

REVIEW

The delicate balance of melanoma immunotherapy

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The strategy of immune modulation for the treatment of cancer is being refined with the introduction of multiple new therapeutic agents into the clinic. Melanoma is a disease where many of these agents have demonstrated efficacy. The mechanisms of action of these agents exploit the counter-regulatory mechanisms of the immune response. However, these agents are also associated with immune-related adverse events (IRAEs), which represent tissue-specific inflammatory responses. These IRAEs highlight the delicate balance of immunologic homeostasis and, with some interventions, may occur more frequently in patients who sustain a therapeutic response. This review will discuss melanoma immunogenicity and immunotherapy. Furthermore, the spectrum and distinction between a reversible immune adverse event and autoimmunity will be highlighted.

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One of the fundamental roles of the immune system is distinguishing self from non-self.¹ Two disease processes that skirt this dichotomy are autoimmunity and cancer. The immune system is designed to prevent autoimmunity, and, in general, autoimmune disease represents a failure of regulatory mechanisms to maintain tolerance to self-antigens. On the contrary, although there are systems in place by which the immune system eradicates malignant cells, increasing evidence points to a multitude of complex mechanisms by which tumors avoid immune recognition. The development of new, effective agents for immunotherapy of cancer has been coupled with the emergence of a new panel of toxicities, which have been termed immune-related adverse events (IRAEs). Although most of these immune events are medically manageable, evidence suggests that some immunotherapies are also eliciting immune-mediated inflammation of normal tissues.

Melanoma is a disease where major insights have been made using immunotherapeutic approaches. This review discusses melanoma tumor antigen expression, associated endogenous antitumor immune responses and mechanisms of action of new immunotherapies. The review further discusses how IRAEs may be related to antitumor responses and how these adverse events reveal mechanistic insights to better understand and utilize novel immunotherapeutics.

EVIDENCE FOR MELANOMA IMMUNOGENICITY

In the late 1950s, Burnet and Thomas introduced the hypothesis of cancer immune surveillance, suggesting that cancers may develop new antigens that could 'provoke an effective immunological reaction with regression of the tumor'.² Burnet³ went on to

describe immunosurveillance as an 'evolutionary necessity' to eliminate mutant cells.

A seminal discovery in the field of tumor immunity by Shankaran *et al.*⁴ introduced the concept of tumor immunoediting, a process where tumors are shaped by the host immune system. The authors demonstrated that carcinogen-induced tumors derived from immunocompetent hosts could be transplanted into syngeneic wild-type littermates, because they had been edited to allow for growth. In contrast, tumors from immunocompromised hosts could only grow after being transplanted into syngeneic immunocompetent hosts half the time.⁴ This observation suggested that tumors arising in immunocompromised mice were not modified by the immune system and remained immunogenic, whereas the tumors arising in immunocompetent mice underwent T-cell-dependent editing, allowing the tumor to be less immunogenic and grow in all animals.⁵ Immunoediting has since been defined as having three key components: elimination (tumor eradication after antigen recognition), equilibrium (maintenance of tumor stability by immune control) and escape (tumor growth).^{5,6}

Approximately 3% of melanoma patients present with metastatic disease without an identifiable primary lesion, also known as melanoma of unknown primary. This phenomenon may represent a clinical illustration of immunoediting (Figure 1).⁷ The hypothesis is that the melanoma initiates an immune response inducing primary tumor 'elimination'. In some patients with melanoma of unknown primary, a patch of vitiligo at the postulated site of the original lesion may represent immune recognition of the melanoma, supporting this hypothesis. However, elimination fails at the site of melanoma metastasis. These cells remain in a state of 'equilibrium' either in a

