Immunotherapy for melanoma

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Abstract

Immunotherapy for the treatment of advanced melanoma has become a primary treatment in the clinic. Current therapies include systemic cytokines, immune checkpoint inhibitors, and localized intratumoral therapies. Checkpoint inhibitors block natural pathways that dampen or inhibit an immune response to stimulus. These pathways include programmed cell death 1 receptor/programmed death-ligand 1 and cytotoxic T lymphocyte antigen-4. Systemic immunotherapies have proven to be effective in clinical trials both as monotherapy and in combination therapy. Oncolytic viruses are used to treat tumor locally and induce an effective immune response. Although some immune-mediated adverse events have been shown to occur with immunotherapy and may cause disease through systemic immune activation, most symptoms are mild to moderate. Overall immunotherapy in advanced melanoma has provided effective and durable responses to treat patients with advanced melanoma.

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The immune system plays a crucial role in controlling and/or enabling cancer to initiate and to invade the body.[1] Malignant cells undergo immunoediting and subsequent immune evasion, in which certain cancer cell clones are able to escape control by the immune system. In doing so, cancer cells co-opt immune checkpoints that can effectively turn off certain aspects of immune cell function. By augmenting preexisting antitumor immunity and preventing tumors from co-opting immune checkpoints, immunotherapy allows the immune system to destroy cancer cells.

Although immunotherapy has changed the way advanced melanoma is treated in the clinic today, the foundations for immune modulation to treat cancer were investigated as early as the 18th and 19th centuries.[2] During this time, superficial tumors were treated with septic dressings to create infection, which was shown to decrease lesion size. Scientists investigated the effect of infection on cancer, known as spontaneous regression, and developed a bacterial inoculation.[3] Bacterial inoculation was used in the early 1900s for the treatment of sarcomas. Early investigations of immune cytokines in mice provided information on the interaction between cancer and the immune system.[4] The use of interferon alpha (IFN-α) was investigated in advanced melanoma in a series of clinical trials. Other studies investigated laboratory-grown T cells named "lymphocyte-activated killer" therapy, with some success in treating advanced melanoma and renal cancer.[5-7] The use of the cytokine interleukin-2 (IL-2) to treat metastatic melanoma was developed at the National Cancer Institute and produced lasting tumor regressions, although the acute toxicity kept investigators searching for a better therapy.[8] More recently, therapies have been developed building on the insight gained from these key studies, which essentially showed the interplay between T-cell-intrinsic and -extrinsic switches that control the activation of T cells and, when manipulated, produce effective antitumor responses in melanoma and other malignancies.

Checkpoint inhibitors

The use of checkpoint inhibitors has proven to be a productive strategy for immunotherapy. These checkpoints (primarily within the adaptive immune system) function to turn off dendritic cell "priming" of T cells or tumor microenvironment-induced T-cell "exhaustion" (Figure). Cytotoxic T lymphocyte antigen-4 (CTLA-4) provided one of the initial targets for checkpoint inhibition immunotherapy. First identified and sequenced as a member of the immunoglobulin superfamily in 1987, CTLA-4 is present on the surface of cluster of differentiation 4 (CD4) and CD8 lymphocytes and acts as a negative regulator of T-cell priming.[9-12] CTLA-4 is also highly expressed on regulatory T cells (T-regs), indicating its involvement in the regulation of the immune response, and is thought to compete with the costimulatory CD28 cell surface protein in binding CD80/CD86 on the surface of dendritic cells to counteract costimulation. CTLA-4 is thought to prevent the uncontrolled stimulation and expansion of the T-cell response. Blocking CTLA-4 with an antibody therefore allows prolonged costimulation and enhances T-cell priming.[5] In addition, in some models, CTLA-4 antibody may delete T-reg cells.[13]

