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Changing landscape of immuno-oncology: CAR-T therapy and PD1/PDL1 blockade

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Thesis  

CHANGING LANDSCAPE OF IMMUNO-ONCOLOGY:  
CAR-T THERAPY AND PD1/PDL1 BLOCKADE  

by  

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CHANGING LANDSCAPE OF IMMUNO-ONCOLOGY:
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NAVEEN KUMAR MUNAGALA REDDY

ABSTRACT

The current field of cancer treatment is undergoing a revolution. The influx of novel therapies derived from basic research on the immune system has shifted the landscape of modern medicine. Immunotherapy seeks to use the body’s own immune system as a medium to terminate neoplastic cells. This is performed by manipulating the immune system into either targeting cancer antigens or breaking down barriers towards T cell infiltration. The former mechanism uses CAR-T cells as an instrument to target specific cancer neo-antigens. CAR-T cells begin as T cells derived from a patient’s immune system. These cells are removed from the body and engineered to express a chimeric antigen receptor (CAR) through a process of viral transduction. This CAR allows the T cell to recognize and bind to a specific antigen of interest. In most cases, the antigen is present on cancer cells. The T cells, now expressing the CAR receptor, are transplanted back into the body of the patient and proceed to target cancer cells. This therapy has been used in hematological malignancies to great effect. Applying CAR-T cells to solid tumors is an ongoing process, but has been difficult to establish due to the immunosuppressive aspects of the tumor microenvironment. As such, combining CAR-T cells with traditional anti-cancer therapies has been proven to be efficacious in treating patients with solid tumors. In general, immunosuppression is a large problem in the treatment of
Cancer. Cancer cells and the tumor microenvironment express receptors that downregulate tumor-targeting actions of the immune system. The discovery of the programmed cell death protein 1 (PD1) allowed researchers to create novel antibodies that inhibit immunosuppression. PD1 located on T cells, binds to PDL1 on cancer and stromal cells. This interaction induces exhaustion and anergy in infiltrating T cells, thereby prevent T cells from targeting cancer cells. As such, the newly approved checkpoint blockade antibodies, Nivolumab and Pembrolizumab, block this interaction and allow T cells to carry out their targeting function. CAR-T cells and checkpoint blockade have both seen immense success in clinical trials and are currently being used the clinic. Nonetheless, development of these therapies for different types of cancers is an ongoing process and one that will require immense effort on behalf of the medical and pharmaceutical establishment.
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LIST OF ABBREVIATIONS

ALL.................................................................................. Acute Lymphoblastic Lymphoma
APC................................................................................. Antigen Presenting Cell
CAF............................................................................... Cancer Associated Fibroblast
CAR............................................................................... Chimeric Antigen Receptor
CD.................................................................................. Cluster Differentiation
CLL.................................................................................. Chronic Lymphoblastic Leukemia
CTLA4................................................................. Cytotoxic T Lymphocyte Activator Protein 4
GVHD............................................................................. Graft Versus Host Disease
MHC.............................................................................. Major Histocompatibility Complex
MSKCC............................................................. Memorial Sloan Kettering Cancer Center
NSCLC........................................................................ Non-Small Cell Lung Cancer
PD1............................................................................... Programmed Cell Death Receptor 1
PDL1.............................................................................. Programmed Cell Death Receptor Ligand 1
SCFV.............................................................................. Single Chain Variable Fragment
TCR................................................................................. T Cell Receptor
TREG............................................................................. Regulatory T Cell
INTRODUCTION

The treatment of cancer has become a prime concern of the modern medical and pharmaceutical establishment. In 2016 alone, more than two million individuals in the United States will be diagnosed with cancer and six hundred thousand patients will die from the disease (1). To put this into perspective, approximately forty percent of all individuals in the United States will contract the disease in their lifetime and more than half of all Americans will eventually succumb to its effects. As such, immense efforts and resources have been poured into cancer therapies. In 2010, national expenditure on cancer treatment totaled 125 billion dollars and is expected to reach 156 billion in 2020 (1). However, there is a positive side to these disconcerting values. The overall cancer death rate has been steadily decreasing (2). Specifically, a two percent decrease in cancer mortality has been shown between 2002 and 2011 (2). This can be attributed to a lower incidence of smoking and the creation of better anti-cancer therapies. However, the increased prevalence of obesity, pollution, and an aging population, all risk factors, pose a challenge to both the current and future political, economical, and medical institutions (1).

As our understanding of cancer has grown, so to has our ability to fight the disease. Cancer, at its core, is a genetic disease that seeks to outmaneuver the body’s ability to recognize abnormal cell proliferation (3). In other pathogenic diseases, the immune system is able to recognize and terminate cells expressing foreign antigens. In cancer, however, the tumor is a propagation of native tissue and thus, does
normally express foreign antigens (3). As such, the tumor can continuously grow under the guise of normal tissue. Most chemotherapy and radiation interventions from the 1920’s and onward focused on killing cancer cells through brute force (3). These therapies targeted all of the body’s highly proliferative cells. As a consequence, the tumor would shrink in size but with side effects of healthy tissue death and bodily sickness. As research into the body’s immune system grew, scientists recognized that immune cells could be reprogrammed and altered to target specific cancer antigens. As such, the modern field of immunotherapy (1980’s onward) provides a novel and powerful means to selectively target and destroy cancer cells (4). This paper will elucidate the history, mechanism of action, efficacy, and future of two promising trends in immunotherapy: CAR-T cells and checkpoint blockade

History Of CAR-T Therapy

CAR-T cells are T cells that have been engineered to detect and eradicate tumor cells. The history of CAR-T cells can be first traced back to 1988 when Dr. Steven Rosenberg discovered that a small subset of tumor-infiltrating lymphocytes (TILs) were immunogenic towards melanoma cancer cells (4). He showed the existence of a population of T cells that specifically targeted melanoma cells without off-target effects. Rosenberg’s group selectively isolated these tumor specific TILs, expanded them ex vivo, and re-inserted them into the patient (4). The response was robust as the patient’s tumor burden dramatically reduced without significant off-
target effects (4). Rosenberg sought to expand this therapy to other cancer types but in 1992, came to the conclusion that TILs are not present in most cancers (4). Moreover, only about half of Melanoma patients have these tumor specific T cells (4). Nonetheless, this initial discovery caused an explosion in the field of cancer immunotherapy. As a first step, in 2003 the genetic code of the T cell receptor (TCR) in patients who responded to TIL therapy was sequenced (5). This was done to determine the specific melanoma antigens that the TILs were targeting. Once the sequence was known, scientists engineered retroviruses and lentiviruses to include the genetic code of the TCRs that were responsive towards tumor antigens. In 2005, these viruses were cultured with a patient’s own cells to generate T cells with reactivity towards tumor antigens in melanoma (5). Initial responses were robust as tumor burden decreased substantially, however, the modified T cells also destroyed normal tissue that expressed the tumor antigen (5). This became known as the “on-target off-tumor” problem as T cells killed cancer cells but also targeted normal tissue (6). Moreover, as increased amounts of modified T cells were engineered to express different tumor antigens, a new problem called the “off-target off-tumor” problem presented itself (6). In 2013, two patients undergoing therapy for melanoma with TCR-modified T cells against the MAGE-A3 antigen died of cardiogenic shock (7). Posthumous analysis revealed that T cells had infiltrated the myocardium and caused death of cardiomyocytes (7). Surprisingly, the MAGE-A3 antigen had a similar structure to a cardiac protein known as Titin. This “off-target off–tumor effect” occurred because the T cells targeting MAGE-A3 cross-reacted
with the Titin receptor, thereby causing destruction of cardiac tissue (7). These TCR-modified T cells are both the precursors and modern partners of the CAR-T cells. As such, they have taught the field of immunotherapy much about the risks that come with engineering T cells to target tumor antigens. Namely, the importance of understanding the full range of off-target and autoimmune effects inherent to engineered T cells.

