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An Investigation into the Impact of Targeting CTLA-4 with Ipilimumab for Metastatic Melanoma Treatment: A Literature Review

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Abstract

Skin cancer in the form of metastatic melanoma (MM) is often accompanied with a poor prognosis, due to its high propensity to spread to other bodily tissues coupled with low remission rates. Although MM is treatable, the five-year survival rate is estimated to be a mere 30%. Common treatment options include radiation, chemotherapy, and immunotherapy. Due to the current limitations associated with cancer treatment such as the use of radiation and chemotherapy, the use of ipilimumab (immunotherapy) as a novel therapeutic intervention serves as a prominent way to treat cancer patients. However, ipilimumab monotherapy is not considered the first line of treatment for metastatic melanoma. Immunotherapy targets T-cells, which are white blood cells that cause an immune response and the apoptosis of cancer cells. Thus, the development of new monoclonal antibodies (ipilimumab) can prove to be an effective way to treat cancer patients with limited risk. Anti-PD-1 therapy can also serve as an alternative treatment option for metastatic melanoma. Specifically, nivolumab, a fully human immunoglobulin G4 PD-1 immune checkpoint inhibitor antibody blocks PD-1 (programmed death-1) and promotes antitumor immunity. It has also been shown to be successful in the treatment of other cancers, such as renal cell carcinoma and small-cell lung cancer. Growing and progressive research indicates anti-CTLA-4 (ipilimumab) and anti-PD-1 (nivolumab) combination therapy serves as a more effective way to treat MM in comparison to anti-CTLA-4 monotherapy via the use of ipilimumab alone. This information can be utilized to advance further research in this field. To conduct our research, specific search terms were created, and relevant articles were screened on Covidence using an inclusion/exclusion criteria. The CRAAP checklist will be used for the quality assessment of the utilized sources.

Keywords: Ipiluminab, CTLA-4, Metastatic Melanoma, Monoclonal Antibody (mAb), T-cells

Introduction

Skin cancer in the form of metastatic melanoma is a particularly aggressive form of cancer, making it difficult to treat. Distant or metastatic melanoma is a stage where the cancer has spread away from its origin-the skin, to other distant parts of the body. According to statistics from the American Cancer Society, the five-year survival rate for distant stage melanoma is a grim 30% [1]. There are multiple treatment options such as radiation, chemotherapy, and immunotherapy. It is widely accepted that radiotherapy does not increase patient survival rates, however it is an effective form of treatment option for symptomatic lesions. Hence, a novel treatment option such as immunotherapy should be considered [2]. Immunotherapy targets T-cells, which are white blood cells that cause an immune response and invoke the apoptosis of cancer cells [3]. The treatment of metastatic melanoma with interleukin-2

(IL-2), a cytokine that stimulates T cells, was approved by the FDA several years ago, but not all melanomas responded [2]. The finding that cancerous melanoma cells express high levels of a T-cell inactivator protein called cytotoxic-T-lymphocyte antigen-4 (CTLA-4) has led to the development of new drugs for melanoma treatment [4].

In the regular immune response to cancer, the first step is the exhibition of tumor-specific antigens by antigen-presenting cells (APCs) [5]. APCs include macrophages, dendrites, and B cells [5]. The major histocompatibility complexes (MHCs) on the surface of APCs bind to cancer antigens, and then bind to T-cell receptors (TCRs) on T-cells, ultimately producing a signal.[5] The T-cells also contain a receptor called CD28 which is expressed on the cell surface.[5] APCs express membrane proteins B7-1 and B7-2

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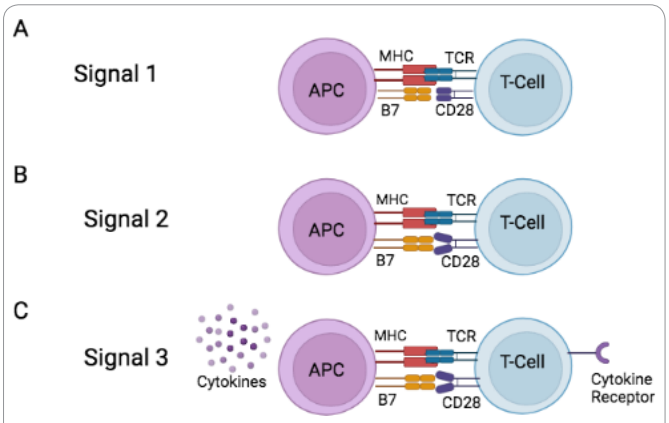

