

CTLA-4 and PD-1/PD-L1 Blockade: New Immunotherapeutic Modalities with Durable Clinical Benefit in Melanoma Patients

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Abstract

Immune checkpoint blockade with monoclonal antibodies directed at the inhibitory immune receptors CTLA-4, PD-1, and PD-L1 has emerged as a successful treatment approach for patients with advanced melanoma. Ipilimumab is the first agent associated with a documented improved overall survival benefit in this patient population. A striking attribute of CTLA-4 blockade is the durability of objective responses, leading to speculation of a possible cure for some patients. Many tumor responses achieved with PD-1 and PD-L1 inhibition were durable in the phase I trials and were seen in a higher proportion of patients with melanoma than typically observed with ipilimumab. Biomarker development to identify the subset of patients with melanoma who will achieve durable clinical benefit with checkpoint blockade is critical; tumor PD-L1 expression has been promising in early studies. The contrast between unprecedented response rates but limited durability of responses achieved with BRAF and MEK inhibition in BRAF^{V600}-mutated melanoma and the impressive durability but relatively low rate of response achieved with immune checkpoint blockade is striking. Preclinical data on potential synergies between CTLA-4/PD-1/PD-L1 inhibition and MAPK-targeted therapy is emerging, and combined immune checkpoint blockade and MAPK inhibition are being explored in clinical trials. Other promising approaches to increase the number of patients with melanoma who benefit from durable responses with immune checkpoint blockade include concurrent or sequenced CTLA-4 and PD-1/PD-L1 inhibition and combination with other immunotherapeutic strategies. *Clin Cancer Res*; 19(19); 5300–9. ©2013 AACR.

Disclosure of Potential Conflicts of Interest

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CME Staff Planners' Disclosures

The members of the planning committee have no real or apparent conflict of interest to disclose.

Learning Objectives

Upon completion of this activity, the participant should have a better understanding of immune checkpoint-blocking antibodies in the treatment of advanced melanoma, the underlying biology explaining the durability of tumor responses associated with these antibodies, and strategies to further improve outcomes, such as combination therapy approaches and the development of biomarkers.

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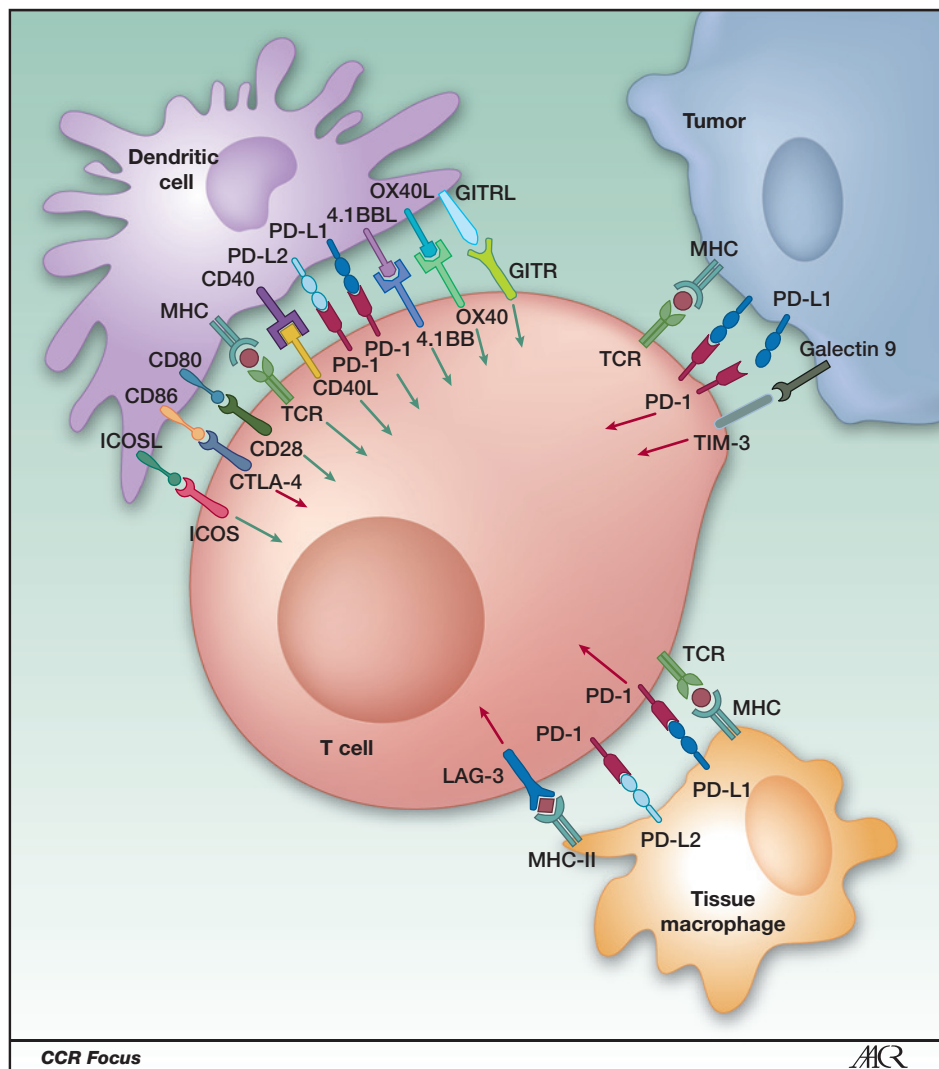
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Introduction

