

Developing Cancer Immunotherapies by the manipulation of Immune Checkpoints

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Abstract—A tumor mass consists of cells that have undergone a large number of mutations, these mutations might provide a diverse set of antigens that are capable of producing an immune response. Therefore cancer cells need to suppress the immune system to form a sustainable tumor mass. Hence, tumors co-opt certain immune checkpoint pathways to suppress the immune response in the tumor microenvironment. Immune checkpoints are the inhibitory pathways used by the immune system to maintain self-tolerance and modulate the immune response in peripheral tissues to minimize the collateral tissue damage, to avoid autoimmune disorders. Most of the immune checkpoints are initiated by ligand-receptor interactions; therefore the blockade of either the ligand or the receptor with the help of the specific antibodies can suppress the activity of these immune checkpoints. Hence one of the most promising approaches to activate antitumor immunity is by the blockade of immune checkpoints. A Cytotoxic T lymphocyte associated antigen 4 (CTLA4) antibody, named Ipilimumab, was the first such immunotherapeutic to achieve US FDA approval. Preliminary clinical findings with blockers of additional immune checkpoint proteins, such as Programmed cell death protein 1 (PD1), indicate broad and diverse opportunities to enhance antitumor immunity with the potential to produce durable clinical responses.

Keywords—APCs, Immune Checkpoints, Objective responses, response rates, TCR, T_{Reg} cells.

I. INTRODUCTION

Conventionally cancers are treated by three methods, which are, Surgery, Chemotherapy and Radiation therapy. As we know that the current conventional therapies are incapable of delivering a solid anti-tumor therapeutic without any pronounced side effects, we are hence in search of a new therapeutic that can eliminate tumors and also have very little to no side effects. Immunotherapy, which basically means to manipulate the immune system in order to obtain a suitable immune response to eliminate the tumor, is currently being developed to be used as a standalone therapeutic in many different types of cancers. Tumor cells are formed from many different types of mutations; these mutations provide a diverse set of antigens that are capable of producing an immune response. So the question comes here, *why is the immune system not able to eliminate the tumors all by itself?* The question can be answered using recent observations that in the tumor micro environment the immune checkpoint receptors and ligands are over expressed and hence suppress the immune response against the tumor cells. Immune checkpoint pathways are those inhibitory pathways used by the immune system to regulate the immune response in peripheral tissues in order to reduce the collateral tissue damage. As most of these immune checkpoint pathways are activated by ligand receptor interactions, blockade of either the ligand or the receptor using specific monoclonal antibodies can stop these immune checkpoint pathways from getting activated, hence we can then see a durable immune response against the tumor cells. In this review, few of the immune checkpoint pathways will be discussed along with the clinical application and status of few antibodies that are being used to block the immune checkpoint pathways.

II. CTLA4 IMMUNE CHECKPOINT

T cell co-stimulatory receptor, CD28, does not affect T cell activation unless the T cell receptor (TCR) is first engaged by its particular antigen. After the antigen is recognized, CD28 signalling amplifies the TCR signalling to activate the T cells. Cytotoxic T lymphocyte associated antigen 4 (CTLA4), also present on T cells, primarily regulates the amplitude of the early stages of T cell activation. CTLA4 shares the same ligands with CD28, CD80 (also known as B7.1) and CD86 (also known as B7.2) [1 to5]. CTLA4 having a higher overall affinity towards these ligands competitively inhibits CD28. Not only does CTLA4 competitively inhibit CD28 but it also has a signalling mechanism that involves the activation of protein phosphatases such as SHP2 (PTPN11) and PP2A that counteract the kinase signals induced by the TCR and CD28 [6 to 12].