Figure 1. *The activation of a T-cell.* On the surface of the APC, the B7 protein (purple) and the MHC (orange) are shown, with an antigen (pink) on the MHC. On the surface of the T-cell is the TCR (yellow) and CD28 receptor (green). Signal 1 shows the binding of the TCR to the MHC. Signal 2 shows the binding of CD28 to the B7 proteins. An additional signal, Signal 3, shows cytokines (gray) being released from the APC, which will then bind to the receptor on the T cell [7].

which can bind to this receptor, producing a second signal, as shown in Figure 1B [6,7]. Together, these signals activate T-cells, leading to the rapid proliferation and attack on tumor-associated cells [7].

CTLA-4 operates as an immune checkpoint, blocking the pathway of T-cell activation to prevent autoimmunity [8]. Upon activation of the naïve T-cells, a signal is generated to cause the upregulation of CTLA-4 on the surface of T-cells [9]. CTLA-4 inhibits T-cell activation by binding to B7 proteins on APCs with a higher affinity than the CD28 receptor [5]. Once the B7 protein is bound, CTLA-4 inhibits the protein kinase B (PI3K/AKT) pathway, which is crucial for T-cell growth and proliferation [10,11]. This, in turn, reduces the immune response due to the decrease in

T-cell activation [12]. Since APCs present multiple B7 binding sites for T-cell receptors, any binding between CTLA-4 and B7 reduces the number of free B7 sites [5]. Therefore, the activation of T cells and their subsequent proliferation is dependent on the number of CTLA-4-B7 bindings (Figure 2A) relative to the CD28-B7 bindings (Figure 2B) [12].

The identification of T-cell regulation by CTLA-4 induces the development of ipilimumab, an anti-CTLA-4 monoclonal antibody [8]. The presence of other therapies that are present indicate that ipilimumab as a monotherapy is not a primary treatment modality for metastatic melanoma.

Monoclonal antibodies are lab synthesized molecules engineered to serve as substitutes for the body's natural antibodies, often designed by utilizing mouse-derived proteins [13]. These antibodies function in a variety of different manners to aid in the immune system response. For example, monoclonal antibodies bind to cancer cell receptors to flag them, trigger cell membrane destruction, block cell growth, and block immune system inhibitors [14]. By interpreting the signals of an immune response to cancer, scientists hypothesized that antibody driven blockades of CTLA-4 would promote tumor suppression [15]. Blockades were examined in syngeneic tumor transplant models and shown to display the predicted antitumor effect [15]. These results led researchers to develop human monoclonal antibodies

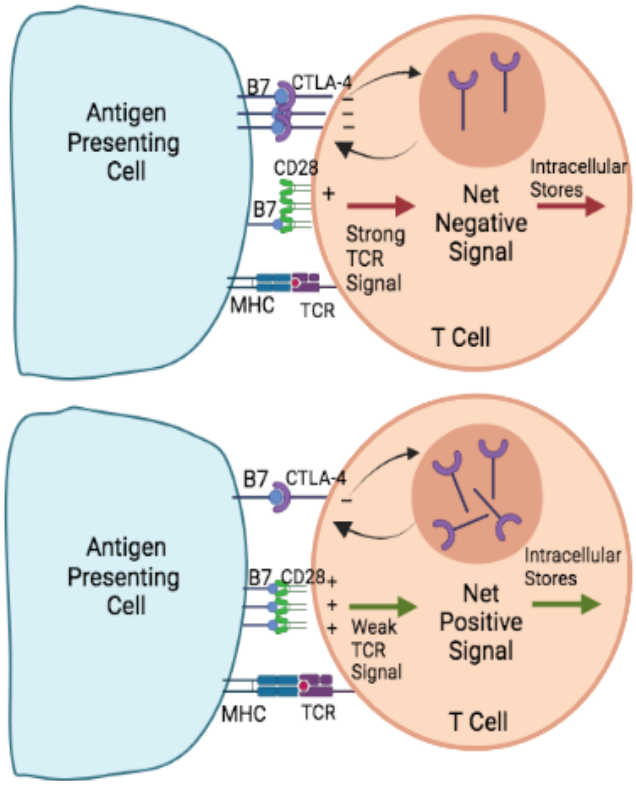

Figure 2. *The CTLA-4 binding mechanism.* (A) shows a net positive signal that promotes cell proliferation, and (B) shows a net negative signal that inhibits cell proliferation [12].