As the preclinical evidence surrounding the potential benefits of checkpoint inhibition expanded, mouse studies investigated the effects of CTLA-4 pathway blockade. In a mouse model of advanced melanoma using adoptive transfer of tumor-reactive $CD4^+$ T cells, CTLA-4 inhibition resulted in a reduction of T-reg accumulation and a larger amplification of effector T cells, indicating that the inhibition of the CTLA-4 checkpoint stimu-

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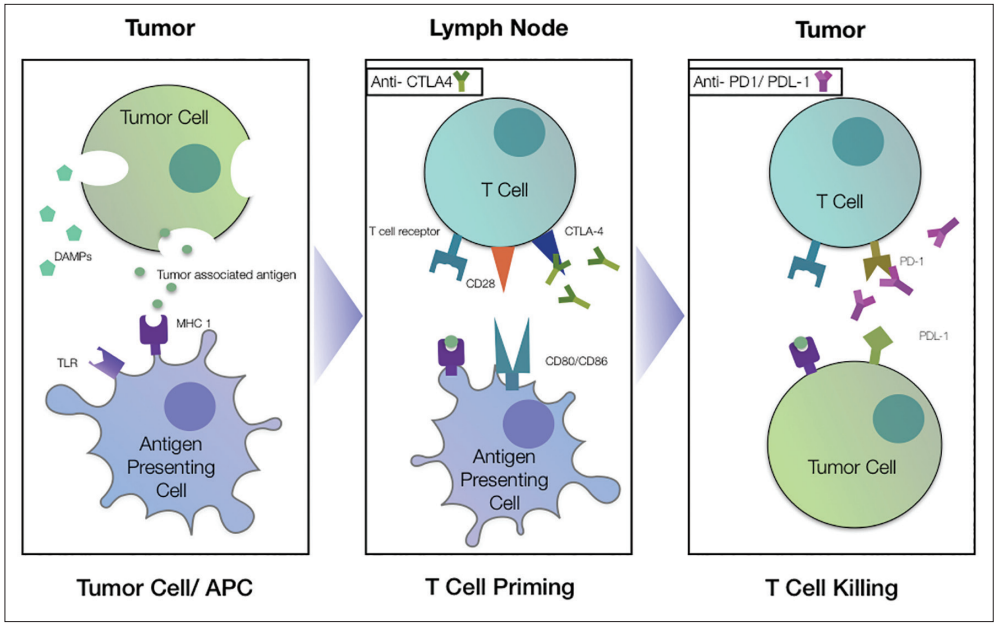

FIGURE. Immunotherapy modulates the interactions of the immune system to initiate an immune response. APCs identify tumor-associated antigen through the MHC; this stimulates antigen presentation to the T cell within the lymph node. In the lymph node, the antigen is presented to the T cell. CTLA-4 inhibition with anti-CTLA-4 antibody allows prolonged T-cell priming. The T cell in contact with the tumor cell can initiate destruction. Anti-PD-1 therapies inhibit the PD-L1 expressed on tumor cells to bind to PD-1 on activated T cells, allowing the immune response to continue. Abbreviations: APC, antigen-presenting cell; CD28, cluster of differentiation 28; CTLA-4, cytotoxic T lymphocyte antigen-4; DAMP, damage-associated molecular pattern; MHC, major histocompatibility complex; PD-1, programmed cell death 1 receptor; PD-L1, programmed death-ligand 1; TLR, toll-like receptorl.

lated an antitumor response.[14] The effectiveness of CTLA-4 as an antitumor therapy was seen in a murine colon carcinoma, in which CTLA-4 blockade reduced tumor size significantly compared with controls.[11] Although CTLA-4 blockade has demonstrated activity in these models, it has also been well documented that the genetic abrogation of CTLA-4 could have profound and harmful consequences throughout the body. In studies involving CTLA-4 knockout (KO) mice, researchers observed the development of lethal autoimmune responses, including lymphoproliferation and gut toxicity.[15] Despite these potential toxicity concerns, the successful preclinical experiments led to the development of an anti-CTLA-4 antibody for use in humans with a humanized immunoglobulin G1 (IgG1) monoclonal antibody.

