Investigation of CTLA4’s Application in Advanced Melanoma with Ipilimumab and Nivolumab

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Abstract. Conventional cancer treatments are less beneficial in melanoma. The immunogenic tumor nature of melanoma allows patients to obtain favorable clinical outcomes from tumor immunotherapy approaches. One of the “paramount achievement” in oncology has been immune checkpoint inhibitors (ICIs) in the last decade. Among various studies and clinical trials, therapies alleviating melanoma have yielded significant progress by incorporating various immune checkpoint inhibitors such as cytotoxic T lymphocyte antigen-4 (CTLA4). CTLA4 is most likely to become a widely used method. Amongst these therapies, ipilimumab and nivolumab have been used most prevalently, and clinical trials have shown that their combined effect is the most effective. However, their combined effect also results in the most severe side effects. The paper will cover an overview of CTLA mechanisms and clinical studies focusing on ipilimumab and nivolumab and their side effects.

Keywords: Melanoma, CTLA4, Ipilimumab, Nivolumab, Clinical Trials.

1. Introduction

In 2020, 324,635 people have been diagnosed with melanoma worldwide. In the US alone, melanoma has an incidence rate of approximately 22.2%, making it the fifth most common in males and females. Melanoma consists of 4 stages, as demonstrated in Figure 1 below [1]. The fourth stage of melanoma is advanced melanoma, where the tumor undergoes metastasis. Although melanoma only accounts for about 1% of all skin cancers diagnosed in the US, it causes most deaths from skin cancer [2]. Despite its high incidence rate, treatments in melanoma have advanced in the past several decades, increasing its 5-year survival rate to 93.3% [3]. In particular, immune checkpoint inhibition (ICI) with cytotoxic T lymphocyte antigen-4 (CTLA4) inhibition has proven to be an effective new strategy in managing patients with both advanced and regular melanoma cancer. However, immune checkpoint therapy is ineffective for more than half of the patients [4]. Hence, it is crucial to consider its side effects and its application to all patients when evaluating the overall effectiveness of ICIs. More specifically, studies have shown that inhibition of CTLA4 induces immune-related adverse events (IRAEs), such as diarrhea, dermatitis, hepatitis, and endocrinopathies, which negates the treatment’s efficacy [5].

![Stages of Melanoma](image)

**Figure 1.** Stages of melanoma [2]

Metastatic melanoma has been infamously hard to treat. Before developing ICIs, melanoma has put up a solid resistance to both chemotherapy and radiotherapy, the two most conventional approaches to advanced cancer [6]. However, researchers saw new light upon the introduction of ICIs.
Due to the nature of melanoma as an immunogenic tumor, manipulation of the immune system produced a favorable clinical result. A variety of immunotherapy strategies, such as the FDA-approved cytokine interleukin-2 (IL-2), CTLA4 blocking monoclonal antibodies, dendritic cell (DC) vaccines, and adoptive transfer of clonally expanded antigen-specific T cells, have now resulted in long-term tumor responses in metastatic melanoma [7]. CTLA4 has the fewest side effects, is non-prescription, and is an immunotherapy reagent that requires no personal changes. Hence, making it the most potential for becoming a widespread approach.

This paper first introduces the research background for CTLA4 along with its potential mechanisms that would assist in therapy; then, the paper discusses several clinical trials which apply ipilimumab and nivolumab either alone or in combination; lastly, the paper addresses side effects regarding immunotherapy, especially ipilimumab and nivolumab, and some challenges with current biomarkers.

2. Overview of CTLA4

CTLA4 is present on T cell surfaces and binds strongly to stimulatory molecules on antigen-presenting cells. CTLA4 inhibits the binding of antigen-presenting cells to T cells, hence lowering T cell activation. The first immune checkpoint molecule, CTLA-4, was identified in 1987 by French researchers Brunet and colleagues, resulting in a revolution in cancer immunotherapy. Following Brunet's discovery, Dr. James Allison provided evidence that CTLA-4 could prevent T cells in the immune system from generating a complete immune response. In his study, Allison illustrated how regression of established tumors in mice were followed by applying CTLA4 antagonistic antibodies [8].