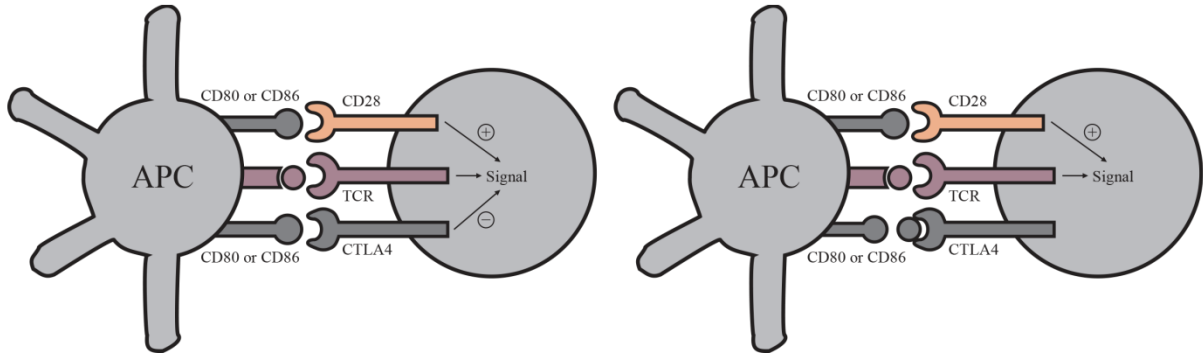


Figure 1. The left portion of the above figure shows how CTLA4 competitively inhibits CD28 (T cell co-stimulatory receptor) in binding to CD80 or CD86 and how the phosphatases counteract the kinase signals produced by the TCR (T cell Receptor) and CD28. The right portion of the figure signifies the CTLA4 blockade by an antibody.

CTLA4 receptors are also known for the removal of CD80 and CD86 ligands from the surface of the Antigen Presenting Cells (APC) [13], to down modulate the Helper T cells and to amplify the immunosuppressive function of the Regulatory T cells (T_{reg}) [14 to 16]. CTLA4 knockout mice were found out to be lethally autoimmune and also the T-cells were hyperactivated [17, 18], therefore the central role of CTLA4 is to keep the T-cell activation in check. T_{reg} cells also express CTLA4, but the mechanism by which CTLA4 enhances the immunosuppressive function of the T_{reg} cells is currently not known. Therefore, CTLA4 blockade could enhance the effector T cell activity and also reduce the T_{reg} cell dependent immunosuppression.

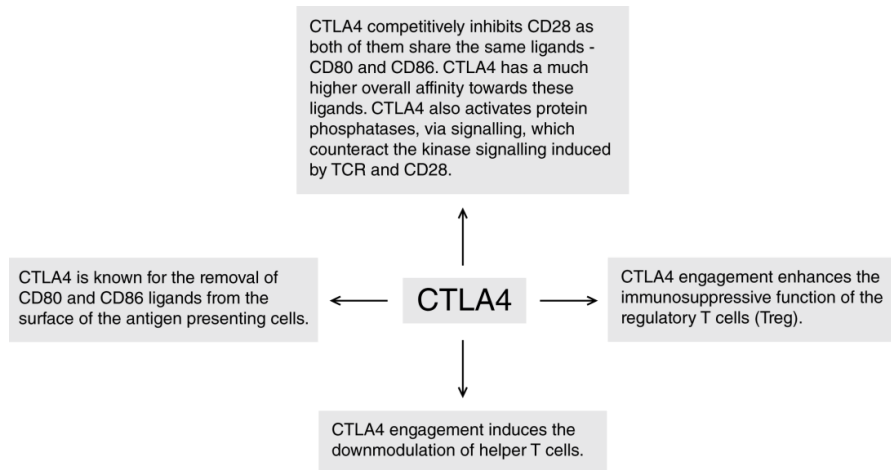


Figure 2. The above diagram represents the working of a CTLA4 immune checkpoint

III. CLINICAL APPLICATION OF CTLA4 BLOCKADE

Preclinical findings encouraged the production of two fully humanized CTLA4 antibodies, Ipilimumab and Tremelimumab, which began testing in 2000. After the phase III clinical trials of Tremelimumab were concluded, it was noted that tremelimumab showed no survival benefits with dose and schedule relative to Dacarbazine (DTIC) [19], which is a standard chemotherapeutic drug used in the treatment of melanoma. Even though the response rates and immune toxicity profiles of both the antibodies in Phase II trials were similar, Ipilimumab was more carefully evaluated at different doses and schedules. A three arm clinical trial [20], that lead to the US FDA approval of ipilimumab is shown in Fig.3.