that block CTLA-4, resulting in the generation of ipilimumab [15]. In clinical trials, when ipilimumab was tested against MM, there was significant tumor suppression, as well as a 32% mortality risk reduction [15]. Ultimately, ipilimumab was FDA approved for metastatic melanoma treatment in 2011, effective due to the immunogenic nature of the cancer [15].

Methods

The first step towards conducting the literature review was to use the keywords mentioned above to conduct a search in Google Scholar, which were then added to an online screening tool called Covidence. Initially a total of 145 articles were added to Covidence from google scholar. There were no duplicates. The screening was completed independently by two of the authors (Patel, Ghai, Sharma) with the third and fourth authors (Das and Joshi) resolving any discrepancies. Any articles that were published before the year 2000, written in a language other than English or lacked scientific data (editorial, conference abstract, trial description) or lacking relevant information (ipilimumab, CTLA-4, Metastatic Melanoma, Monoclonal Antibody (mAb), T-cells) were excluded. In the first round of screening, the two reviewers (Sharma and Ghai) screened the abstracts of the articles to exclude any non-eligible articles. This step resulted in the exclusion of 68 articles, leaving 77 articles, out of the previously added 145 articles, for further screening. In the second step, the full body texts of the remaining articles were screened to determine their eligibility in terms of the presence of relevant information. After the thorough screening, only 27 articles were left that were utilized to extract information and

data needed to conduct this review. This was followed by the Currency, Relevance, Authority, Accuracy, and Purpose (CRAAP) test, which allowed us to evaluate, assess and eliminate bias from the reviewed article with the help of a list of questions [5] .

Mechanism of Action of Ipilimumab

As previously mentioned, ipilimumab is a fully human monoclonal antibody [5]. Antibodies consist of a general structure of two heavy chains and two light chains, each of which consists of a variable and constant region [16]. The variable region can be further divided into three regions known as the complementarity determining regions (CDRs), called HCDRs and LCDRs for heavy and light chains, respectively [16]. These are the regions where specific antigens will bind to their corresponding antibody [16]. In this case, ipilimumab binds to CTLA-4, forming approximately 13 hydrogen bonds between the two of them [17]. This enhances T-cell activation and proliferation, such that T-cells can then target cancerous cells for destruction (Figure 3) [5].

CTLA-4's extracellular domain is comprised of two β-sheet faces: the front A'GFCC'C'' β-sheet face and the back ABED β-sheet face (Figure 4) [17,18]. The front β-sheet participates in

binding to the three regions of each of the HCDRs and the LCDRs in ipilimumab (Figure 5 & Table 1) [17]. Ipilimumab surrounds the front β-sheet of CTLA-4 with LCDR1 and LCDR3 on one side, and HCDR1 and HCDR2 on the other. LCDR1 and LCDR3 participate in hydrogen bonding interactions along the G strand [17]. The F and G β-strands participate in stacking interactions with HCDR1 and HCDR2 [17]. This results in both hydrogen bonds and hydrophobic interactions between these regions, whereas HCDR3 participates solely in hydrophobic interactions [17]. LCDR2, while involved in binding, does not play as significant of a role as the other CDRs [17]. The only possible interaction of LCDRR2 occurs between the hydroxyl group of tyrosine 50 of LCDR2, and serine 44 of CTLA-4 [17]. Another important interaction occurs with the FG loop of CTLA-4 [17]. The FG loop, also known as the (^{99}MYPPPY^{104}) loop, is a proline rich motif that connects the F β-strand to the G β-strand (Figure 4) [17]. This loop is important in allowing CTLA-4 to bind to B7 [17]. Ipilimumab interacts with the FG loop by binding to it and sterically obstructing it [17]. The binding is evident