**Design of CAR-T Cells**

CAR-T cells combine the binding properties of monoclonal antibodies with the lytic function of Effector T cells. The core structure of a CAR-T cell has stayed consistent throughout the many generational advancements in CAR-T cell design (4). It is important to recognize that the engineering of CAR-T cells occurs through transduction of viral plasmids (8). Significant effort is required to generate plasmids that contain the specific genes that generate CAR-T cells and simultaneously do not interfere with normal T cell function. Moreover, there exists a high risk of gene insertion that may cause activation or deactivation of oncogenes and tumor suppressor genes, thereby leading to uninhibited cell proliferation (8). These outcomes have yet to be seen in clinical trials but will always exist due to the inherent randomness of gene insertion (8). Once transduction occurs, T cells translate the chimeric antigen receptor (CAR). The CAR consists of an extracellular variable light and heavy region (scFv) derived from a monoclonal antibody, a hinge
region, a transmembrane domain, an activation domain, and the TCR intracellular signaling domain (8) (Figure 1).

**Figure 1- Core structure of the chimeric antigen receptor. Source: Jensen at al.**

First, the scFv affinity of the ectodomain predicts the binding affinity of the specific CAR (9). As such, the specific affinity of the monoclonal antibody from which the scFv is derived will determine the ability of the CAR to recognize and target cancer cells. Many of the scFv regions used in today’s CAR-T cells are derived from murine monoclonal antibodies and thus, include a risk of rejection through anti-idiotype and anti-murine antibodies derived from the host (9). Two solutions have been proposed to this problem. First, researchers have increased efforts to determine the smallest amount of scFv ectodomain necessary to induce immunogenicity (10). Second, scientists are in the process of humanizing the scFv domain so as to eliminate the risk of host rejection (10). Both solutions hold promising directions for future generations of CAR-T cells. Currently, however, the risk of host rejection is still significant (10).
Implied in the previous discussion of the scFv domain is the notion that the CAR-T cell does not undergo the normal process of activation through the APC/MHC complex (7). This is another important distinction between normal effector T cells and CAR-T cells. Regular T cells funnel through a process that leads to specific effector functions. First, antigen presenting cells (APCs) digest a pathogenic organism and express a portion of the pathogen on their MHC I/II complex (7). Next, a T cell (CD4 for MHC II and CD8 for MHC I) binds to the APCs MHC complex with their T cell receptor (7). This gives the T cell information on the characteristics of the pathogen present in the body. However, this does not fully activate the T cell for effector function. The second step requires the APC to license the T cell for killing. The APC expresses costimulatory molecules (B7 complex) that bind to receptors on the T cell (CD 28) and thereby fully activate the T cell for effector function (7). The T cell would become anergic without the second step of costimulatory activation (Figure 2). The T cell can now destroy pathogens (Cytotoxic CD8 T cell) or release cytokines that attract macrophages, neutrophils, and lymphocytes to an area of interest (Helper CD4 T cell) (7). CAR-T cells, on the other hand, do not require MHC activation as they are directly activated when the TCR complex binds to the target antigen (6). This type of MHC independent activation holds important consequences for CAR-T activation and killing. First, CAR-T cells can be engineered to respond to antigens that would not normally activate effector T cells (6). APCs do not have the ability to present numerous cancer antigens and thus, cannot prime T cells for cancer-specific killing. CAR-T cells, on the other hand, do not require MHC activation
and can be engineered to target certain cancer antigens that are not involved in APC-MHC presentation. Second, cancer cells have the ability to protect themselves from the immune system by down-regulating MHC complexes (6). This allows the tumor to evade the immune system by prohibiting T cell activation by APCs. CAR-T cells circumvent this problem by binding directly to target antigens through an APC independent manner (6). As such, the ability of a tumor to down-regulate MHC expression does not affect CAR-T effector functions. However, the lack of CAR-T interaction with APCs precludes the important costimulatory pathway (B7, CD28).

**Figure 2-Mechanism of T cell Activation. Derived from Gotsman et al.**

As a consequence, the first generation of CAR-T cells lacked full effector function and quickly became anergic once activated by cancer antigens (9). Researchers have partly remedied this problem by including costimulatory molecules in the TCR intracellular signaling domain of the CAR. This aspect of CAR-T function will be more fully elaborated upon in the section dealing with intracellular
signaling of CAR-T cells. As can be elucidated from the above discussion, the lack of MHC activation in CAR-T effector function forms many of the unique aspects inherent to CAR-T cells.

Below the scFv region lies the hinge domain, which tethers the ectodomain to the plasma membrane (8). The hinge domain influences the length and elasticity of the CAR and thereby effects CAR-T cell function. However, studies on hinge characteristics have been altogether inconclusive. For example, Hudecek et al. has shown that short hinges in certain RORI-specific CARs increase effector function while long hinges in CD19-specific CARs decrease effector function (10). The outcome of hinge length on CAR-T function has yet to be standardized and most likely will vary on which type of antigen a specific CAR-T recognizes. As such, more experiments will be required to reach conclusions regarding hinge function.

The extracellular hinge is connected to the intracellular signaling domain through the transmembrane domain (11). As such, the transmembrane domain is moreso a structural feature of the CAR rather than a recognition or signaling motif (11). Nonetheless, the type of transmembrane domain used affects the stability and function of the CAR-T cell. First generation CAR-T cells used domains from CD3-ζ, CD4, CD8, or CD28 molecules (11). Stability tests were performed on CARs made from these varied receptors. Results showed that the CD3-ζ and CD28 transmembrane domains had higher structural stability when compared to CD4 and CD8 molecules (11). Subsequent tests have also shown that CD28 molecules provide higher membrane integrity compared to the CD3-ζ (11). However, there is a trade
off between CD28 and the CD3-ζ. CD3-ζ transmembrane domains undergo a surprising and beneficial reaction when inserted into T cells. The CD3-ζ molecule dimerizes with the T cell’s endogenous TCR, which results in increased effector function of the resultant CAR-T cell (11). CD28 domains do not express this trait. Thus, the scientist engineering the CAR-T cell has to weigh the options of increased stability or increased effector function. Nonetheless, these studies have shown that CAR transmembrane domains can affect CAR-T function on a high level and thus, have led researchers to experiment with various transmembrane domain structures.

