Published in final edited form as:

Clin Cancer Res. 2019 September 01; 25(17): 5191–5201. doi:10.1158/1078-0432.CCR-18-1550.

Immunotherapy of Melanoma: Facts and Hopes

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Abstract

Melanoma is among the most sensitive of malignancies to immune modulation. Although multiple trials conducted over decades with vaccines, cytokines and cell therapies demonstrated meaningful responses in a small subset of patients with metastatic disease, a true increase in overall survival within a randomized phase 3 trial was not observed until the development of anti-CTLA-4 (ipilimumab). Further improvements in overall survival for metastatic disease were observed with the anti-PD-1-based therapies (nivolumab, pembrolizumab) as single agents or combined with ipilimumab. A lower bound for expected five-year survival for metastatic melanoma is currently approximately 35% and could be as high as 50% for the nivolumab/ipilimumab combination among patients who would meet criteria for clinical trials. Moreover, a substantial fraction of long-term survivors will likely remain progression-free without continued treatment. The hope and major challenge for the future is to understand the immunobiology of tumors with primary or acquired resistance to anti-PD-1 or anti-PD-1/anti-CTLA-4 and to develop effective immune therapies tailored to individual patient subsets not achieving long-term clinical benefit. Additional goals include optimal integration of immune therapy with non-immune therapies, the development and validation of predictive biomarkers in the metastatic setting, improved prognostic and predictive biomarkers for the adjuvant setting, understanding mechanisms of and decreasing toxicity, and optimizing the duration of therapy.

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Conflicts of Interest:

Dr. Weiss: *Consultant for:* Array BioPharma, Magellan Rx; *Research support:* NIH (NCI) K12 CA215110–01A1

Dr. Wolchok: *Consultant for:* Adaptive Biotech; Advaxis; Amgen; Apricity; Array BioPharma; Ascentage Pharma;Astellas; Bayer; Beigene; Bristol Myers Squibb; Celgene; Chugai; Elucida; Eli Lilly; F Star; Genentech; Imvaq; Janssen; Kleo Pharma; MedImmune; Merck; Neon Therapuetics; Ono; Polaris Pharma; Polynoma; Psioxus; Puretech; Recepta; Trieza; Sellas Life Sciences; Serametrix; Surface Oncology; Syndax. *Research support:* Bristol Myers Squibb; Medimmune; Merck Pharmaceuticals; Genentech. *Equity in:* Potenza Therapeutics; Tizona Pharmaceuticals; Adaptive Biotechnologies; Elucida; Imvaq; Beigene; Trieza. *Patents:* Xenogeneic DNA Vaccines; Alphavirus Replicon Particles Expression TRP2; Myeloid-derived suppressor cell (MDSC) assay; Newcastle Disease Viruses for Cancer Therapy; Genomic Signature to Identify Responders to Ipilimumab in Melanoma; Engineered Vaccinia Viruses for Cancer Immunotherapy; Anti-CD40 agonist mAb fused to Monophosphoryl Lipid A (MPL) for cancer therapy; CAR+ T cells targeting differentiation antigens as means to treat cancer; Immune modulating antibodies to CTLA-4, PD-1, GITR for use in cancer therapy.

Dr. Sznol: *Consultant for:* Abbvie, Agonox, Allakos, Almac, Anaeropharma, Arbitus, Array, Astra-Zeneca/Medimmune, Baxalta-Shire, Biodesix, Bristol-Myers, Celldex, Genentech-Roche, Genmab, Gritstone, Hinge, Immunocore, Incyte, Innate Pharma, Inovio, Janssen/Johnson and Johnson, Kyowa-Kirin, Lilly, Lion Biotechnologies (Iovance), Merck, Modulate Therapeutics, Molecular Partners, Nektar, Newlink Genetics, Novartis, Pfizer, Pierre Fabre, Seattle Genetics, Therevance, Vaccinex; *Scientific Advisory Boards*: Adaptimmune, Lycera, Omniox, Pieris, Symphogen, Torque; *Stock Options*: Actym, Adaptive Biotechnologies, Amphivena, Intensity, Torque

Keywords

melanoma; immunotherapy; survival; PD-1; CTLA-4

INTRODUCTION

Incidence rates of melanoma have doubled over the past 30 years due to an aging population, increased ultraviolet (UV) sunlight exposure, ongoing tanning bed use, and improved awareness and detection [1]. Although melanoma represents only one percent of all skin cancers diagnosed, it is by far the most fatal with an estimated 10,000 deaths in the United States in 2018 [2]. Because of UV light exposure and possibly the biology of melanin, the DNA of melanoma cells in most patients contains a relatively large number of mutations [3]. The mutations result in altered protein sequences, a subset of which are processed and presented as 'foreign' peptides on surface MHC molecules and therefore recognized by a host T-cell response. Melanosomal proteins such as MART-1, gp100 and tyrosinase can also be recognized by host T-cell responses, possibly because of molecular mimicry between the peptides presented on cell surface MHC molecules and peptides from pathogen-associated proteins [4]. In addition, melanomas often re-express developmental proteins such as the cancer-testes antigens which can be recognized by host immune responses [5]. Multiple studies have shown that T-lymphocytes can be grown ex vivo from tumor-infiltrating lymphocytes (TIL) of metastatic melanoma lesions, and in most patients, a subset of these TIL specifically recognize autologous melanoma. Consistent with the latter observations, clinical activity was observed with a variety of local or systemically administered immune therapies including interleukin-2 (IL-2) [6], interferon-alfa [7, 8], and adoptive cell therapy (ACT) [9–11]. Objective response rates (ORR) in metastatic melanoma ranged from approximately 15% for cytokines to up to 50% for ACT with expanded TIL, but only 5–20% of patients achieved long-term complete responses (CR). Nevertheless, the durable CRs provided proof of concept for immunotherapy efficacy in melanoma and supported further development of novel immune modulators in melanoma and other malignancies.

