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New therapies in the treatment of melanoma

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Abstract

Introduction: Therapies targeting immune checkpoints (CTLA-4) and the MAP kinase signaling pathway (RAS/RAF/MEK/ERK) have transformed the treatment of advanced melanoma in the past year. Agents aimed at other therapeutic targets of interest are being actively evaluated in the clinic.

Areas covered: Areas of active therapeutic interest in melanoma include immunotherapy, molecularly targeted therapy and chemotherapy; combinations of these modalities are now under systematic exploration.

Expert opinion: The evaluation of patients with melanoma now includes the molecular profiling of tumor mutations in the BRAF, as well as c-Kit, NRAS and other genes that have been discovered to be drivers of different subsets of the disease. The analysis of the host immunological response to melanoma is equally important, as a basis for the development of immunotherapies that have been of value to melanoma patients in the adjuvant arena, as well as for therapy of metastatic disease. The understanding of these two facets of the disease will provide a more rational basis for the delivery of individualized therapy for the disease both in its advanced setting, and in the adjuvant arena, in the future.

Keywords

adoptive cell transfer; Bcl-2; BRAF; MEK; CTLA-4; emerging; HERV-K; immunotherapy; KIT; melanoma; new therapies; vaccines

1. Introduction

Although amenable to surgical cure when found early, advanced melanoma is an aggressive disease accounting for 9000 deaths in the USA in 2011 alone [1]. With a rapidly increasing incidence especially among younger patients, this disease exacts a disproportionate economic toll compared with other cancers. Until recently, the only Food and Drug Administration (FDA) approved therapies available for advanced melanoma were dacarbazine (DTIC® Dome) and high-dose IL-2 (HD IL-2, Aldesleukin® Prometheus). DTIC therapy is associated with low response rates (~ 10%) with overall survival (OS) benefits of approximately 6 – 8 months. Although associated with response rates of 16%, HD IL-2 was approved on the strength of the durable complete responses (CR) seen in 6% of patients with advanced inoperable disease.

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Improved understanding of the mutations underpinning melanoma tumorigenesis has resulted in a series of novel agents that selectively inhibit key steps in the pathways activated by these mutations. Similarly, increased knowledge of the mechanisms of immune surveillance and escape has resulted in the development of new classes of immunologic agents that exploit cellular immunity to exert potent antitumor effects. In 2011, two new agents developed on the basis of these principles were licensed by the FDA for the treatment of metastatic melanoma, each with clear evidence of improved survival of the treated patients who received the new agents in registration Phase III trials: i) the selective inhibitor of V600E BRAF vemurafenib (Roche/Plexxikon, Zelboraf®) and ii) the cytotoxic T-lymphocyte antigen-4 (CTLA-4) blocking monoclonal antibody ipilimumab (Bristol-Myers Squibb/Medarex, Yervoy®) [2–5]. Each of these new classes of agent has significant benefits, and significant limitations: vemurafenib has shown unprecedented antitumor response rates, but a relatively short duration of response secondary to the tumor's acquisition of resistance with overall median survival of approximately 16 months; ipilimumab has the obverse attributes, with durable responses that have improved survival by 10% past 2 years in two Phase III trials conducted at different dosages in different populations but with low overall response rates (ORR) on the order of 10.9 – 15.2%. Novel agents and novel combinations of existing and new agents are desperately sought to overcome limitations of current treatments. In this review, the authors detail recent advances and examine potential strategies to overcome the hurdles posed by existing therapies in the treatment of melanoma.

2. Current understanding of melanoma tumorigenesis and therapeutic implications

Like many cancers, the development of cutaneous melanoma requires sequential mutations that activate proto-oncogenes and inactivate tumor suppressor genes resulting in deregulation of cellular growth, differentiation and apoptosis. Different clinical subtypes of melanoma (lentigo maligna, superficial spreading, acral, nodular, mucosal and uveal) harbor distinct sets of genomic alterations [6]. These alterations are critically located in key signaling networks such as - mitogen-activated protein kinase pathway (MAPK–RAS/RAF/MEK/ERK) or phosphoinositide 3-kinase (PI3K/PTEN/AKT) pathway or in tumor suppressors such as CDKN2A (p16 and p14ARF) separately but surely drive melanoma tumorigenesis [7].

Differential gene expression profiling of nevi, primary melanomas, lymph node metastases and distant metastases has shed light on the patterns of gene expression at various stages of melanoma progression. Separately, pathway analysis of differential gene expression profiles between primary melanomas and sentinel lymph node metastases suggests that genes down-regulated in association with sentinel lymph node metastasis were involved in cell cycle regulation, cell adhesion, protease inhibitory activity and keratinocyte-associated functions while up-regulated genes tended to be oncogenes or tumor promoters [8]. These results have been synthesized in several review articles including a review of genetic drivers of melanoma progression based on gene expression profiling [9,10], a review of the primary genetic events required to initiate melanoma tumorigenesis [11], a meta-analysis

of melanoma microarray data [12] and an online open access database [13]. A thorough evaluation of genetic and signaling alterations implicated in melanoma transformation, growth and progression is beyond the scope of this review. Readers are directed to these articles as well as general reviews on the topic for this information [14,15].

Drug development in patients with advanced melanoma has entered a new era, with the paradigm for initial selection now defined by the molecular insights of the past decade. Previously, options in advanced melanoma were restricted to one cytotoxic agent DTIC or HD IL-2. Responses were uncommon and generally for cytotoxic agents fleeting, averaging 10% for DTIC and 16% with HD IL-2 although durable CRs were noted in one-third of responders to IL-2. Improved understanding of melanoma subtypes and the distinct molecular and genetic lesions associated with the various phenotypes have now spawned trials evaluating a number of specific inhibitors targeting the RAS/RAF/MEK/ERK, PI3K/PTEN/AKT, c-KIT, MTOR and GNAC signal transduction pathways. Currently, agents targeting BRAF, MEK and c-KIT are furthest along, with BRAF inhibitor vemurafenib already approved and approval anticipated for the MEK inhibitor trametinib based on Phase III trial data demonstrating improved progression-free survival (PFS) and OS [16]. In the following sections, the authors detail the advances made in targeted and immunomodulatory therapies and discuss combinations being evaluated in the clinic.

3. Targeted therapy

3.1 Targeted therapy: BRAF inhibitors

Activating mutations are present in the BRAF gene of approximately 40 – 60% of cutaneous melanomas, of which 80 – 90% are V600E mutations in which glutamic acid has substituted for valine at the V600 locus, and the remainder consist generally of alternate substitutions at the V600 locus (principally V to K) [17]. BRAF phosphorylates regulatory serine residues on MEK1 and MEK2 and mutation of BRAF results in activation of the RAS/RAF/MEK/ERK pathway, leading to cellular proliferation and a series of anti-apoptotic and potentially immunoregulatory events that culminate in the progression of this tumor.