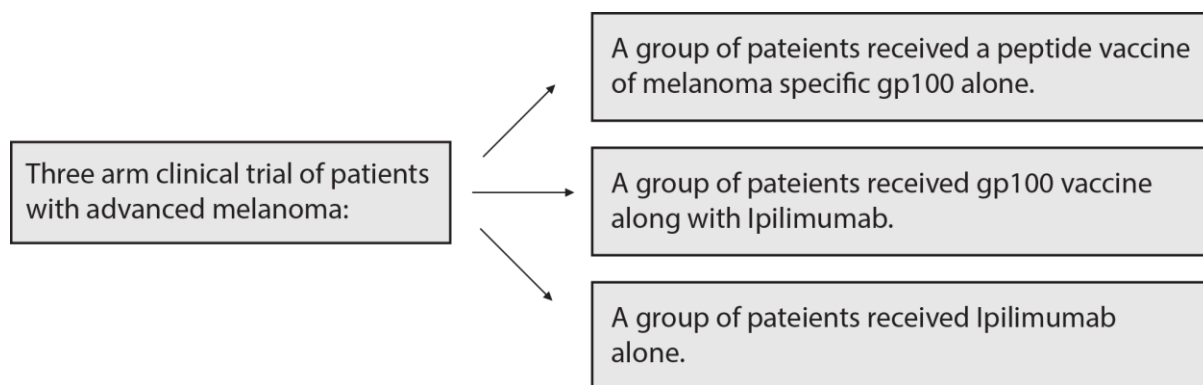


Figure 3. The above figure shows the three arm clinical trial conducted in patients suffering from advanced melanoma. A group of patients received a peptide vaccine, gp100, which stimulates the immune system against the tumor. The next group received gp100 along with ipilimumab and the last group received ipilimumab as a single therapeutic agent alone.

In the above randomized three arm clinical trial of patients with advanced melanoma, shown in Fig. 3, patients treated with ipilimumab with or without the gp100 vaccine showed a mean 3.5 months survival benefit. Dacarbazine was approved by the US FDA on the basis of response rates but has not been shown to provide a survival benefit in patients with melanoma. Therefore, as Ipilimumab was the very first therapy to show a survival benefit, it was approved by the US FDA for the treatment of advanced melanoma in 2010. More impressive than the mean survival benefit was the effect of Ipilimumab on long term survival, that is, the proportion of long term survivors was higher than the proportion of objective responders [20]. Hence this finding of ongoing responses and survival long after completion of a relatively short course of therapy (four doses of 10mg per kg over 3 months) support the concept that immune based therapies might reeducate the immune system to keep tumors in check after the completion of therapeutic intervention [21].

IV. PD1 IMMUNE CHECKPOINT

The major role of PD1 is to limit the activity of T cells in the peripheral tissues at the time of an inflammatory response to infection and to restrict autoimmunity [22 to 28]. Within the tumor microenvironment, this translates into a major immune resistance mechanism [29 to 31]. PD1 expression is induced when the T cells are activated. When engaged by one of its ligands, PDL1(B7-H1) and PDL2(B7-DC) [32 to 34], PD1 through help of the SHP2 phosphatase inhibits kinases that are involved in the T cell activation [32], it is shown in Fig.4. PD1 engagement also inhibits the TCR stop signal which could modify the duration of T cell-APC or T cell-target cell contact [35]. Similar to CTLA4, PD1 is highly expressed on T_{reg} cells, where it may enhance their proliferation in the presence of a ligand [36]. PD1 is more broadly expressed than CTLA4, as it is induced on other activated non T cell subsets, including B cells and the Natural Killer (NK) cells [37, 38]. Therefore, although PD1 blockade is typically viewed as enhancing the activity of effector T cells in the tumor microenvironment, it probably enhances NK cell activity and may also enhance the antibody production either indirectly or through direct effects on PD1+ B cells [39].