Subsequent development of monoclonal antibodies targeting the immune checkpoints cytotoxic T-lymphocyte antigen-4 (CTLA-4) (ipilimumab, approved by FDA in 2011) and programmed death 1 (PD-1) (nivolumab, pembrolizumab, approved by FDA in 2014) drastically transformed the management of advanced melanoma and of melanoma at high risk for distant recurrence after resection of the primary and regional nodal disease (Table 1). Average life expectancy for a patient with metastatic melanoma ranged from six to twelve months before introduction of the immune checkpoint inhibitors (ICI); 3-year overall survival (OS) rates in clinical trials of anti-PD-1 alone or in combination with ipilimumab now exceed 50% [12]. Five-year survival rates for anti-PD-1 alone could approach 35–40% [13], and the 4-year survival rate for nivolumab plus ipilimumab exceeded 50% [14]. Although not well documented in the current trials, our substantial institutional experiences with these agents indicate that a large fraction of the 5-year survivors are off treatment and have no active disease, having required only the immune therapies and in some cases additional radiation or surgical resection of residual oligometastatic disease.

However, despite the substantial advances, roughly half of all melanoma patients treated with ICI will demonstrate primary or acquired resistance [15, 16]. No highly accurate predictive biomarkers exist and there are limited effective treatment options available once resistance develops, except for targeted BRAF + MEK inhibitors in tumors expressing driver mutations in the BRAF gene. While adverse effects from immune therapies (irAE) are manageable in most patients, they cause significant morbidity in a subset and may require treatment discontinuation. Finally, ICIs are expensive agents with important individual and societal economic implications, problems which must be addressed with more refined dosing schedules, optimization of treatment duration, and rational patient selection in the future [17, 18].

IMMUNE CHECKPOINT INHIBITORS IN MELANOMA

Before 2011, standard of care immune therapy for melanoma was limited to interferon-alfa for primary/regional disease at high risk for recurrence and high dose IL-2 for advanced/metastatic disease. High dose IL-2 produced ORRs of up to 16% and a CR rate of 6%, based on data obtained before ICI moved to front-line treatment of melanoma [19]. No randomized trials of high dose IL-2 versus chemotherapy were conducted. Recognition that T-cell activation through the T-cell receptor (TCR) was modulated by ligand-receptor costimulatory and co-inhibitory signals provided additional targets for immune intervention. CD28 was the first co-stimulatory molecule identified in 1986 and binds to CD80/CD86 expressed on APCs, but can be countered by induced cell surface expression of CTLA-4, which competitively binds to CD80/CD86 with higher affinity than CD28 [20]. PD-1 is another co-inhibitory receptor induced by T-cell activation and has two known ligands, PD-L1 and PD-L2 [21]. PD-L1, also known as B7-H1, was found on the cell surface of melanoma cells, on other immune cells within the tumor microenvironment and on dendritic cells. Multiple other T-cell costimulatory and co-inhibitory ligand-receptor interactions have been discovered [22]. The immunobiology of these pathways is complex, could influence various immune cell subsets including regulatory T cells, and may have roles in naïve T-cell priming as well as in expansion and function of effector T cells in the tumor microenvironment. Blocking monoclonal antibodies against both CTLA-4 and PD-1 were shown to produce clinical activity that surpassed any of the prior available therapies and revolutionized the care of melanoma patients [23]. A major challenge for improving therapy is to fully understand the baseline host anti-tumor immune response and post-therapy evolution of the response that results in anti-tumor activity.

Anti-CTLA-4

Ipilimumab and tremelimumab ORRs are in the same range as high dose IL-2, and responses can also be quite durable [24, 25]. Several important lessons were learned during anti-CTLA-4 development, including management of the induced irAEs, and the varying patterns of response kinetics, for example the observation of clear clinical disease progression of existing and new lesions in the first 6–12 weeks of treatment followed in some cases by dramatic disease regression, or pseudoprogression, which occurs in an estimated 10% of patients [26, 27]. Growing experience with anti-CTLA-4 demonstrated that the standard radiographic Response Evaluation Criteria in Solid Tumors (RECIST) may underestimate

clinical benefit from ICI. Since then and with development of anti-PD-1, multiple iterations of modified RECIST and immune related response criteria for patients receiving ICI have been developed, however RECIST is still the most common criteria in use [28–32]. There have also recently been reports of rapid progression, termed hyperprogression, in some patients treated with checkpoint blockade [29, 30, 33]. Further study of this important area is needed to better understand underlying biology.

Anti-CTLA-4 was active in patients who had progressed on prior IL-2. Ipilimumab improved median OS compared to a gp100 peptide vaccine (10 vs. 6.4 months) in previously-treated patients with advanced melanoma and was the first ICI to be approved by FDA for any malignancy in 2011 [24]. Follow-up revealed a 3-year OS of 22% and a plateau of the survival curve for up to 10 years, consistent with the observation of durable responses [34]. Although a randomized study showed ipilimumab 10 mg/kg produced superior survival to the approved 3 mg/kg (median 15.7 vs. 11.5 months) [35], the outcomes are still inferior to studies of single agent anti-PD-1 (nivolumab and pembrolizumab) [36].

Anti-PD-1

Both nivolumab and pembrolizumab are superior to ipilimumab based on single agent trials and randomized studies [16, 36]. When comparing results for similar groups of patients, nivolumab and pembrolizumab produce nearly identical rates of adverse events, objective response, progression-free survival (PFS) and OS. In one trial, single agent pembrolizumab demonstrated superior PFS and 2-year OS rates (55% vs. 43%, crossover was allowed) compared to ipilimumab [36]. Three and four-year survival rates for pembrolizumab and nivolumab in previously untreated patients are 51% [37] and 42% [38]. Five-year survival for pembrolizumab in treatment-naïve patients is 41% [13]. Five-year survival for nivolumab in previously treated patients was estimated at 35% [39]. Both pembrolizumab and nivolumab produce much lower rates of irAEs than ipilimumab, although types of irAEs are similar. PD-1 inhibition became the standard of care first-line therapy for metastatic melanoma after FDA approval in 2014 [36]. Of note, patients with or without tumor PD-L1 expression receive survival benefit from anti-PD-1 compared to a non-effective treatment such as dacarbazine [37]. Anti-PD-1 has also shown clinical benefit for several specific melanoma subgroups, for example in patients with desmoplastic melanoma, a rare histologic variant with a high mutation burden [40], and for untreated brain metastases in which pembrolizumab yielded a brain metastasis response rate of 26% and 2-year OS of 48% [41].