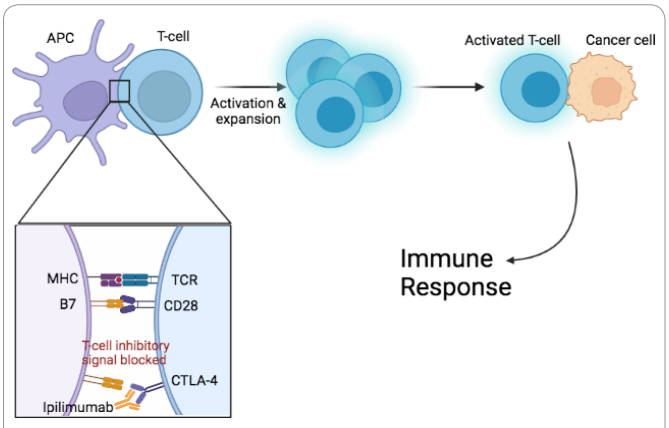

Figure 3. *The overall mechanism of ipilimumab.* Ipilimumab binds to CTLA-4, and the TCR binds to the antigen (fuchsia) found on MHC. This allows CD28 to bind to B7. The result is T cell activation and then proliferation, such that the T-cells can attack the cancer cells [5].

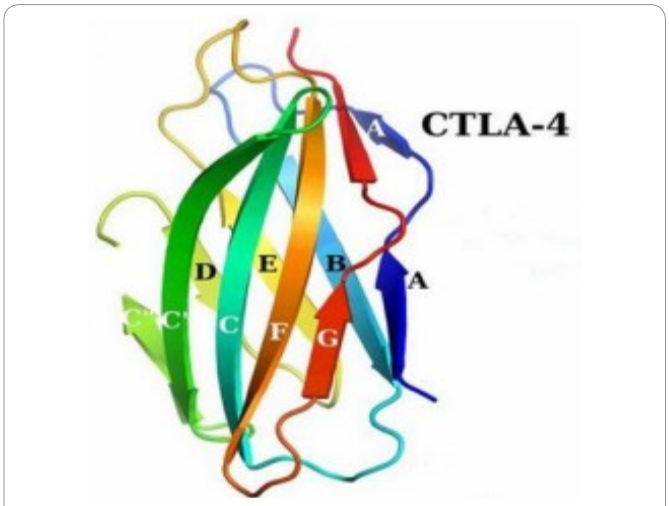

Figure 4. *The outer structure of CTLA-4.* Rainbow representation of the front and back β-sheet faces of CTLA-4's structure. The strands that make up the front β-sheet face are labeled in white lettering, whereas the strands that make up the back β-sheet face are labeled in black lettering [17].

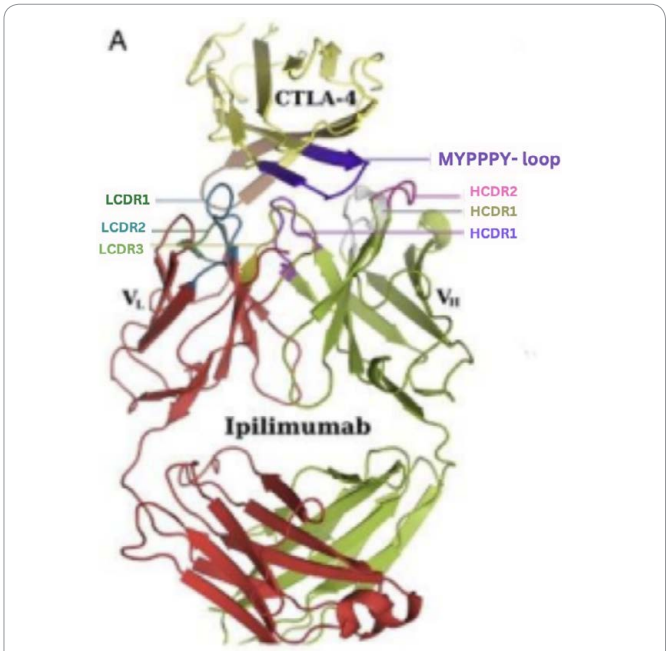

Figure 5. *Ipilimumab and CTLA-4 binding.* The top portion contains CTLA-4, with the FG loop and CC' loop labeled. The ipilimumab ribbon diagram is shown bound to it, containing LCDR1, LCDR2, and LCDR3, as well as HCDR1, HCDR2, and HCDR3 [17].