4.2 Anomalous Behaviour of CD80

CD80 which acts as a ligand for the T cell co-stimulatory receptor (CD28) and CTLA4 was recently found to function as a receptor when engaged to PDL1, delivering inhibitory signals and reducing the effector T cell functions [40, 41].

4.3 Blockade of PDL1

On the surface of solid tumor cells, the major ligand expressed is PDL1. Based on the known interactions of PD1 ligands, it is theoretically possible that a PD1 antibody would have a distinct biological function compared to a PDL1 antibody. A PD1 antibody would block the interaction of PD1 with both PDL1 and PDL2, but it cannot block the interactions of PDL1 and CD80. In contrast, the PDL1 antibody would block the PDL1 interaction with PD1 and CD80, but cannot block the interaction of PD1 and PDL2. Thus, depending on which type of interaction dominates in a particular cancer, antibodies for PD1 or PDL1 may be used. There is also a theoretical probability of PD1 and PDL1 antibodies to show synergistic effects, when used together.

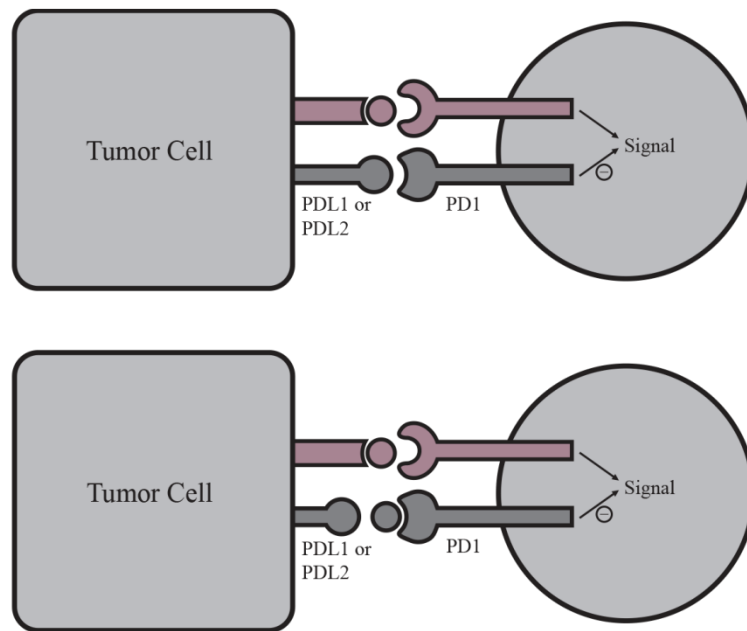


Figure 4. The upper portion of the above figure shows how the production of phosphatases, which are produced via a signaling mechanism when the PD1 receptor is engaged with its ligand, counteract the kinase signaling of the effector T cell. The lower portion of the figure shows the enhancement of the effector T cell signals when the PD1 receptor is blocked by an antibody that is optimized to specifically bind to the PD1 receptor.

V. CLINICAL APPLICATION OF PD1 BLOCKADE

The clinical experience with PD1 antibodies is currently much less extensive than with CTLA4 antibodies, but the initial results do look extremely promising. The initial results from a clinical trial, extending the treatment with anti-PD1 to two years, objective responses were observed in 16 out of 39 patients with advanced melanoma, and an additional 14 patients achieved either a partial response or disease stabilization [42]. Similar response rates have been observed in renal cancer [43], and there is an ongoing evaluation of anti-PD1 in lung cancer. As the frequency of immune related toxicities from anti-PD1 therapy seem to be less than in anti-CTLA4 therapy, and with the promising results of the initial clinical trials, we can conclude that anti PD1 immunotherapy could indeed be a potential immunotherapy that can be standardized for the treatment of multiple cancers.