Based on Allison's findings, CTLA4 inhibiting monoclonal antibodies have been produced and investigated as a possible treatment for various malignancies but have been most widely utilized in melanoma patients. Two completely human CTLA4 inhibiting monoclonal antibodies are currently used: nivolumab with ipilimumab [9].

3. Potential mechanisms of CTLA4

The expression of CTLA4 dampens the activation of T-cell receptor (TCR) signaling and regulates the TCR signal amplitude. CTLA4 competes with co-stimulatory molecule CD28 for the B7 ligands B7-1 (CD80) and B7-2 (CD80) due to its high affinity and avidity (binding strength of an antibody to a binding site) (CD86). Because B7-1 and B7-2 release positive co-stimulatory signals through CD28, competition between CTLA4 and CD28 effectively suppresses TCR signaling, as seen in Fig. 2

![Figure 2. Molecular mechanisms of CTLA4 attenuation of T-cell activation](image)
Aside from regulating the activation of TCR signaling, CTLA4 also attenuates TCR signal amplitude. The degree of CTLA4, contained in intracellular vesicles that travel to the immunologic synapse, directly correlates with the TCR signal strength. When CTLA4 travels to the immunologic synapse, it binds with B7, resulting in its accumulation. The accumulation of CTLA4 outcompetes CD28. Through the trafficking mechanisms of CTLA4, it can reduce the positive signaling by CD28 resulting in “robust regulation” TCR signaling7.

Additionally, CTLA4 can also regulate TCR signaling in a cell-extrinsic pathway. Most CTLA4 cell-extrinsic pathways are mediated through Tregs, a specialized T-cell subpopulation that suppresses the immune response. The loss of CTLA4 activity in Tregs causes aberrant T-cell activation, resulting in reverse signaling to cells expressing B7 co-stimulatory molecules. Anti-CTLA4 antibodies can inhibit Treg activation and the B7 co-stimulatory molecules activation that ensues [10]. By inducing cancer cell apoptosis, CTLA4 engaging reagents have demonstrated a direct effect on the CTLA4-positive melanoma cells. CTLA4-positive melanoma cells were selectively treated with CTLA4 recombinant versions (CD80 and CD86) [11].

4. Clinical trials applying CTLA4 with melanoma

Several investigations over the last decade have attested to the biological and clinical actions of CTLA4 inhibiting monoclonal antibodies. In particular, clinical studies focused on Ipilimumab and Nivolumab, two of the most prevalent immunotherapy drugs for treating melanoma. The results of some selected complete trials are summarized below in Table 1. The overall response rate has been relatively low for Ipilimumab mono-therapy groups, ranging from 0% to 20%. However, the combination of Ipilimumab and Nivolumab produces a better response, ranging from 11% to 60%. Though there is still space for improvements and enhanced treatments, these pioneering clinical trials provide a proof-of-concept that the anti-CTLA4 pathway performed in mice can bring objective benefits to melanoma patients.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Combination</th>
<th>Antibody dose</th>
<th>Scheduled dose</th>
<th>Number of Patients</th>
<th>Objective tumor response (%)</th>
<th>Ref</th>
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<tbody>
<tr>
<td>Ipilimumab</td>
<td>None</td>
<td>3mg/kg</td>
<td>Single dose</td>
<td>17</td>
<td>18</td>
<td>[12]</td>
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<td>Single dose</td>
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<td>0</td>
<td>[13]</td>
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<tr>
<td>Ipilimumab</td>
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<td>3mg/kg</td>
<td>Q3 weeks</td>
<td>100</td>
<td>18</td>
<td>[14]</td>
</tr>
<tr>
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<td>3mg/kg</td>
<td>Q3 weeks</td>
<td>10</td>
<td>20</td>
<td>[17]</td>
</tr>
<tr>
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<td>Q2 weeks</td>
<td>11</td>
<td></td>
<td>[18]</td>
</tr>
<tr>
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<td>gp100 peptides</td>
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<td>Q3 weeks</td>
<td>56</td>
<td>13</td>
<td>[15, 16]</td>
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<td>Q3 weeks</td>
<td>36</td>
<td>22</td>
<td>[21]</td>
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<tr>
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<td>3mg/kg</td>
<td>Q3 weeks</td>
<td>98</td>
<td>39</td>
<td>[14]</td>
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<tr>
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<td>Ipilimumab</td>
<td>1mg/kg</td>
<td>Q3 weeks</td>
<td>9</td>
<td>56</td>
<td>[16]</td>
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<tr>
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<td>1mg/kg</td>
<td>Q3 weeks</td>
<td>73</td>
<td>60</td>
<td>[18]</td>
</tr>
</tbody>
</table>