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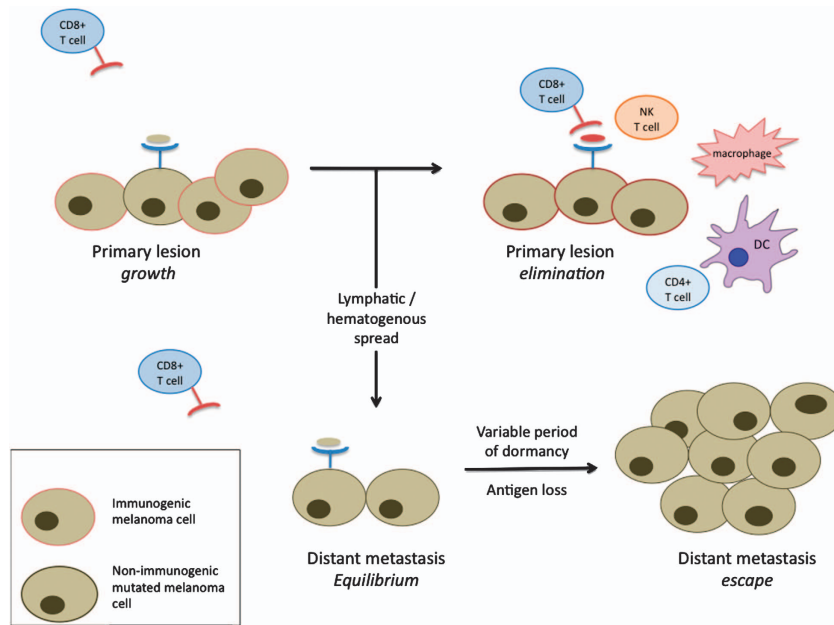


Figure 1 Suggested mechanisms of immunoediting in melanoma of unknown primary.

regional lymph node or at a distant site until a further event allows the tumor to ‘escape’. At this point, the patient presents with clinically significant metastatic disease. Whether the tumor that escapes has an ‘edited’ antigen profile remains unknown as the primary is usually undetectable at the time of discovery of the metastasis.

The unpredictable natural history of many patients with melanoma is also suggestive of immunoediting. Late recurrences and distant metastases in the setting of patients with early-stage melanoma suggest prolonged periods of tumor equilibrium. The strongest clinical evidence for melanoma immunogenicity comes from a small number of cases of melanoma transmission to organ transplant recipients via their engrafted organs.^{8–11} In one of these cases, the organ donor had been disease-free from a localized (non-metastatic) melanoma for 16 years.¹¹ Both kidneys were donated, and immunosuppression of the recipients after transplantation allowed the malignant cells held in equilibrium for decades to escape. One recipient developed rapid widespread metastatic disease and, despite cessation of immuno suppression and interferon (IFN) therapy, died 22 months post transplant. The second recipient developed a localized mass at the site of the transplanted kidney 2 years post transplant. The transplanted kidney was removed following systemic therapy demonstrating necrotic melanoma; this patient remained disease-free 2 years later. The different trajectories of these two transplant recipients further demonstrates the complex interaction of the immune system with tumor antigens.

The reason underlying the immunogenicity of melanoma is unclear. One hypothesis relates to the high mutation rate seen within melanomas compared with other tumor types.¹² A recent study investigating the mutational landscape in melanoma found a median of 171 mutations in melanomas of sun-exposed regions compared with nine mutations in sun-shielded regions.¹³ This compares to approximately 80 mutations in an average colon or breast tumor.¹⁴ The majority of these mutations are passenger mutations that are non-essential for the survival of the tumor but offer an opportunity for immune recognition. Each breast and colon cancer develops on average 10 and 7 novel and unique HLA-A 0201 epitopes,¹⁵

respectively, while in melanoma, where the mutation rate is higher, the chance of generating a mutation with the capacity to bind major histocompatibility complex could also be higher. Others have argued that the extensive research in melanoma immunology is largely opportunistic and stems from the failure of standard chemotherapeutic agents, leaving a therapeutic void for patients with metastatic disease.¹⁶

Vitiligo and melanoma

Vitiligo is a cutaneous disorder manifested by areas of hypopigmentation due to melanocyte loss. The pathogenesis of this depigmentation is likely autoimmune with autoantibodies against melanocyte-differentiation antigens such as tyrosinase found in the serum of a significant proportion of these patients.^{17,18} Even stronger evidence comes from *in vitro* studies demonstrating that sera from patients with vitiligo are able to destroy cultured human melanocytes¹⁹ as are CD8⁺ cytotoxic T cells extracted from cutaneous lesions.²⁰ A recent genome-wide association study looking for susceptibility loci in patients with generalized vitiligo identified novel single-nucleotide polymorphisms (SNPs) involving the major allele of the TYR gene encoding for tyrosinase.²¹ By contrast, SNPs in the TYR gene associated with susceptibility to melanoma are found in the minor allele.^{22,23} These findings may help explain the threefold lower lifetime prevalence of melanoma in patients with vitiligo.²⁴

