. **In the peptide injection area**, although the initial results have been relatively mild compared to the TIL and CAR areas, a new trend is to inject peptide mixtures. Some treatments have produced longer patient survival, but relatively few long-term full cancer remissions. **For the monovalent antibody area**, although the treatments sometimes prolonged a patient’s life, full cancer remissions were not common. The reported “complete responses” (full immune involvement) are not the same as “complete cancer remissions”, as have been seen recently with TIL and CAR vaccines.

3. **The bivalent antibody area** has seen some excellent success stories, especially for the CD19 x CD3 Blinatumomab antibody, where one study with 189 patients with B-cell acute lymphoblastic leukemia (B-ALL) showed that after two treatments, 81 of the 189 patients (43%) had complete cancer remissions. In a few cases, the patients showed serious side effects from the complete loss of their B-cells (and one patient died from an opportunistic infection), but the low percentages of these cases far outweighed the complete remissions seen from these diseases with very poor prognoses.

4. **The immune checkpoint inhibitor area** shows great promise for re-activating TIL cells that have infiltrated a patient’s tumor but have become inactivated by the tumor. The future likely will involve combining these immune stimulators with other antibody or T-cell vaccines. In this category, the patients should also be monitored closely for autoimmune or inflammatory reactions while the immune system becomes activated. Although grade-3 and 4 side effects were often seen with these treatments, they were transient and treatable, and pale compared to their prognosis without treatment.

5. **For the DC cancer area**, the results so far are somewhat lukewarm compared to those obtained with TIL and CAR cells, including for Provenge one of the best characterized vaccines in this area. But future approaches will likely include combining the DC vaccines with checkpoint antibodies, combining the vaccines with immune stimulatory hormones (like IL-12), using a personalized medicine approach to sequence the exomes of a patient’s tumor to identify neo-antigens unique to that patient for priming the vaccine against (within this area it is especially important to identify neo-antigens that are immuno-dominant and more likely to produce an
immune response), improving the speed at which this DNA sequencing can be done to decrease the time to therapy, using adjuvants such as poly-(I:C) to boost the immune response, using pre-injections of a “recall antigen” such as tetanus toxoid Td for skin injections of DC cells (which has been shown to increase DC migration to lymph nodes to improve the immune response), identifying biomarkers for which type of patient is most likely to respond to a vaccine, supplementing the vaccines with other cellular components of the immune system, such as natural killer cells (NKs) (which have recently been shown to help other cells present antigens).

6. **For the TIL and CAR T-cell vaccine area**, this area has provided some of the most spectacular successes in the entire field of cancer vaccines, including one study where 30 patients (children and adults) with relapsed acute lymphoblastic leukemia (ALL) were treated with CD19-directed CAR cells, and 27 of 30 patients (90%) showed complete remission at 6 months! Although all 30 patients developed cytokine-release syndrome (CRS) (27% with severe CRS), the problem was effectively treated with anti-interleukin-6 receptor antibody (tocilizumab), and the patients remained in cancer remission. With respect to the future, the best patient survivals appear to occur in patients with the highest T-cell load in the tumor, so this underscores the importance of producing large numbers of T-cells for each vaccine. Some of the best cancer remissions occurred by enriching the TILs from a patient with for a subset committed to neoantigens, so in the future although this approach is labor intensive, it should be tested further. Scientists have identified neoantigens in all tumors analyzed for far, so the neoantigen approach should work for each tumor type. The speed of the neoantigen identification technology appears to be improving, with one scientist indicating he has already lowed the time from tumor biopsy to vaccine to only 6 weeks, which is sufficient for treating all but the very worst cases of cancer. So, this critical neoantigen identification process appears to be improving with advances in DNA sequencing and synthetic chemistry. It is also important for the neoantigen to be present on most of the patient’s tumor cells, not just a few, or the cancer cells will not be eliminated.