4.1. Mono-therapy

Tchekmedyian et al. conducted the first phase I study in malignant melanoma in 2002, which marked the first use of CTLA4 blocking monoclonal antibodies in patients by single-dose infusions of ipilimumab. This pioneering study only yielded an 18% objective tumor response in 17 patients,
meaning that only 2 had had objective tumor responses [12]. Following Tchekmedyan et al., Dana Farber Cancer Institute released the first full-length manuscript in 2003 with a single-dose infusion of ipilimumab. Again, the study did not observe any objective tumor response amongst the 7 patients using 3mg/kg of ipilimumab. Nevertheless, these two early experiences provided evidence of tumor regression in patients treated with 3mg/kg ipilimumab. Such evidence laid a solid foundation for the development of future clinical trials [13].

A 2018 study by Parker Institute for Cancer Immunotherapy (Pici) revisited single doses of ipilimumab with increased dosage frequency every three weeks. However, the clinical study only yielded a 20% objective tumor response rate, which is not a significant improvement compared with the 2002 Tchekmedyian et al. study, which yielded an 18% response rate. These two studies across the decade suggest that ipilimumab itself may not be effective enough to target the CTLA4 pathway [14].

4.2. Combined therapy

Following these initiatives, a series of studies from the National Cancer Institute's (NCI) Surgery Branch increased their patient number to 56 patients and improved their immunotherapy therapies in dose frequency and antibody combination. In terms of dosage frequency, the NCI study increased the frequency of dosage from a single dose of ipilimumab to 3mg/kg dose every 3 weeks. In terms of antibody combination, the study forgoes the single ipilimumab treatment for a combination of ipilimumab with two gp100 peptide vaccines. Although the study did not display a significant increase in objective tumor response rate, it marked a trend of increased combined immunotherapy application [15, 16].

Amongst the trend, most clinical studies have focused on comparing the objective tumor response rate between ipilimumab alone and ipilimumab combined with nivolumab. In a 2022 published study, Pici investigated the objective tumor response rate between patients taking 3mg/kg of ipilimumab alone and patients taking 1mg/kg of nivolumab combined with 3mg/kg of ipilimumab. In the ipilimumab mono-therapy group, the patients received ipilimumab every three weeks, and out of the 10 patients participating, 2 were objective responders, yielding a 20% objective tumor response rate. Again, this is consistent with results from previous ipilimumab mono-therapies. However, when the patients took the combination of ipilimumab and nivolumab every three weeks, their response rate increased to 56%, with 5 patients being objective responders out of the 9 patients in the trial [17]. Similar results were obtained from Bristol-Myers Squibb's 2021 published study. Bristol-Myers Squibb used the same dosage and schedule on a larger patient population. Out of the 37 patients treated with ipilimumab, 4 were objective responders, producing an objective tumor response rate of 10.8%. Meanwhile, 44 of the 73 patients treated with ipilimumab and nivolumab combination treatment were objective responders, producing a 60.3 percent objective response rate. Overall, these two studies suggest that a combination of ipilimumab and nivolumab would yield the most effective response [18].

Other clinical trials have attempted combined trials with different antibodies, namely, studies from Amgen et al. and Maker et al. have combined ipilimumab with talimogene laherparepvec (T-VEC) and IL-2 respectively. In Amgen et al.’s study, they performed trials on ipilimumab with or without T-VEC. In their clinical trials, when ipilimumab was applied as mono-therapy, 18 out of 100 patients demonstrated objective responses, yielding an 18% objective response rate which does not vary significantly from previous studies. When ipilimumab was coupled with talimogene laherparepvec, 38 out of 98 patients were objective responders, producing a 38.8 percent objective response rate, indicating that the usage of T-VEC during early progression in conjunction with checkpoint inhibitors might give beneficial outcomes [14, 19]. However, not all combined therapy yielded such significant improvements in objective tumor response rates. In the research by Maker et al., ipilimumab was administered at a dose of 0.1 to 3mg/kg every 3 weeks, along with a high dose of IL-2. The combination therapy was administered to 36 patients, with 8 objective responders, for a 22% objective tumor response rate. Based on their findings, Maker and colleagues concluded that a synergistic
impact of IL-2 and anti-CTLA4 antibodies (ipilimumab) is not substantiated because treatment with either medication alone would result in a comparable observed rate, yielding no additive benefit [20].