Advanced melanomas carrying BRAF mutations appear to be associated with truncal primaries, an earlier age at onset, and may lack the findings of chronic UV skin damage; clinically, the disease that is associated with BRAF mutation has been shown to follow a more aggressive clinical course with shorter OS for patients not treated with BRAF inhibitors [18]. Other authors have reported that BRAF mutated melanoma is more likely to be ulcerated and to have a more advanced stage at initial diagnosis compared with patients with wild-type tumors [19].

Early clinical trials of sorafenib – a small molecule RAF kinase inhibitor – in melanoma resulted in negative trials when tested both as a single agent and as part of a combination with other chemotherapeutic agents despite its success in treating metastatic renal and hepatocellular carcinomas [20]. Recently however, the highly specific and potent BRAF V600E inhibitor PLX-4032 – designated vemurafenib – has shown significant promise when used in the Phase II and Phase III settings. In the Phase III registration trial, when

compared with DTIC, vemurafenib use resulted in a 63% decrease in hazard of death ($p < 0.0001$), 74% decrease in hazard of tumor progression ($p < 0.0001$) with a complete response rate (CRR) of 48% and benefit being noted in all subgroups including the high-risk M1c and lactate dehydrogenase (LDH) cohorts with an ORR of 48% [21]. These results are summarized in Table 1 [4,22,23].

Dabrafenib (GSK2118436 GlaxoSmithKline®), a 4-(3-aminosulfonylphenyl)-5-(pyrimidin-3-yl) thiazole, is a highly selective and potent adenosine triphosphate (ATP)-competitive BRAF inhibitor with > 100-fold selectivity for mutant BRAF over wild-type BRAF in cell lines. Additionally, dabrafenib displays dose-dependent inhibition of MEK and ERK phosphorylation in BRAF mutant cell lines, and induces tumor regression in melanoma xenografts. Dabrafenib was first clinically tested in study BRF112680 (NCT00880321), a Phase I first-time-in-human dose escalation study designed to evaluate clinical efficacy, safety and pharmacokinetics (PK). Preliminary analysis of the initial Phase I trial and an expanded Phase II cohort indicated that dabrafenib was active in the treatment of intracerebral melanoma metastases, with commensurate extracranial activity. Clinical activity at doses of 150 mg twice daily (b.i.d.) with objective tumor responses judged using RECIST criteria was observed as soon as 8 weeks after initiation of treatment in 21 of 34 (62%) subjects with V600E mutant BRAF melanoma, although the corresponding response rate in melanomas carrying the V600K mutation was considerably lower at 19% [24].

An ongoing Phase II trial (BRF113929/NCT01266967) is attempting to assess the efficacy of dabrafenib in treating brain metastases. Patients are being enrolled in two cohorts, the first containing patients who had not yet received any local therapy for brain metastases and the second containing patients who had received prior therapy for brain metastases including surgery, radiotherapy and radiosurgery.

While the up-front use of BRAF inhibitors results in objective antitumor responses in a majority of treated patients, most of these responses are limited in time, and relapses are noted at a median of 6 – 7 months. Moreover, chronic BRAF inhibitor therapy is associated with secondary keratoacanthomas and cutaneous squamous cell carcinomas (SCC) in up to 24% of patients treated in the pivotal vemurafenib trial known as BRIM-3. These may be due to activation of the MAPK pathway and enhanced growth through the CRAF pathway in these non-melanocytic tissues of the skin as a consequence of single-agent BRAF inhibitor use [25].

Synergistic benefits of therapy combining BRAF inhibition with other targeted therapies may overcome resistance and increase the degree and duration of responses with BRAF inhibitors. It has been demonstrated that resistance to BRAF inhibition occurs through MAP kinase-dependent and MAP kinase-independent mechanisms. MAP kinase-dependent pathways of resistance include secondary NRAS mutations, elevated expression of COT kinase, RAS/CRAF activation and acquired MEK1 mutations [26]. MAP kinase-independent pathways include alternative receptor tyrosine kinases activation including AXL, ERBB4 and IGF1R, PDGFR up-regulation, PI3K/AKT signaling activation and PTEN loss [26]. Concurrent pharmacologic blockade of downstream targets in the MAP kinase pathway may improve response rates viz. either agent singly in addition to abrogating resistance. Pre-

clinical data suggest that the vemurafenib/MEK inhibitor combination may be synergistic in colorectal cancer cell lines [27]. A Phase I/II trial of the oral MEK1/2 inhibitor trametinib (GSK1120212, GlaxoSmithKline) in combination with dabrafenib was conducted in patients with BRAF V600 mutation positive advanced solid tumors [23]. Forty-five patients (43 melanoma, all BRAF inhibitor naive) were treated with 13 partial responses (PR) and 3 patients with stable disease (SD) noted in 16 evaluable patients for an ORR of 81% – more than either drug singly – and a randomized Phase II trial of the combination has been under-taken (NCT01072175). Other slated combinations include vemurafenib/PI3K inhibitor (NCT01512251) and vemurafenib/VEGF-R antagonist (NCT01495988) which are in Phase I testing.

Other groups have demonstrated that BRAF inhibition results in increased expression of melanocyte differentiation antigens (MDA) and increased antigen-specific T-cell recognition, with tumor infiltration by T cells suggesting that BRAF inhibition may rationally be combined with immunotherapeutic approaches such as IFN-α, IL-2 or CTLA-4 blocking antibodies [28]. This approach is being investigated in Phase I/II trials of vemurafenib with ipilimumab (NCT01400451) and has been proposed for IL-2 and IFN-α (Cytokine Working Group and University of Pittsburgh SPORE in Melanoma and Skin Cancer, respectively) with results being eagerly awaited.

3.2 Targeted therapy: MEK inhibitors

In the MAP kinase signaling pathway, MEK lies directly downstream of RAF and is an attractive therapeutic target as MEK inhibition would target BRAF mutant and RAS mutant melanomas. Currently, three MEK inhibitors are being actively investigated in the clinic: AZD6244/ARRY-142886 (selumetinib, Array BioPharma/AstraZeneca), GSK1120212 (trametinib, GlaxoSmithKline) and MEK162 (Novartis Oncology).

Although pre-clinical and Phase I studies suggested that selumetinib (AZD6244) would be promising in melanoma, the randomized Phase II study comparing selumetinib with temozolomide (TMZ) showed no difference in either PFS or ORR between the two groups [29,30]. Possible explanations for these results include the differential crossover between TMZ/selumetinib groups (61% in TMZ arm vs 25% in selumetinib arm) and imbalances in BRAF/NRAS mutations that may have occurred in this study which was designed before the wide availability of BRAF mutation testing that now exists. Upfront mutation testing may be vital to selumetinib efficacy given the results of a Phase I study of selumetinib-based combination therapy that suggested melanoma patients with BRAF mutations were more likely to respond, and have improved time to progression (TTP), establishing BRAF mutation status as a therapeutic biomarker for this therapy [31]. Currently, selumetinib is being evaluated in the Phase II setting in combination with DTIC in BRAF mutant melanoma patients (NCT00936221) and in combination with the Akt inhibitor MK-2206 (NCT01510444) in patients with BRAF-resistant tumors, based on the MAP kinase dependence of acquired BRAF resistance [32]. Separately, given the preponderance of activating GNAQ/GNA11 mutations that have been documented among patients with uveal melanoma (80%), selumetinib is being investigated in the setting of advanced uveal melanoma compared with TMZ (NCT01143402).