In the first clinical trial of anti-CTLA-4 antibody, previously treated advanced melanoma patients were administered a single infusion of ipilimumab (Yervoy; Bristol-Myers Squibb, Princeton, NJ) and, even at the starting dose, showed extensive tumor necrosis and infiltration of lymphocytes and granulocytes, indicating an immune response stimulation.[16] Although objective responses were not achieved in this initial trial, the induction of lesion-specific immune responses did provide evidence for further investigation. To characterize the most effective dose and schedule, a phase II study of ipilimumab investigated the effects of 0.3 mg/kg, 3 mg/kg, and 10 mg/kg in patients with advanced melanoma. Results from this study identified best overall response rates in patients dosed with 10 mg/kg every 3 weeks; however, this group also saw the highest rates of adverse events.[17] In a subsequent phase III trial, 3 mg/kg ipilimumab administered every 3 weeks was compared to glycoprotein 100 peptide vaccine (gp100) or a combination of both gp100 and ipilimumab in previously treated metastatic melanoma patients. Patients receiving either ipilimumab or ipilimumab plus gp100 demonstrated greater median overall survival compared with gp100 (10.1, 10.1, and 6.4 months, respectively).[18] A second phase III trial investigating the higher 10 mg/kg dose found that ipilimumab combined with dacarbazine, a cytotoxic drug, prolonged overall survival over dacarbazine alone. In addition, the higher dosing also increased grade 3 or higher adverse events to 53% occurrence, reducing the enthusiasm for the higher dose especially in combination with chemotherapy. Due to its increased efficacy compared with previous therapies, ipilimumab was approved in the United States in 2011 for use as a treatment for metastatic melanoma by the Food and Drug Administration (FDA).

The success of ipilimumab stands in contrast to another anti-CTLA-4 therapy, tremelimumab (Pfizer, New York City, NY). Early trials demonstrated antitumor activity and safety in patients with melanoma and renal cell and colon cancers, but the development of tremelimumab was terminated after the drug failed to demonstrate improved outcomes over chemotherapy in a phase III trial involving treatment-naïve unresectable advanced melanoma patients.[19,20]

Although ipilimumab was effective, patients receiving it had a high number of immune-mediated adverse events, raising concerns over the drug's tolerability.[1] As new therapeutic pathways were explored, one of the most effective checkpoint targets identified was programmed cell death 1 receptor (PD-1). PD-1 is up-regulated on activated T cells and inhibits signaling downstream of the T-cell receptor, limiting T-cell effector functions (Figure). Its ligands include PD-L1 and PD-L2. Whereas PD-L2 is expressed on the surface of antigen-presenting cells (APCs), PD-L1 is expressed on the surface of cancer cells and

tumor-infiltrating macrophages. PD-L1 is expressed in 20% to 50% of cancers.[21]

Unlike murine CTLA-4 KO models, PD-1-deficient mice did not show severe toxicity due to autoimmune activation, suggesting the improved tolerability of PD-1 blockade. Clinical trials were initiated with a humanized PD-1 antibody as well as a PD-L1 antibody, and in 2012, a multitumor dose escalation phase I clinical trial was reported on the anti-PD-1 antibody nivolumab (Opdivo; Bristol-Myers Squibb, Princeton, NJ). Investigators found that the objective response rate was approximately 17% in previously treated patients with advanced melanoma.[22] This study also demonstrated evidence of stable disease lasting longer than 24 weeks in 27% of patients, and a prolonged median overall survival of 16.8 months in patients with advanced melanoma.[22] The nivolumab phase I trial 1-year survival rate of 62% was more surprising given that half of all patients participating in this trial had shown no response during the previous 2 to 5 therapies.[23]