Long-term survival is an important concern to most patients diagnosed with metastatic cancer. In melanoma, we have known for decades that high-dose interleukin-2 (IL-2; refs. 1, 2) can induce durable tumor responses in approximately 5% of patients with metastatic disease; it has been suggested that some of these patients may have been cured. Adoptive transfer with autologous tumor-infiltrating

Figure 1. Costimulatory and coinhibitory ligand–receptor interactions between a T cell and a dendritic cell, a tumor cell, and a macrophage, respectively, in the tumor microenvironment. Figure adapted from Sharma (88) and Melero et al. (89).



T cells can also induce long-lasting tumor responses in patients with melanoma (3). As a proof of principle, these successes in a small fraction of patients indicate that eradication of metastatic disease can be achieved by manipulating a patient's immune system. However, the toxicity profile of high-dose IL-2 excludes many patients as potential candidates for this therapy, and the drug has never been compared with standard therapy in a randomized trial (1, 2).

To mediate antitumor responses, T cells must be specific for cancer cells and be activated into an effector state. In addition to the antigen-specific signal mediated by the T-cell receptor (TCR), a second costimulatory signal is required for full activation of T cells (4). Costimulation is mediated by a tightly controlled interplay of stimulatory and inhibitory receptor and ligand pairs. A number of inhibitory receptors and ligands expressed on T cells, antigen presenting cells (APC), and tumor cells have recently been identified as targets for cancer immunotherapy as they are critical mediators of immune suppression in the tumor microenvironment (ref. 5; Fig. 1). Because of their biologic role as

regulators of T-cell activation, these receptor/ligand pairs have been termed "immune checkpoints". Blockade of these checkpoints has emerged as a successful treatment concept. Specifically, inhibition of cytotoxic T lymphocyte antigen-4 (CTLA-4), an inhibitory receptor expressed on T cells, with the fully human monoclonal antibody ipilimumab has shown antitumor activity in patients with advanced melanoma, leading to improved overall survival in patients with advanced melanoma (6, 7). Furthermore, targeting the inhibitory receptor/ligand axis PD-1/PD-L1 with monoclonal antibodies has shown striking antitumor activity in patients with melanoma and other cancers in large phase I studies (8, 9).

One of the hallmarks of CTLA-4 blockade has been the durability of objective tumor responses that can be achieved in approximately 10% of patients with melanoma. Monoclonal antibodies targeting PD-1 and PD-L1, which are earlier in their clinical development, seem to follow a similar pattern: The objective responses in patients with advanced melanoma in phase I trials occurred in about one

third of patients, and the majority of these responses lasted at least 1 year, with many of them ongoing at the time of the initial data analysis (8).

Overall survival improvement with CTLA-4 inhibition likely results from the durability of clinical benefit in a relatively small subset of patients

CTLA-4 outcompetes the stimulatory receptor CD28 for binding to its ligands (CD80/CD86) due to its higher binding affinity and delivers a negative signal into the T cell, leading to inhibition of T-cell activation and expansion (Fig. 2). It thus modulates an immune response and averts autoimmunity (10–12). CTLA-4 regulates T cells predominantly during initial activation by dendritic cells and other APCs (priming phase; refs. 13–15). Furthermore, CTLA-4 is expressed by regulatory T cells (Treg) and memory CD4 cells and these cells may also be targeted by CTLA-4 blockade (16). Ipilimumab and tremelimumab are fully human monoclonal antibodies targeting CTLA-4 (17–20).

In phase III studies, response rates were between 11% with ipilimumab alone in previously treated advanced patients with melanoma and 15% with ipilimumab plus dacarbazine in treatment-naïve patients (6, 7). Nevertheless, improved overall survival with ipilimumab was clearly shown in both of these trials, as evident by reductions of the probability of death by 34% (compared with vaccine) and 28% (compared with dacarbazine alone). When ipilimumab was combined with dacarbazine, the median duration of best overall response was 19.3 months compared with 8.1 months with

dacarbazine monotherapy. The consistent separation of the survival curves by approximately 10% until almost 4 years of follow-up suggests that the impact on overall survival with ipilimumab is mainly driven by the long-lasting clinical benefit achieved in a small proportion of patients.

Notably, tremelimumab was also shown to induce durable objective responses in patients with metastatic melanoma; in the phase III trial, the median response duration was almost three times as long as the response duration with chemotherapy (35.8 vs. 13.7 months, $P = 0.0011$; refs. 21, 22).