Interestingly, the development of vitiligo in patients with melanoma is associated with an improved prognosis both in the setting of early and advanced disease.^{25,26} The majority of cells infiltrating both the tumor and the patches of vitiligo in these patients are CD8⁺;²⁷ however, there may also be a role for Th17 cells.²⁸ Mouse models suggest that the development of vitiligo in the setting of primary tumor resection-induced antitumor immune response is associated with an effector memory phenotype. This could be associated with protection against a secondary tumor challenge, as compared with mice without vitiligo that displayed a central memory phenotype.²⁹ These effector memory T cells home preferentially to peripheral

tissues, maintain specificity to tumor antigens and are able to provide long-term protection against a secondary tumor challenge.²⁹

Melanoma antigens

Many crucial discoveries in the role of immunity in cancer immunosurveillance have been made using melanoma models. In patients with melanoma, T cells were identified specifically targeting tumor antigens,³⁰ and the role of interleukin-2 (IL-2) in promoting this response was also elucidated.³¹ Furthermore, the first specific tumor antigen (MAGE-1), targeted by human reactive T cells, was characterized in melanoma.³² Melanoma antigens can be categorized into four main groups: germ cell/cancer testis antigens (silenced in somatic cells but reactivated in melanoma cells), differentiation antigens (expressed on normal melanocytes), overexpressed antigens (mutated self-antigens) and sequestered antigens (ubiquitous self-antigens that are usually hidden from immune detection) (Box 1). Not all melanomas express all melanoma antigens and, over time, variability in levels of antigen expression can be seen within a tumor.³³ Tumor-infiltrating lymphocytes (TILs) within melanomas contain both effector and regulatory T cells (T reg) with specificity for the expressed tumor antigen.³⁴ The expansion of the latter may explain the consistent failure of vaccine strategies aimed at these melanoma-specific antigens.

Immune escape

Tumors utilize a number of pathways to avoid immune detection. Antigen expression and presentation mechanisms may be suppressed through decreased major histocompatibility complex class I expression.³⁵ Tumors are also able to limit an immune response by releasing immunosuppressive paracrine mediators including adenosine, transforming growth factor- β , vascular endothelial growth factor-A and indoleamine 2,3-dioxygenase (IDO) to suppress T-cell activation. Dampening of T-cell activity also occurs through the regulatory pathways such as upregulation of cytotoxic T-lymphocyte antigen-4 (CTLA)-4 on T cells, or engagement of programmed death-1 (PD-1), an inhibitory T-cell co-receptor, with its ligand, B7-H1 (PD-L1) on tumor cells (Box 2). Finally, tumors further create an immunosuppressive microenvironment by recruiting other cell populations, such as T regs and myeloid-derived suppressor cells to the tumor microenvironment.

The same mechanisms that the immune system employs to prevent autoimmunity can also limit effective antitumor responses. Research has demonstrated multiple reasons that anti-melanoma responses can be ineffective. T cells within melanomas often have low levels of self-reactivity, partly because they are positively selected during thymic maturation.³⁶ As a result of this weak T-cell receptor affinity, there may be tolerance to the tumor antigen and priming is restricted.³⁷ This post-thymic tolerance is driven in part by CD4⁺ CD25⁺ FoxP3⁺ T regs that are recruited to tumors to dampen the local immune response by a number of immunosuppressive mechanisms. These include release of chemoattractant cytokines (CCL2 and CCL22),³⁸ immunosuppressive cytokines (transforming growth factor- β and IL-10)³⁹ and upregulation of IDO expression causing T-cell anergy.⁴⁰ Antigen-presenting cells (APCs) including plasmacytoid dendritic cells can also express IDO in order to recruit mature T regs, further contributing to tumor tolerance.⁴¹ The importance of these local factors in inhibiting the immune response is suggested by the success of adoptive T-cell therapy. In this model, the infiltrating T cells are removed and cultured *ex-vivo* in the presence of growth factors. Reinfusion after local ablative therapy with chemotherapy or total body irradiation results in response rates > 50%.⁴²