Trametinib (GSK1120212) is a selective potent allosteric MEK inhibitor with a long half-life and significant anticancer activity observed in multiple tumor model systems, even administered once daily. Notably, the greatest effects have been observed in tumors bearing mutant RAF/RAS [33]. Phase I studies established recommended Phase II dosage (RP2D) 2 mg/day and maximum tolerated dose (MTD) 3 mg/day [34]. Reversible dose-limiting toxicities (DLT) observed included rash, diarrhea and retinopathy. Of the 20 melanoma patients in the study, responses were seen in 8/11 (3 PR, 5 SD) BRAF mutant patients and 5/9 (2 PR, 3 SD) BRAF wild-type patients. Additionally, responses have been reported in patients with KRAS mutated colorectal and pancreatic malignancies [35]. The Phase II study also reported responses in V600 E/K mutated melanoma [36]. In the recently published Phase III METRIC trial of trametinib in advanced BRAF mutated melanoma, investigators reported that treatment with trametinib significantly increased PFS and OS compared with chemotherapy (either dacarbazine or paclitaxel) [16]. Although ORR were low at 22%, treatment with trametinib significantly improved PFS by 3.3 months and reduced risk of death by 14% at 6 months, especially notable as trial design allowed for crossover to receive trametinib if patients progressed on chemotherapy. Notably, no secondary cutaneous neoplasms were noted and toxicity did not significantly limit drug delivery, no reports of treatment stoppage were secondary to toxicity.

Both RAS/RAF/MEK/ERK and PI3K/AKT pathways are found to be activated in a number of cancers including melanoma. Concurrent inhibition of the MAPK and PI3K/AKT pathways has therefore been pursued. Data from the Phase I portion of a planned three-part Phase I/II study assessing the combination of trametinib with the oral AKT inhibitor GSK2141795 were recently presented [37]. Of the 13 patients evaluable, 3 PRs (2 ovarian and 1 endometrial) were observed. In patients with BRAF mutated melanoma, the Phase II trial is complete and results are awaited (NCT01037127). Ongoing trials in melanoma include trametinib versus chemotherapy (NCT01245062) and the combination of trametinib with the BRAF inhibitor dabrafenib (GSK2118436) (NCT01072175).

MEK162 is an oral, highly selective MEK inhibitor that has demonstrated significant activity in tumor cell lines and animal models [38]. The Phase I trial involving patients with biliary tract cancer established a recommended Phase II dosage of 60 mg b.i.d. [39].. A Phase I trial of single agent MEK162 demonstrated activity in BRAF/NRAS mutated melanoma as reported at ASCO 2012 and the follow-up Phase II study (NCT01320085) is underway [40]. Other trials on the horizon include combinations of MEK162 with PI3K/mTOR inhibitor BEZ235 (NCT01337765) and PI3K inhibitor BKM120 (NCT01363232). The results of Phase I/II trials testing the efficacy of MEK inhibitors are summarized in Table 2 [29,30,34,37].

3.3 Targeted therapy: c-KIT inhibitors

c-KIT is a cell surface protein tyrosine kinase encoded by the proto-oncogene KIT that is an upstream activator of the MAP kinase pathway. Mutations and/or increases in c-KIT copy number result in kinase activity independent of its ligand (the cytokine stem cell factor) and have been described in 15.6 – 21% of mucosal melanomas, 11 – 23% of acral melanomas and 16.7% of cutaneous melanomas [41]. Unlike NRAS/BRAF mutations which tend to be

found in melanomas in skin without chronic sun-induced damage, KIT mutated melanomas tend to be found in chronic sun-damaged skin and acral/mucosal sites suggesting divergent mechanisms of tumorigenesis in different melanoma subtypes. Although KIT mutations in GIST (gastrointestinal stromal tumors) tend to be insertions or deletions at exon 17, the c-KIT mutations in melanoma are generally point mutations that do not correlate well with KIT copy number/CD117 expression and involve the juxtamembrane domain at exons 11/13 and the kinase domain at exon 17 [42,43]. Recent publications have presented divergent data on the frequency of KIT mutations in melanomas arising from genital mucosal compared with sinomucosal sites. A recent European publication noted KIT mutations in melanomas from genital mucosal sites but not sinomucosal sites [44]. Although the overall numbers were low (11 vulvo-vaginal lesions and 12 sinonasal lesions), the difference was statistically significant. However, a Chinese series documented KIT staining in 24 out of 28 (85.7%) cases of sinonasal melanoma [45]. Although a larger analysis may shed light on the matter, given the rarity of sinomucosal melanoma, it is unlikely that this will be definitively answered. However, if true, this suggests differential origins of the melanocyte populations in the genital and sinomucosal areas.

Given the success that has been seen in patients with chronic myelogenous leukemia (CML) and in GIST with KIT inhibition, several trials have tested the use of KIT inhibitors in melanoma. Imatinib mesylate is a first-generation tyrosine kinase inhibitor (TKI) with activity against c-KIT, ABL (Abelson cytoplasmic tyrosine kinase) and platelet-derived growth factor receptor (PDGFR).

A British study reported in 2006 enrolled patients with metastatic disease in whom c-KIT (or PDGFR-a/b or c-ABL) positivity was not required for inclusion [46]. Of the 26 patients enrolled, immunohistochemical (IHC) staining was performed in 17 of whom 3 showed tumor that stained moderately for c-KIT. The authors did not observe any clinical responses among 25 evaluable patients. An MD Anderson study subsequently reported in 2008 enrolled 21 patients whose tumor expressed at least one protein tyrosine kinase (c-KIT, PDGFR, c-ABL or ABL-related gene) as assessed by IHC [47]. Notably, the one patient in the study with a dramatic response (PR lasting 12.8 months) had an acral primary that had the highest c-KIT expression observed in the study.

More recently, two Phase II studies were conducted in cohorts screened for c-KIT mutations by PCR assays. The Chinese study enrolled 43 patients with mostly acral and mucosal melanomas to receive 400 mg/day (increased to 800 mg/day for progression) [48]. These investigators reported a median PFS of 3.5 months, median OS of 14.0 months and ORR of 23.3%. Notably, 11 of the 27 responders had exon 11 mutations. In a multicenter American study, investigators selectively recruited 28 patients with melanomas arising from acral, mucosal, and chronically sun-damaged sites and treated them with imatinib mesylate 400 mg b.i.d. in 6-week cycle [49]. Median TTP reported was 3 months, median OS of 11.5 months and durable ORR of 16%.