Table 1: Important amino acids involved in binding for CTLA-4 and ipilimumab 17 [27]

CTLA-4		Ipilimumab	
β-strand/loop	**Amino Acids**	**Type of Chain**	**Amino Acids**
C	^{3}Glu, ^{35}Arg,^{39}Leu	LCDR1	^{31}Ser,^{33}Tyr
C'	^{46}Val	LCDR3	^{93}Gly, ^{94}Ser, ^{95}Ser, ^{97}Trp
F	^{93}Ile, ^{95}Lys,^{97}Glu	HCDR1	^{28}Thr,^{29}Phe, ^{30}Ser
G	108 Ile	HCDR2	^{52}Ser, ^{53}Tyr, ^{57}Asn, ^{59}Tyr
FG	^{99}MYPPPY^{104}	HCDR3	^{101}Trp,^{102}Leu

Note that the order of the type of β-strand loop and the type of chain does not necessarily mean that they bind. Also note that LCDR2 does not contribute significantly to the binding.

through hydrogen bonding with amino acids such as serine 95 found on LCDR3, which contributes two hydrogen bonds and a tyrosine 104 residue, which contributes one hydrogen bond, and proline 103, which also contributes one hydrogen bond [17]. Furthermore, tyrosine 59 of HCDR2 contributes a hydrogen bond by interacting with the carbonyl oxygen of methionine 99 of the FG loop [17]. The steric obstruction of the FG loop, this is caused by the stacking interactions from the aromatic residues of ipilimumab [17].

The effectiveness of ipilimumab in inhibiting CTLA-4 from binding to B7 is due to ipilimumab having a higher binding affinity for the CTLA-4 receptor than the B7 protein [10]. Ipilimumab therefore outcompetes CTLA-4 in binding to B7 [19]. Ipilimumab's binding affinity for CTLA-4 is given by an equilibrium dissociation constant (KD) of 18 nM, which is far lower and better than that of B7-1, given by KD = 420 nM [19]. This can be explained structurally, as ipilimumab extends its interaction with CTLA-4 to the CC' loop, but B7 does not [19]. The binding of ipilimumab to the CC' loop causes CTLA-4 to have concave front β-sheet face [17].

In comparing CTLA-4 and CD28, it is important to note that they share approximately 30% sequence identity [17]. Further, the FG loop amino acid sequence is highly conserved for these two receptors [17]. Therefore, ipilimumab needs to be capable of differentiating between the two structures such that it will not bind to CD28 [17]. One reason why it is believed that ipilimumab can differentiate between the two is because CTLA-4 contains leucine 39 and isoleucine 93, which interact with tryptophan 101 and leucine 103 of HCDR3 of ipilimumab [17]. Comparatively, the histidine and phenylalanine at residues 38 and 93 of CD28 interact with HCDR3 [17]. Another difference is that CD28 contains an insertion of glutamate on the G strand.[17] This results in the β bulge of the G strand sticking out even more, making it easier for ipilimumab to differentiate between the two homologs [17].

Impact of Ipiluminab: Results

Upon interacting with CTLA-4, ipilimumab allows T cells to mediate an antitumor response. Multiple clinical trials over the last few decades have shown increased survival rates in patients with advanced distant melanoma. This is why ipilimumab with a dosage of 3 mg/kg is now utilized commercially for the treatment of metastatic melanoma. Typically 75% of the patients diagnosed with advanced melanoma survive less than a year [20]. However, when patients who have previously received MM treatment, were given 3 mg/kg and 10 mg/kg ipilimumab, their 5-year-survival rates increased by 16.5% and 18.2% respectively [20]. People who have received ipilimumab treatment have shown significantly increased overall survival compared to treatment with gp100 melanoma peptide vaccine alone. In a study, when ipilimumab was administered with chemotherapeutic agent dacarbazine (DTIC), the risk of disease progression reduced by 24% along with increased survival, in comparison to treatment with DTIC only [20]. However, this specific treatment regime has a higher incidence of adverse effects than treatment with ipilimumab alone, which happens due to the combination with DTIC, which alone can cause low level hepatotoxicity [20]. Another study found that the five year recurrence free survival rate was 40.8% and overall survival was 65.4% if a previously treated patient was given 4 doses of 10 mg/kg ipilimumab every 3 weeks and then every 3 months for 3 years or until disease recurrence [20]. However, with such high doses, there are also a lot of associated adverse effects. While the vast majority of AEs were resolved within 4 to 8 weeks, there are some that take as long as 54 weeks or a long term hormone replacement therapy [20].