Combinations of anti-PD-1 and anti-CTLA-4

In CheckMate-067 which compared combination ipilimumab and nivolumab or nivolumab to ipilimumab alone, the combination demonstrated 3 and 4-year OS rates of 58% and 53%, compared to 52% and 46% for nivolumab and 34% and 30% for ipilimumab [12, 14]. The combination produced substantially greater rates of toxicity than single agent nivolumab, although manageable and reversible in almost all patients. Nearly 40% of patients discontinued treatment in the combination arm. Outcome in those experiencing severe toxicity and requiring steroids or other agents to reverse toxicity was not compromised [42]. Based in part on improvement in ORR and PFS in the post-hoc comparison of the combination to nivolumab, the combination was approved by FDA in 2015 [12, 16]. Of note,

patients experiencing toxicity from the combination were not allowed to receive nivolumab alone after resolution of toxicity, which may have negatively affected the OS in that arm.

In subsequent single arm studies and a small randomized phase 2 trial, a lower dose of ipilimumab (1 mg/kg) was combined with the more standard single agent dose of either nivolumab or pembrolizumab, resulting in lower rates of severe toxicity [43] and activity appears similar. For example, a phase Ib trial of pembrolizumab 2 mg/kg combined with low dose ipilimumab (1 mg/kg) reported an ORR of 61% [44]. The effects of the altered dose ratios on PFS and OS can only be accurately assessed in larger randomized trials, but based on current data, differences would likely be small and therefore only detectable in very large trials. CheckMate-064 assessed whether sequential administration of ipilimumab followed by nivolumab or the reverse sequence could decrease toxicity and maintain similar efficacy to combined ipilimumab and nivolumab. Treatment-related AEs were similar between the two study arms. Patients in the nivolumab followed by ipilimumab group had higher response rates at week 25 (41% vs. 20%) and improved 12-month OS rates (76% vs. 54%) compared to the ipilimumab followed by nivolumab group [45]. Ipilimumab alone and ipilimumab plus anti-PD-1 have shown activity in patients unresponsive to or with acquired resistance after single agent anti-PD-1 [46, 47]. Current data cannot exclude the possibility that sequential anti-PD-1 followed by ipilimumab alone or ipilimumab/anti-PD-1 combination could produce similar survival to the combination therapy given first-line.

Both clinical and laboratory features have been assessed to identify the subset of patients that clearly benefit from the addition of ipilimumab to anti-PD-1. In Checkmate-067, PFS and OS were improved by the combination in the subset with PD-L1 negative tumors (at the <5% level in stratification, or at the <1% level in post hoc analysis) but the difference was not statistically significant. Exploratory analyses using time-dependent receiver-operating characteristic curves also determined that PD-L1 expression could not reliably predict OS [14].

Melanoma brain metastases, a common occurrence and therapeutic challenge, are typically treated with local therapy such as stereotactic radiosurgery. There is now evidence for use of ICI which appears to provide benefit in a subset of patients with asymptomatic, small, untreated brain metastases. In a single arm phase 2 study, the combination of ipilimumab/nivolumab demonstrated significant activity against baseline untreated brain metastases (similar to activity against non-CNS metastases) [48], and in a similar brain metastases population, the combination appeared superior when randomized against anti-PD-1 alone, although sample size was very small [49]. The results of a small randomized study in the stage III neoadjuvant setting also suggested superior results for ipilimumab/nivolumab over nivolumab alone [50]. In certain populations, such as metastatic disease from mucosal primaries, retrospective analyses show that the combination is superior to nivolumab alone, but the advantage occurs in the group with PD-L1 negative tumors (which represents most of the patients) [51]. Development of effective immunotherapeutic approaches for metastatic uveal melanoma also remains a challenge and most clinical trials exclude this population due to its distinct tumor biology [52]. Only a small subset of uveal melanoma patients respond to ipilimumab [53] or anti-PD-1 [54], and data are not yet available on the activity of ipilimumab plus nivolumab. An integrative analysis of uveal melanomas from the Cancer

Genome Atlas suggests that an inflammatory molecular subgroup does exist, but patient selection is still an issue [55]. Extrapolating from clinical data to date, combination ipilimumab and nivolumab may represent a preferred first line therapy for patients with PD-L1 negative tumors, elevated LDH, mucosal primaries, and/or untreated brain metastases.

Cross-study comparisons suggest an advantage in median and overall survival for first-line anti-PD-1 based therapy over BRAF/MEK inhibitors in melanoma harboring a BRAF V600 mutation. However, for certain disease presentations in which very rapid clinical response is required or immune therapies are contra-indicated, targeted molecular therapies should be given first [56]. This question is being formally addressed by an ongoing phase III trial randomizing patients with BRAF V600 mutant melanoma to targeted therapy with dabrafenib and trametinib followed by ipilimumab and nivolumab at time of disease progression, or vice versa (NCT02224781).

Adjuvant Immunotherapy

Prior to development of ICI, the majority of patients with completely resected melanoma at high risk for recurrence could be offered adjuvant interferon-alfa or pegylated-interferon, however the agents were associated with bothersome and chronic adverse effects during therapy and only provided modest recurrence-free survival (RFS) benefit and a small OS advantage [57]. A randomized trial of ipilimumab at 10 mg/kg versus placebo for completely resected stage III melanoma improved RFS and OS, however caused a high rate of grade 3 and 4 AEs (54%) [58]. Adjuvant anti-PD-1 quickly replaced ipilimumab in 2017 after CheckMate-238 showed improved 12-month RFS rates for nivolumab compared to ipilimumab (70.5% vs. 60.8%), with lower rates of high grade toxicity (14.4% vs 45.9%) in patients with resected stage IIIB, IIIC, or IV melanoma. The hazard ratio for disease recurrence or death was 0.65, however, survival results have not yet been reported [59]. Pembrolizumab also improved RFS compared to placebo [60]. In the latter trial, effects on OS are eagerly awaited because all placebo patients were offered pembrolizumab at time of recurrence, which could address the value of treatment in the adjuvant setting versus waiting to treat until disease recurrence. Accrual to Checkmate-915 (NCT03068455) was recently completed in which nivolumab plus low dose ipilimumab was compared to nivolumab monotherapy in patients with completely resected stage IIIB/C/D or stage IV melanoma. Patients with stage IIIA-IIIC (AJCC VII) resected melanoma whose tumors contain a BRAF V600 mutation are also eligible to receive dabrafenib plus trametinib which was FDA approved in 2018 for use in the adjuvant setting [61]; however, targeted therapies have not been compared to ICI in the adjuvant setting.