Box 1 Examples of melanoma-specific antigens

1. Cancer testis antigens—NY-ESO, MAGE, BAGE and GAGE.
2. Differentiation antigens—tyrosinase, tyrosinase-related protein 1 (TYRP1), gp100, melan-A/MART-1 and dopachrome tautomerase (DCT).
3. Mutated antigens—mutations in β -catenins and cyclin-dependent kinase 4 (CDK4).
4. Sequestered antigens.

Box 2 Co-inhibitory mechanisms

Cytotoxic T-lymphocyte antigen 4 (CTLA-4) is normally found in intracellular stores within T cells and is transported to the cell surface upon T-cell activation via the T-cell receptor (TCR).¹²⁶ Once expressed on the cell surface, CTLA-4 competes with the co-stimulatory molecule CD28 for CD80 and CD86 (B7.1 and B7.2) on APCs to deliver a negative signal to the TCR.^{127,128} The ligand for CTLA-4 is not expressed on tumor cells.

By contrast, programmed death-1 (PD-1) is expressed on chronically stimulated activated T cells, B cells and monocytes, and its ligands PD-L1 (B7-H1) and PD-L2 (B7-H2) are expressed on APCs, tumor cells and non-hematopoietic cells within tumors.⁹⁰ Upon ligation, PD-1 is phosphorylated leading to downregulation of TCR signaling. The expression of the ligand for PD-1 on tumor cells suggests an adaptive immune tolerance.

LAG 3 is highly expressed on T regs and has an immunosuppressive role by enhancing T reg function. It also has an independent role in inhibiting CD8⁺ effector T-cell function.¹²⁹

Tim 3 is expressed on T cells and inhibits T-helper cell responses. It binds to galectin 9, which is expressed in a number of cancer types.¹³⁰

In mouse models, T reg depletion can enhance melanoma immunity.^{43–45} Furthermore, in patients with melanoma, an increased fraction of circulating T regs is seen in peripheral blood compared with healthy controls.³⁹ These circulating T regs recognize numerous melanoma-associated antigens of different classes.⁴⁶ Studying the tumor microenvironment demonstrates a worse outcome in patients with an increased proportion of T regs within TILs.^{47,48}

Recent evidence suggests that PD-L1 expression by tumors is an adaptive 'escape' mechanism to counter an antitumor immune response.⁴⁹ Not only are TILs found to colocalize with PD-L1 expressing tumor cells, but laser capture microdissection suggests that the PD-L1 expression is driven by IFN- γ release from these TILs. Patients with high expression of PD-L1 in metastatic melanoma deposits had longer survival than those without, suggesting that these tumors elicit an antitumor response and may have been held in immune equilibrium for some time. These findings differ from observations in other tumor types where PD-L1 expression is a poor prognostic indicator.^{50–52} This seeming contradiction may reflect the increased role of immune regulation in melanoma and the heavy pre-treatment of these melanoma patients with immunotherapy. However, it also highlights the complicated interplay between factors within a tumor.

These immunological insights have accompanied the development of a number of novel therapeutic agents targeting specific aspects of the immune system. These agents are described below with a discussion of lessons learned from IRAEs.

MELANOMA IMMUNOTHERAPY—NOVEL AGENTS AND CHANGING PARADIGMS

Physicians for over a century have been enamored with the idea of utilizing immune defenses to eradicate cancer. William Coley, a surgeon at the Memorial Hospital in New York from 1892 to 1936, was the first major proponent of such therapy, infecting patients with live and subsequently killed bacteria, later known as Coley's toxin.⁵³ Despite some successes, the unpredictability of this therapy and the increasing availability of external beam radiotherapy sidelined immunotherapy for much of the next half century.