The last motif of the CAR is the endodomain. The endodomain has undergone the most changes from generation to generation due to its central importance in activating effector function in CAR-T cells (12). The MHC independent activation of CAR-T cells plays an integral role in endodomain construction. Namely, the lack of costimulatory input from the B7 complex on antigen presenting cells precludes full activation of CAR-T effector function. It was previously stated that costimulatory input from APCs acts as a license for T cells to target pathogenic organisms in the body. Without this costimulatory input, T cells become anergic and lie dormant. This intrinsic mechanism evolved to prohibit autoimmunity as APCs only present their B7 costimulatory complex when they have ingested a pathogen. Without this mechanism, T cells would be free to target self-antigens that APCs erroneously ingested. As such, the presence of this mechanism, while protective against autoimmunity, hinders CAR-T function. This is due to the ability of CAR-T cells to be
activated without APC input. However, the lack of costimulatory input from an APC can cause CAR-T cells to become anergic after binding to their target antigen. Moreover, the tumor and tumor microenvironment lack costimulatory receptors and actively downregulate mechanisms that lead to APC-B7 activation. As such, many first generation CAR-T cells lacked efficacy in clinical trials due to hyporesponsiveness of CAR-Ts towards tumor antigens (12). Further experimentation concluded that the lack of costimulation led to decreased efficacy of CAR function (12).

This discovery led to a second generation of CAR-T cells that were engineered with co-stimulatory endodomains (12). For example, second generation CAR-T cells included CD134/OX40, 4-1BB, and CD2 (12). These molecules are analogous to the intracellular signaling events involved in costimulation of normal effector T cells (12). Third and fourth generation CAR-Ts have included the B7 complex molecules involved in direct APC costimulation (12). Moreover, an added benefit of engineered endodomains is the ability of each molecule to affect different downstream signals. As such, multiple endodomains can be included within a single CAR-T cell and thereby, maximize the stimulation of a CAR when reacted against a tumor antigen. (Figure 3) However, the use of multiple signaling moieties increases the probability of T cell exhaustion (12). As a consequence, further research is underway to engineer CAR-T cells that are less resistant to anergy and exhaustion (12). The previous discussion has highlighted the central importance of endodomain construction on CAR-T function. As techniques for CAR engineering become
increasingly refined, specific endomains can be singled out for certain cancer lineages and thus, create highly potent and targeted therapies on a patient specific basis (12).

Figure 3- Evolution of CAR-T cell structure. Derived from Maude et al.

Clinical Efficacy in Hematological Malignancies

Second and third generation CAR-T cells have shown to be extremely effective against certain types of hematological malignancies. However, this was not the case with first generation CAR-T cells. Initial trials with CAR-T cells in 2008 and 2010 resulted in disappointing clinical outcomes (13). Specifically, two patients who received CD20 CAR-T cells and four patients who received CD19 CAR-T cells showed no cancer remission (13). Analysis showed that the number of CAR-T cells in the
body was drastically reduced after one week of therapy (13). Moreover, two patients developed antibodies directed against the CAR receptor (13). This lack of persistence coupled with immune rejection led to disappointing clinical outcomes in patients with hematological disorders. Similar results were also seen in patients with solid tumors. For example, Lamers et al. engineered first generation CAR-T cells against the CAIX antigen present in renal cell carcinoma patients (14). 66% of the patients developed hepatitis due to on-target off-tumor effects (14). The majority of the patients also developed antibodies against the CAR receptor (14). As such, the CAR-T imparted no clinical benefit to the patients (14). These negative results were seen in many other clinical trials conducted with first generation CAR-T cells. Due to these failures, scientists engineered second-generation CAR-T cells with endodomains that provided costimulation and increased activation of effector function (discussed above).
The second-generation CAR-T cells invariably shifted the landscape of modern cancer therapy (Table 1). The first trials of second generation CARs almost ubiquitously involved patients with acute lymphoblastic leukemia (ALL) and chronic lymphoblastic leukemia (CLL) (9). These malignancies were chosen due to the CD19 receptor on B cells. The CD19 receptor was chosen as a target due to its

<table>
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<tr>
<th>Target antigens</th>
<th>Cancers</th>
<th>Receptor</th>
<th>Year reported</th>
<th>Number of patients</th>
<th>Responses</th>
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<td>1998</td>
<td>16</td>
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Table 1- CAR-T clinical trials. Taken from Kershaw et al. Abbreviation of relevant data-CR: Complete Remission, PR: Partial Response, NI: No Information, SD: Stable Disease, OR: Objective Response
almost universal expression on cancerous B cells in ALL and CLL (9). Moreover, the risk of on-tumor off-target effects was minimal, as CAR-T toxicity towards healthy B cells did not lead to dangerous side effects (9). As such, ALL and CLL provided an attractive model for initial clinical trials on second-generation CAR-T cells. Many of the pioneering trials were conducted at Memorial Sloan Kettering Cancer Center (MSKCC), University of Pennsylvania (UPenn), and the National Cancer Institute (NCI) (9). Initially, a small trial was conducted on one patient in 2011 diagnosed with ALL at MSKCC (9). Treatment with CD19 CAR-T cells led to complete remission of the patient’s malignancy. After a two year follow-up, the cancer was still absent from the patient’s body (9). This initial success led to trials with larger cohorts. Sixteen patients at MSKCC with ALL underwent treatment with CAR-T cells in 2012 (15). Outcomes indicated an 88% cancer remission rate in the cohort. Moreover, a phase 1 clinical trial at MSKCC conducted with 32 patients showed a 91% complete remission rate (15). Many of these phase 1 patients were treated with refractory disease (15). This disease type is notoriously hard to treat as cancer cells become immune to classic chemotherapy and radiation (15). As such, the remission rates seen in these patients were unprecedented. Similar results were seen in cases of pediatric ALL at UPenn (15). Published results in 2014 from this phase 1 trial showed 91% complete remission in a 30 patient cohort (15). These robust responses were also seen at the NCI in a cohort of children and young adults (15). The CAR-T cells used in the three trials varied in their CAR architecture, and yet, similar results were seen across all cohorts. These trials have demonstrated the
remarkable effect of CAR-T therapies on ALL and allude to a functional cure in the near future.

As previously stated, initial trials of second-generation CAR-T cells were conducted on patients with CLL. Compared to CAR-T efficacy on ALL, CLL showed less robust responses. In 2011, six patients with CLL were given CD19 CAR-T cells (9). Results showed that persistence of CAR-T cells in the blood was low in patients that were not previously treated with a lymphodepleting agent such as Cyclophosphamide (9). These patients all had progressive disease after treatment with CAR-T therapy (9). However, four patients pre-treated with lymphodepleting agents either had stable disease or partial remission (9). Another trial at UPenn showed similar results with 20 out of 44 patients achieving complete remission (15). These observations show that treatment of CLL with CAR-T cells is not as efficacious when compared to ALL due to the lack of cell persistence in-vivo (15). However, CAR-T cells still provide a much stronger alternative to traditional chemotherapy and will continue to improve as more research is done on CLL.

