REVIEW ARTICLE

Immune Checkpoint Inhibitors Targeting CTLA-4 in Cutaneous Malignancies: Recent Updates

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ABSTRACT

With the gradual rise of the occurrence of cutaneous and other malignancies, and due to lack of an effective-most therapeutic means free of associated adverse effects, **target protein inhibition-based immunotherapy has gradually become the need of the day.** Among all types of malignancies, skin cancers have been reported to be profoundly sensitive to immunotherapy. As of now, immune checkpoint inhibition, targeting CTLA-4, is a highly specific type of immunotherapeutic technology. Reported research on monoclonal antibodies and other anticancer drugs used as anti-CTLA-4 immune checkpoint inhibitors, prove this novel technology as a means to provide potential therapeutic benefits to patients with severe cutaneous malignancies. These discoveries have fueled the thought that immunotherapy involving immune checkpoint inhibitors like anti-CTLA-4 ligands, would be one of the most effective therapeutic means to combat skin and other cancers. Future advancements in anticancer immunotherapy, with special reference to therapy based on anti-CTLA-4 inhibitors, will depend on the success of the use of a combination of target-specific anticancer drugs targeting various active immune regulatory pathways and thereby alter the immunological responses in the host, in a coordinated and synergistic manner.

Keywords: Cancer, CTLA-4, Immune checkpoints, Immunotherapy, Skin cancer.

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INTRODUCTION

Many epidemiological data have shown over the last half-century that the incidence of cancer has risen rapidly. One of the most prevalent classes of human malignancies is skin cancer. The two major classes of skin cancer are *I.* Melanoma and *II.* Non-melanoma skin cancers (NMSCs). The latter class further has two parts, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Due to the lack of an effective-most therapeutic means free of associated adverse effects, target protei inhibition-based immunotherapy has become the modern cornerstone for combating skin cancer. In 2013, cancer immunotherapy has been named as "landmark of the year" in oncology.[1] Clinical studies and successful research on antibodies and other ligands blocking the immunological checkpoints like cytotoxic T lymphocyte antigen-4 (CTLA-4), programmed cell death protein-1 (PD-1), and programmed death-ligand 1 (PD-L1) proteins, have generated a lot of successful advancements in the field of oncotherapy.[2] T cell-mediated immune responses are mainly regulated by stimulatory and inhibitory mechanisms, ensuring a perfect equilibrium of activities on antigens and self-tolerance.[3-5] Immune checkpoints are important components of the immune system's negative-regulatory pathways, which protect the host from autoimmunity and hence, sustain self-tolerance.[6-11] During the initial phases of anti-tumor T cell responses, antibodies that block the immune checkpoints, target these co-inhibitory pathways successfully overcoming tumor immune repression in the context of cancer. Immune checkpoint blockade therapy has revolutionized the skin cancer treatment with antibodies targeting immune checkpoints by eliciting responses that can be remarkably durable and presently advancing to combat other malignancies.

In the last decade, cytotoxic T-lymphocyte protein 4 (CTLA-4) has been targeted by monoclonal antibodies (mAbs), resulting in a revolutionizing approach for the treatment of advanced cancers.[2] Tumor degradation by immune-mediated mechanisms has long been a possible therapeutic pathway. A better understanding of tumor progression and the complexity of the tumor microenvironment (TME) have discovered several mechanisms by which growths can avoid immune destructions and actively quash the immune responses.[12] As a result of an *ex vivo* study, tumor-induced immunosuppression can mainly be reversed using tumor-emitted chemokines to activate FoxP3+ regulatory T cells (Treg).[13,14] CTLA-4 is a crucial checkpoint