The first agent to show a significant activity in the adjuvant setting in patients with high-risk melanoma was IFN- α 2b.⁵⁴ The mechanism of action is multifaceted with a definite immunomodulatory effect^{55,56} as well as an antiangiogenic effect.⁵⁶ IFN- α 2b has been studied extensively and delays relapse-free survival consistently. However, further studies failed to demonstrate a consistent overall survival benefit of IFN, which has been more recently shown in a meta-analysis.⁵⁷ Only recently, long-term follow-up suggested a possible benefit of treatment in the subset of patients with early-stage, ulcerated melanomas.⁵⁸ The clinical activity of this drug is associated with significant systemic toxicity, which prompts thoughtful discussions between patients and physicians to weigh the risk-benefit profile, especially in the adjuvant setting.^{57,59}

A major breakthrough in the development of immunotherapy was the discovery of the T-cell growth factor IL-2.⁶⁰ This was the first targeted agent used in humans to utilize systematic immune manipulation to treat patients with metastatic melanoma.⁶¹ As has been demonstrated with multiple immune modifiers since, the radiographic response rate to IL-2 therapy is low with an objective response rate of approximately 16%;⁶² however, a subset is durable.^{62,63} This trend is in clear distinction from cytotoxic chemotherapy and molecular targeted therapy where durable responses are rare. Based upon this activity, IL-2 was approved by the Food and Drug Administration in 1998. Management of toxicities, including capillary leak syndrome, during administration of IL-2 requires hospitalization and close surveillance.

Checkpoint blockade

The latest clinical advances in melanoma immunotherapy have targeted immune response by blocking critical inhibitory checkpoint molecules. The best characterized of these are CTLA-4 and PD-1, which have been used with great success in melanoma as discussed below. Other checkpoint molecules being studied included lymphocyte activation gene 3 (LAG3), T-cell membrane protein 3 (Tim 3), B7H3 and others (summarized in Pardoll⁶⁴) (Box 2). As the field evolves, additional therapies are being investigated for use including OX40 agonists⁶⁵ and GITR agonists.⁶⁶ It is important to remember that although blockade of a negative signal has been met with success, potentiation of a positive co-stimulatory signals with CD28 agonist was associated with severe cytokine storm,⁶⁷ and an agonist antibody to CD137 (4-1BB) was associated with fatal hepatotoxicity in early-dose-ranging study.⁶⁸

Cytotoxic T-lymphocyte antigen-4

CTLA-4 is a negative regulator of the immune system. It is expressed on T regs and within intracellular compartments in resting effector T cells. Following activation, CTLA-4 is expressed at increased levels on the extracellular surface of T cells. The cell intrinsic action of CTLA-4 is through competitive inhibition of the co-stimulatory molecule CD28, for the B71 and B72 ligands.^{69–72} CTLA-4 has also been shown to have a cell extrinsic role resulting in removal of B7 molecules from

APCs.^{73,74} The critical role of CTLA-4 in immune modulation is illustrated by CTLA-4 null mice, which die within 4 weeks of birth due to systemic lymphoproliferation.⁷⁵

The exact mechanism leading to tumor rejection by CTLA-4 blockade remains unclear. Initially, CTLA-4 blockade was thought to act primarily via CD8⁺ T cells,⁷⁶ as treated mice experienced both tumor rejection and vitiligo that were dependent on CD8⁺ T and natural killer cells. Additional studies have shown that blockade of CTLA-4 on both T-effector and T reg compartments is important in antitumor immunity.⁷⁷ More recently, mechanistic insights have been gained through the conditional knockout (KO) of the CTLA-4 gene from T regs in mice.⁷⁸ These mice develop a multiorgan immune infiltrate after 7 weeks of age associated with fatal autoimmune cardiomyopathy. Although the autoimmunity in this conditional KO model is less severe than the CTLA-4 KO mice, it highlights the role of CTLA-4 in T reg function. Further investigation has suggested a role of Fc-dependent depletion of intratumoral T regs.⁷⁹ Anti-CTLA-4 antibodies that bind Fc receptors lead to a dramatic reduction in intratumoral T regs and an associated increase in the intratumoral T-effector to T reg ratio compared with non-Fc-binding mutant antibodies.