Long-term follow-up was recently reported on 177 patients with advanced melanoma who were treated on three of the earliest ipilimumab trials conducted at the Surgery Branch of the National Cancer Institute (23–27). In these previously published studies, the median duration of objective responses was up to 88 months (Table 1). The authors document that some of the tumor responses initially reported as partial responses evolved into complete responses over a follow-up of several years. On average, complete responses were not achieved until 30 months after treatment initiation; one patient was on treatment for 6 years until a complete response was achieved. The durability of complete responses in the long-term analysis is impressive: Fourteen of 15 complete responses were ongoing at data cutoff (all 6 complete responses in patients who were treated with concurrent ipilimumab and IL-2 and 8 of 9 complete responses in patients who were treated with ipilimumab and gp100 vaccine). The durability of

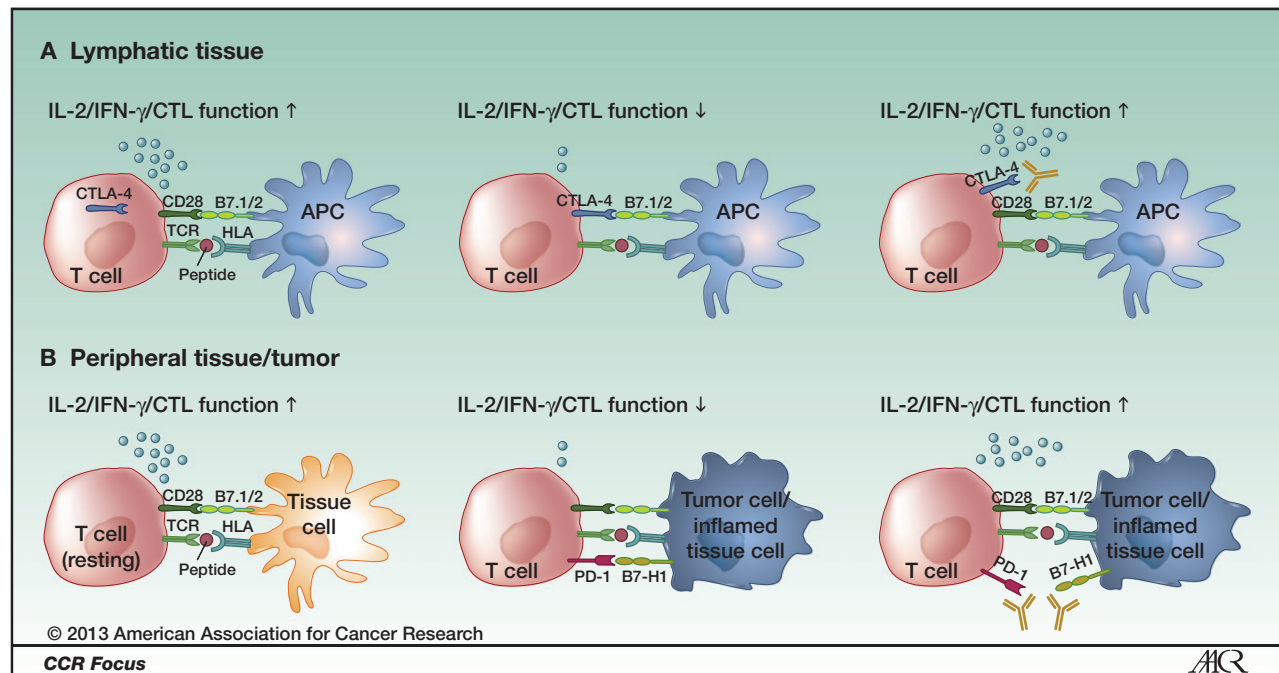


Figure 2. CTLA-4 and PD-1 modulate different aspects of the T-cell response: A, CTLA-4 is upregulated after antigen-specific activation of a naïve or memory T cell in lymphatic tissue, leading to decreased effector function (early activation phase). B, PD-1 is mainly expressed on antigen-experienced memory T cells in peripheral tissues cells. The immune modulation mediated by this pathway ensures protection of tissue from collateral damage during an inflammatory response. Tumor cells use this regulatory mechanism to evade a tumor-directed T-cell response by upregulating the PD-1 ligands B7-H1 and B7-DC.

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Table 1. Durable responses with CTLA-4, PD-1, and PD-L1 inhibition in individual clinical trials