OTHER IMMUNOTHERAPIES FOR METASTATIC MELANOMA

In looking forward for approaches to improve therapeutic outcomes, reviewing past development efforts of other immune modulators is instructive. Because of its presumed immunogenicity, most immune modulators were tested initially in metastatic melanoma. Using objective response as the measure of clinical activity, most agents were either inactive or at best demonstrated low response rates. Immune modulators tested in clinical trials

included cancer vaccines, cytokines, co-stimulatory receptor agonists, and multiple types of cell therapies.

Many types of cancer vaccines progressed to clinical development, immunizing against shared melanosomal proteins or cancer testes antigens, or against antigens contained in autologous tumor or allogeneic tumor cells. Multiple antigen delivery approaches and immunologic adjuvants were employed in the vaccine trials, including gene modified cells, peptides or proteins with adjuvant, antigen loaded onto autologous dendritic cells, and delivery of defined antigens by viral vectors or DNA plasmids [62]. Rare responses of small volume distant metastatic disease were observed in some of these trials. Vaccine development is currently focused on immunization against autologous neoantigens defined by whole exome sequencing or RNAseq combined with bioinformatics analyses to predict binding of peptide sequences containing the mutation to the patient's HLA molecules [63, 64]. All older vaccine trials in the adjuvant setting have failed to improve RFS or OS.

Intratumoral immunization efforts began with substances such as BCG and progressed over time to include cytokines delivered by various means, oncolytic viruses, toll-like receptor agonists, and STING agonists. A replicating herpesvirus containing GM-CSF, T-VEC or talimogene laherparepvec, was approved by the FDA in 2015 for intratumoral administration after demonstrating modest ORR compared to GM-CSF (26% vs. 6%) in a phase III trial for patients with unresected stage IIIB-IV melanoma [65]. Most responses occurred in injected lesions and regional non-injected disease, with rare responses in distant non-injected small volume disease [66]. T-VEC has also been studied in a phase II trial in combination with ipilimumab compared to ipilimumab alone (ORR 39% vs. 18%) and in a phase III trial of pembrolizumab plus T-VEC vs. pembrolizumab alone which is ongoing (NCT02263508) [67].

In addition to IL-2 and interferon-alfa, many cytokines were also tested including type II interferons, IL-4, IL-6, IL-12, IL-18, and IL-21, FLT-3 ligand and M-CSF. Pegylated-IL-10 and several forms of IL-15 are currently in clinical trials [68]. Although several of the cytokines produced low rates of objective responses, development as single agents has not yet proceeded beyond phase 2 [69, 70].

T-cell costimulatory antibodies targeting CD-137 (4–1BB), OX40, ICOS, and GITR have entered the clinic. Low rates of response were observed with urelumab (CD137 agonist antibody), but doses higher than 0.1 mg/kg were associated with liver toxicity [71]. Phase 2 studies of other agents have not been reported. Notably, a phase 1 trial of agonist anti-CD40 produced objective responses in 4/15 (27%) on an intermittent dosing schedule but was inactive when given weekly [72], and until recently, was not pursued further as a single agent for melanoma.

Predictive biomarkers are not available for any of the above-cited agents, and it remains possible that several could be active in subsets of patients with disease progression after exposure to anti-PD-1 +/− anti-CTLA-4. Many of the agents were also developed before anti-PD-1 or anti-CTLA-4 were available. Preclinical studies indicate that several of the agents when combined with anti-CTLA-4 or anti-PD-1 (or both) could address mechanisms

of resistance to ICI in subsets of patients. Anti-CTLA-4 may be important to allow optimal expansion and broadening of T-cell responses following immunization, and release of inhibitory effects on T-cells by anti-PD-1 may optimize the anti-tumor effect of tumor antigen-specific T-cells induced, expanded and driven to the tumor microenvironment by other agents. While most combination studies include a PD-1/PD-L1 antagonist, it is important to emphasize that meaningful anti-tumor activity has been observed in melanoma with high dose IL-2, anti-CTLA-4, and TIL ACT, suggesting that alternate combinations or approaches may drive T-cell activation to a threshold beyond sensitivity to PD-1 pathway inhibition.

MECHANISMS OF RESPONSE AND NON-RESPONSE TO PD-1 PATHWAY ANTAGONISTS

Approximately 50% of all advanced melanoma patients presenting for treatment will demonstrate primary or acquired resistance to anti-PD-1 based therapies [12, 36]. At the time of presentation, melanoma metastases have co-evolved with the anti-melanoma immune response for long periods, possibly many years. The immune response to tumor is shaped by the tumor but also by host genetic factors and environmental factors such as prior pathogen exposures and the microbiome. The immune response itself is complex and involves the interaction of many types of immune cells and many molecular interactions between the cells and include stimulatory and inhibitory signals and actions. It is within this complex and heterogenous host tumor immune relationship that physicians apply relatively narrow therapeutic interventions in the hope of altering the threshold for productive anti-tumor immune reaction. Given the relatively limited access to human tissue at baseline and after an intervention, and the technological limitations in measuring the many variables simultaneously, critical mechanisms for response and non-response are difficult to define, particularly for individual patients.