Nilotinib and dasatinib are second-generation dual Src/Bcr-Abl TKIs while masitinib inhibits KIT/PDGFR/FGFR3 and the FAK pathway with greater activity, and shows selectivity against KIT compared with imatinib *in vitro* [50]. Dasatinib has demonstrated

activity in c-KIT mutated melanoma and both the single agent, and combinations of this agent are being evaluated in acral/mucosal melanomas and in combinations, for example, dasatinib/bevacizumab (NCT00792545) [51]. Phase I trials investigating nilotinib and masitinib are also underway.

3.4 Targeted therapy: Bcl-2 inhibitors

The Bcl-2 gene family comprises genes that code for pro- and anti-apoptotic proteins; Bcl-2 is an anti-apoptotic protein that has been linked with chemotherapy resistance in many cancers including melanoma. Oblimersen (Genasense®, Genta Pharmaceuticals) is an antisense compound that has been reported to selectively target Bcl-2 RNA for degradation by RNase H, reducing transcription and down-regulating target Bcl-2 protein.

Pre-clinical studies suggested that oblimersen enhanced tumor response to chemotherapy and the Phase I/II trial of oblimersen suggested that oblimersen might be synergistic with DTIC [52]. The subsequent randomized Phase III trial that compared oblimersen/DTIC with DTIC alone in 771 patients reported favorable increases in PFS (2.6 vs 1.6 months) and RR (13.5 vs 7.5%), although the OS increase (9.0 vs 7.8 months) did not meet statistical significance [53]. Subset analysis suggested that patients with normal LDH at baseline had improved survival benefit possibly because elevated LDH correlates with a more aggressive tumor type that may be less amenable to antisense therapy, but trials in populations selected according to these factors have not been confirmatory [54].

Currently, oblimersen is being pursued in combination with other chemotherapeutic agents carboplatin/paclitaxel in the treatment of metastatic uveal melanoma (NCT01200342) and paclitaxel/TMZ in advanced melanoma (NCT00409383).

3.5 Targeted therapy: ERBB4 inhibitors

ERBB4/HER4 is a receptor protein tyrosine kinase that has broad roles in cell signaling and signal transduction, regulating cell growth and development through its downstream targets p85 subunit PI3K, GRB2, STAT5 and Shc [55]. ERBB4 mutations are seen in approximately 20% of melanomas and unlike KIT mutations may be seen in concert with BRAF mutations [56]. Once mutated, melanoma cells appear to be highly dependent on ERBB4 signaling (oncogene addiction) making this a highly interesting therapeutic target.

Lapatinib is an oral inhibitor of HER1 and HER2 that is approved for the treatment of HER2-positive advanced breast cancer. Pre-clinical data suggest lapatinib although not ERBB4-specific exerts a profound negative inhibition of growth [2]. A NCI Phase II trial evaluating the use of lapatinib in ERBB4 mutated melanoma after failure of standard therapy is underway (NCT01264081). If successful, it may be reasonable to pursue synergistic combinations with inhibition of other oncogenes such as BRAF.

3.6 Targeted therapy: siRNA-based therapy

RNA interference (RNAi) is a form of post-transcriptional gene silencing by which either microRNAs (miRNAs) or small interfering RNAs (siRNAs) induce the degradation of cellular mRNA resulting in the reduction or loss of gene activity. miRNAs are genomically

encoded non-coding RNA sequences that associate with cellular proteins to form a complex that down-regulates genes pre-transcriptionally, RNA-induced transcriptional silencing (RITS). siRNAs are generated by the cleaving of double-stranded RNAs (dsRNAs) to generate 20 – 25 base-pair fragments by the ribonuclease protein Dicer. siRNAs associate with the RNA-induced silencing complex (RISC) and bind to target mRNA, inducing cleavage to prevent subsequent translation of the mRNA into the specified protein product. These processes are extensively reviewed in the literature elsewhere [57].

RNAi is profoundly involved in translational repression and embryonic development and can be exploited therapeutically to 'knockdown' the expression of a particular gene. Naked miRNA has limited clinical utility as it is unstable and prone to rapid degradation prior to cellular internalization. Although highly selective and minimally toxic, the major factor precluding more widespread adoption of this technology was the lack of an effective vector for delivery. Prior clinical trials attempted to overcome this limitation through local administration with good results, aerosolized ALN-RSV01 to treat respiratory syncytial virus infections in lung transplant patients and intravitreous injections of Sirna-027 (siRNA targeting VEGFR-1) for the treatment of macular degeneration [58,59].

A number of *in vitro* and *in vivo* murine experiments have indicated the potential feasibility of this approach to target genes relevant to melanoma progression (BRAF V600E, AKT, MYC, STAT3, BIRC7 and CD147) thereby inhibiting melanoma proliferation [60–64]. A first-in-human Phase I clinical trial is evaluating the safety and efficacy of CALAA-01, a siRNA targeting the M2 subunit of ribonucleotide reductase (R2), in a variety of advanced solid tumors including melanoma (NCT00689065). Another Phase I DC vaccine study is evaluating the use of siRNA methods to generate enhanced antimelanoma immune responses and is in active accrual (NCT00672542).

4. Immunotherapy

4.1 Immunotherapy: IL-2 and IFN

Multiple observations associated with melanoma, including its spontaneous regressions, the discovery of melanoma-specific antigens and the antitumor activity of melanoma-specific T cells have prompted the pursuit of immunotherapeutic strategies. Despite grade III/IV toxicity, and modest antitumor response rates (16%) and high costs of delivery in the hospital, HD IL-2 received regulatory approval in 1998 for treatment of metastatic melanoma on the basis of the durable responses observed in 6% of patients. No Phase III study has ever been conducted to more rigorously establish the benefit of this agent, and to date no predictive biomarkers have been advanced that would allow more refined patient selection.

Toxicity may be minimized and efficacy enhanced if biologically active agents could be engineered to selectively target tumor tissue. The development of antibodies specific for tumor tissue antigens and their conjugation with immunologically active agents has led to the development of a new class of agent termed 'immunocytokine'. These include L19-IL-2 that targets tumor stromal fibronectin and Hu14.18-IL-2 (EMD273063) that targets GD2 disialoganglioside. Hu14.18-IL-2 has been tested in a Phase I trial and Phase II studies

are in progress (NCT00590824) [65]. Phase I study of single agent L19-IL-2 in patients with advanced solid tumors defined RP2D of 22.5 MIU IL-2 equivalent with efficacy in metastatic renal cell carcinoma (RCC), 83% SD [66]. An open-label non-randomized Phase II study assessed the safety and efficacy of L19-IL-2/DTIC combination in patients with metastatic melanoma reporting an ORR of 28% (8 of 29 patients) with more than 60% of patients alive 12 months from study initiation [67]. The controlled Phase IIb trial of this combination (NCT01055522) is underway.