Trial and reference	N	Median response duration (mo)	RR (%)	Complete response (%)	Partial response (%)	SD (%)	Median OS (mo)	
CTLA-4 Inhibition (ipi)								
Phase I	Ipi + gp 100 ²³	56	88	13	7	6	NR	14
	Ipi + IL-2 ²³	36	79	25	17	8	NR	16
	Ipi DE +/- gp100 ²³	85	42	20	6	14	NR	13
Phase II	Ipi 10 mg/kg (PT) ³⁰	155	NR	5.8	0	5.8	21	10.2
	Ipi 10 mg/kg (PT) ²⁹	71	NR	11.1	2.7	8.3	18.1	11.4
	Ipi 3 mg/kg (PT) ²⁹	71	NR	4.2	0	4.2	22.2	8.7
	Ipi 10 mg/kg (PT + TN) + budesonide ³¹	58	NR	12	2	10	19	17.7
	Ipi 10 mg/kg (PT + TN) + placebo ³¹	57	NR	16	0	16	19	19.3
Phase III	Ipi + dacarbazine ⁷	250	19.3	15.2	1.6	13.6	18	11.2
	Ipi + gp100 ⁶	403	11.5	5.7	0.2	5.5	14.4	10
	Ipi + placebo ⁶	137	Not reached	11	1.5	9.5	17.5	10.1
CTLA-4 Inhibition (treme)								
Phase II	Treme (10–15 mg/kg) ²²	84	NR ^a	9.5	2.4	7.1	31	10
Phase III	Treme (15 mg/kg) ²¹	328	35.8	11	3	8	NR	12.6
PD-1 Blockade								
Phase I	Nivolumab (0.1–10 mg/kg) ⁸	94	12.0 (73.1% ongoing)	28	0	28	6	16.8
	Lambrolizumab (2–10 mg/kg) ⁴⁷	135	81% ongoing ^b	38				
PD-L1 Blockade								
Phase I	BMS-936559 (0.3–10 mg/kg) ⁹	52	16.2 (55.5% ongoing)	17	0	17	27	NR

Abbreviations: Ipi, ipilimumab; Treme, tremelimumab; NR, not reported; TN, treatment naïve; PT, prior treatment; OS, overall survival.

^aSeventy-five percent of objective responses ongoing at 15+ to 28+ months.

^bAt 11 months median follow-up.

complete responses observed even in the trials not including IL-2 led the authors to speculate that a cure may be achievable with this drug. The long-term clinical benefit in a subset of patients with melanoma was also confirmed in earlier phase II ipilimumab studies, documenting flat survival curves between the 4- and 5-year marks (28–31).

Durable objective tumor responses in patients treated with ipilimumab are likely driven by endogenous tumor-reactive T cells and limited by other immunosuppressive mechanisms

In the phase III trial, clinical activity was identical whether patients received ipilimumab alone or in combination with the peptide vaccine gp100, suggesting that the vaccine was not effective. Lymphocytic tumor infiltrates, including CD8 and CD4 cells, along with tumor necrosis have been clearly identified in biopsies of melanoma metastases obtained after treatment with ipilimumab (32, 33). Although NY-ESO-1 was recently implicated as a potential target antigen, the specificity of these endogenous tumor-reactive T cells remains largely unknown (34). Numerous tumor antigens are probably targeted by the "anti-CTLA-4-enhanced" T-cell response, likely including antigens derived from tumor mutations. Indeed, the plasticity of the immune response, enabling it to adapt to a changing tumor (in contrast with

newly acquired mutations resulting in resistance to a targeted drug), may to some extent explain the durability of tumor responses achieved with immune checkpoint blockade.

Multiple layers of immune suppression are operational in the tumor environment, including other coinhibitory molecules expressed on T cells such as PD-1/PD-L1 (35), Tim-3 (36), and LAG-3 (37), Tregs, myeloid-derived suppressor cells (38), and soluble immunosuppressive mediators such as IDO (indoleamine 2,3-dioxygenase), arginase, prostaglandin E2 (PGE2), IL-6, IL-10, VEGF, and other cytokines and chemokines. Furthermore, there are many potential limitations intrinsic to the antibody therapy, such as tumor penetrance and drug concentration in the tumor, antibody stability, and receptor occupancy (39). Given the multitude of mechanisms that may impede tumor responses to checkpoint blockade, it is quite remarkable that CTLA-4 blockade as monotherapy induces partial and complete tumor responses that can last for many years. It also indicates that CTLA-4 is the predominant inhibitory driver of preexisting, endogenous tumor-specific T cells in these responding patients.

The observation that a subset of patients had delays before achieving best overall response underlies the characteristic response kinetics associated with ipilimumab. Immunologically, such delay likely reflects the interval

during which the endogenous, melanoma-specific T cells after uncoupling from the CTLA-4–mediated inhibitory signal are being activated, and then expand and infiltrate into the tumor. The observation of ongoing or even improving tumor response many months or years after the last dose of ipilimumab suggests that the (transient) anti-CTLA-4 blockade allows for sufficient activation and expansion of tumor-reactive T cells to control the tumor.

PD-1/PD-L1 blockade induces durable clinical benefit in patients with advanced melanoma

Whereas CTLA-4 is operational during early activation of T cells in lymphatic tissues, another regulatory molecule expressed on T cells, programmed death-1 (PD-1), functions during the effector phase of T-cell activation (Fig. 2). The interaction of PD-1 with its two ligands, B7-H1 and B7-DC (PD-L1 and PD-L2), occurs predominantly in peripheral tissues including the tumor microenvironment and leads to apoptosis and downregulation of T-cell effector function. The engagement of PD-1/PD-L1 interaction decreases the risk of collateral tissue damage by T cells (40–43). PD-L1 is expressed on many different cell types, including hematopoietic and epithelial cells, and is upregulated in response to proinflammatory cytokines, such as IFN- γ and IL-4 (35, 44), whereas PD-L2 expression is restricted largely to APCs. PD-L1 upregulation in metastatic melanoma was found to be colocalized with tumor-infiltrating lymphocytes and IFN- γ production, suggesting a resistance mechanism by the tumor against the endogenous immune response (45).