that serves as a negative T cell migration regulator and carries tumor located Tregs that have been subjected to *in vivo* studies in a range of conditions, including CTLA-4 deficient mice.[15] In *in vivo* studies, programmed death-ligand 1 (PD-L1) expressed by tumor cells, has been found to promote suppression of T cell activities, in mouse malignancies.[16] The overexpression of PD-L1 thus enhances the development of FoxP3+ Tregs when interacting with the T cell-associated checkpoint receptor programmed cell death protein-1 (PD-1), also known as CD279.[17] Immune checkpoint blockade therapy has been discovered as a potential therapeutic target for overcoming TME-mediated immunosuppression and restoring anti-tumor immunity.[18] The present review focuses on the current scenario and prospects of CTLA-4 blockade therapy and FDA-approved CTLA-4 inhibitors as anticancer drugs, as well as how immune checkpoint inhibitors have already revolutionized malignant growth immunotherapy.

PROMISING BIOMARKERS FOR PREDICTING RESPONSES TO IMMUNOTHERAPY

Immune checkpoint inhibitor (ICI) therapy has sparked an innovative age in anticancer treatment, with persistent responses and substantial survival seen in various cancer patients. Subsequently, further researches have led to the discovery and development of prescient biomarkers for the identification of various ICIs, and understanding of the tumor genome and neoantigen. Various promising biomarkers for predicting responses to immunotherapy have been described in Figure 1.[19]

THE CYTOTOXIC T LYMPHOCYTE ANTIGEN (CTLA-4)

CTLA-4 (CD125) is a protein receptor and member of the growing immunoglobin superfamily of co-inhibitory receptors.[20] It is also the first protein receptor molecule identified as a negative stimulatory molecule, which is upraised initially when the T cell is activated. CTLA-4 has been found to sustain the homeostases of lymphocytes and anti-autoimmune response regulation in mice, due to the

early death of these mice because of lymphoproliferation and autoimmunity.[21,22] CTLA-4 gathers among T-cells and antigen-presenting cells (APCs) at the immunological synapses where CTLA-4 is stabilized by binding with CD80 ligands.[23,24] CTLA-4 competes with the costimulatory molecule CD28 due to its higher affinity for CD80/CD86, contributing to the negative regulation of activated T cells.[25-27] This strength of negative signs brings about decreased T cell multiplication and diminished interleukin (IL)-2 productions.[28,29] Research has shown that by inhibiting CTLA-4 and thereby freeing the B7 proteins to interact with the co-stimulatory particle CD28, tumors have been eliminated and immunity to a second tumor challenge has been generated.[30] Regulatory T cells (Tregs) are a subclass of T cells that aid the prevention of autoimmunity by improving self-antigen immunological tolerance. They regularly express CTLA-4 and use a variety of strategies to inhibit CD4+ and CD8+ autoreactive T cells, including the generation of regulatory cytokines.[31]

James Allison of Berkeley (1996) California, led research that had shown CTLA-4 antagonistic antibodies to slow the progression of tumor formation in mice.[30] More extensive tests in murine models had shown that a single immunogenic tumor responded to a single antibody that inhibited CTLA-4, while a less immunogenic tumor required the development of multiple drugs, such as tumor transplantation and regulatory T cells (Treg).[32-37] In 2000, ipilimumab (Yervoy®) and tremelimumab were the main antibodies used to block the CTLA-4 receptor and keep the action of certain T-cells in patients with advanced diseases, particularly in malignant melanoma.[38] CTLA-4 blocking had achieved a radiological response in 15% of patients with metastatic MM since its inception, with some patients experiencing a long-term response of over ten years.[38,39] In two phase-3 investigations, it was discovered that individuals treated with ipilimumab had better endurance than those treated with normal chemotherapy, dacarbazine, or glycoprotein 100 peptide antibody. The FDA had approved it for the treatment of advanced malignant melanoma in 2011, followed by the approval by the European Medicines Agency (EMA), a few months later. Tremelimumab showed no significant differences when compared to chemotherapy in a phase 3 study, therefore it wasn't approved for use in malignant melanoma. However, its usage in other types of malignancies was further being studied. The biological details of CTLA-4 have been enlisted in Table 1.