Future Considerations: Safety and Developments

While the blocking of CTLA-4 by ipilimumab and subsequent T-cell proliferation is advantageous for cancer treatment, it can have unfavorable side effects. Extreme increases in T cell activity may lead to autoimmune disorders, such as pruritus and organizing pneumonia [21,22]. These are classified as immune-related adverse events (irAEs), which are triggered by antigens common between cancerous and healthy cells, resulting in T-cell attacks on both [23]. For instance, research has shown there are about nine candidate shared antigens for dermatology related irAEs, which are the most common type of irAEs following treatment with ipilimumab [23]. It may also lead to immunotherapy toxicities such as diarrhea, nausea, vomiting, colitis, thyroiditis, and fever which are most common. More rare ones include back pain, hypotension, neuropathy, and heart failure. Also, an analysis of fourteen studies evaluating the effects of ipilimumab doses showed that 64.2% of patients experienced an irAE [24]. Another trial with 10 mg/kg, showcases 22% of patients who experienced an irAE. In contrast, the toxicities of PD-1 blockade with nivolumab are similar for doses that were ranging from 3 to10 mg/kg. In another study, 5% of the patients in one trial who received nivolumab from 3-10 mg/kg suffered through a grade 3 to 4 irAEs (for example, colitis and optic neuritis). As such, several clinical trials are currently exploring ipilimumab treatment with chemotherapy, targeted therapy, and vaccines, aiming to reduce irAE occurrence [25]. They are also testing the effects of reduced doses or a greater duration between doses, such as 1 mg/kg every three to four weeks, relative to the current standard of 3 mg/kg [25].

As of now, the combination of ipilimumab with another checkpoint inhibitor, nivolumab, has the most success for metastatic melanoma treatment, giving patients an estimated five-year survival rate of 52% [20]. Most notably, the frequencies of Ki-67+ cells were comparably elevated in both PD-1− and

PD-1+ CD8 T cells following combination ipilimumab plus nivolumab therapy, consistent with our preclinical findings [20]. This suggests that monotherapies are sufficient to induce the proliferation of already activated PD-1+ CD8 T cells and combination therapy is able to induce the proliferation of PD-1+ and PD-1 low CD8 T cells [20].

However, this combination treatment is associated with an increase in irAEs, and about half of patients still do not respond [27,28]. Given this, other treatments, namely T cell receptor (TCR) therapy, are being explored [26]. This therapy relies on genetically modifying a patient's T-cells to recognize neoantigens, which are the mutated antigens expressed strictly on cancer cells [26]. Limited information on clinical outcome is available at this point, but a combination approach of immune checkpoint inhibitors and TCR therapy is starting to be tested, with the goal of making tumors more permissive to T-cell attack [26]. TCR therapy will also continue to be tested in patients who have shown progression in immune checkpoint inhibitors [26].

Conclusion

Ever since its approval nearly a decade ago, the use of the anti-CTLA-4 monoclonal antibody ipilimumab has been crucial in the treatment of metastatic melanoma. CTLA-4, a checkpoint protein expressed on T-cells, prevents autoimmune responses by binding to the B7 costimulatory protein more frequently than the activation-inducing CD28 receptor does. This inhibits T-cell activation, which may also prevent T-cells from attacking cancer cells. As ipilimumab binds to CTLA-4, it prevents CLTA-4 from binding to B7, and CD28-B7 binding is promoted. This binding cascade ultimately allows for

T-cell proliferation, resulting in cancer cell death. Recent research on nivolumab and anti PD-1 therapy indicates that this treatment can be deemed more plausible as opposed to anti-CTLA-4 monoclonal antibody therapy. Nivolumab alone can block PD-1 checkpoint leading to adaptive tumor growth regulation. Fortunately, ipililuminab and nivolumab consolidation showcases the ability to promote the proliferation of PD-1 CD8 T-cells. This treatment prolongs the 5 year life expectancy of patients by approximately 52%, which is far greater than solely ipilimumab therapy. Although the ipilimumab and nivolumab combined therapy posits severe side effects, metastatic melanoma should continue to be explored in the hopes of improving response, survival rates and the potential of discovering a more effective and safe form of treatment for metastatic melanoma.

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