VI. COMBINATION THERAPIES

Preclinical models validate the dramatic synergy between tumor vaccines and blockade of the immune checkpoint receptors such as CTLA4 and PD1. Given below, in Fig.5, is a diagrammatic explanation of the working of combination immunotherapies. In some instances when the tumor is poorly immunogenic, then the anti-tumor immune response, enhanced by the immune checkpoint blockers, would not be effective enough to eliminate the entire tumor. Therefore anti tumor immunity enhancing vaccines must be administered before the blockade of the immune checkpoints in order to see an effective anti tumor immune response.

So, the future immunotherapies must be developed by keeping in mind the synergy between immune enhancing vaccines and immune checkpoint blockers.

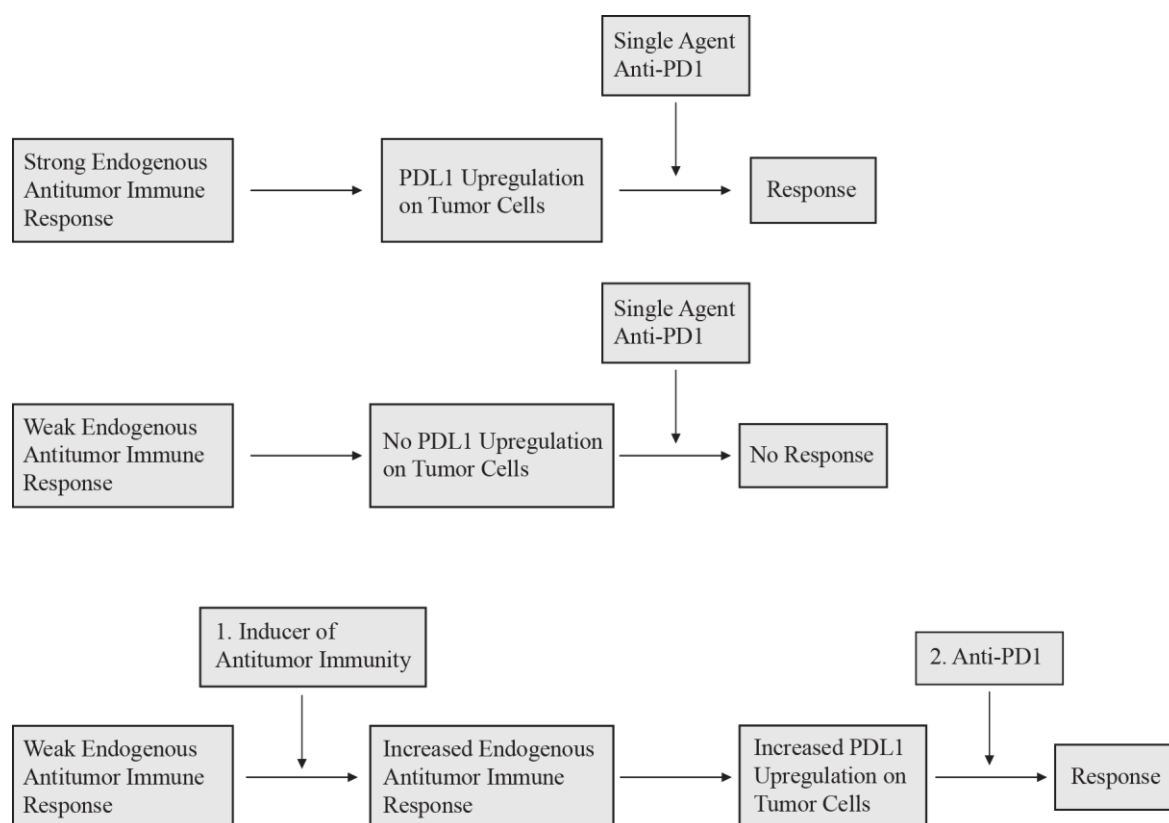


Figure 5. The above figure shows the significance of combination therapies. When a tumor is strongly immunogenic, then even if an antibody against the single immune checkpoint receptor is used as a single agent, we can see a good response. Whereas if the tumor is poorly immunogenic then the endogenous immune response will not be able to eliminate the tumor if an antibody against an immune checkpoint receptor is used as a single agent therapy. Therefore an antitumor immunity enhancer must be used in order to boost the endogenous immune response and later an antibody to block the immune checkpoint pathway can be used in order to see a good response.