STRUCTURE AND BINDING SITES OF CTLA-4

CTLA-4 and CD28 share 30% amino acid sequence identity in their extracellular domains, which are made up of a single immunoglobulin variable domain.[40] They have two β-sheet faces, one with the A0GFCC0 strand in the front and the other with the ABED strand at the back.[41] The front A0GFCC0 sheet supplies the key residues for CTLA-4's interaction with its B7 ligands or therapeutic antibodies, according to the complex structures of CTLA-4.[42,43] The F and G strands of CTLA-4's front face contain the overlapping binding surface for B7, ipilimumab, and tremelimumab. To detect B7 ligands, CTLA-
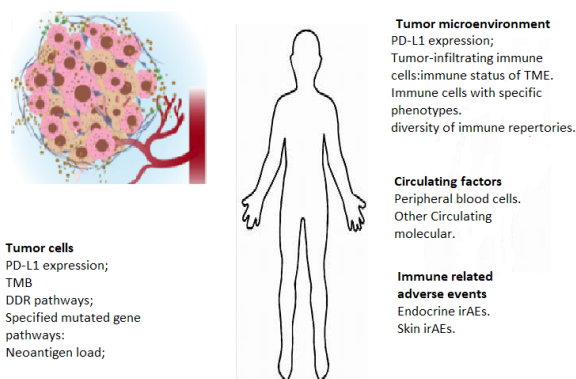

Figure 1: A summary of Promising Biomarkers for Predicting Response to Immunotherapy[19]

Table 1: Biological details of CTLA-4

Description	CTLA-4
Gene location	Chromosomes2q33
Synonyms	CD_{152}
Protein details	Amino acids #223 Type 1 transmembrane glycoprotein belonging to lg super family Dimer Domains: a single peptide, an extracellular ligand-binding domain, a transmembrane domain, and a short cytoplasmic tail
Signaling motif	Cytoplasmic tail
Cells expressing receptor	Effector T-cells & Tregs
Ligands	CD_{80} (B_{7-1}), CD_{86} (B_{7-2})
Cells expressing ligands	APC_S

4's FG loop interacts with the MYPPPYY sequence (residues 99–105). The three sequential proline residues inside the FG loop assume a unique cis-trans-cis conformation and offer important interactions with the B7 ligands, according to crystal structures of the apo form of CTLA-4 and its complex with B7-1 or B7-2.[41-43] Indeed, the FG loop mutation resulted in a 90% reduction in binding affinity for B7 ligands.[44] The FG loop is implicated in the interaction with antibodies in the complex structures of CTLA-4 with ipilimumab and tremelimumab. The apo form and B7-bound CTLA-4, on the other hand, have no discernible conformational differences, showing that this loop region is rigid and ready to bind to ligands or antibodies. Ipilimumab and tremelimumab have total buried surface areas of 1880 and 1802, respectively, while the total buried surface areas of CTLA-4/B7-1 and CTLA-4/B7-2 are 1255 and 1212, respectively. The B7 ligand and antibody binding affinities to CTLA-4 are different, which explains the differences in total hidden surface area when binding CTLA-4. The binding affinities of ipilimumab (Kd = 18 nM) and tremelimumab (Kd = 5.9 nM) molecules are substantially higher than those of B7-1 (Kd = 420 nM) molecules.[45] As a result, ipilimumab and tremelimumab effectively compete with B7 ligands for CTLA-4 binding.

CTLA-4-Blocking Monoclonal Antibodies: Clinical Response and Molecular Mechanisms

There is evidence for a variety of possible anticancer activity pathways when CTLA-4-blocking monoclonal antibodies are administered.