The safety and activity of a different anti-PD-1 antibody, pembrolizumab (Keytruda; Merck, Kenilworth, NJ)—formerly lambrolizumub—was studied in a landmark phase I trial of patients with advanced melanoma and showed effectiveness with an objective response rate of 38%.[24,25] In this trial, referred to as keynote 001, pembrolizumab showed both antitumor activity and increased tolerability, with grade 3/4 adverse events less than 20%. Pembrolizumab became the first FDA-approved anti-PD-1 treatment, receiving approval in 2014. Data collected during the follow-up of keynote 001 demonstrated that pembrolizumab displayed treatment durability. Specifically, in patients who attained a complete response, 90% were disease free at the 24-month follow-up time point.[26] Following the phase I trial, pembrolizumab was compared to standard of care chemotherapy in the keynote 002 trial. Doses of 2 mg/kg and 10 mg/kg pembrolizumab were evaluated for their effectiveness compared with investigator-selected chemotherapy.[27] Overall, the 6-month progression-free survival of patients receiving 2 mg/kg and 10 mg/kg pembrolizumab was significantly higher than patients receiving chemotherapy treatments (34% and 38% versus 16%, respectively), and phase III trials were initiated. Keynote 006 aimed to compare the effectiveness of pembrolizumab at 2 different schedules to the CTLA-4 inhibitor ipilimumab. Compared with ipilimumab dosed at 3-week intervals, both 2-week and 3-week schedules of pembrolizumab showed superior overall survival rates. Pembrolizumab administration at 2- and 3-week interval groups showed 55% survival at 24 months, while ipilimumab monotherapy was 43%.[28] Pembrolizumab also showed less toxicity, with 10% to 13% severe adverse event rates compared with the 19.9% shown with ipilimumab. Ultimately, these clinical trials demonstrated both the superior efficacy and durability of pembrolizumab over ipilimumab in patients in the first-line setting and have cemented the role of PD-1 in the first-line setting for melanoma.

As both anti-CTLA-4 and anti-PD-1 therapies have demonstrated significant clinical success, these treatments have been combined to treat patients with advanced melanoma to investigate whether the effects of these therapies are additive. In a phase I combination trial of ipilimumab and nivolumab, an objective response rate of 40% was attained in 53 treatment-naïve patients with advanced melanoma.[29] Eventually, a phase III trial was performed to understand whether nivolumab and ipilimumab would have complimentary effects on treatment-naïve patients with advanced melanoma. Patients were randomized into 3 arms: nivolumab monotherapy, ipilimumab monotherapy, and a combination of the two. Patients receiving nivolumab monotherapy or combination therapy had progression-free survival rates of 11.5 and 6.9 months, compared with 2.9 months with ipilimumab alone.[30] More recently, the combination of ipilimumab and nivolumab (CheckMate 204) was investigated in melanoma patients with brain metastasis. An interim analysis revealed an objective response rate in the brain lesions of 55%, with 21% of patients experiencing a complete response. Moreover, these interim results also showed treatment durability, with 65% progression-free survival at 6 months.[31] The profound effectiveness of checkpoint inhibitors was shown both in monotherapy and in combination therapy.

Current trials are identifying clinical markers to determine prospective responders and nonresponders to checkpoint inhibition. Tumor mutation burden, the expression levels of PD-L1 on tumor cells and tumor immune-infiltrating cells, tumor burden to $CD8^+$ ratio,[32] the site of metastasis,[33] and the host gut microbiome[34] have all been shown to influence response to checkpoint immunotherapy. In the future, these factors will likely be used to personalize treatment and predict best response.

Intratumoral therapy

Systemic therapies have the advantage of global treatment of tumor including metastases, but intratumoral immunotherapies are able to treat a lesion without the risk of systemic toxicity and are possibly able to increase inflammation within the tumor microenvironment for noninflamed tumors. Although systemic treatment with anti-PD-1 therapy is effective, many patients do not respond, and for these noninflamed tumors, a local therapy may help.