The anti-PD-1 monoclonal antibody nivolumab, (also known as MDX-1106 or BMS-936558) in a phase I trial at doses from 0.1 to 10 mg/kg induced objective responses in 26 of 94 patients (28%) with advanced, previously treated melanoma (8). Importantly, a significant number of these objective responses seemed to be durable. Thirteen of 18 patients (72%) who had received the drug for more than 1 year had responses that lasted for 1 year or longer. Furthermore, 19 of 26 reported responses in patients with melanoma were ongoing at the time of the data cutoff, indicating that the median duration of responses induced by nivolumab may be substantial. Of note, in addition to the objective responses, 6 of 94 patients with melanoma achieved stable disease that lasted ≥ 24 weeks. In a follow-up analysis of this study, 16 responses lasting more than 1 year were seen in 29 responding patients who had started treatment ≥ 1 year before data analysis; the median overall survival was 16.8 months for all dose cohorts and 20.3 months at the 3 mg/kg dose (46).

In another phase I study, 9 of 55 patients with advanced, previously treated melanoma had objective responses (3 complete responses and 6 partial responses) after being treated with the anti-PD-L1 monoclonal antibody MDX-1105 (BMS-936559; ref. 9). Five of these responses lasted at least 1 year and 5 were ongoing at the time of data analysis. Furthermore, 14 of 55 patients had SD that lasted ≥ 24 weeks.

Several other efforts are under way in the development of antibodies that target the PD-1/PD-L1 pathway. Objective responses with another PD-1–directed inhibitory antibody, lambrolizumab, were recently reported in 44 of 117 (38%) patients with advanced melanoma treated in a phase I study (47). An additional 8 of 117 patients had unconfirmed responses at the time of data cutoff. Most of the responses were durable, and 81% of the patients were still receiving treatment at the time of data analysis. Of note, previous treatment with ipilimumab did not have a large impact on the probability to achieve clinical benefit with lambrolizumab.

Interactions within the PD-L1/PD-1 pathway are complex, illustrated by the fact that PD-L1, in addition to PD-1, exerts an inhibitory signal to T cells through B7.1 (48). An antibody directed against PD-1, which only blocks interactions between PD-1 and its two ligands PD-L1 and PD-L2 (B7-DC), does not block the inhibitory signal through B7.1. Similarly, an antibody directed against PD-L1 blocks the inhibitory signals through PD-1 and B7.1, but does not affect the PD-L2-PD-1 pathway. The different biology that is likely associated with inhibition of these distinct sets of receptor–ligand interactions should be taken into consideration when designing and evaluating clinical trials with PD-1- and PD-L1–directed antibodies (14). For example, lack of PD-L2 expression has been associated with potentially tumor promoting T_H2 inflammation, suggesting that PD-L2 blockade may be an unwanted collateral effect of PD-1, but not PD-L1 inhibition (49–51).

Distinct sites of action for CTLA-4 and PD-1–resulting spectrum of clinical events

PD-1 interaction with its ligands mostly occurs in peripheral tissues and organs, upon re-presentation of antigens to memory T cells (Fig. 3). To be capable of extravasating to various tissues organs or tumors these T cells must have encountered their antigen previously. This initial encounter or priming takes place in lymphoid organs. Naïve T cells are endowed with a set of adhesion molecules that allow them to extravasate through high endothelial venules (HEV) and to reach T-cell–dependent areas of the lymph nodes where antigens are brought via afferent lymphatics, processed, and carried by APCs (52). If the antigen is presented to the T cell harboring the corresponding receptor, T-cell activation will occur, implicating the costimulation molecules as described in Fig. 3. Replacement of CD-28 by CTLA-4 downregulates T-cell activation after 24 to 48 hours. Inhibiting CTLA-4 can thus, in theory, lead to an important, diffuse, and unspecific T-cell activation. This is concordant to what is observed in the clinic in patients treated with the anti-CTLA-4 antibody ipilimumab with an important increase of T-cell subpopulations and the emergence of frequent and various immune-related adverse events involving the gut, the skin, the endocrine glands, the liver, and other organs (6, 53, 54).