- Anti-CTLA-4 immunotherapies inhibit the main inhibitor of T cell capability, allowing T cells to become activated. CTLA-4 is a surface-expressed protein receptor found on activated T cells. It successfully competes with CD28, CD80, and CD86's constitutive positive receptor for costimulatory atoms. CTLA-4 commitment by a costimulatory molecule on the exterior of T cells decreases IL-2 and IFN production after TCR commitment.[46-48] Blocking this negative regulation with CTLA-4-inhibiting monoclonal antibodies can result in increased initiation

development of initiated T cells, which can lead to anticancer action.[30]

- When T cells have surface CTLA-4, hyperactive cells collaborate with cells that deliver their associated antigen in limited ways.[49,50] An imperfect setting off with TCR flagging has the accompanying impact. To stimulate T-cell effector capacities such as cytokine production or cytotoxic granule release, more stable interactions are required[51] Starting T cells and cancer cells may be able to collaborate for longer if CTLA-4 is blocked with antibodies, lowering the threshold for TCR identification, and inducing cancer cell death.[52]

- CTLA-4 signaling suppression on plasmacytoid dendritic cells can lower Treg cell functional activation or decrease indolamine 2,3-dioxygenase (IDO) (pDCs). Treg cells are dominant suppressor cells in peripheral tissues that play an important role in autoimmune response regulation.[53] CTLA-4 is continuously generated in these cells, providing reverse signaling that causes them to express B7 costimulatory molecules and activate the immune-suppressive enzyme IDO in pDCs.[54-56] Anti-CTLA-4 antibodies can deplete or reduce Treg activity by inhibiting CTLA-4-mediated reverse signaling to pDCs and decreasing IDO production.

- It has been suggested that CTLA-4 connecting components have an immediate effect on CTLA-4 expressing melanoma cells, resulting in disease cell apoptosis. The treatment of CTLA-4-expressing cancer cells with recombinant forms of the CTLA-4 ligands CD80 and CD86 activated the cell's apoptotic apparatus, resulting in the direct killing of the cancer cells.[57] The primary distinction between these recombinant B7 costimulatory particles and the anti-CTLA-4 antibodies now being developed in clinical trials is that these antibodies were chosen for their ability to reduce CTLA-4 flagging after being restricted to the CTLA-4 atom. As a result, using CTLA-4-blocking monoclonal antibodies to kill cancer cells directly may not be feasible, regardless of whether useful quantities of CTLA-4 are expressed on the surface of melanoma cells *in vivo*.[52]

Melanoma Treatment with CTLA-4 Blockade

- Ipilimumab is endorsed for advanced melanoma. The combination of nivolumab and ipilimumab brings about more elevated levels of survival contrasted with ipilimumab alone.

- Skin melanoma, despite its expanding event and lower pervasiveness contrasted with other cutaneous malignancies, is quite possibly the most forceful type of disease. There is a strong surgical prognosis for Non-invading (melanoma in situ), yet advanced melanoma needs remedial treatment alternatives.[58]

- In light of this body of evidence, CTLA-4-blocking monoclonal antibodies are being studied as a treatment for a variety of malignancies, but they are currently being studied most intensively in patients with advanced malignant melanoma. Ipilimumab (formerly known

as MDX010), a human CTLA-4-blocking monoclonal antibody developed by Medarex Inc. in collaboration with Bristol-Myers Squib, and tremelimumab (formerly known as CP-675,206, more commonly known as ticilimumab), a human CTLA-4-blocking monoclonal antibody developed by Pfizer Inc. Preliminary statistics reveal that these CTLA-4-hindered antibodies are effective in several metastatic melanoma patients, and two CTLA-4 blockers have begun key preliminaries (Table 2).[52]

Merkel Cell Carcinoma (MCC)

MCC is a rare and deadly neuroendocrine tumor with a 30% higher fatality rate than metastatic melanoma.[68] In most cases, it is linked to a polyomavirus and has a low gene mutation burden, not like it is induced by ultraviolet radiation, which has no link to a polyomavirus and is associated with a greater number of genetic disorders.[69] The first-line treatment for primary MCC is surgery followed by radiation. Chemotherapy has been the only option for treating MCC before immunotherapy. As per the literature outcomes, pembrolizumab, nivolumab, and avelumab are right now the favored choices in metastatic melanoma patients. There is evidence that this treatment provides a longer-lasting response than standard chemotherapy. (where cancer-free lifespan is only 3 months).[68-71]