**CAR-T In Solid Tumors**

Solid tumors are the most difficult of all cancer types to be treated with CAR-T cells. Even though solid tumors were the first to be treated with CAR-T cells, no substantial clinical efficacy has been produced from many first, second, and third generation CAR-T cells (16). The challenges posed with solid tumors are numerous due to the unique characteristics of the tumor microenvironment. Moreover,
specific antigens unique to tumor cells are not readily abundant. This poses a challenge as over-expressed receptors on tumors are readily available on non-tumor tissue, thereby exacerbating on-tumor off-target effects (16). The same problem is not seen in ALL and CLL since the body can exist without normal B cells for an extended period of time (15). To combat this problem, some scientists have engineered CARs to notice multiple tumor antigens before activation of effector function (17). Scientists have also engineered inhibitory CARs (iCAR) onto traditional CAR-T cells. These iCARs act as termination signals if the CAR-T cell interacts with an antigen present on healthy tissue (17).

Another difference between hematological malignancies and solid tumors is the location of the neoplasia. In ALL/CLL, the disease is blood borne so the CAR-T cell can act on the cancer after direct intravascular injection. In solid tumors, CAR-T cells cannot simply traffic to the site of interest since neoplastic cells are located outside the vasculature (16). As such, the CAR-T cells have trouble homing to the tumor site and thereby cannot eliminate the cancer. Multiple partial solutions have been developed to combat this problem. First, intratumoral delivery through surgical methods has been shown to increase CAR-T penetration and persistence (17). A surgeon can open the tumor to direct injection of CAR-T cells and thereby, directly improve CAR-T homing. This has been shown to work in clinical trials but cannot solve the problem of metastases (17). Metastasis is the process by which tumor cells disseminate to other organs within the body (3). This is the main cause of cancer mortality and is notoriously difficult to treat (3). Surgical injection of CAR-
T cells into primary tumor sites cannot solve the problem of metastasis since the cells will be localized to the primary tumor. As such, intravenous injection of CAR-T cells still provides a more ideal means of delivery to multiple tumor sites. A clever solution has been applied to partially solve this problem. Research on tumor biology has found that cancer cells release chemokines into vasculature at an extremely high rate (16). To take advantage of this unique characteristic, CAR-T cells have been engineered to express chemokine receptors that bind to specific chemokines released by a tumor (16). This would allow a CAR-T cell to traffic to the tumor site with the aid of chemokine signaling.

Anti-angiogenic drugs also assist in CAR-T trafficking to tumor sites (18). Tumors release Vascular Endothelial Growth Factor (VEGF) to stimulate blood vessel growth (18). This results in tumor vasculature that has different properties compared to normal vasculature. Namely, these vessels are immunosuppressive and are not conducive towards T cell trafficking (18). To combat this, anti-angiogenic drugs can be used to standardize tumor vasculature and thereby promote CAR-T infiltration into tumor sites (18).

 Trafficking of CAR-T cells to tumor areas still does not allow for full efficacy of effector function. The tumor microenvironment, composed of cancer associated fibroblasts, pericytes, extracellular matrix, stellate cells, immune cells, and endothelial cells, creates both an immune and physical barrier to CAR-T activation and infiltration (19). The following paragraphs will list a specific negative aspect of
the tumor microenvironment and elucidate mechanisms by which scientists have engineered CAR-T cells to bypass each challenge.

Figure 4- Components of the tumor microenvironment. Derived from Joyce et al.

First, tumor stroma, composed of cancer-associated fibroblasts (CAFs), provides a physical barrier against CAR-T infiltration (18). CAFs also secrete extracellular matrix proteins that sequester T cells away from cancer cells (18).
However, research has shown that CAFs over-express a protein called Fibroblast Activating Protein (FAP) (18). As a consequence, anti-FAP drugs used in combination with CAR-T cells have been shown to control tumor growth (18). Moreover, second generation CAR-T cells have been engineered to target the FAP protein on stromal cells (18). These studies have shown a decrease in tumor burden and may be useful if used with cancer specific CAR-T cells.

Second, the metabolic environment of the tumor microenvironment is not conducive for CAR-T persistence. Once neoplasia has been activated, the tumor cell stops producing ATP through oxidative phosphorylation and switches to aerobic glycolysis (19). As a consequence, the tumor environment becomes acidic since lactic acid waste from aerobic glycolysis lowers the pH of the surrounding area (19). This is known as the “Warburg Effect” as the pH drops from 7.4 to 6.5 or less (19). Normally, CAR-T cells switch to a glycolytic state when posed with sustained effector function (13). However, the acidic pH disallows this switch as the high lactic acid gradient stops membrane transport of lactic acid out of the CAR-T cells (19). Thus, the CAR-T cells cannot sustain their effector function. One solution to this problem is to use a proton pump inhibitor to increase the pH of the tumor microenvironment and allow for CAR-T activity (19). However, research into metabolic manipulation of the tumor microenvironment is lacking and more research still has to be done to increase CAR-T efficacy in acidic locations.

Lastly, the tumor microenvironment creates a state of hypoxia that further produces immunosuppression (4). Tumor cells produce a molecule named Hypoxia
Inducible Factor alpha (HIF1-alpha) when presented with a hypoxic environment (4). HIF1-alpha acts to decrease CAR-T function by attracting regulatory T cells (Tregs) to a tumor site (18). Tregs suppress the immune response and will thereby restrict CAR-T activation. Hypoxia also facilitates the release of microRNA from tumor cells that can degrade mRNA in CAR-T cells (18). Many more examples of hypoxia-related immunosuppression exist in the tumor microenvironment that will not be covered in this paper. As such, targeting hypoxia in the microenvironment can diminish the suppression of CAR-T activity. Studies have shown that simply having patients inhale high concentrations of oxygen can increase oxygen content in the tumor microenvironment (17). This increase in oxygen actually improves T cell infiltration and eventually decreases tumor burden (17). The previous discussion has shown that solid tumors provide an immense challenge for CAR-T cells. The physical characteristics of the microenvironment coupled with its immunosuppressive effects diminish both CAR-T infiltration and effector function (Figure 5). As such, CAR-T cells are being engineered to bypass or target the tumor microenvironment through both autonomous and combination therapies. Nonetheless, many more creative strategies will need to be employed to improve CAR-T function in solid tumors.
Future of CAR-T Therapy

As research into CAR-T therapy has exploded, so has the potential for combination immunotherapies. The ability to combine CAR-T therapy with checkpoint inhibitors (discussed below) and conventional chemotherapies holds promise towards higher tumor eradication. The tumor microenvironment presents infiltrating T cells with an immune blockade composed of PDL1 and CD80/86 (11). These ligands, expressed on tumor cells and tumor macrophages, bind to PD1 and CTLA4 receptors on T cells (11). As a consequence, T cells lose their effector function and become senescent. Many promising studies and trials are underway to test the efficacy of checkpoint inhibitors (PD1 and CTLA4) against various types of cancers. The results from these trials have been immensely promising, as tumor burden has drastically decreased in patients (11). As such, combining checkpoint
blockade with CAR-T cells would unleash the full potential of modern immunotherapy. Checkpoint blockade, by itself, allows the body’s own immune system to kill cancer cells. This would augment the already powerful ability of CAR-T cells to target neoplastic cells and thus, form a multiplicative effect on tumor lysis.