Our overall conclusion is that some types of cancer vaccines, especially the more recent innovations, appear to work well, and are worth further research funding. In some cases, the CAR T-cell vaccines have shown as high as 90% full cancer remissions in medium sized (30 patient) studies. The literature and our interviewees were full of exciting ideas for moving the cancer vaccine field forward, and we hope that funding continues to support research in this area.
APPENDIX

Example Questions for Cancer Vaccine Experts:

1. **Antibodies Against CD19 on B-Cells**: One of the recent success stories in cancer vaccine research is the use of antibodies against CD19 on the surface of B-cells to fight B-cell cancers like leukemia. These antibodies seem to be very well suited for removing early stage B-cells in leukemia, and have caused several total cancer remissions.
   a. In your opinion why is CD19 such a good vaccine candidate? Is it because it is not located on healthy cells, but is located on most (if not all) leukemia cells?
   b. Does the CD19 antibody also deplete mature B-cells that produce antibodies? If so, is that a serious problem, or can that be remedied? It is our understanding that patients can live without B-cells.

2. **Bi-Specific Antibodies**: Another successful area of cancer vaccine research is the use of bi-specific antibodies, where one arm of the antibody molecule recognizes a tumor antigen (like CD19 in leukemia), while the other arm of the antibody recognizes an immune system activator (like CD3 on T-cells) to bring the tumor cell together with the T-cell that lyses it.
   a. If your lab works with bi-specific antibodies, has your lab combined the bi-specific antibody approach (and its idea of cell coupling) with the use of a second normal antibody (i.e. against immune stimulators PD-1 or CTLA-4) to try a combined vaccine approach?

3. **Antibodies Against PD-1 or CTLA-4**: These antibodies have helped induce several complete cancer remissions by stimulating the immune system to over-ride the suppression caused by the tumor.
   a. If your lab uses this approach, have you tried combining these stimulatory antibodies with other antibodies against tumor specific antigens in a combined approach?
   b. Some labs have observed side-effects caused by the immune stimulation, such as autoimmunity (where the patient’s immune system reacted against the body’s own tissues) or inflammation (the body’s crude innate immune response). When doing immune stimulations, did your lab assay for patient autoimmunity? Are these side effects treatable?

4. **Neo-Antigens**: Neo-antigens are new proteins (or portions of proteins) created on the surface of tumor cells by DNA mutations that form as the tumor grows.
   a. It is our understanding that antibodies and T-cells directed against tumor neo-antigens help facilitate tumor cell removal, and the presence of such cells in a patient correlates positively with improved patient prognosis. Do you think that T-cell responses against tumor-specific antigens are important for vaccine success?
   b. In some cases, a tumor may no longer express a neo-antigen that was expressed earlier. It is our understanding that any immune vaccine would no longer be effective against that patient’s tumor. Do more experiments need to be performed to
understand this loss of antigenicity? Should the vaccines be designed against multiple antigens if possible in case one neo-antigen is lost?

5. **Personalized Medicine**: It is our understanding that the type of neo-antigens formed by a cancer are different for each patient, which requires a type of personalized medicine to be performed. In this case, the patient’s exome (portion of DNA encoding the proteins) is sequenced by new rapid DNA sequencing technologies to determine surface neo-antigens, and then bioinformatics is used to predict which neo-antigen might be the best candidate to design the patient’s immunotherapy against.
   a. **Cost**: Such an intensive approach must be expensive? Are you aware of the costs of this type of procedure?
   b. **Speed**: Some types of cancer develop faster than others. For example, malignant melanoma if diagnosed in stage-IV can kill a patient within weeks, while prostate cancer usually develops slowly over several years. So, when treating patients with rapidly growing tumors, it seems that speed is critical. How long does such an intensive personalized approach take? Is it fast enough to treat a fast growing tumor with the patient’s life at stake?
   c. **Bioinformatics**: It is our understanding that only a portion of the neo-antigens formed by a tumor are actually antigenic to the body. Do we need better bioinformatics to identify which neo-antigens we should make the vaccine against?