Once T cells have been primed and have acquired an immunologic memory, they also acquire a distinct set of

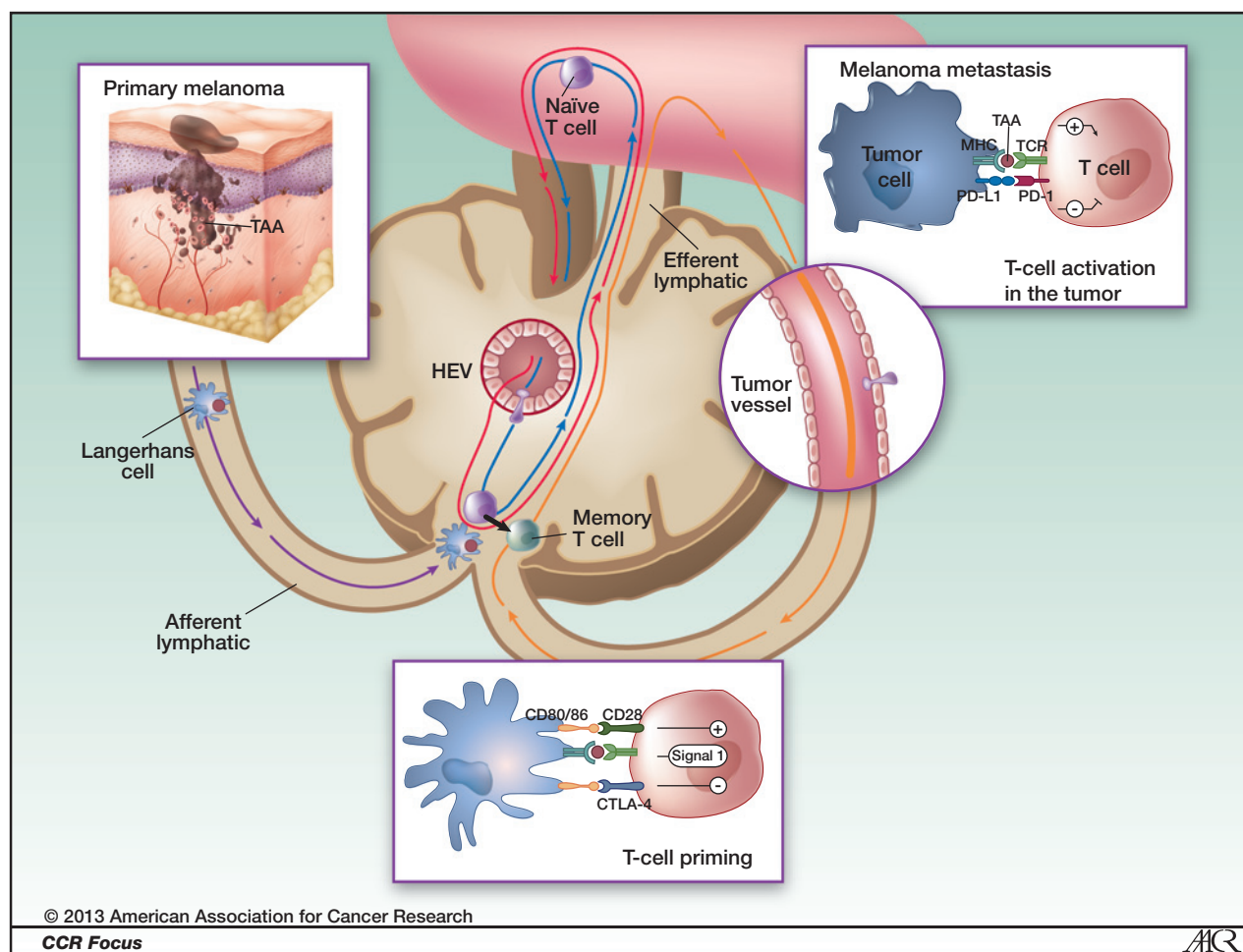


Figure 3. The figure shows a primary melanoma and tumor-associated antigens (TAA) that are taken up by an APC (a Langerhans cell; left). APCs then migrate via afferent lymphatics to the sentinel skin-draining lymph node, where they present TAA to naïve T cells. These naïve T cells continuously screen the lymphoid organs, extravasating through HEV until their corresponding peptide is presented to them in the context of the MHC. When a TAA is presented in the T-cell-dependent area of a lymph node to its specific naïve T cell (center), the latter requires signal 1 (antigen presentation to the TCR) and 2 (costimulation) for full activation. It will then proliferate and acquire "antigen memory" and a distinct and different set of adhesion molecules that will allow it to navigate outside of the blood vessels and the lymphoid organs, to the peripheral tissues and organs, like the melanoma metastases where it will be able to be reactivated upon re-presentation of the same TAA. However, in the lymph node, T-cell activation is interrupted when signal 3, mediated through interaction between CTLA-4 and CD80/86, takes over CD28 and CD80/86 interaction. This occurs 24 to 48 hours after the initiation of T-cell priming. When the memory T cell is recruited to the melanoma metastasis (right) and activated upon TAA re-presentation, the effector activation is decreased when PD1 is engaged with its ligand PDL-1 that can be expressed on the tumor cell constitutively or in the context of inflammation. The figure is adapted from the C. Robert and C. Verjat figure collection, Institute Gustave Roussy (Villejuif-Paris Sud, Paris, France).