As there is no such promising Anti-CTLA-4 agent for MCC. Some therapeutic combinations of CTLA-4 blockade monoclonal antibody ipilimumab[72] are presently being considered and studied. Ipilimumab is being tested as an adjuvant treatment after extraction versus perception in stage II randomized preliminary experiment. A case study that includes 5 metastatic MCC patients which is performed in 2017 with ipilimumab (3 mg/kg body weight at regular intervals) revealed questionable outcomes for the drug in advanced MCC. Only one report on a patient who showed a reduction in cutaneous MCC lesion following a combination of ipilimumab and chemotherapy had been shown before that case scenario.[73] Other immune checkpoint blockade therapies have shown to be tolerable and effective against MCC. These are PD-1 and PD-L1 inhibitors, such as avelumab and pembrolizumab, which have been approved for MCC treatment. Nivolumab has been found to be efficacious against MCC in a recent study, as well as the drug has a favorable safety profile also. Before chemotherapy, the NCCN recommends avelumab, pembrolizumab, and nivolumab as first-line treatments for advanced MCC.[58]

CTLA-4's Role in Carcinoma of Cutaneous Squamous Cells (CSCC)

CSCC is an immunogenic tumor that, like MCC, can be treated with immunotherapy. It's a keratinocyte tumor, and it's the world's second most frequent human malignancy[74] with over 700,000 cases identified each year in the United States. Because PD-L1 and PD-L2 are thought to have a role in tumor progression, they could be helpful targets.[75,76] Personal experiences and the preliminary findings of a few clinical trials are the only evidences of immunotherapy in oncology in this malignancy, so more research is needed.[77,78] The prevalence of CSCC has increased in recent decades in the United States and is expected to continue to rise in the future.[74] Despite the good prognosis, 4% of CSCC patients are unable to be resected, and 1.5 percent die from the disease.[74] There is no clarity on how to treat advanced CSCC until recently. The use of CPIs in CSCC has received a lot of attention, because CSCC has a number of mutations and is more common in immunocompromised people and that no promising anti-CTLA-4 agent exists for CSCC till date.

Cancer Toxicities Associated aith CTLA-4 Blockade Therapy

Apart from the therapeutic benefits obtained so far with anti-CTLA-4 treatment, another significant aspect that has emerged from the early preliminaries with immune checkpoint inhibitors is the potential for undesirable (and previously unknown) side effects. Immune responses against its healthy cells or prompting organ-specific inflammation have been accounted as adverse effects of CTLA-4-obstructing monoclonal antibodies. In the biggest publicly known sample of patients given ipilimumab at 3 mg/kg every three weeks, 25% (14 patients out of 56) experienced grade 3 or 4 toxicity.[63] The dosing routine is connected to the pace of evaluation 3 and 4 toxicities levels with tremelimumab. Though immune checkpoint inhibitors don't target just tumor-explicit T cells,

Table 2: Outline of Complete Reports Describing Clinical Response of Metastatic Melanoma Patients concerning Anti-CTLA-4 Monoclonal Antibodies

Antibody	Combination	Monoclonal antibody dose (mg/kg)	Schedule	Patients data of objective tumor response	Patients data of measurable metastic melanoma	References
Ipilimumab	None	3	Single dose	2	17	[59]
Ipilimumab	None	3	Single dose	0	7	[60]
Ipilimumab	Gp100 peptides	3	Q3 weeks	7	56	[61,62]
Ipilimumab	Gp100,tyrosinase, MART-1 peptides	0.3–3	Q4 weeks	0	0	[63]
Ipilimumab	None	0.1–3	Q3 weeks	8	36	[64]
Ipilimumab	None	3–9	Q3 weeks	5	46	[65]
Tremelimumab	None	0.01–15	Single dose	5	29	[66]
Tremelimumab		10–15	Q1–3 months	5	30	[67]