Encouraging preclinical data led to the development of two fully human monoclonal antibodies to CTLA-4—ipilimumab and tremelimumab. Two phase III randomized controlled trials using ipilimumab demonstrated a significant improvement in overall survival in patients with metastatic melanoma with a reduction in risk of death in ipilimumab-treated patients of 28–34%.^{80,81} These were the first large-scale trials designed to specifically target an immune inhibitory pathway. Long-term follow-up of patients from some of the earliest phase I studies of ipilimumab underscores the durability of responses; the majority of patients who experienced a complete response (14/15) continued to be disease-free at 54–99 months of follow-up.⁸² The results of a phase III trial of tremelimumab were not as impressive; however, a number of long-term responses were seen, and the general pattern of clinical activity and adverse events resulting from tremelimumab treatment were similar to those shown by ipilimumab.⁸³

Efforts to identify markers of response to CTLA-4 blockade have identified inducible costimulator (ICOS), a marker of activated T cells⁸⁴ as a potential candidate. Induction of CD4⁺ ICOS^{hi} T cells in peripheral blood from patients with metastatic melanoma treated with ipilimumab correlates significantly with clinical response at 24 weeks as well as with overall survival.⁸⁵ Another predictor of clinical response is the absolute lymphocyte count measured in peripheral blood after the first two doses of ipilimumab. Patients with absolute lymphocyte count of $>1000 \mu\text{l}^{-1}$ had a significantly improved clinical benefit rate as well as an improved overall survival.^{86,87}

Programmed death-1

The primary role of the PD-1/PD-L1/2 pathway is to limit T-cell activity in the setting of an inflammatory response.^{88,89} PD-1 is expressed on activated T, B and natural killer cells, whereas its ligands PD-L1 (B7-H1) and PD-L2 (B7-H2) are expressed on immune cells as well as on many non-hematopoietic cells, including tumors.⁹⁰ PD-1 is highly expressed on TILs as well as on circulating melanoma antigen-specific T cells,^{91,92} and tumor expression of PD-L1 is able to induce T-cell anergy and immunosuppression.⁹³ In chronic viral illness, PD-1 is upregulated on viral specific T cells that display an 'exhaustive' or anergic phenotype, which was reversed by PD-1, but not CTLA-4 blockade.⁹⁴ Together, this data suggested a role for PD-1 blockade in antitumor immunity.

Based upon robust preclinical data, several PD-1- or PD-L1-blocking antibodies have been developed. Clinical activity for three of these reagents, nivolumab (BMS, Princeton, NJ, USA),⁹⁵ MK-3475 (Merck, Whitehouse Station, NJ, USA),⁹⁶ and BMS-936559,⁹⁷ have been reported so far. Topalian *et al.*⁹⁵ in a phase I study of a specific anti-PD-1 antibody (Nivolumab; BMS-936558) revealed a response rate of 28% in patients with metastatic melanoma, with 50% of these responses lasting more than 1 year. These data were recently updated and presented at the European Society for Medical Oncology reporting a 31% response rate in advanced melanoma.⁹⁸

USING IRAES TO PREDICT RESPONSE AND GUIDE THERAPY

As described earlier, an immune response against melanoma can be associated with autoimmune-type manifestations such as vitiligo. Not surprisingly, many immunotherapies induce IRAEs. Hypothyroidism,⁹⁹ hyperthyroidism, the antiphospholipid syndrome,¹⁰⁰ vitiligo and other syndromes have all been described. A large prospective study of patients receiving high-dose IFN by Gogas *et al.*¹⁰¹ found that about one quarter of patients developed autoantibodies or clinical manifestations of autoimmunity, and these patients experienced a statistically significant improvement in relapse-free and overall survival when compared with patients without evidence of autoimmunity. Significantly, a higher number of patients were noted with biochemical autoimmunity than with clinical symptoms. Similarly, an association exists between IRAEs and a response to IL-2, with tumor regression noted in 71% of patients who developed biochemical hypothyroidism compared with regression in only 19% of patients who remained euthyroid.^{102,103} In a large retrospective series of patients treated with IL-2 therapy, 33% of patients with vitiligo demonstrated a clinical response compared with only 10% of patients without ($P < 10^{-6}$).¹⁰³ In a small study of patients being treated with IL-2 together with CD8 adoptive cell therapy, all patients who developed vitiligo demonstrated tumor regression.¹⁰⁴ These observations are highly suggestive of a true association, yet detailed mechanistic explanations are lacking. It is also possible that the association is related to lead time bias, with patients responding to therapy having time to develop autoimmune side effects with multiple treatments.¹⁰⁵