5. Side effects

While immune checkpoint inhibitors have been generally well tolerated by most patients and frequently used in clinical trials, as demonstrated above, they may still be associated with an array of side effects [21, 22]. Although most side effects occur in the first twelve weeks of study, the adverse effects can be delayed to several months after the treatment has paused.

Immune-related adverse events (IrAEs) result from immune responses to the body’s own tissues, most commonly affecting the intestine (diarrhea, colitis), the skin (rash, cutis), the liver (hepatitis), endocrine organs, and lungs. The severity degree of IrAEs varies from patients and from treatments. Although, in general, when treated with corticosteroids or other immunosuppressants, most IrAEs are reversible, over half of the patients receiving Ipilimumab treatments require immunosuppressive therapy to minimize the severity of IrAEs. Furthermore, inflammation of endocrine organs (thyroid) frequently leads to irreversible function loss, necessitating long durations of hormone treatment [21].

In general, the European Medicines Agency (EMA) undertook a pooled review of data from 1,551 patients who took part in the pivotal CheckMate 037, 066, 067, and 069 trials for immunological checkpoints.

The total incidence of irAEs among these patients is 95% for Ipilimumab + Nivolumab, 86 percent for Ipilimumab alone, and 78% for Nivolumab alone. 54% of patients treated with Ipilimumab + Nivolumab experienced severe adverse events (CTCAE grade 3–4), 27% of patients treated with Ipilimumab alone experienced it along with 14% of patients treated with Nivolumab alone. These severe side effects lead to many discontinuing the trial. However, their discontinuation did not demonstrate a correlation with the effectiveness of the trial, which remained relatively high. According to recent research, lowering the dose of Ipilimumab may minimize such severe side effects [23].

In particular, for anti-CTLA4 antibodies, adverse effects seem to occur at specific periods [24]. In the first 3-4 weeks, skin-related adverse effects usually occur. Following that, in the next 6-7 weeks, gastrointestinal and hepatic adverse effects would occur. Then, around 9.2 weeks, endocrine adverse effects can be seen. Months after the treatment, hypophysitis may be seen. It is important to note that these side effects will occur earlier with the combination of Ipilimumab and Nivolumab.

6. Current biomarkers

Recent advancements in immunotherapies have greatly improved the clinical management of metastatic melanoma. With the help of immunotherapies, such as anti-CTLA4 antibodies, many patients can receive durable and even complete remissions. Despite the fact that immunotherapy is becoming more effective in treating melanoma, more than half of patients do not react to it. Hence, in order to resolve such a problem, scientists have focused on identifying biomarkers that are able to predict a patient’s response prior to the treatment. Such prediction would maximize the therapeutic efficacy, avoid undesirable adverse effects and save the patients from spending unnecessary treatment fees.

6.1. LDH

Previously, doctors primarily employed the TNM staging method as a diagnostic and prognostic marker. Lactate dehydrogenase (LDH) was included in the AJCC recommendations in 2009 after it displayed predictive abilities of survival rate in melanoma [25]. Due to the increased glycosides in cancer cells’ rapid metabolism, a large quantity of LDH is released. [26]. Hence, it can serve as an indicator of tumor burden. Increased LDH levels in immunotherapy clinical trials are an unfavorable prognostic indication for individuals receiving Ipilimumab and Pembrolizumab. [27].
6.2. S100

S100 is used as an indicator during the advanced clinical disease stage. In a Kelderman et al. research, he and his colleagues established that S100 might predict patients' response to anti-CTLA4 therapy [28]. S100, like LDH, has a similar shortcoming in that it appears to be primarily a proxy for the melanoma cancer stage, capable of identifying individuals who are unable to react to immunotherapy due to high levels of tumor burden but unable to suggest or predict responses to immunotherapies. The same limitation is mirrored in another possible biomarker presented by Diem et al. to stratify patients prior to CTLA4 treatment by counting the number of organs involved: the number of organs involved [29].