adhesion molecules that permit their homing to various peripheral tissues and organs, including tumors (52). Their extravasation through tissue postcapillary venules is facilitated by inflammation. It is precisely in this context of subsequent encounters with their antigens in a peripheral tissue, in an inflammatory environment, that PD1 interaction with its ligands is playing a role by decreasing the magnitude of the immune response. By targeting more specifically T cells that are already engaged in an ongoing effector T-cell response, PD1 blocking will have a more restricted spectrum of T-cell activation compared with CTLA-4 blocking. This is likely the reason why immune adverse events seem less frequent with anti-PD-1 or anti-PDL-1 antibodies (around 12–18% of grade 3/4 adverse

events than with ipilimumab (8, 9). This also can explain why responses to CTLA-4 antibodies may be delayed while we can expect to see early clinical responses with PD-1 antibodies in patients with metastases heavily infiltrated with T cells.

Successful retreatment with anti-CTLA-4 or PD-1 blockade after tumor recurrence in patients who achieve initial clinical benefit

We have recently shown that retreatment with ipilimumab in patients who initially achieved clinical benefit (objective responses or stable disease) lasting ≥ 3 months can induce objective tumor responses and disease stabilization (55). The data suggest that tumor-specific T cells that

mediate the initial responses may no longer control the tumor because of its changing antigen repertoire as a result of the attack by the initially relevant T cells (immune editing; refs. 56, 57). By this hypothesis, clinical response to retreatment with ipilimumab would indicate activation and expansion of a new set of T cells specific to the new antigen repertoire. Durable responses lasting for several years off therapy as well as successful reinduction in patients with cancer, including a patient with melanoma, were recently also reported with PD-1–directed antibodies. These observations suggest that in the context of immunoediting: (i) PD-1 blockade, similar to CTLA-4 inhibition, has the potential to establish a favorable equilibrium between adequate T-cell response against the tumor and immune evasion by the tumor; (ii) tumor recurrence after successful treatment with PD-1 blockade indicates disruption of this equilibrium; and (iii) retreatment with PD-1 inhibition after recurrence can reset this balance (58).

Biomarkers

The identification of the relatively small proportion of patients who attain durable clinical benefit from treatment with ipilimumab has been challenging. There is a critical need for a biomarker for clinical benefit; however, a reliable predictive marker for response to ipilimumab has so far been elusive. An increase of the absolute lymphocyte count after the initial doses of ipilimumab and the presence of combined antibody and CD8⁺ cell responses to the cancer/testis antigen NY-ESO-1 before treatment were associated with clinical benefit (34, 53). More general signatures for immunocompetence have recently been associated with responses to immunotherapy, such as NRAS mutational status in patients with melanoma treated with high dose IL-2 and an 84 gene expression signature in melanoma and in patients with non-small cell lung cancer (NSCLC) vaccinated with MAGE-A3 (59, 60).

PD-L1 expression by the tumor is actively being pursued as a predictive marker for tumor activity of PD-1– and PD-L1–directed antibodies. In a retrospective analysis of a small subset of patients who were treated in the phase I nivolumab study (the majority with melanoma, renal cell carcinoma, and NSCLC), none of the patients with tumors lacking PD-L1 expression had a tumor response (8). This observation has led to the selection of patients based on tumor PD-L1 expression in some of the ongoing anti-PD-1 and anti-PD-L1 clinical development programs. It is important to emphasize that the data from the nivolumab study are preliminary and that only tumor responses (and not disease stabilization) were captured as a clinical benefit endpoint.

Furthermore, PD-L1 expression may undergo changes driven by alterations in the tumor microenvironment, for example, infiltrations with immune cells, potentially limiting its use as a clear-cut predictive biomarker as opposed to, for example, BRAF^{V600} mutational status. Furthermore, reliable PD-L1–specific antibodies have been difficult to develop, and an agreement on acceptable criteria for the definition of PD-L1 positivity such as appropriate staining

patterns (membranous vs. cytosolic) and cutoffs for percentages of staining cells has so far been elusive. Prospective randomized trials using validated immunohistochemical assessment of PD-L1 expression are needed for further exploration.

Future strategies: Maximizing the number of patients benefiting from durable disease control

The long-term experience with CTLA-4 blockade and early promising data on durable responses observed with PD-1/PD-L1 inhibition suggest that long-term disease control may be within reach for a subset of patients. Arguably the most critical challenge is to substantially increase the proportion of patients with melanoma who can enjoy the durable clinical benefit that can be achieved with immune checkpoint blockade. Almost certainly, combinatorial strategies of some sort will be needed to achieve this goal (Fig. 4). In BRAF^{V600} mutant tumors, BRAF/MEK inhibition has also revealed promising antitumor activity (61–69). Importantly, as discussed elsewhere in this *Clinical Cancer Research* Focus section (70), there is emerging evidence for favorable effects of BRAF/MEK pathway inhibition on the endogenous tumor immune response as well as for potential synergies between MAPK-targeted therapy and immunotherapy (71–79); clinical trials combining MAPK pathway inhibition with CTLA-4 and PD-L1 blockade in patients with melanoma are under way (80).