A major challenge to CAR-T therapy concerns cost and logistics. At the core of the problem is the necessity for CAR-T cells to be personalized for each patient. Providing CAR-T cells for a patient requires cell collection, viral construction, transduction, viability assays, ex vivo cell proliferation, and continuous clinical observation. This is no easy feat for non-academic institutions and small hospitals. As such, scientists are in the process of developing “off the shelf” CAR-T cells that can be given to a patient without personalization (21). This would greatly simplify the engineering process and thereby lower cost and increase access of therapy. However, the main barrier to this is the high probability of Graft Versus Host Disease (GVHD). GVHD occurs when donor cells (graft) recognize the body’s tissue (host) as foreign and proceed to attack host cells (Figure 6) (22). CAR-T cells that are not personalized would possess a TCR that can recognize and target host HLA molecules. As such, “off the shelf” CAR-T cells would have to be engineered to reduce the possibility of GVHD (21).
Many proposals are in the process of being tested but the most promising includes using progenitor cells as the medium for CAR-T construction (23). Progenitor T cells are not fully developed and thus, cannot discern the difference between self and non-self antigens (23). Therefore, progenitor T cells cannot induce GVHD due to their inability to recognize HLA molecules. As a consequence, they can function as the raw material for “off the shelf” CAR-T cells as they do not have the potential to induce GVHD when given to different hosts (23). Once the progenitor CAR-T is given to the patient, the cells will mature into normal effector T cells in the thymus and develop standard T cell functions (23). Once fully matured, the progenitor CAR-T cells can fulfill their tumor targeting function. One study has been
conducted on these progenitor CAR-T cells with positive results (23). Induced pluripotent stem T cells were transduced with a CD19 CAR and were given to mice implanted with human cancer cells (xenograft) (23). Results showed that these progenitor CAR-T cells were able to effectively target CD19 expressing cancer cells (23). This type of CAR-T cell holds promise for a future where CAR-T cells can be safe, highly effective, and affordable to all patient populations. This is only one example of the promise CAR-T cells hold for the future of cancer treatment. As with any new therapy, caution must be taken to validate trials and understand significant side effects. A deliberate and rigorous method must be implemented to mitigate the hysteria that comes with such powerful technology. Nonetheless, the future of medicine has been unequivocally changed by the introduction of these chimeric antigen receptor T cells to the widening medical landscape.

**Immune Checkpoints**

At the core of modern immunotherapy is the T cell. This fraction of the immune system holds the immense responsibility of carrying a large proportion of the cytotoxic functions of the body. Thus, the T cell holds an enormous amount of power when a patient develops a tumor. Either the T cell can target the tumor or it can stay quiet and let the tumor breed. As such, the tumor goes to immense lengths to disrupt the T cell from targeting neoplastic cells. The tumor hides itself behind a wall of stroma and extracellular matrix. It cloaks itself under the guise of normal tissue by under expressing activation markers. It disseminates throughout the body
looking for fertile soil where it can create anew. And lastly, it forces the body’s own troops to become lethargic and exhausted (Figure 7) (4). Every point in this multi-pronged attack by the tumor is being targeted by modern cancer therapies. Antibodies against tumor stroma and extracellular matrix proteins have been developed to disrupt the tumor microenvironment. Molecular libraries are being probed to determine neo-antigen expression on cancer cells. Anti-angiogenic drugs stop formation of blood vessels that allow for metastasis. Lastly, checkpoint inhibitors combat the ability of tumors to induce T cell exhaustion. This last point is where modern developments in immunotherapy come into play.

Figure 7- Cancer cells adapt to their environment and proliferate by developing a combination of these hallmarks. Derived from Weinberg et al.
The immune system has evolved multiple degenerate mechanisms to preclude the possibility of auto-immunity. This manifests itself in the form of immune checkpoints (24). Immune checkpoints decrease the probability of auto-immune disease and shield normal tissue from damage during immune activation (24). However, there is a delicate balance between immune tolerance and immune activation. This is elegantly exposed in the case of the T cell. The T cell receptor recognizes a foreign antigen and undergoes a process where numerous downstream effectors are activated (ZAP70, FYN, LCK, LAT etc) (25). The quality and quantity of downstream activation is based upon the input the T cell receives from costimulatory and inhibitor molecules. These signals are normally located on antigen presenting cells (monocytes, macrophages, dendritic cells), B cells, NK cells, and normal tissue (24). However, in the case of neoplasia, cancer cells hijack the system for their own ends. This is manifested in the expression of markers that induce T cell exhaustion (25). Receptors and ligands for T cell activation are normally not over-expressed in cancer relative to normal tissue (25). Inhibitory receptors towards T cell activation, on the other hand, are highly expressed on both cancer cells and supporting cells in the tumor microenvironment (25). This discovery has allowed scientists to develop antibody therapies that reduce on-tumor off-target effects. Most of the conventional therapies used in cancer target the cancer cells themselves and thereby circuitously boost the immune system’s ability to target neoplastic cells. However, in the case of checkpoint blockade, modern therapies target the receptors and ligands on the T cell rather than the tumor (24).
This directly activates the immune system and allows it to kill cancer cells in a safe and targeted manner, without conventional side effects. CTLA4 will be used as an example to illustrate this concept.

Cytotoxic T Lymphocyte Associated Protein 4 (CTLA4) was the first inhibitory receptor to be identified and targeted (26). It is primarily located on T cells and is involved in inhibition of effector function (26). It does this through inhibiting co-stimulatory activation by CD28. As previously discussed, antigen presenting cells (APCs) license a T cell to kill by providing costimulatory input through the B7 complex. The B7 complex, composed of CD80 and CD86, binds to the CD28 receptor on T cells and allows the cell to carry out its effector function. This only occurs once the T cell has been presented with a pathogenic antigen by an APC. CTLA4 is inhibitory because it competes with the normal costimulatory pathway (27). It binds to CD80 and CD86 and thereby disallows CD28 interaction with the B7 complex (27). This occurs because CTLA4 has a higher affinity for the B7 complex compared to CD28 (27). CTLA4 expression also directly inhibits T cells function by catalyzing an inhibitory cascade within the T cell (27). As such, CTLA4 has a dual inhibitory role. It insulates CD28 from the B7 complex and directly inhibits T cells activity through intracellular signaling pathways (27). CTLA4 plays an integral role in the body's ability to stop autoimmune events. This is exemplified in knockout studies where the gene for CTLA4 (Ctla4) is removed from a mouse's genome (26). In these cases, the mouse experiences an immense hyper-activation of its immune system which eventually leads to death (26). Interestingly, CTLA4 is more so a factor
in CD4 helper T cells and Tregs than in CD8 cytotoxic T cells (28). CD4 T cells recognize MHC II antigens on APCs and release cytokines that mediate B cell and CD8 T cell activation (28). Tregs are a subset of CD4 cells that inhibits immune function (28). CTLA4 expression on helper T cells suppresses their ability to activate the immune system through the dual inhibitory mechanism previously discussed. The mechanism by which CTLA4 activates Tregs is currently unknown (28). This paradoxical function illustrates the complexity of the immune system's response to a certain signal. One signal can activate a certain pathway in a cell but inhibit the same pathway in a different cell. However, when seen from a higher vantage point, CTLA4 expression leads to overall immunosuppression by inhibiting CD4 T cells and activating Tregs (28). Thus, it follows that blocking CTLA4 through an antibody would unleash CD4 activity and reverse Treg suppression (Figure 8). This is precisely what happened in clinical trials involving CTLA4 blocking antibodies (29). Initially CTLA4 as a single agent did not prove successful as tumor burden was not drastically reduced (29). However, a small subset of patients reacted very positively to the treatment (29). These patients had tumors that were immunogenic. Immunogenic tumors are characterized by lymphocyte infiltration and general immune activation in the tumor area (29). CTLA4 only decreased tumor burden in patients that already had an endogenous tumor response (29). This observation follows closely in line with our previous discussion. CTLA4 inhibits CD4 activation and boosts Treg suppression. As such, CTLA4 blockade would only work in instances where CD4 cells and Tregs are already present in tumor areas. Since it
cannot activate immune infiltration, it cannot work in tumors that are non-immunogenic. Thus, CTLA4 can only boost an ongoing immune response (29). These studies led to the development of clinical CTLA4 inhibitory antibodies by pharmaceutical companies. Bristol-Myers Squibb eventually won the race and released the FDA approved ipilimumab (Trade name Yervoy) in 2010 for the treatment of melanoma (25). This date is significant as it represents the first FDA drug approved for use in checkpoint blockade (25). In general, CTLA4 blockade did not live up to its initial expectations due to the lack of pronounced tumor regression and unpronounced objective clinical response (25). However, Ipilimumab has gone on to be used in combination with conventional chemotherapy regimens with moderate success (25). This initial foray into checkpoint blockade paved the way for
therapies targeting the PD1/PDL1 axis.