7. Future biomarkers

7.1. Whole exome sequencing (WES)

When assessing a tumor’s genomic landscape, whole exome sequencing (WES) and RNA sequencing (RNAseq) are utilized in tandem. While WES may only capture exonic gene regions, RNAseq can provide information about the whole transcriptome of a single sample and is thus used to create gene signatures. WES is very effective in detecting neoantigens in melanoma tumors when used in immunotherapies. Melanoma cancer has one of the fastest mutation rates among all malignancies. Hence it is more likely to develop neoantigens. Because it is only expressed by tumors and not by the body’s tissue, neoantigens are an attractive target for immunotherapies.

Many researchers have used WES and RNAseq to explore the CTLA4 pathway gene expressions in patients and mutation profiles. Snyder et al. were the first to do WES on 64 anti-CTLA4 treated patients [30]. They assumed that a neoantigen signature is associated with clinical benefit. In their conclusion, they found that although their assumption was correct, it was only partially true since the patients’ response to CTLA4 depends on the specific types of neoantigens expressed. Following Snyder et al.’s study, another study of WES on anti-CTLA4 was performed by Van Allen et al. on 110 patients. They were able to confirm Snyder’s conclusion that neoantigen mutations are associated with clinical benefits for anti-CTLA4 treatments [31]. They would not, however, identify the neoantigen signature indicated in Synder et.al. Van Allen and colleagues determined that therapeutically advantageous neoantigens are most likely unique to each patient, but recurring neoantigens in the general population are extremely rare.

7.2. T-Cell Receptor (TCR) Profiling

TCR repertoire profiling can investigate intratumoral T-cell responses since antigen recognition by T-cells is dependent on tumor-specific T-cell production and clonal amplification. In terms of immunotherapy, TCR repertoire profiling is used as a potential biomarker to enhance the recognition of cancer cells by the immune system.

Several researches have been carried out to examine the TCR repertoire profiling in anti-CTLA4 therapy. In 2014, Robert et al. compared pre-and post-treatment peripheral blood mononuclear cell (PBMC) samples from melanoma patients treated with anti-CTLA-4. The study finds that anti-cTLA4 therapy promotes TCR repertoire diversity, although in an unspecified way. Following Robert et al.’s discovery, Cha and his colleagues conducted more research on the possible mechanism of anti-CTLA4 therapy. Cha et al.’s investigation was likewise conducted on melanoma patients, but it focused on changes in TCR repertoire in PBMCs between baseline and 4 weeks of therapy [33]. Their study confirmed Robert et al.’s conclusion that anti-CTLA4 treatment results in changes in clonotype frequency, and, on top of that, demonstrated that diversification is induced by increased levels of new clonotypes. Additionally, Cha and colleagues showed that non-naïve T-cells clones expanded most in response to therapy, conveying that patients who have pre-primed T-cells prior to the treatment will respond to the anti-CTLA4 therapy. Postow et al. hypothesized that the shape of the TCR repertoire before the beginning of CTLA4 therapy might alter the likelihood of a response to the
treatment, based on recent findings by Robert et al. and Cha et al. He performed his studies on 4 responders and 8 non-responders’ blood samples in an anti-CTLA4 treatment. The TCR repertoire’s richness and evenness were assessed in blood samples. Postow and colleagues discovered that a low richness or evenness of the TCR repertoire correlates with a poor response to anti-CTLA4 therapy [34]. Their findings imply that when a TCR repertoire consisted of fewer varied clones or leaned towards a small number of specific clones, it will be correlated with a reduced or non-response to anti-CTLA4 therapy.

8. Conclusion

Despite the advancements in both investigation of CTLA4’s mechanisms and clinical trials using ipilimumab, challenges remain when therapies attempt to alleviate and eradicate melanoma. Namely, these challenges arise when current biomarkers cannot efficiently and effectively identify the patients best suited for immunotherapy using CTLA4 pathway and other biomarkers are not ubiquitous enough to be offered to all patients. In the future, in order to achieve ubiquitous monumental success, suitable biomarkers for all patients must be identified. In summary, this review combined the current clinical results of ipilimumab and nivolumab, their side effects, and overall challenges in oncology.

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