Studies of pre-treatment tumor biopsies suggest that potential biomarkers of melanomas most responsive to anti-PD-1 based immunotherapy include increased CD8 T-cell infiltration [73], an interferon-gamma gene signature, or expression of PD-L1 on tumor cells or immune cells [74]. While these biomarkers are challenging to incorporate into meaningful clinical practice at this time, new biomarkers are in development. The type of CD8 T-cell within tumors may be important, for example those expressing markers of earlier differentiation such as CD28 or the TCF7 transcription factor [75]. The correlation of tumor mutation burden with response for melanoma is logical but there are outliers in terms of precise association and the features of mutation encoded neo-antigens leading to functional immune responses are not clearly established [76–78]. Lack of response has been associated with a specific transcriptional signature associated with epithelial to mesenchymal transition, myeloid cells and angiogenic factors such as VEGF. Several factors outside of the tumor microenvironment appear to influence anti-PD-1 response including species of bacteria within the gut microbiome [79, 80], and various circulating protein classes such as complement, the acute phase response and wound healing [81]. Viewed another way, the mechanisms responsible for lack of response to anti-PD-1 based therapies may be grouped into several categories: lack of prior priming of naïve T-cells to produce tumor antigen

specific T-cells; exclusion of T-cells from the tumor; lack of supportive cytokines or co-stimulation within the tumor; T-cell suppression caused by co-inhibitory ligand-receptor interaction, by cytokines and other soluble ligands for inhibitory receptors on T-cells, by suppressive myeloid cells or regulatory T cells (Tregs), or by adverse metabolic conditions such as low oxygen or glucose; and loss of tumor recognition by T-cells, for example downregulation of surface MHC molecules, antigen processing and presentation defects, or simply loss of antigen expression. Because of the many possible mechanisms, biomarkers should be prospectively incorporated into future clinical trials and validated to ultimately guide treatment for individual patients. Single agent therapies are unlikely to address resistance alone due to the high degree of tumor heterogeneity and the complexity of the host immune-tumor relationship. Therefore, most development has focused on combination therapies.

COMBINATION IMMUNOTHERAPY STRATEGIES

Many combination trials are in progress or are in development, most combining with anti-PD-1 or anti-PD-L1 and a smaller number in combination with anti-CTLA-4. Targeted, chemotherapeutic, antiangiogenic, and immunotherapeutic agents have all been combined with standard ICIs. Trials have been developed for previously untreated patients, or for patients with primary or acquired resistance. In the context of single arm phase 2 trials conducted in patients without prior exposure to either agent in the combination, activity is often compared to historical controls receiving the ‘standard’ agent, either anti-PD-1 or anti-CTLA-4. Interpretation of data from the phase 2 trials can be confounded by unknown biases in patient selection. Caution is warranted when concluding that a combination is superior or inferior to single agent therapy from uncontrolled phase 2 trials, although the results of these studies are used to proceed to and design the larger confirmatory randomized trials. Activity signals are possibly more reliable for combinations studied in acquired or primary resistance to anti-PD-1 or to anti-PD-1/anti-CTLA-4, but even in this setting low rates of late response or pseudo-progression from prior therapy, or the potential for re-response when disease progresses after an interval off treatment, can lead to an overestimation of the combination partner’s activity [82, 83]. Attention to pharmacodynamic or mechanistic activity of the combinatorial partner can be very informative, even if additional clinical activity is not observed [84]. It is important to consider this point before abandoning a novel combination approach which may be enhanced with additional agents or alterations in dosing.

Although it is outside the scope of this review to describe all the ongoing combinations, several approaches to address potential major mechanisms of non-response to anti-PD-1 or anti-PD-1/anti-CTLA-4 combination are illustrative of broader efforts. The approaches include blockade of other co-inhibitory ligand-receptor pathways, blockade of various other T-cell inhibitory mechanisms in the tumor microenvironment, modulation of inhibitory immune cells, delivery of key proliferative or other agonist signals to T-cells, and approaches to increase or broaden the antigen-specific T-cell response including immunization or ACT.

LAG-3

Lymphocyte activation gene-3 (LAG-3) is a T cell-associated inhibitory checkpoint molecule co-expressed with PD-1 that regulates immune tolerance and T cell homeostasis. Preclinical studies have demonstrated that dual PD-1 and LAG-3 blockade synergistically stimulate T-cell responses and decrease tumor burden more than either agent alone [85–87]. LAG-3 was the third inhibitory receptor, after CTLA-4 and PD-1, to be targeted with monoclonal antibodies in clinic trials starting in 2013 and multiple LAG-3 inhibitors are now in development (BMS-986016, LAG525, and MK-4280) [88].

A phase I/II trial studying anti-LAG-3 (BMS-986016) 80 mg plus nivolumab (NCT01968109) in patients with advanced melanoma whose disease progressed on anti-PD-1/PD-L1 demonstrated ORR of 11.5% (1 CR, 6 PR). ORR was 3.5-fold higher in patients who tumors had greater than or equal to 1% positivity for LAG-3 expression, compared to those who were LAG-3 negative (ORR 18% vs. 5%) but was unrelated to PD-L1 status. Treatment was well-tolerated, with only a 4% rate of grade 3 or 4 treatment-related adverse events [89]. Based on this data, a phase II/III study of relatlimab (BMS-986016) plus nivolumab versus nivolumab alone is now recruiting treatment-naïve patients with advanced melanoma (NCT03470922).

IDO

Indoleamine 2,3-dioxygenase 1 (IDO1) is an interferon-inducible enzyme that catabolizes tryptophan and promotes tumor-mediated immunosuppression. IDO1 is overexpressed in cancers including melanoma and inhibition of IDO1 is thought to shift the tumor microenvironment from a tumor-promoting inflammatory state to one of immune stimulation [90]. The selective IDO1 inhibitor epacadostat was combined with pembrolizumab in the phase I/II ECHO-202/KEYNOTE-037 study in multiple tumor types including treatment-naïve patients with advanced melanoma [91].