VII. OTHER PROMISING IMMUNE CHECKPOINTS AND THEIR CLINICAL STATUS

Given below in Table 1 are a few immune checkpoint receptors and their ligands. They are currently being studied extensively. The blockade of these immune checkpoints also holds a lot of potential.

Table 1. Few promising immune checkpoint receptors and their ligands

Immune Checkpoint Receptor	Ligand
Lymphocyte Activation Gene 3 (LAG 3)	MHC class II
B and T Lymphocyte Attenuator (BTLA)	Herpes Virus Entry Mediator (HVEM)
T cell membrane protein 3 (TIM3)	Galectin 9
Adenosine A2a receptor (A2aR)	Adenosine
Receptor not yet known	B7-H3
Receptor not yet known	B7-H4

Preclinical mouse models of cancer have shown that blockade of many of these individual immune checkpoint ligands or receptors can enhance antitumor immunity, and dual blockade of coordinately expressed receptors can produce synergistic antitumor activities. Inhibitors for a number of these immune checkpoint targets are either entering the clinic or are under active development. The clinical status of few of antibodies used to block the immune checkpoint receptors are given below in Table 2.

Table 2. Current clinical status of few antibodies used to block immune checkpoint pathways

Target	Antibody	Clinical Status
CTLA4	Ipilimumab	US FDA approved for melanoma in 2010. Phase II and Phase III trials ongoing for multiple cancers.
PD1	MDX-1106 (BMS-936558)	Phase I and Phase II clinical trials ongoing in patients with melanoma, renal and lung cancers.
PDL1	MDX-1105	Phase I clinical trials ongoing in multiple types of cancers.
LAG3	IMP321	Phase III clinical trials ongoing in patients with breast cancer.
B7-H3	MGA271	Phase I clinical trials ongoing in multiple cancers.

VIII. IMMUNOSUPPRESSION IN THE TUMOR MICROENVIRONMENT BY THE REGULATORY T CELLS (T_{REG})

CD4⁺ CD25⁺ T_{Reg} cells are crucial for the maintenance of self-tolerance. They play a major role to suppress the effector T cell functions and reduce the collateral tissue damage during immune responses and hence play a major role in the regulation of autoimmune disorders. T_{Reg} cells were found to accumulate in the tumor micro environment and are thought to represent a major immune resistance mechanism that helps the cancer cells to form a sustainable tumor mass. They express the forkhead transcription factor FOXP3. They do not express cell surface molecules that are unique to them, but they do express high levels of multiple immune checkpoint receptors. Genes encoding for some of these immune checkpoint receptors, such as CTLA4, are actually the target genes for the forkhead transcription factor FOXP3 [44, 45]. Although, these immune checkpoint receptors inhibit the effector T cell functions, they enhance the T_{Reg} cell activity or proliferation. An antibody that specifically binds to the T_{Reg} cells has not yet been made, many of the monoclonal antibodies currently in clinical testing block the immune checkpoint receptors that are present on the T_{Reg} cells, which might reduce the activity or proliferation of the T_{Reg} cells, which eventually leads to the enhancement of the effector T cell functions.

IX. CONCLUSION AND FUTURE IMPLICATIONS

Cancer immunotherapies have made significant strides in the past few years due to the improved understanding of tumor biology and immunology. Therapeutic antibodies currently provide clinical benefit to patients with cancer and have been established as standard care agents for several highly prevalent human cancers. There will be many advances in the field of cancer immunotherapies, over the next decade, that will arise in the form of identification of new targets, manipulation of the immune checkpoints and optimization of the antibody structure to promote the amplification of antitumor immune responses.

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