IRAEs are common with ipilimumab therapy and include colitis, pruritis, dermatitis, hepatitis, hypophysitis and uveitis.^{86,106} These IRAEs are independent of the underlying tumor burden and reflect tissue-specific inflammation occurring in a dose-dependent manner.⁸⁶ A similar, but somewhat milder, IRAE profile is seen in patients treated with PD-1 blockade.⁹⁵ Increasing experience has allowed clinicians to better manage these through early diagnosis and treatment if required. It is important to note that temporary blockade of CTLA-4 and PD-1 for cancer treatment is associated with short-lived immune events, the majority of which can be reversed by cessation of therapy combined with corticosteroids or

rarely TNF-blocking of other immunosuppressive agents.¹⁰⁷ This is in strong contrast to autoimmune disease, where even chronic immunosuppression often does not cure the individuals.¹⁰⁸

An association between IRAEs and response was first described by Attia *et al.*,¹⁰⁹ with 36% of patients with IRAE demonstrating a response to ipilimumab therapy compared with 5% without IRAE. In another study, clinical benefit was seen in 60% of patients at 24 weeks with grade 3 or 4 IRAE compared with only 22% for patients with grade ≤ 2 IRAEs ($P < 0.01$).⁸⁵ Furthermore a dose-dependent relationship between CTLA-4 blockade and IRAEs has been suggested.⁸⁶ Whether this is merely a reflection of patients who were doing well and received more drugs or truly represents a causal relationship between dose and toxicity is unknown. It is important to note that all of the above studies also describe durable responses in patients without any IRAEs.

The relationship between IRAEs and response to CTLA-4 blockade is belied by increasing evidence of the role of co-inhibitory molecules such as CTLA-4 and PD-1 in preventing autoimmune disease. CTLA-4 has been associated with a number of autoimmune diseases.¹¹⁰ This association has been exploited to prevent autoimmunity in mouse models by generating a fusion protein composed of the extracellular domain of CTLA-4 and the constant region of IgG to block the CD28/B7 interaction.^{111,112} A similar protein has been generated for use in humans (abatacept; BMS) and has shown efficacy in patients with autoimmune diseases including rheumatoid arthritis,¹¹³ juvenile idiopathic arthritis¹¹⁴ and psoriatic arthritis.¹¹⁵

SNPs in the CTLA-4 gene have been linked to a variety of autoimmune diseases and to an increased susceptibility to a number of cancer types.¹¹⁶ The CTLA-4 49A>G SNP has been particularly well studied, and it is a non-synonymous Thr17Ala substitution in exon 1 that encodes for the cell membrane signal peptide.¹¹⁷ The CTLA-4 49A allele has been shown to be protective against development of autoimmune thyroid disease¹¹⁸ and type 1 diabetes mellitus.¹¹⁹ By contrast, in a meta-analysis of cancer susceptibility of almost 15 000 patients and controls, the CTLA-4 49A>G SNP was associated with increased susceptibility to numerous cancers.¹¹⁶ These associations are not surprising given the enhanced receptor–ligand interaction and stronger ability to downregulate T-cell proliferation with the CTLA-4 49A>G SNP.¹²⁰ Even more interestingly, in a study of 152 patients with metastatic melanoma treated with an anti-CTLA-4 antibody, the CTLA-4 49 allele was strongly predictive of response with 78.3% of responders having the A allele ($P = 0.009$).¹¹⁷

Combination therapies and IRAEs in the clinic

To increase the efficacy of immunotherapy, combination therapies are currently being investigated (Table 1).^{121,122} These aim to cause immunogenic cell death and associated antigen presentation either by using cytotoxic chemotherapy (such as fotemustine), molecular targeted therapy (such as vemurafenib) or radiation therapy.