6. **Side-Effect Problems**: In some human experiments, the patients showed grade-3 and 4 (serious) side-effects that appear to be caused by the cancer vaccine.
   a. In your clinical trials, have you observed any serious side effects? Were they treatable?
   b. Were the side-effects transient?
   c. We assume the side-effects are far less of a problem than the original cancer?

7. **TIL Vaccines Against Melanoma**: Tumor infiltrating lymphocytes (TILs) are T-cells that have migrated to a tumor site (presumably to help fight the tumor). But in the body, these cells often become inactivated by the tumor, allowing the tumor to grow. Several cancer remissions have recently been achieved by isolating TIL cells from a patient’s melanoma tumor to get the TILs away from the suppressing environment, expanding them outside the body, and then perfusing them back into the patient.
   a. Why were most of the early TIL studies performed with melanomas? Is that because Steve Rosenberg (a pioneer of the field at the National Cancer Institute) mostly studies that particular cancer? Is there something special about melanomas that work well with TIL treatments?
   b. It is our understanding that TILs have also been used to treat other types of cancer, such as epithelial and ovarian cancers. Are you aware of any other types of cancers treated with TIL cells?
   c. Some of the best remissions seem to have occurred in patients with a high TIL load. So, is the rate-limiting problem here the ability to amplify the TIL cells? Don’t high TIL loads also cause side-effect problems?
   d. Some of the best remissions occurred by enriching the TILs for a subpopulation of cells active against tumor-specific neo-antigens, so should this type of enrichment be done routinely, or just for resistant cases?
8. **Best Correlates of Protection:** It is not clear to us what causes the best cancer remissions.
   a. For TIL and CAR experiments, some studies have indicated that the best cancer remissions correlate with patients having the highest surviving TIL or CAR cell numbers. Other studies show the worst side effects associated with the highest cell doses. What is your opinion?
   b. Do the patients with the longest survival have TIL or CAR cells that target **multiple** tumor antigens, or just one important tumor antigen?

9. **More Patients:** The CAR success stories are so recent that relatively few patients have been followed for more than a year with this approach.
   a. Do we need more CAR studies to show the long-term effectiveness of this technique?

10. **Patient Pre-Treatments:** Recent clinical trials have demonstrated that chemotherapy treatment of leukemia patients **prior** to T-cell transfer improves the patient’s prognosis.
    a. What is the basis of the success? Does the loss of lymphocytes simply allow more room for the TIL or CAR therapy to expand? Does the pre-treatment reduce the size of the tumor as much as possible prior to cell therapy?
    b. Would this type of pre-treatment work for most tumors?

**Example Questions for Cancer Experts Not Using Immune-Vaccines:**

1. Are you aware of some of the recent success stories with cancer vaccines?
2. Do you think the recent data is finally strong for this approach?
3. Do we need more long-term survival studies? The technology is relatively new so it appears that not many long-term studies have been done, especially for the CAR-type vaccines. Or is cancer remission for 1 year a sufficient time to declare a success?
4. Cancer vaccines have worked in patients that have not responded to any other traditional treatments. Do you think the cancer vaccine approach should be used as a back-up approach to the traditional therapies, or as a front-line approach?

**INTERVIEW PREAMBLE**

We are a group of students from the Worcester Polytechnic Institute in Massachusetts, and for our research project we are conducting a series of interviews to investigate problems associated with cancer vaccines which have recently shown some strong successes.

Your participation in this interview is completely voluntary, and you may withdraw at any time. During this interview, we would like to record our conversation for later analysis. We will also be taking notes during the interview on key points. Is this okay with you?

Can we also have your permission to quote any comments or perspectives expressed during the interview? This information will be used for research purposes only, and we will give
you an opportunity to review any materials we use prior to the completion of our final report, which will be published on-line in WPI’s archive of projects.

If the subject does not agree to be quoted, we will respond as follows: “Since you would not like to be quoted during this interview, we will make sure your responses are anonymous. No names or identifying information will appear in any of the project reports or publications.”

Your participation and assistance is greatly appreciated, and we thank you for taking the time to meet with us. If you are interested, we would be happy to provide you with a copy of our results at the conclusion of our study.