One approach to this has been local injection of cytokines such as IL-2, IFN-α, and granulocyte macrophage colony-stimulating factor (GM-CSF). Another approach has been to use oncolytic viruses. Oncolytic viruses are, in some cases, naturally occurring viruses such as herpes or coxsackie viruses either unmodified or modified to add immune-stimulatory genes such as for GM-CSF. These viruses work by exploiting virus replication selectively within tumor tissue lysing the infected cells and causing tumor regression.[35] Talimogene laherpervic (T-VEC; Imlygic; Amgen,Thousand Oaks, CA) is a modified type 1 herpes simplex virus that is administered intratumorally. T-VEC works to replicate and lyse cells in the lesion while in-

creasing the expression of GM-CSF to improve antigen presentation of dendritic cells for T-cell priming and response.[35] After demonstrating safety and tolerability in phase I studies, a phase II trial of T-VEC investigated injection of lesions every 2 weeks for up to 24 treatments; the overall response rate in 50 patients was 26%.[36,37] Additional analysis characterized the systemic effects of the therapy.[38] In a phase III Oncovex Pivotal Trial in Melanoma (OPTiM) study of 436 patients with advanced melanoma, the overall response rate was 26.4% with T-VEC compared with 2.1% in GM-CSF injections alone, demonstrating the therapeutic benefit of T-VEC to treat metastatic melanoma.[39] In a retrospective study of the OPTiM trial, 64% of T-VEC-treated lesions were reduced ≥50%, while untreated lesions also showed a 34% decrease, supporting previous findings of systemic therapeutic immune effects from the phase II trial.[40] A phase Ib combination trial with T-VEC and pembrolizumab investigated the targeted therapy and systemic therapy. The overall response rate to the combination therapy was 62%, and complete response rate was 33% in 21 patients.[41] Although T-VEC has the limitation of requiring skin metastasis, or palpable injection areas, it has shown efficacy and a durable response as a monotherapy and combined with systemic therapy.[42] Additional therapies such as intratumoral cytokine therapy without viruses using electroporation are also being investigated.

Adverse events

Adverse events in immunotherapy are distinctive in their spectrum and are often delayed. Initial indications of adverse events were shown in CTLA-4 KO mice that show a distinctive syndrome with lymphoproliferative disease and T-cell infiltration into healthy tissues causing inflammation and destruction.[43] The results seen in CTLA-4 KO mice demonstrated the effect of releasing the brakes on this immune pathway. The adverse events seen with anti-PD-1 therapy were milder, and PD-1-deficient mice had later-developing autoimmune responses. These differences in adverse events are thought to relate to the method of action; the involvement of CTLA-4 is broader and more proximal in the T-cell immunity cycle, while PD-1 activity is more specific to T cells that are already primed and interacting with tumor cells.[44] Turning from the preclinical data to the clinical trials, many patients develop adverse events although most are mild to moderate in severity. Although we are still learning about the factors that initiate and/or potentiate adverse events with immunotherapy, it is thought that preexisting autoreactive T cells or autoantibodies and an increase in inflammatory cytokines may play a role.[44] Commonly seen adverse events in systemic checkpoint inhibitors are inflammatory responses inducing skin rashes, gastrointestinal disease, endocrine disorders, and abnormal liver function.[45] Short-term glucocorticoid therapy is commonly used to suppress the immune system and attenuate the inflammatory response underlying the adverse event. The onset of immune-related events can occur any time after the treatment and can even occur after treatment has been discontinued.[44] Future directions of research will be to better understand and predict adverse events in patients. Some endocrine side effects such as autoimmune diabetes and hypophysitis appear to be permanent. Rare but serious side effects such as myocarditis and neurological adverse events such as Guillain-Barre syndrome have been reported.

Conclusion

Although the immune system is a complex and multifaceted system, our understanding currently allows us to use potent therapeutics that not only target it but also produce long-lasting responses. This durability of response is unique to immunotherapy and offers a compelling advantage to patients. Current therapies and future therapies for which the foundation has been laid will continue to give hope to patients and encouragement to treating oncologists, not only in the treatment of metastatic melanoma but also other forms of cancer.

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