Table 3: Tumor response correlation with toxicity grade

Treatment								
	Ipilimumab 3 mg/kg + gp100 (Attia[a])		Ipilimumab 3 mg/kg + IL-2 (Maker 2005[a])		Ipilimumab 3–9 mg/kg (Maker 2006[a])		Tremelimumab 10–15 mg/kg (Reuben[a])	
Toxicity grade	0-II	III-IV	0-II	III-IV	0-II	III-IV	0-I	II-IV
No response	40	9	25	3	28	13	17	8
Response	2	5	6	2	2	3	1	4
p-value	0.008*		0.3*		0.32*		0.045**	

[a] first author
*fisher exact test
**Chi-square test

their capacity to release wanted anti-tumor resistant reactivity can be trailed by accidental initiation of non-tumor-explicit safe reactions. The main objective of this is to communicated with self-antigens on non-infective cells. Immune system-related adverse effects have been accounted in various organs because of anti-CTLA-4[52] therapy, with various dermatological conditions like pruritis and mucositis. Gastrointestinal inconvenience like loose bowels and colitis are additionally widespread side effects found in up to 40% of ipilimumab-treated patients. Various unpredicted immune-related adverse events (IrAEs) associated with cancer immunotherapy may be hepatic toxicity, endocrinopathies, and pneumonia, and sometime pulmonary embolism. Renal harmfulness, pancreatitis, neural and cardiovascular toxicities are also potential side effects. There have been hematological anomalies as well as ocular manifestations. These side effects, as well as existing guidelines for their treatment, have been published, management has recently been thoroughly analyzed elsewhere[79,80] CTLA-4 blockade, on the other hand, is linked to a higher risk of cancer.[81] Several dose-dependent design clinical research have shown that monoclonal antibodies have a dose related impact on the development of various toxicities.[82-84] Accordingly, higher dosages or longer exposure to antibodies cause greater poisonousness and increments the likelihood of antitumor reactions (Table 3). The various research is to find out the comparitive impacts toxicities related to autoimmune toxicities and induced immunotoxicity.[85,86] Regardless, two clinical preliminaries are the basis for a reaction, or whether increased levels of circulating monoclonal antibodies cause more systematic exposure, which interacts with the two hazardous levels and response independently, as recently noted.

CONCLUSION

As our understanding of the immune system's regulatory mechanisms are increasing, new therapeutic choices for cancer patients are also being developed. Even though in ICB treatments, especially anti-CTLA-4 antibodies are being effectively tried in various cutaneous malignancies, it stays to be completely perceived why the inhibition of CTLA-4 shows effective activity against some restricted tumor types. Ipilimumab, which blocks CTLA-4, has become the benchmark for all immune checkpoints related to T-cells which are

being studied in melanoma and various other malignancies. Combination therapies in which ipilimumab (CTLA-4 blocker) and nivolumab (PD-1 blocker) have been used, shows better response than CTLA-4 blockade monotherapy. There are currently no prognostic indicators for the long-term implications of CTLA-4 suppression. As a result of a greater understanding of this particular immune checkpoint and its role as a crucial inhibitor of T-cells, immunotherapeutic methods focusing on CTLA-4 are being more and more explored. Ipilimumab and tremelimumab suppress CTLA-4, resulting in a longer lifetime for cancer patients. Positive results from the ipilimumab in 2011 (approved in this year by the FDA for the treatment of melanoma) have sparked interest in a new cancer treatment strategies (in addition to surgery, chemotherapy, and radiotherapy), namely cancer immunotherapy, which has been declared the breakthrough in the last few years.

Dermatologists working together on a multidisciplinary basis include surgeons, surgical oncologists, radiation oncologists and so on to handle the advanced and deathly diseases like CSCCs, MCC, MM and others. Patients at high risk are more likely to need multiple treatments of medication to best control their condition over time. For ongoing monitoring for the recurrent CSCC, the side effects of the treatments should be managed.

ACKNOWLEDGEMENT

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