Table 1 Response rate and IRAEs

		n	Ipi dose (mg kg ⁻¹)	CR (%)	PR (%)	SD (%)	DCR	PD (%)	Any IRAE	Grade 3/4 IRAE
Hodi ⁷⁹	Ipi alone	137	10	1.5%	9.5%	17.5%	28.5%	51.1%	61.1%	14.5%
	Ipi + gp100	306	10	0.2%	5.5%	14.4%	20.1%	59.3%	58.2%	10.2%
Robert ⁸⁰	Ipi + DTIC	196	3	1.6%	13.6%	18.0%	33.2%	44.4%	77.7%	41.6%
Di Giacomo ¹²⁰	Ipi + fotemustine	86	10	6.9%	22.1%	17.4%	46.4%	53.4%	71.0%	28.0%
Patel ¹²¹	Ipi + temezolemid	64	10	16.0%	16.0%	42.0%	74.0%	23.0%	88.0%	31.0%

Abbreviations: CR, complete response; DCR, disease control rate; DTIC, dacarbazine; Ipi, ipilimumab; IRAE, immune-related adverse events; PD, progressive disease; PR, partial response; SD, stable disease.

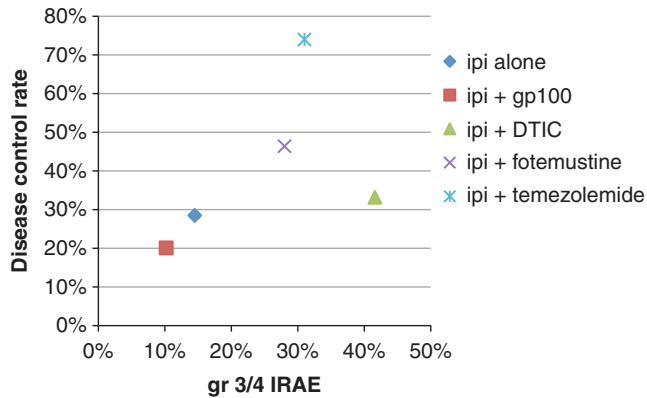


Figure 2 Association between Grade 3&4 IRAE and disease control rate in ipilimumab combination trials compared with ipilimumab therapy alone.

Alternatively, increased efficacy is being sought by combining immune therapies to target multiple steps in the immune modulatory pathway. Mouse models using B16 melanomas demonstrated that combined antibody-based CTLA-4 and PD-1 blockade lead to a higher T-effector to T reg cell ratio within tumors than when either agent was used alone.¹²³

Clinical trials of these combination therapies are in early stages. Understanding the mechanisms and side effects of each drug is paramount in order to combine regimes safely. This is highlighted by the significant rates of hepatotoxicity reported in the initial cohort of patients receiving concomitant ipilimumab and vemurafenib.¹²⁴ Other studies published to date also suggest that combination therapies have increased response rates and also increased grade 3 and 4 adverse events (Figure 2).^{80,81,121,122} Most recently, the results of a phase I study combining PD-1 blockade in escalating doses with CTLA-4 blockade were reported. The response rate was highest in patients receiving concurrent therapy (65%). In all, 40% of patients had a response, with a majority of the responses resulting in $\geq 80\%$ tumor reduction. This response is clearly beyond what has been seen with either agent alone. Although the incidence of grade 3 or 4 adverse events was also increased in patients undergoing concurrent therapy (56%), the majority of these were reversible with anti-inflammatory treatment. Interestingly, although the response rate was higher in patients with PD-L1 expression on the tumor, many patients without PD-L1 expression still responded to therapy.¹²⁵ It will be important to see whether these observations hold true in larger phase III trials that are currently being planned. Importantly, now that clinicians know how to identify and treat these events, the long-term consequences of IRAEs are often minimal.

CONCLUSION

This review describes the complex interplay of immune tumor surveillance, immunotherapy and autoimmunity. Major strides have been made in cancer immunotherapy in recent years; however, in the clinical setting with currently available therapies, response rates are low. Lessons learned from initial experiences are highly relevant to increase the efficacy of immunotherapy and to identify predictors of response.

CONFLICT OF INTEREST

CEA and JDW serve on advisory boards for Bristol-Myers Squibb. JDW is also a consultant for Merck and receives research funding from Bristol-Myers Squibb. The remaining authors declare no conflict of interest.

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