Early clinical data suggest that combined CTLA-4 and VEGF inhibition can induce durable responses in a substantial proportion of patients with advanced melanoma (81). Furthermore, a durable complete response rate of

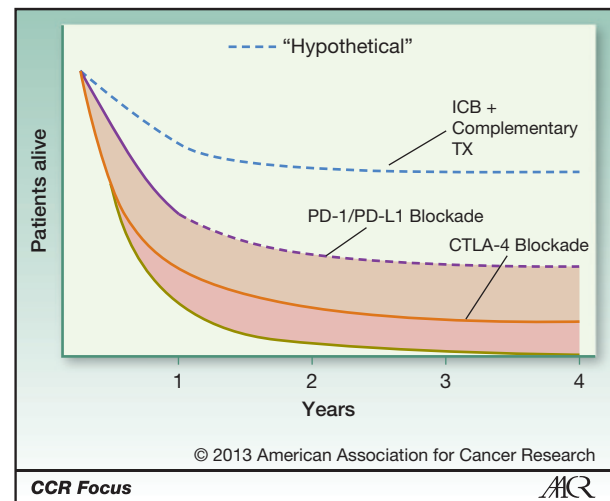


Figure 4. Ipilimumab induces durable objective responses in a relatively small proportion of patients, leading to a plateau towards the end, but no shift of the early portion of the survival curve. The higher objective responses rates and response durability observed in phase I trials suggest that PD-1/PD-L1 blockade impacts both aspects (early shift and plateau towards the end). Innovative, biomarker-driven combinatorial strategies building on immune checkpoint blockade as monotherapy may further improve on these advances.

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17% was seen with the combination of ipilimumab and high-dose IL-2 in a selected population of patients with advanced melanoma (23). A noteworthy rate of durable objective response, including 4 complete responses, was also seen with the combination of tremelimumab and IFN- α in a group of 37 patients with previously treated advanced melanoma (82). Additional potential complementary immune therapies include adoptive T-cell transfer (83), vaccines, suppression of Tregs (84, 85) or the blockade of other inhibitory checkpoints such as Tim-3 (36) LAG-3 (37), and B7-H3, among others.

There is also a strong scientific rationale for concurrent CTLA-4 and PD-1/PD-L1 blockade. The distinct roles of CTLA-4 and PD-1 in immune regulation (early T-cell activation phase in lymphoid tissue vs. antigen experienced T cells in peripheral/tumor tissue) suggest that the two pathways are nonredundant immune checkpoints. In fact, combined blockade with CTLA-4, PD-1, and PD-L1 blocking antibodies acts synergistically in a B16 melanoma model. Importantly, in this model, inhibition of CTLA-4 leads to a higher proportion of tumor-infiltrating cells (TIL) expressing PD-1, whereas PD-1 blockade leads to upregulation of CTLA-4 on TIL (86). These data suggest that antitumor activity of CTLA-4 blockade may be dampened by suppression of tumor-specific T effector cells through the PD-L1/PD-1 pathway in the tumor and that this immune suppression is partially mediated by the CTLA-4 blockade itself. Conversely, the efficacy of PD-1 blockade may be compromised by the lack of full activation of tumor-specific effector T cells mediated by CTLA-4, which also may be aggravated by upregulation of CTLA-4 induced by the PD-1 inhibition itself. Therefore, monotherapy with either CTLA-4 or PD-1 blockade leaves the other critical immune checkpoint unopposed and may induce further upregulation of a compensatory regulatory pathway. Substantial synergy may therefore arise by blockade of both checkpoints. Impressive proof

for the efficacy of combined checkpoint blockade in the clinic was recently reported in a phase I study, in which 53% of patients with melanoma in the highest dose cohort achieved objective responses after treatment with nivolumab and ipilimumab, the majority of which were rapid in onset and deep (>80 tumor reduction; ref. 80).

With such exciting prospects, the therapeutic window for these combinatorial approaches will need to be better delineated. The phase I combination trial of ipilimumab and vemurafenib was halted because of liver toxicity, and more than half of patients treated concurrently with ipilimumab and nivolumab experienced treatment related grade 3 or 4 adverse events (80, 87).

Immune checkpoint blockade has established a new standard for the treatment of cancer with prospects for clinical benefit durability in patients with melanoma. Our improved understanding of the mechanisms involved with immune regulation now provides a strong foundation for the development of combinatorial approaches of immune therapeutic, small molecule targeted, and antiangiogenic strategies to improve patient outcomes.

Authors' Contributions

Conception and design: P.A. Ott, F.S. Hodi, C. Robert

Development of methodology: P.A. Ott, F.S. Hodi, C. Robert

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): P.A. Ott

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): P.A. Ott, C. Robert

Writing, review, and/or revision of the manuscript: P.A. Ott, F.S. Hodi, C. Robert

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): P.A. Ott

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