As of October 2017, ORR for 50 patients enrolled on the phase II study was 62% (9 CR, 22 PR) with responses observed in both PD-L1-positive and negative patients (ORR 70% vs. 56%). Twelve-month PFS and OS rates were 63% and 92%, respectively and treatment was well tolerated [92]. These promising results of similar efficacy to dual ICI with lower toxicity led to the Phase III ECHO-301/KEYNOTE-252 study in which 706 treatment-naïve advanced melanoma patients were randomized 1:1 to pembrolizumab combined with either epacadostat or matched placebo. Unexpectedly, there were no differences between the epacadostat and placebo arms for ORR (34% vs. 31%) or 12-month PFS (37% for both) [93]. This disappointing data resulted in cancellation and/or downsizing of multiple clinical trials studying IDO inhibition in melanoma, although more work is needed to identify the specific subset of patients that may respond due to specific dependence on the IDO pathway for escape from immune surveillance.

CSF-1R and CD40

Immunotherapies including CSF1R inhibitors (CSF1Ri) and CD40 agonists (CD40α) target innate immune cells such as macrophages. Pre-clinical studies have supported the hypothesis that tumor-associated macrophages (TAM) may confer resistance to ICI [94]. Macrophage

colony-stimulating factor 1 (CSF-1) is chemotactic signal that stimulates monocyte tumor infiltration and macrophage differentiation [95, 96]. Increased CSF-1 and CSF1R expression has been associated with a poor prognosis [97]. CD40 is expressed on macrophages and other antigen presenting cells (APC) and binds to CD40L on T-cells. CD40 agonists increase the tumoricidal activity of macrophages and stimulate maturation of APCs. In a poorly immunogenic melanoma mouse model, combination CSF1Ri and CD40α suppressed tumor growth more than either agent alone and did so in a T-cell independent fashion [98]. A phase 1/1b trial (NCT03502330) is currently studying the safety and efficacy of the CSF1Ri cabiralizumab combined with the CD40α APX005M with or without nivolumab in patients with advanced melanoma, renal cell carcinoma, or non-small cell lung cancer whose disease has progressed on anti-PD-1/PD-L1.

4–1BB

4–1BB (CD137/TNFSF9) is a co-stimulatory receptor and member of the tumor necrosis factor (TNF) receptor family that is expressed on both innate and adaptive immune cells [99]. 4–1BB agonism promotes CD8+ T-cell proliferation, enhances TCR signaling, and induces immunologic memory [100, 101]. Therapeutic approaches combining a 4–1BB agonist with and without ICI have been established in pre-clinical models [101, 102]. A phase 1 dose escalation study of BMS-663513 (anti-4–1BB, urelumab) in advanced solid malignancies enrolled 83 patients of whom 54 had melanoma and demonstrated clinical activity including 3 PRs in melanoma patients [103]. However, the follow-up phase 2 study of second-line BMS-663513 for melanoma was terminated early due to an increased incidence of grade 4 hepatitis. This resulted in withdrawal of several other trials that planned to study 4–1BB agonists at that time [100], but retrospective analyses revealed that hepatic toxicity was dose related, and trials of urelumab were re-initiated at a dose of 0.1 mg/kg [71]. Data from pre-clinical models have suggested that irAEs are significantly reduced when 4–1BB agonists are combined with ICIs [104]. A phase I/II trial combining urelumab and nivolumab in patients with advanced melanoma reported a 50% ORR (SITC 2016) and several studies are planned or currently recruiting that are studying combinations of 4–1BB with other immunomodulatory approaches.

NKTR-214

NKTR-214 is a CD122 agonist and prodrug composed of IL-2 conjugated to 6 releasable polyethylene glycol (PEG) chains that increases T-cell and NK cell proliferation and enhances PD-1 expression. In melanoma mouse models, NKTR-214 increased anti-tumor efficacy and decreased toxicity compared with aldesleukin [105]. NKTR-214 monotherapy demonstrated minimal clinical activity in a phase I/II trial but led to PIVOT-02, a phase I/II trial of NKTR-214 and nivolumab in patients with locally advanced or metastatic tumors including melanoma (NCT02983045). As of May 2018, ORR was 50% for the immunotherapy-naïve melanoma cohort in the stage 2 portion of the trial. ORR for PD-L1 negative and positive patients was 42% and 62%, respectively. Eighty-percent of patients had a normal LDH, one-third had liver metastases, and disease stage in most patients was M1b or M1c. Data from PIVOT-02 for the immunotherapy-refractory melanoma patients is not yet available. PIVOT-02 is also now recruiting patients treated with NKTR-214 in

combination with nivolumab and ipilimumab. Randomized trials are planned and will be necessary to determine the contribution of NKTR-214 to the baseline effect of anti-PD-1.

TLR agonists

Toll-like receptor (TLR) stimulation can enhance antigen presentation and stimulate immune activation [106]. ILLUMINATE-204 is a phase II study (NCT02644967) of the TLR-9 agonist IMO-2125 administered intratumorally in combination with ipilimumab or pembrolizumab in PD-1 refractory advanced melanoma patients. A preliminary ORR of 47% in 15 evaluable patients merits further evaluation and accrual is ongoing [107]. A phase III trial of IMO-2125 plus ipilimumab vs. ipilimumab alone in patients with anti-PD-1 refractory melanoma (ILLUMINATE-301) is also currently recruiting patients (NCT03445533).

In the same refractory population, CMP-001, a CpG-A oligodeoxynucleotide and TLR9 agonist is being studied by intratumoral injection in combination with pembrolizumab in a phase Ib trial [108]. Preliminary data show ORR of 40% with tumor reduction occurring in both injected and non-injected lesions, with most responses lasting over 6 months. These studies suggest that intra-tumoral injection of TLR9 agonists, and possibly other agents such as oncolytic viruses or STING agonists, could induce antigen presentation and systemic T-cell responses in patients whose tumors have little or no baseline immune infiltrate.