Programmed Cell Death Protein 1 (PD1) was first cloned and isolated in 1992 (30). There are two ligands that activate PD1: PDL1 and PDL2. PD1 is mainly expressed on mature T cells located in peripheral tissue (30). This contrasts to CTLA4, which is expressed on naïve T cells after antigen presentation by an APC (26). This is an important distinction and one that will further an understanding of PD1 blockade’s higher clinical efficacy when compared to CTLA4. PD1 regulates T cell activity within tumor tissue while CTLA4 is involved in initial T cell activation (27). CTLA4 is up-regulated in naïve or resting T cells at the time of antigen presentation (27). More specifically, CTLA4 inhibits continuous activation of immature T cells by sequestering the CD28 costimulatory molecule away from an APC’s B7 complex (27). This inhibits continuous activation of effector function and allows the T cell to target one antigen at a time. CTLA4 is expressed once the T cell receptor has been activated (27). CTLA4 expression increases with stronger TCR activation (27). PD1, on the other hand, is not involved in initial T cell activation by an APC (30). Rather, it responds to global levels of inflammation (30). This can be illustrated with Interferon gamma. Interferon gamma is a cytokine released by T helper 1 cells during inflammatory events in peripheral tissue (30). As such, interferon gamma interaction with a T cell induces the expression of PD1 (30). This contrasts to CTLA4, where APC interaction with a naïve T cell induced expression of CTLA4 (27). The CTLA4 pathway allows for a consistent level of T cell activation in the presence of ligands with varying affinities (27). The PD1 pathway decreases T
cell function in response to heavy and chronic inflammation (30). Thus, PD1 upregulation occurs in response to global levels of inflammation in peripheral tissue while CTLA4 activation mediates T cell activation in non-peripheral tissue. Moreover, PD1 is expressed on a greater variety of cells when compared to CTLA4 (27). CTLA4 is expressed almost exclusively on naïve or dormant T cells (26). PD1, on the other hand, it present on T cells, B cell, NK cell, and some myeloid populations (31). This also accounts for the higher efficacy of PD1 blockade as PD1 blocking antibodies might also unleash NK cell and B cell activity in tumor sites (31).

Activation of PD1 induces exhaustion and anergy in T cells (31). As such, it plays an important role in mitigating the tissue damage that comes with chronic infection (31). This mechanism evolved to protect the body from unnecessary tissue damage during inflammatory states. However, cancer, once again, hijacks this autoimmune mechanism for its own survival. In this case, cancer cells upregulate PD1 ligands (PDL1 and PDL2) that bind to and immobilize tumor infiltrating T cells (TILs) (31). PDL1 is the major PD1 ligand used by cancer cells. Moreover, it is also expressed on myeloid cells (macrophages and dendritic cells), fibroblasts, and other components of the tumor microenvironment (31). PDL2 is also expressed on tumor cells in B cell lymphomas and Hodgkin's disease (31). However, PDL2 does not play as significant a role in patient survival as PDL1 (31). Many studies have shown that the expression of PDL1 in patients with cancer results in a poorer prognosis when compared to patients without PDL1 expressing tumors (31). This observation agrees firmly with the mechanism of PD1 exhaustion previously discussed. As such,
the question arises: How is PDL1 upregulated by tumors? Two theories have been developed to address this question (Figure 9).

Figure 9- Competing mechanisms of PDL1 upregulation. Derived from Pardoll et al.

The first theory uses an internal genetic approach to the T cell. Cells become neoplastic due to the activation of proto-oncogenes or deactivation of tumor suppressor genes (32). These mutations cause changes in intracellular signaling pathways that eventually lead to uncontrolled cell proliferation. Thus, at its core, cancer is a genetic disease (4). The expression of PDL1 might also follow this pathway of abnormal expression. This theory, termed “innate immune resistance,” proposes that internal oncolytic driver mutations eventually result in upregulation of PDL1 on cancer cells (32). In glioblastomas, the continuous internal oncolytic stimulation causes deletion of the PTEN gene (32). The lack of PTEN unleashes the
PI3K-AKT pathway, which leads to uncontrolled cell proliferation and PDL1 expression (32). The same goes for patients with lymphoma and lung cancer. The deletion of a certain gene leads to continuous activation of the ALK-STAT3 pathway, which causes expression of PDL1 (32). The idea behind the “innate immune resistance” theory looks at cancer cells in a vacuum. Meaning, the expression of checkpoint molecules will undoubtedly occur after a certain amount of oncolytic mutations. This theory does not take into account the mechanisms by which cancer cells interact with non-cancerous immune components.