Following recent data suggesting improved outcomes with the combination of IL-2 and a peptide vaccine against GP-100, several authors have attempted to combine HD-IL-2 with other agents. These include radiotherapy (SBRT/IL-2, NCT01416831), chemotherapeutic agents such as DTIC (NCT00553618) and low-dose TMZ (NCT01124734) and biological options including vaccination (rec-MAGE-A3 + AS15 ASCI, NCT01266603), VEGF-trap aflibercept (NCT01258855) and ipilimumab (NCT01480323). The latter is a Phase II study based on the premise that intratumoral IL-2 generates melanoma-specific immune responses that may be potentiated by ipilimumab resulting in a systemic melanoma-specific immune response [68]. A NIH study of a similar combination demonstrated a CR rate of 17% with the vast majority of CRs achieving durable responses [69].

In the adjuvant setting, high-dose IFN-α (HDI) received regulatory approval on the basis of consistently observed improvement in relapse-free survival (RFS) across virtually all published studies and meta-analyses to date. However, significant toxicity and inconsistently observed OS benefit have provided an impetus to develop biomarkers of response to improve patient selection and to accelerate the development of new agents or combinations to improve on the benefit of IFN in the adjuvant therapy of operable high-risk disease.

Data from an individual patient data analysis conducted by Wheatley *et al.* and more recent European trials suggest that node-positive disease arising from ulcerated primary melanoma treated with IFN-α accrue greater benefit [70]. Primary lesion ulceration has been studied as a component of multivariable analyses performed in the context of several US Cooperative Group trials where this factor has not previously been observed to predict improved response in these US intergroup studies of HDI. EORTC 18081 has been designed to prospectively enroll patients with ulcerated melanomas and microscopic sentinel lymph node involvement, randomizing study subjects to pegylated IFN-α versus observation. This study is awaited to properly evaluate the adjuvant therapy of the importance of primary tumor ulceration in guiding therapy with IFN. Autoimmune clinical manifestations and/or the development of serum auto-antibodies have been linked with improved outcome and sensitivity to IFN-α treatment in ECOG studies E2696 and E1694 following the identification of autoimmune response as a predictor of IFN adjuvant benefit in the Hellenic Oncology Group trial HeCOG13A/97 [71,72]. The prospectively conducted Greek trial HeCOG13A/97 provides the best evidence that the induction of autoimmune responses correlate with the therapeutic benefit of IFN-α adjuvant therapy [73]. Other studies that have utilized retrospective serological analysis without the clinical evaluation of autoimmune manifestations have yielded conflicting data regarding the question whether autoimmunity is a biomarker of IFN therapeutic response against melanoma, but this question remains under active evaluation. Several other candidate biomarkers include tumor-associated antigen 90 immune complex

(TA90IC), methylthioadenosine phosphorylase (MTAP) expression, KL-40, S100B and melanoma-inhibiting activity (MIA), however, prospective data to rigorously assess these markers are lacking at this time.

Efforts to improve on the benefit of IFN-α are being actively evaluated. In the adjuvant setting, a Phase I study (NCT00861406) is assessing the combination of pegylated IFN and GP-100 peptide vaccination in patients with resected intermediate thickness node-negative melanoma at low to intermediate risk of recurrence. Given the expectedly low event rate in this patient population, demonstrating efficacy would be a challenging task in later phase trials. IFN combined with the anti-GD3 monoclonal antibody KW2871 (NCT00679289) is being evaluated in the metastatic setting in a Phase II trial that is presently in active accrual (NCT00679289).

4.2 Immunotherapy: checkpoint inhibition

Although melanoma is considered one of the more highly immunogenic solid tumors of the human, the disease appears to have developed the ability to thwart the immune system by down-regulating MHC class I antigen expression on tumor cells, secreting immunosuppressive factors, inducing tolerance and inhibition of T-cell co-stimulatory function [74]. CTLA-4 also known as CD152 is expressed on the surface of $CD4^+$ T-helper cells and is a member of the B7-CD28 immunoglobulin superfamily. While the interaction between B7 (CD80/86) on antigen-presenting cells (APCs) and CD28 on $CD4^+$ T-helper cells is a stimulatory signal that up-regulates the adaptive immune response, CTLA-4 transmits an inhibitory signal possibly mediated by de-phosphorylation of TCR-proximal signaling molecules by secondary signaling moieties such as PI3-K, SHP-2, AP-1/AP-2 that bind to the YVKM motif found on the cytoplasmic side of CTLA-4 [75]. By competing with CD28 for binding sites on CD80/86 on APCs, CTLA-4 serves as a key inhibitory checkpoint in regulating the adaptive immune response. Intracellular CTLA-4 is found in $CD4^+$ $CD25^+$ T-regulatory cells though its function in this setting is unclear.

Ipilimumab (Medarex Inc./Bristol-Myers Squibb) and tremelimumab (Pfizer/MedImmune) are two CTLA-4 blocking antibodies that have been evaluated in clinical trials. The early phase trials utilizing single-agent tremelimumab at the 15 mg/kg dose every 3 months observed ORR of 6.6%, which does not differ significantly from the antitumor response rate of the recently approved agent ipilimumab. The responses observed with tremelimumab, like those induced with ipilimumab, were frequently durable and longer than 6 months [76]. Unfortunately, the Phase III trial of this agent compared it in an open-label trial against chemotherapy (TMZ or DTIC) in patients with advanced melanoma, and did not meet its objectives, showing OS improvement at interim analysis (OS tremelimumab 11.76 months vs chemotherapy 10.71 months) that led to closure for futility [77]. Several aspects of the design of this trial are notable, including its open-label nature and the post-trial access to other expanded access programs offering another anti-CTLA4 blocking antibody that culminated in the occurrence of systematic crossover and may have diminished the apparent treatment benefit. Currently, further evaluation of this agent is evaluating combinations of tremelimumab with chemotherapy in patients with metastatic melanoma, bladder cancer and prostate cancer. A Phase I trial of tremelimumab with the CD40 agonist CP-870,893

(NCT01103635) that has previously demonstrated efficacy in melanoma is under current study [78].

Ipilimumab is a fully humanized monoclonal CTLA-4 blocking antibody that garnered the FDA and European regulatory approval for treatment of metastatic melanoma in both the first- and second-line settings following successful trials against vaccine and chemotherapy comparators, the results of which are summarized in Table 3 [2,3,79–84]. The earlier Phase III MDX010–20 trial compared ipilimumab alone (3 mg/kg), ipilimumab plus a peptide vaccine and vaccine plus placebo in the metastatic second-line setting while the subsequent BMS-024 study compared ipilimumab (10 mg/kg) plus DTIC (850 mg/m^2) with DTIC with placebo in previously untreated patients with metastatic melanoma [2,3]. In the adjuvant setting, separate European and the US intergroup studies are evaluating ipilimumab against placebo (EORTC 18071) and HDI (ECOG 1609), respectively in an attempt to translate the benefit observed. EORTC 18071 has completed accrual whereas accrual for E1609 is active and results are anticipated in 2014.