Adoptive Cell Therapy

Given the high rate of activity of the ICI as first-line therapies, and the clinical and technical challenges of ACT, current studies of ACT are primarily focused on patients resistant or non-tolerant to the ICIs. For TIL ACT, clinical responses are limited by the quality and quantity of tumor resident antigen specific T-cells, and after ex vivo expansion, their ability to reach and infiltrate the tumor and subsequently overcome immunosuppressive factors in the tumor microenvironment [109]. In a phase II trial (NCT02360579), 9 ICI-resistant patients treated with ACT had ORR of 33% after an albeit short median follow-up of 3.6 months [110] and the trial is ongoing. In a separate single institution study, 74 patients treated with ACT had ORR of 43%. When responses were grouped based on prior treatment, ORR was 51% in treatment-naïve patients and 33% in patients who received prior ipilimumab, who also had decreased OS post-ACT (24.6 vs. 7.7 months). There were not enough patients to analyze impact of prior anti-PD-1 monotherapy on outcomes [111]. In the first trial of TIL produced by shipment to and from a central facility, ORR was 38% among 47 patients, most of whom had received prior anti-PD-1 alone or in combination with ipilimumab [112]. Activity of TIL in this setting is encouraging and provides the foundation for future approaches that combine with ICI or improve cell properties through genetic engineering such as with TCR engineered T-cells targeting differentiation and cancer-testis antigens [113].

Targeted Agents

Of note, ICI are also being studied in combination with inhibitors of the mitogen activated kinase (MAPK) pathway (NCT02027961, NCT02967692, NCT02908672, NCT03273153). Controversy exists over the impact of adding MAPK pathway inhibitors to ICI. While

several preclinical studies initially reported that MAPK inhibitors can positively modulate the immune microenvironment [114], more recent data have demonstrated that PD-1 resistant melanomas have a transcriptional signature consistent with innate anti-PD-1 resistance (IPRES), defined as having upregulation of genes modulating mesenchymal transition, cell adhesion, angiogenesis, and extracellular matrix remodeling. This IPRES signature is very similar to that induced by combined BRAF/MEK or BRAF inhibition, suggesting that these drugs may mediate resistance to anti-PD-1 [81]. The phase 2 KEYNOTE-022 study randomized patients with BRAF mutant melanoma to dabrafenib and trametinib plus pembrolizumab or placebo. The primary outcome of PFS was 16 months for the pembrolizumab arm and 10.3 months for the placebo arm (HR 0.66), but this outcome did not reach significance for the pre-specified HR goal. Additionally, the triplet combination was more toxic with 58% of patients experiencing grade 3–5 adverse events [115].

There is also evidence that MEK inhibition alone may improve T-cell function and enhance antigen presentation and thereby may improve the effect of anti-PD-1/PD-L1 therapies [116]. Using this rationale, a phase 1b trial reported ORR of 45% for combined atezolizumab and cobimetinib in patients with BRAF mutant and wild-type advanced melanoma [117]. Ultimately, optimal dosing and complex sequencing issues for ICI and MAPK inhibitors will need to be addressed in future studies.

FUTURE DIRECTIONS AND CONCLUSIONS

The ultimate goal of immunotherapy treatment in patients with advanced melanoma is to eradicate the disease and/or produce long-term durable responses. Given the complexity of the anti-tumor immune response, combination rather than single agent strategies will likely dominate the investigational trial landscape.

For those who respond to ICI, optimal duration of treatment is unknown but is crucial to understand from quality of life, toxicity, and health economics perspectives. Multiple studies suggest that a limited rather than indefinite course of ICIs may be sufficient to provide meaningful durable responses [12]. For example, 90% of patients who developed a CR on pembrolizumab remained disease-free after a two-year median follow-up from drug discontinuation [118]. This can be true even for patients who do not develop a CR. In KEYNOTE-006 after median 9 month follow-up of patients who completed pembrolizumab treatment, PFS rates were 95%, 91%, and 83% in patients with a CR, PR, and SD [119]. It is highly encouraging that even those patients without a CR who discontinue ICIs can be free of disease progression. Besides clinical implications, length of treatment raises broader economic concerns. Drug costs alone for 6 months of anti-PD-1 can reach $145,000 per patient, costs rise steeply with dual ICI and subsequent toxicity management, and this will become financially unsustainable as more patients with many different malignancies have access to ICIs [17, 18].

For those patients who develop resistance to ICI, new combinatorial strategies are in high demand and must be rationally based on biologic mechanisms of resistance. Particularly important is how to overcome a non-inflamed tumor microenvironment and specific targets

are currently under study from multiple mechanistic angles. For example, intratumoral STING and TLR agonists are being used to promote innate immunity, anti-CD40 agonists and CSF1R inhibitors to bridge innate and adaptive immunity, STAT3 inhibitors to inhibit immunosuppressive oncogene pathways, probiotics to reverse immunosuppression in the microbiome, and many more, often in combination with a PD-1 backbone, representing the next wave of treatment approaches in immuno-oncology [120]. Optimizing clinical outcomes for special populations such as patients with mucosal and ocular melanomas, which are less responsive to ICI, is also needed.

Tremendous scientific progress has been made in the past 10 years in understanding how to manipulate the immune system to improve outcomes in melanoma and has translated into unprecedented clinical success. Despite the major hurdles of resistance to ICI, the challenges are defined and are being actively investigated. Ultimately, predictive biomarkers will need to personalize and guide treatment decisions for each individual patient with melanoma.

Acknowledgements:

S. Weiss acknowledges NIH (NCI) research support from K12 CA215110.

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Table 1.

Practice-Changing Trials for Immune Checkpoint Inhibitors in Locally Advanced and Metastatic Melanoma