The second theory of PDL1 upregulation, named “adaptive immune resistance,” involves both the cancerous and non-cancerous cells (32). It takes into account the inflammatory conditions present in the tumor microenvironment. Cancer has classically been called “the wound that never heals” due to the observations that tumor progression exhibits similar characteristics to the process of wound healing (33). These similarities include growth of new blood vessels (angiogenesis), the shifting of the extracellular matrix around stromal cells, changes in cell adhesion, upregulation of specific genes, and inflammatory cytokine signaling (33). In the case of a wound, the body terminates the immune reaction after wound closure and pathogen removal (33). However, in cancer, the immune activation continues endlessly due to progressive actions by the tumor (33). The theory of “adaptive immune resistance” follows from this notion. PDL1 on cancer cells has shown to be upregulated in response to actions by the immune system (32). Normally, cells express PDL1 during times of chronic inflammation and infection.
This stops T cells from destroying tissue located peripheral to the site of infection. Cells know to express PDL1 when approached by inflammatory cytokines. In most cases, the inflammatory cytokine is interferon gamma. The T cell releases interferon gamma, which interacts with STAT3 in unwounded tissue. This eventually leads to upregulation of PDL1 on normal tissue, which then stops the T cell from releasing interferon gamma. The negative feedback mechanism is important for combating continuous T cell activation. In cancer, the same defense mechanism occurs due to T cell infiltration into tumor sites. However, in this case, the release of interferon gamma does not induce a brief increase in PDL1 expression. Rather, the tumor hijacks the transient upregulation for its own ends and expresses PDL1 in a constitutive fashion. This theory is supported by observations concerning tumor infiltration and variability in PDL1 expression on different areas within a tumor. Studies on PDL1 expression in melanoma have shown that PDL1 expression is not homogenous within a single tumor. Rather, PDL1 is upregulated only in areas of lymphocytic infiltration. This supports the “adaptive” theory as immune infiltration is a pre-requisite for PDL1 expression. The infiltrating T cells release interferon gamma that upregulates PDL1 on cancer cells permanently. It follows that PDL1 should not be expressed in areas that lack lymphocytic infiltration due to the absence of inflammatory cytokines. This is precisely what is observed. As such, the “adaptive immune resistance” more fully encompasses the complexity inherent in the tumor microenvironment while simultaneously accounting for clinical observations concerning non-uniform PDL1
expression in tumors. Implicit in this discussion is the notion that both immune and non-immune cells contain the ability to express PDL1 (31). Any tissue stimulated by interferon gamma will express PDL1 to mitigate the damage caused by T cells during inflammation (31). This contrasts with PD1, which is mainly expressed on T cells (31). As such, the potential for ubiquitous expression of PDL1 holds the key to understanding the discrepancy of safety profiles between PD1 and PDL1 inhibitors.

Therapies composed of PDL1 inhibition lead to higher incidences of side effects and autoimmune events (34). This observation follows from the notion that PDL1 can be expressed on many cell types (30). PDL1 is critical for the maintenance of an immunosuppressive environment during inflammation (30). Providing PDL1 inhibition for a patient deactivates mechanisms of systemic immuno-suppression. This increases the potential for inflammatory damage at sites peripheral to the tumor (30). PD1 inhibition, on the other hand, mainly affects T cells and thus, does not predispose the body to unwanted autoimmune events. As a consequence, PD1 blockade has been more efficacious when compared to PDL1 due to the lower incidence of side effects and greater specificity of immune cell targeting (34). This will be more fully elaborated on in the following discussion concerning clinical trials of PD1 and PDL1 blockade.

**Clinical Trials of PD1/PDL1 Blockade**

As can be seen from the previous discussion, the PD1 and PDL1 axis is implicated in the inability of the immune system to target cancer cells. The binding
of PDL1 to PD1 leads to exhaustion in tumor infiltrating lymphocytes and thereby allows unabated growth of tumors. As such, disrupting the PD1/PDL1 axis carries great potential in unleashing the immune system upon cancerous cells (Figure 6). This method of cancer therapy has a much lower risk of adverse side effects when compared to conventional chemotherapy and radiation. Chemotherapy and radiation simultaneously target tumors and all highly proliferative cells (35). Hair follicles, epidermal/dermal cells, and various gastrointestinal cells are susceptible to damage by conventional therapies due to their highly proliferative nature (35). As such, side effects like hair loss, skin disorders, fatigue, and nausea are a function of the non-targeted mechanism by which conventional cancer therapies rid the body of neoplastic cells (35). PD1/PDL1 blockade, on the other hand, would only target cells that express these specific receptors. The majority of PD1 in the body is expressed on a specific subset of mature T cells and thus, the potential for off-target interactions are significantly diminished (30). This notion has been manifested in the formation of small molecule inhibitors against PD1 and PDL1. Several PD1 inhibitors have been approved for use in specific types of cancers. The following discussion will expand upon the clinical efficacy of FDA approved PD1/PDL1 blockade in melanoma and non-small cell lung cancer (NSCLC).
Malignant melanoma is the fifth most prevalent cancer in the United States and the past thirty years has seen a growing incidence of melanoma in the American population (36). Approximately, 132,000 new cases of melanoma are diagnosed every year across all countries (36). In the United States, roughly 10,000 individuals will succumb to the disease in 2016 (36). Nonetheless, melanoma is known to be a relatively curable disease when diagnosed in early stages. 91% of patients with localized melanoma survive for five years after initial diagnosis (36). However, this rate drops once melanoma approaches higher stages of dispersion. 16% of patients survive once melanoma has progressed to distant sites and metastasis (36). This is where checkpoint blockade comes into play. More specifically, the anti-PD1 inhibiting antibodies, nivolumab and pembrolizumab, have shown to be highly
efficacious in the treatment of advanced melanoma.

Nivolumab, a human monoclonal antibody targeting PD1, was approved by the FDA on December 14, 2014 for treatment of unresectable (immune to surgery) and metastatic melanoma (37). The first phase II trial on nivolumab was carried out with a cohort of 418 patients diagnosed with unresectable and malignant melanoma. 25% of participants treated with nivolumab exhibited partial tumor response (37). Median objective survival of patients after one year was 73%. This was a drastic improvement in response when compared to the 42% objective survival in the standard chemotherapy group (dacarbazine) (37). Moreover, the safety profile of nivolumab was much higher than that of standard chemotherapy. 11% of patients in the nivolumab group exhibited significant side effects compared to the 18% in the chemotherapy regimen (37). Another study using nivolumab and ipilimumab (CTLA4 inhibitory) showed the power of combining PD1 blockade with classic immunotherapy (38). The study, composed of 86 patients, showed that the combination of nivolumab and ipilimumab led to a 40% objective response rate while the monotherapy group exhibited a 20% objective response (38). As such, the combination therapy resulted in a higher response rate and higher median one-year survival when compared to monotherapy (38). These results are drastic improvements over conventional therapy and have allowed PD1 blockade to become a first line therapy in the treatment of advanced melanoma (38).

Pembrolizumab is another humanized monoclonal antibody targeting the PD1 receptor (39). The efficacy of pembrolizumab was established in a phase I study
composed of 135 patients (39). The patients were divided into three cohorts (39). The first cohort received 10mg/kg every two weeks, the second 10mg/kg every three weeks, and third 2mg/kg every two weeks (39). Overall response rate was 38% across all three cohorts (39). However, results showed that patients given 10mg/kg of pembrolizumab every two weeks exhibited a 52% response rate (39). Moreover, these patients had a progression free survival of seven months (39). This study laid the foundation for pembrolizumab’s remarkable efficacy in advanced melanoma and facilitated its designation as an FDA “fast track” drug (39). Another phase one trial conducted on 173 patients with refractory melanoma showed similar results (39). Refractory melanoma is more difficult to treat due to the adaptive mechanisms that the tumor employs to bypass the immune system (39). This trial contained two cohorts (39). The first was administered 2mg/kg every three weeks while the second was given 10 mg/kg every three weeks (39). The overall response rate was 26% while the progression free survival for the 2mg/kg and 10mg/kg groups were 22 weeks and 14 weeks respectively (39). These results coupled with low amounts of adverse events allowed pembrolizumab to be approved by the FDA for use in melanoma patients on September 4, 2014 (39).