The regulatory success of ipilimumab owes much to its induction of durable long-term remissions (1-year survival rate of approximately 45% and 2-year benefit over GP-100 control of 10%) rather than the increment in median OS (2.1 – 3.6 months) or the response rates (ORR 6 – 11% and disease control rate (DCR) 30%). Strategies to improve response rates through combination therapy and the effort to identify clinically relevant predictors of response are vital to maximize therapeutic gains with this agent. Analogous to HDI, the occurrence of immune-related adverse events (IRAEs) appears to correlate with response [85]. Additionally, elevated pre-treatment levels of tumor infiltrating lymphocyte (TIL), regulatory T cells (Tregs) and indoleamine 2,3-dioxygenase (IDO) in tumor biopsy specimens [86] have been of particular interest. The observation that the rise in absolute lymphocyte counts (ALC) during treatment in the MDX010–20 study data has led to the hope that biomarkers will be identified that serve to predict which patients are most likely to benefit from this modality with improved outcomes.

Ipilimumab is being combined with other agents to increase the degree and duration of clinical benefit. In the Phase III ipilimumab/DTIC study, the response rates observed for the combination were greater than that observed in the earlier ipilimumab/GP-100 vaccine study (15.2 vs 10.9% for single-agent ipilimumab vs 5.7% for ipilimumab/GP-100 vaccine) [2,3]. Interestingly, despite the different response rates observed, the 1/2-year survival rates were similar in these three groups: 47.3/28.5 (ipilimumab/DTIC) vs 43.5/21.6 (ipilimumab/GP-100) vs 47.3/28.5 (ipilimumab alone) suggesting that the proportion of patients who accrue benefit tend to behave in a similar fashion although the studies were not powered for this conclusion.

Two other studies – the Phase I combination of ipilimumab/bevacizumab and the Phase II study of the combination of ipilimumab/TMZ – recently presented preliminary data including an overall DCR of 67%, which was greater than expected for the single agent therapy and suggesting that ipilimumab may be combined with chemotherapeutic agents to show synergism [87,88]. Currently, ipilimumab combinations are areas of active investigation and include: targeted therapy (concurrent BRAF

inhibition (NCT01400451)); immunotherapy (GM-CSF (NCT01363206), DC vaccination (NCT01302496)); intratumor IL-2 (NCT01480323); concurrent IL-21 (NCT01489059) and pegylated IFN (NCT01496807)); chemotherapy (combination biochemotherapy (NCT01409174) and fotemustine (NIBIT-M1 trial)) and radiotherapy (concurrent stereotactic radiation RADVAX (NCT01565837)). Results from these trials are eagerly awaited.

While CTLA-4 negatively regulates T-cell activation during the initial phase of antigen presentation, the programmed death 1 (PD-1) receptor is expressed by T cells during long-term antigen exposure and regulates the effector phase of T-cell responses. PD-1 has two ligands: PD-L1 (B7-H1/CD274) and PD-L2 (B7-DC/CD273) that are primarily expressed within inflamed tissues and the tumor microenvironment. Interaction between T-helper PD-1 and PD-L1 generates a negative co-stimulatory signal that maintains immune tolerance such as in cancer and chronic viral infections. PD-1 blockade has been shown to promote the generation of antigen-specific cytotoxic T cells (CTL) and overcome Treg-mediated suppression [89].

Recently, the Phase I trial results of the anti-PD-1 (BMS-936558) and anti-PD-L1 (BMS-936559) antibodies were reported at ASCO 2012 and subsequently published [90,91]. The earlier dose-escalation study of the PD-1 blocking antibody BMS-936558 (MDX-1106/ONO-4538) in 39 patients with advanced solid tumors reported modest antitumor response rates: 3 responses among 23 evaluable patients [92]. However, MTD (10 mg/kg) was achieved without significant DLTs and spurred further evaluation. The subsequent Phase I study enrolled 296 patients with a variety of advanced solid tumors to receive PD-L1 antibody as an infusion every 2 weeks of an 8-week cycle in three dose escalations (1, 3 and 10 mg/kg) with response assessments after each cycle [90]. Topalian *et al.* have now reported objective responses in multiple tumor types including the traditionally non-immunogenic non-small-cell lung cancer (NSCLC) with an ORR of 24.1%. Tumor surface PD-L1 expression was not a pre-specified end point and this information was only available on 61 tumor specimens from 42 patients. However, when responses were broken down by tumor PD-L1 expression, objective responses were noted in 9 of the 25 PD-L1 positive patients with none in the 17 PD-L1 negative patients. Responses were durable – exceeding 1 year in 20 of 31 patients who had greater than 1 year of follow-up – and consistent with patterns of immune-related response previously described in patients treated with ipilimumab [93].

A concurrently published study assessed the PD-L1 blocking antibody BMS-936559 that inhibits the binding of PD-L1 to both PD-1 and CD80 [91]. Patients with advanced solid tumors were enrolled to receive escalating doses of BMS-936559 in a standard 3 + 3 design. Based on initial response, several dose-expansion cohorts were enrolled for a total of 207 patients treated at doses ranging from 0.3, 1, 3 to 10 mg/kg. Similar to the BMS-936558 trial, responses were noted in multiple tumor types including ovarian cancer and NSCLC with an ORR of 12.6% in the 135 patients in whom response was evaluable, lower than that observed with BMS-936558.

Both these studies suggest the potential for checkpoint inhibition as a therapeutic option in advanced malignancies. These agents are associated with durable responses in advanced malignancies including traditionally non-immunogenic cancers such as NSCLC and ovarian cancer and have relatively limited overall toxicity, with a reported incidence of grade III/IV toxicity of 14 and 9% in the anti-PD-1 and anti-PD-L1 trials, respectively. Although not directly comparable, PD-1 blockade has achieved perhaps more striking results than PD-L1 blockade, underscoring that these drugs are equivalent, anti-PD-1 blocks interactions between PD-1 and PD-L1/PD-L2 while anti-PD-L1 blocks interactions between PD-L1 and PD-1/CD80. However, MTDs were not defined in either trial although relative dose intensity of > 90% was achieved in most patients suggesting that additional Phase II dose-ranging trials may be required. Investigators have already planned Phase II trials (NCT01354431 and NCT01358721) and Phase III trials in melanoma, renal cell cancer and NSCLC are being considered. Emerging evidence suggests that B7-H1 expression in the tumor microenvironment may contribute toward adaptive resistance mechanisms evolved by the tumor [94]. Ultimately, combinations of checkpoint inhibitors such as PD-1/PD-L1 antagonists and agents stimulating antitumor immunity may be required to achieve lasting therapeutic benefit.