Drug	Trial	Phase	Population	Treatment Arms	Primary Outcome		95% CI	HR	p-value
UNRESECTABLE/METASTATIC									
Ipilimumab (FDA approved 2011)	Hodi et al. (2010, ref. 24)	III	Unresectable stage III/IV Previously treated	Ipilimumab 3mg/kg × 4 + gp100 vaccine	Median OS (months)	10	8.5 to 11.5	0.68 (vs. gp100)	<0.001
				Ipilimumab 3 mg/kg		10.1	8 to 13.8	0.66 (vs. gp100)	0.003
				gp100 vaccine		6.4	5.5 to 8.7	-	-
Pembrolizumab (FDA approved 2014)	Robert et al. (2014, ref. 121) (KEYNOTE-001)	I	Ipilimumab-refractory	Pembrolizumab 2 mg/kg q3wk	ORR (%)	26	-	-	0.96
				Pembrolizumab 10 mg/kg q3wk		26	-	-	-
	Ribas et al. (2015, ref. 122) (KEYNOTE-002)	II	Ipilimumab-refractory	Pembrolizumab 2 mg/kg q3wk	Median PFS (months)	2.9	2.8 to 3.8	0.57 (vs. chemo)	<0.0001
				Pembrolizumab 10 mg/kg q3wk		2.9	2.8 to 4.7	0.5 (vs. chemo)	<0.0001
				Investigators' choice Chemotherapy		2.7	2.5 to 2.8	-	-
	Schachter et al. (2017, ref. 36) (KEYNOTE-006)	III	Unresectable stage III/IV Up to 1 prior treatment (excluding anti-CTLA4, PD-1/PD-L1 agents)	Pembrolizumab 10 mg/kg q2wk	12-month OS rate (%)	74.1	-	0.63 (vs. ipi)	0.0005
				Pembrolizumab 10 mg/kg q3wk		68.4	-	0.69 (vs. ipi)	0.0036
				Ipilimumab 3 mg/kg q3wk × 4		58.2	-	-	-
Nivolumab (FDA approved 2014)	Weber et al. (2015, ref. 123) (CheckMate-037)	III	Unresectable or metastatic Progression on ipilimumab and BRAF inhibitor if BRAF mutant	Nivolumab 3 mg/kg q2wk	ORR (%)	31.7	23.5 to 40.8	-	-
				Investigators' choice Chemotherapy		10.6	3.5 to 23.1	-	-
	Robert et al. (2015, ref. 124) (CheckMate-066)	III	Metastatic Previously untreated BRAF WT	Nivolumab 3 mg/kg q2wk	1-year OS rate (%)	72.9	65.5 to 78.9	0.42	0.001
				Dacarbazine 1000 mg/m2 q3wk		42.1	33 to 50.9	-	-
Ipilimumab plus Nivolumab (FDA approved 2015)	Wolchok et al. (2013, ref. 23)	I	Unresectable stage III/IV Previous therapy with T-cell modulating Abs (excluding ipilimumab for pts in the sequenced-regimen cohorts)	Nivolumab 0.3 mg/kg + Ipilimumab 3 mg/kg	ORR (%)	21	5 to 51	-	-
				Nivolumab 1 mg/kg + Ipilimumab 3 mg/kg		53	28 to 77	-	-

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Drug	Trial	Phase	Population	Treatment Arms	Primary Outcome		95% CI	HR	p-value
				Nivolumab 3 mg/kg + Ipilimumab 1 mg/kg		40	16 to 68	-	-
				Nivolumab 3 mg/kg + Ipilimumab 3 mg/kg		50	12 to 88	-	-
				All		40	27 to 55	-	-
	Postow et al. (2015, ref. 125) (CheckMate-069)	II	Unresectable stage III/IV Treatment naive	Ipilimumab 3 mg/kg + Nivolumab 1 mg/kg × 4 followed by Nivolumab 3 mg/kg q2wk	ORR (%) BRAF WT BRAF mutant	6152	49 to 72 31 to 73	-	-
				Ipilimumab 3 mg/kg + Placebo × 4 followed by Placebo q2wk	BRAF WT BRAF mutant	11 10	3 to 25 0 to 45	-	-
	Larkin et al. (2015, ref. 16) (CheckMate-067)	III	Unresectable stage III/IV Treatment naive	Nivolumab 3 mg/kg q2wk + Placebo	Median PFS (months)	6.9	4.3 to 9.5	0.57 (vs. ipi)	<0.001
				Nivolumab 1 mg/kg + Ipilimumab 3 mg/kg × 4 followed by Nivolumab 3 mg/kg q2wk		11.5	8.9 to 16.7	0.42 (vs. ipi)	<0.001
				Ipilimumab 3 mg/kg q3wk × 4 + Placebo		2.9	2.8 to 3.4	-	-
	Wolchok et al. (2017, ref. 12) (CheckMate-067)	III	Unresectable stage III/IV Treatment naïve	Nivolumab 3 mg/kg q2wk + Placebo	3-year OS (months) (3-year OS rate %)	37.6 (52)	29.1 to NR	0.65 (vs. ipi)	<0.001
				Nivolumab 1 mg/kg + Ipilimumab 3 mg/kg × 4 followed by Nivolumab 3 mg/kg q2wk		NR (58)	38.2 to NR	0.55 (vs. ipi)	<0.001
				Ipilimumab 3 mg/kg q3wk × 4 + Placebo		19.9 (34)	16.9 to 24.6	-	-
ADJUVANT									
Nivolumab (FDA approved 2017)	Weber et al. (2017, ref. 59) (CheckMate-238)	III	Resected stages IIIB-IV (AJCC 7th ed.)	Nivolumab 3 mg/kg q2wk up to 1 year	1-year RFS rate (%)	70.5%	66.1 to 74.5	0.65	<0.001
				Ipilimumab 10 mg/kg q3wk x4, then q12 wk up to 1 year		60.8%	56 to 65.2	-	-
Pembrolizumab (FDA approved 2018)	Eggermont et al. (2018, ref. 60)	III	Resected stages IIIA (>1 mm micrometastasis)-IIIC (AJCC 7th ed.) Completion lymphadenectomy required	Pembrolizumab 200 mg IV q3wk up to 1 year	1-year RFS rate (%)	75.4%	71.3 to 78.9	0.57	<0.001
				Placebo up to 1 year		61%	56.5 to 65.1	-	-

Drug	Trial	Phase	Population	Treatment Arms	Primary Outcome		95% CI	HR	p-value
Ipilimumab plus Nivolumab	NCT03068455 (CheckMate-915)	III	Resected stage IIIB-IV (AJCC 8th ed.)	Nivolumab 240 mg q2wk + Ipilimumab 1 mg/kg q6wk + Placebo	RFS	Results not yet available			
				Nivolumab 480 mg q4wk + Placebo					
				Ipilimumab 10 mg/kg q3wk x4, then q12 wk (this arm was subsequently removed)					

CI = Confidence Interval; HR = hazard ratio; OS = overall survival, PFS = progression-free survival, ORR = objective response rate; RFS = recurrence-free survival; WT = wild-type; NR = not reached