Trials on PDL1 targeting antibodies have achieved similar or slightly lower response rates when compared to PD1 antibodies (37). However, the toxicities of these drugs have been a cause of concern. For example, MSB0010718C, a humanized monoclonal antibody targeting PDL1, is currently being tested for safety, tolerability, and pharmacokinetics in a phase I study (40). 27 patients with solid
tumors were chosen for the trial (40). Two patients had to discontinue treatment due to adverse events while three patients developed grade three to four adverse events (40). These toxicities are much higher than those seen in PD1 patients. The rationale for this discrepancy involves the high expression levels of PDL1 in non-immune tissue (previously discussed). Nonetheless, the use of anti-PDL1 antibodies still shows promise as an immunotherapy and further research is ongoing to both reduce toxicity and enhance its tumor targeting ability. Treatment of melanoma with anti-PD1 and PDL1 antibodies has proven to be successful in patients with unresectable and advanced stages of the disease (39–40). These melanoma trials facilitated the FDA approval of nivolumab and pembrolizumab. Consequently, this has laid the foundation for the usage of these antibodies in a greater variety of cancer types. Most recently, this has expanded into trials on patients with lung cancer.

Non-small cell lung cancer (NSLC) accounts for the plurality of cancer deaths worldwide (41). Most patients are diagnosed at advanced disease progression and metastasis (41). These patients have eight to ten months of survival after diagnosis, which is low compared to the large majority of cancers (41). Median overall survival increases in individuals with specific driver mutations as targeted therapies have been developed to treat these actionable patients (41). However, only 25% of cases contain these mutations and thus, the majority of patients are treated with conventional chemotherapy and platinum-based medicine (41). As such, novel therapies are in the process of being tested on individuals with NSCLC. The most
promising has been PD1 blockade. A phase one trial conducted with nivolumab on
129 patients with NSCLC showed a 17% overall survival rate (42). This result is
especially noteworthy in that 54% of participants were thrice treated with
conventional therapies (42). Moreover, these responses were stable with median
response durations of seventeen months. Trials with pembrolizumab have shown
similar robust responses. In a phase one trial, 495 patients with advanced or
metastatic NSCLC given pembrolizumab produced a response rate of 19.4% (42).
This trial also contained individuals who previously underwent at least three
regimens of chemotherapy (42). Treatment with pembrolizumab was well tolerated
by patients. Only 9.5% of patients exhibited adverse events with one treatment
related death (42). Moreover, trials have been conducted comparing PD1 blockade
with conventional second line therapy (docetaxel) (42). Patients given nivolumab
responded with a median overall survival of nice months compared to docetaxel’s
six months (42). One-year survival with nivolumab was 42% compared to 24% with
docetaxel (42). These robust responses have elevated PD1 blockade’s status to that
of the primary second-line therapy in NSCLC (Table 2) (31).
Table 2- Summary of clinical trials targeting PD1. Derived from Nguyen et al

**Conclusion**

Cancer has become the prime medical challenge of the modern era. The conventional therapies for cancer do not provide the means to effectively tackle this problem due to their non-targeted and toxic approach to tumor killing. As such, the modern field of immunotherapy has brought upon a revolution in the establishment's approach to cancer treatment. CAR-T cells provide the means to
target specific cancer antigens by engineering a patient’s endogenous immune cells. Moreover, CAR-T cells have been shown to be safe in clinical settings. They have been proven to be efficacious in hematological malignances and are currently being engineered for use in solid tumors. Checkpoint blockade has also been used to tremendous effect. Basic research on immune regulation has led to the development of therapies that can negate the suppressive effects of the tumor microenvironment. PD1 inhibitors have been shown to be effective in patients with advanced melanoma and non-small cell lung cancer. Moreover, trials are currently underway to broaden the scope of checkpoint blockade to other cancer types. The next decade will see the manifestation of these discoveries in the wider populace. However, such powerful technology does not come without a price. The current transition brings about many important questions regarding the economics and logistics of medical treatment. How much do these therapies cost? Who will be able to afford them? How will current policy changes within the medical system affect the production and usage of these treatments? These questions cannot be comprehensively answered with current information. As such, policy makers, pharmaceutical companies, and physicians must be diligent and cautious in their approach to such powerful technology. The potential for exploitation is high due to the immense hope immunotherapy gives to patients. Thus, the current medical establishment must proceed with the interests of the patient acting as their prime motivator. Only through such a method will the full potential of immunotherapy realized.


Curriculum Vitae

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Education

<table>
<thead>
<tr>
<th>Institution</th>
<th>Address</th>
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<th>Date</th>
</tr>
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<tbody>
<tr>
<td>Boston University</td>
<td>Boston, Massachusetts</td>
<td>Masters of Medical Sciences</td>
<td>May 2016</td>
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<tr>
<td>University of California Los Angeles</td>
<td>Westwood, California</td>
<td>Bachelor of Science, Neuroscience</td>
<td>June 2014</td>
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Work Experience

**University of California Los Angeles: Cardiac Arrhythmia Center**,  
September 2011 – June 2014  
*Research Associate*

- Developed clinical interventions to remedy cardiac arrhythmias post-myocardial infarction
- Stellate Ganglion extraction and analysis of neurotransmitter respecification
- Analysis of activation recovery intervals and development of Scaldyn ARI algorithms
- Journal article writing and editing – Multiple Publications

**University of California Los Angeles: Department of Surgical Anesthesiology**,  
January 2012 – June 2014  
*Surgical Assistant*

- Developed procedures for use in porcine survival surgeries
- Trained in stellectomy, vagal stimulation, spinal cord transection
- Analysis of hemodynamic data derived from surgeries

**University of California Los Angeles, Easton Alzheimer’s Center**,  
July 2010 – June 2011  
*Research Associate*

- Methodized early identification of Alzheimer’s and Dementia through neuropsychological examinations
- Administered WAIS, IQ, and WCST examinations to individuals with neurodegenerative disorders
- Analyzed test data through SPSS and developed cognitive models of executive and memory function

**Ronald Reagan Medical Center**,  
December 2012– June 2014  
*Volunteer Intern*

- Assisted with minor procedures including drawing blood, ABG measurements, intravenous line insertions, and suturing
- Improved safety standards by providing feedback on clinical interventions and physician hygiene
Research Skills

Histological Procedures (Thyroxine, Tyrosine Hydroxylase, H and E), ELISA assay, UV-VIS spectroscopy, mass spectroscopy, multiple kinetic rate analysis methods, analysis, Lab Safety and Regulated Chemicals (OSHA), SPSS, CardioLab, Definiens Analyzer, Scaldyn ARI, iLife Suite (Garage Band, iMovie, iPhoto, iWeb, Microsoft Office Suite (Excel, PowerPoint, Word), Windows OS support, R

Conversational Spanish and Telugu

Activities

Project RISHI, UCLA Chapter, International Medical Volunteer Organization, Vice-President
UCLA Radio, UCLA Chapter, Member, Disc Jockey
Neuroscience Society, UCLA Chapter, Treasurer
Hope Collaborative, Boston Chapter, Member