Other T-cell targets of interest are OX44 and CD137 (4–1BB) that may provide alternative avenues to up-regulate the immune response. Agonistic antibodies such as anti-OX44 and anti-CD137/(4–1BB) increase the T-cell response against melanoma and may have a role in the treatment of advanced disease. BMS-663513 is an anti-CD137/4–1BB agonist monoclonal antibody and Phase I trials suggested activity in broad variety of tumor types including melanoma [95]. Further evaluation is planned.

5. Conclusion

In conclusion, there has been explosive progress in the understanding of the molecular biology of melanoma and the pathways relevant to its transformation and progression, as well as the immunological lesions that appear to be associated with melanoma progression, over the past year. Therapeutic agents that target the pathways relevant to melanoma have been brought to bear on melanoma, along with new immunomodulators that may reverse the processes of tolerance and restore immune response to this tumor. These new advances have demonstrated significant clinical benefits, but the rational application of these agents individually and in combination with other agents that selectively address the specific lesions associated with melanoma progression are the next goal for advanced melanoma and for patients with high-risk operable melanoma where adjuvant intervention may achieve goals that eclipse the benefits of treatment for advanced disease.

6. Expert opinion

The evaluation of patients with melanoma now requires the molecular profiling of tumor mutations in BRAF, c-KIT, NRAS and other genes that have been discovered to be drivers of different subsets of the disease. The analysis of the host immunological response to melanoma is equally important, as a basis for the development and application of immunotherapies that have been of value to melanoma patients in the adjuvant arena, as

well as for therapy of metastatic disease. Improved understanding of the mutations driving melanoma tumorigenesis and the host immunological response to melanoma have resulted in the development of novel therapies and new classes of immunologic agents that have demonstrated clear evidence of improved survival in registration Phase III trials. Although these transformational agents have altered the treatment landscape in metastatic disease, they are not without significant limitations: the BRAF inhibitor vemurafenib has shown unprecedented antitumor response rates, but has a relatively short duration of response secondary to the rapid acquisition of resistance with median OS of approximately 16 months; conversely, ipilimumab is associated with durable responses in two Phase III trials conducted at different dosages in different populations but has a low ORR of 10.9 – 15.2%.

Recently presented data on the BRAF inhibitor dabrafenib and MEK inhibitor trametinib suggest promising clinical activity in metastatic melanoma and the FDA approval for this indication is pending. Phase I data on the use of PD-1/PD-L1 blocking antibodies suggest that immunotherapy may have applicability beyond melanoma and RCC with responses being seen in ‘non-immunogenic’ tumors such as NSCLC.

A complete understanding of the tumor’s molecular heterogeneity and host–tumor immune interactions will provide a more rational basis for the delivery of individualized therapy for the disease both in its advanced setting, and in the adjuvant arena, in the future. Novel agents and novel combinations of existing and new agents will help overcome limitations of current treatments.

Declaration of interest

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Article highlights.

- Where there have been only a single biological, and a single chemotherapy approved for metastatic melanoma, and one biological approved for adjuvant therapy over the past 25 years, in the past year two new agents were approved for therapy of metastatic disease on the basis of improved survival: the CTLA-4 (cytotoxic T-lymphocyte antigen-4) blocking antibody ipilimumab and the BRAF inhibitor vemurafenib. Recent advances in Phase III registration trials suggest that a new BRAF inhibitor dabrafenib and the MEK inhibitor trametinib may reach regulatory approval in this year.
- As a consequence of the molecular specificity of the BRAF inhibitors, and potential adverse effects of these agents in patient whose tumors are wild-type (lacking the relevant mutation), evaluation of patients with advanced melanoma should now include molecular profiling of tumor tissue for BRAF mutations, as well as c-KIT and NRAS mutations as genetic drivers of tumorigenesis.
- Effective immunotherapy against melanoma is hampered by the tumor's ability to subvert host immune responses through tumor escape mechanisms. A more thorough understanding of the host–tumor immune interaction is required, and feasible through neoadjuvant trials in which tumor tissue is obtained both before and after introduction of the study agent(s).
- Combinations of targeted and immunotherapeutic approaches are likely to provide yet further improvement in the control of metastatic melanoma, and may in the future also be applicable to the adjuvant therapy of this disease. Information from molecular classification of the drivers of melanoma, as well as the bases of resistance to these agents, along with a deeper understanding of the host–tumor immune interaction should illuminate more effective combined modality therapy tailored to the relevant lesions of this disease.

Table 1.

Phase II/III studies of BRAF-I inhibitors in melanoma.

Study reference	Number of patients eligible for analysis	Study design	Primary end point	Dose and schedule - treatment arm	Responses	Survival	HR (95% CI)
BRIM 2 [22]	132	Phase II, open label	BORR	Vemurafenib (PLX-4032) 960 mg b.i.d. orally	BORR: 52.3% CR: 2.3% PR: 50%	6.2 months	N/A
BRIM 3 [4]	675	Phase III, randomized, double blind	OS	Vemurafenib (PLX-4032) 960 mg b.i.d. orally	PLX-4032: 84% (6 months) DTIC® alone: 64% (6 months)	PLX-4032: 5.3 months DTIC alone: 1.6 months	Death 0.37 (95% CI 0.26 – 0.55) Progression 0.26 (95% CI 0.20 – 0.33)
Infante *et al* [23]	16 (45)	Three-part Phase I/II Part 1: PK drug-drug interaction study Part 2: dose escalation to find MTD followed by expansion Part 3: randomized Phase II trial in untreated stage IV melanoma	Part 1: PK/PD Part 2: OR	Dabrafenib (GSK2118436, BRAF inhibitor) 75 – 150 mg b.i.d. + GSK1120212 (oral MEK1/2 inhibitor) 1.0, 1.5 or 2.0 mg q.d. RP2D: GSK2118436 150 mg b.i.d. + GSK1120212 2 mg q.d.	ORR: 81% (13 PR + 3 SD)	N/A	N/A

CI: Confidence interval; HR: Hazard ratio; N/A: Not applicable; ORR: Overall response rate; OS: Overall survival; PD: Pharmacodynamics; PFS: Progression-free survival; PK: Pharmacokinetics; PR: Partial response; RP2D: Recommended Phase II dosage; SD: Stable disease.

Table 2.

Phase I/II studies of c-MEK inhibitors in melanoma.

Study reference	Number of patients eligible for analysis	Study design	Primary end point	Dose and schedule - treatment arm	Responses	Survival	Toxicity
Agarwal *et al.* [29],	28 (8 with melanoma)	Two-part Phase I dose escalation	Part A: 3 + 3 dose escalation Part B: relative bioavailability of the two formulations	AZD6244 hydrogen sulfate formulation 25 – 100 mg b.i.d.	Not reported	N/A	Rash; fatigue; nausea/ vomiting; diarrhea; dyspnea; peripheral edema (% not available)
Kirkwood *et al.* [30]	200	Phase II, open-label, multicenter, 1:1 randomized, parallel-group study	PFS	Selumetinib 100 mg b.i.d. OR TMZ 200 mg/m^2/day D1 – 5 of each 28-day cycle	ORR (all PR): 5.8% (MEK-I) vs 9.4% (TMZ) BRAF mutant cohort: 11.1% (MEK-I) vs 10.7% (TMZ)	PFS: not calculated Time to death: 9.5 months (MEK-I) vs 12.3 months (TMZ)	Rash 59.6% (G3/412.1 %) Diarrhea 56.6% (G3/44.0%) Nausea 50.5% (G3/43.0%) Edema 40.4% (G3/41.0%)
Infante *et al.* [34]	84 patients (29 with melanoma, 20 evaluable)	Three-part Phase I/II study	Part 1: dose escalation Part 2: expansion in selected tumor types to evaluate RP2D Part 3: PK/PD assessment using tumor biopsies or FDG-PET	GSK1120212 in escalating doses RP2D 2 mg/day MTD 3 mg/day	ORR (all PR): 20% (3 mutant, 2 wild type) CBR (PR + SD): 73% (8/11 mutant) vs 56% (5/9 wild type)	N/A	Rash 77% (G3 5%) Diarrhea 45% (G3 3%)
Kurzrock *et al.* [37]	23 (13 evaluable)	Three-part Phase I/II study	Part 1: dose escalation Part 2: expansion in selected tumor types to evaluate RP2D Part 3: PK/PD assessment using tumor biopsies or FDG-PET	GSK1120212 + AKT inhibitor GSK2141795 in escalating doses RP2D GSK1120212 2 mg/day MTD GSK2141795 75 mg/day RP2D/MTD for combination not identified	ORR (all PR): 23% (3/13)	N/A	Nausea 26% (G3/40%) AST elevation 22% (G3/49%) Fatigue 22% (G3/40%) Rash 22% (G3/40%)

AST: Aspartate aminotransferase; CBR: Clinical benefit rate; FDG-PET: Fluorodeoxyglucose positron emission tomography; MTD: Maximal tolerated dose; N/A: Not applicable; ORR: Overall response rate; PD: Pharmacodynamics; PFS: Progression-free survival; PR: Partial response; PK: Pharmacokinetics; RP2D: Recommended Phase II dosage; SD: Stable disease.

Table 3.

Phase II/III studies of CTLA-4 blockade in melanoma.

Study reference	Number of patients eligible for analysis	Study design	Primary end point	Dose and schedule - treatment arm	Responses	OS/PFS	HR (95% CI)
BMS 008 [79]	155	Phase II, open-label, single arm, advanced melanoma	Dose-finding	IPI 10 mg/kg	47% (1 year)	N/A	N/A
BMS 022 [80]	217	Phase II, randomized, double-blind, advanced melanoma	Efficacy of three dose levels of IPI	IPI at varying doses (0.3, 3, 10 mg/kg) Induction 0.3/3/10 mg/kg q3weeks for 4 doses Maintenance 0.3/3/10 mg/kg q6weeks until week 48 then q12weeks afterward	48% (1 year)	N/A	N/A
BMS 007 [81]	115	Phase II, randomized, double-blind, advanced melanoma	Rate of grade II + diarrhea	IPI 10 mg/kg	51% (1 year)	N/A	N/A
Medarex MDX010–20 [2]	676	Phase III, randomized, double-blind, advanced melanoma	ORR, subsequently amended to OS	IPI 3 mg/kg + GP-100 peptide vaccine vs IPI alone vs GP-100 peptide vaccine alone	IPI alone: 10.9% IPI + GP-100: 5.7% GP-100 alone: 1.5%	OS rates (1/2 year): IPI alone: 45.6/23.5% IPI + GP-100: 43.5/21.6% GP-100 alone: 25.3/13.7% OS duration: IPI alone: 10.1 months IPI + GP-100: 10.0 months GP-100 alone: 6.4 months PFS duration: IPI alone: 2.86 months IPI + GP-100: 2.76 months GP-100 alone: 2.76 months	IPI alone (compared with GP-100 alone): 0.66 (95% CI 0.51 – 0.87) IPI + GP-100 (compared with GP-100 alone): 0.68 (95% CI 0.55 – 0.85)
BMS 024 [3]	502	Phase III, randomized, double-blind, advanced melanoma	OS	IPI + DTIC: induction 10 mg/kg + DTIC (850 mg/m^2) q3weeks for four doses Maintenance 10 mg/kg + DTIC (850 mg/m^2) q12weeks	IPI + DTIC: 33.2% (DCR), 15.2% (ORR) DTIC alone: 30.2% (DCR), 10.3% (ORR)	OS rates (1/2/3 year): IPI + DTIC: 47.3/28.5/20.8% DTIC alone: 36.3/17.9/12.2% OS duration: IPI + DTIC: 11.2 months DTIC alone: 9.1 months PFS duration: IPI + DTIC: 2.8 months DTIC alone: 2.6 months	IPI + DTIC: OS 0.72, PFS 0.76
Sarnaik *et al.* [82]	75	Phase II, single-arm, open-label, resected high-risk melanoma	40% rate of tolerable IRAEs	HLA A*0201 positive: IPI 3 or 10 mg/kg q8weekly for 12 months + multipeptide (MART-1/GP-100/ tyrosinase) vaccine HLA A*0201 negative: IPI 10 mg/kg q8weekly for 12 months	Not reached (29.5 months follow-up)	Resected stage IV: 40.5 months Resected stage IIIc: not reached (29.5 months follow-up)	Not reached (29.5 months follow-up)

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Study reference	Number of patients eligible for analysis	Study design	Primary end point	Dose and schedule - treatment arm	Responses	OS/PFS	HR (95% CI)
Margolin *et al.* [83],	72	Phase II, single-arm, open-label, advanced melanoma with brain metastases 2 cohorts: cohort A (asymptomatic; no steroids) and cohort B (symptomatic, on stable dose of steroids)	DCR	IPI: induction 10 mg/kg q3weeks for 4 doses Maintenance 10 mg/kg q12weeks	Cohort A: 18% Cohort B: 10%	N/A	N/A
NIBIT-M1 [84]	84	Phase II, single-arm, open-label, advanced melanoma	Immune-response DCR using IRRC	IPI + fotemustine: IPI induction 10 mg/kg q3weeks for 4 doses Maintenance 10 mg/kg + DTIC (850 mg/m^2) q12weeks Fotemustine 100 mg/m^2 qweekly for 3 weeks then q3weekly	IRORR - 29.1% OS at 1 year 51.8% (median OS not reached)	Median IRPFS 5.3 months	Not reported

CI: Confidence interval; DCR: Disease control rate; HR: Hazard ratio; IPI: Ipilimumab; IRAEs: Immune-related adverse events; IRRC: Immune-related response criteria; N/A: Not applicable ORR: Overall response rate; OS: Overall survival; PFS: Progression-free survival.