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Advanced stage melanoma therapies: Detailing the present and exploring the future

Chelsea Luther[a], Umang Swami[b], Jun Zhang[b], Mohammed Milhem[b], Yousef Zakharia[b,*]

[a] Department of Dermatology, Henry Ford Hospital, Detroit, MI, United States
[b] Department of Internal Medicine, Division of Hematology, Oncology and Blood and Marrow Transplantation, University of Iowa Hospitals and Clinics, Iowa City, IA, United States

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ABSTRACT

Metastatic melanoma therapies have proliferated over the last ten years. Prior to this, decades passed with only very few drugs available to offer our patients, and even then, those few drugs had minimal survival benefits. Many treatment options emerged over the last ten years with diverse mechanisms of action. Further, combination regimens have demonstrated superiority over monotherapy, especially for targeted agents. Each therapeutic combination possesses different advantages and side effect profiles. In this review, we outline the United States Food and Drug Administration–approved melanoma treatment agents and therapies currently in clinical development, focusing on combination approaches.

1. Introduction

In less than one decade, there has been considerable progress in the treatment of metastatic melanoma (MM). Scarcely ten years ago, there was little to offer patients with this devastating disease. Dacarbazine remained the standard of care from the 1970s to the early 2000s despite the fact that this drug failed to demonstrate survival prolongation (Korn et al., 2008; Yang and Chapman, 2009). IL-2 therapy yielded grueling side effects and often required inpatient monitoring, yet with modest overall response rate (ORR) and survival benefit (Petrella et al., 2007). Finally, a paradigm shift transpired in the 2010s with the advent of ipilimumab and its United States Food and Drug Administration (FDA) approval in 2011. This drug marked the forefront of tolerable, innovative therapies in melanoma with proven increases in survival.

Abbreviations: MM, metastatic melanoma; ORR, overall response rate; FDA, Food and Drug Administration; GITR, glucocorticoid-induced tumor necrosis factor-related gene receptor; mAb, monoclonal antibody; CTLA-4, cytotoxic T-lymphocyte-associated antigen-4; APCs, antigen-presenting cells; gp100, glycoprotein 100; OS, overall survival; DOR, prolonged duration of response; AEs, adverse effects; MAPK, mitogen-activated protein kinase; ERK, extracellular-signal-regulated kinase; SCC, squamous cell carcinoma; PD-1, programmed death 1; TVEC, talimogene Laherparepvec; HSV1, herpes-simplex-1; GM-CSF, granulocyte-macrophage colony-stimulating factor; COT, Cancer Osaka Thyroid; CDKN2A, Cyclin-Dependent Kinase Inhibitor 2A; DLTs, dose-limiting toxicities; DCR, disease-control rate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; CPK, creatine phosphokinase; TNFRSF, TNF receptor super family; Tim-3, T-cell immunoglobulin and mucin containing protein-3; IDO, indoleamine 2, 3-dioxygenase; TDO, tryptophan 2,3-dioxygenase; AHR, aryl-hydrocarbon receptor; CpG, cytosine guanine dinucleotide; ODN, oligodeoxyneucelotides; pDC, plamacytoid dendritic cells; MTD, maximum tolerated dose; CRS, cytokine release syndrome; LAG-3, Lymphocyte activation gene-3; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma; MAP2, microtubule-associated protein

* Corresponding author at: Division of Hematology, Oncology and Blood and Marrow Transplantation, University of Iowa Hospitals and Clinics, 200 Hawkins Drive, 5984 JPP, Iowa City, IA, 52242, United States.
E-mail address: Yousef-zakharia@uiowa.edu (Y. Zakharia).

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Since 2009, advanced melanoma one-year overall survival rates have more than doubled from 16 to 30% to rates now reaching 50% to over 70% (Balch et al., 2009). Before the new therapies, median survival time for MM patients was a heinously short 6.2 months (Korn et al., 2008). Following ipilimumab, the development of targeted therapies, immunotherapies, cancer vaccines, and other small molecules have opened even more doors for MM treatment. These drugs have since demonstrated superiority to ipilimumab, especially if used in combination.

Since 2011, the FDA has approved ten melanoma treatment agents. Here, we summarize these therapies as well as major melanoma therapy agents undergoing clinical trials. Further, we outline the multitude of combination therapies established or under exploration for melanoma today and the proposed mechanisms of therapy synergism.

A literature search traversed PubMed, Medline, and the American Society of Clinical Oncology abstracts between 1970 and June 30, 2017. Search terms included melanoma immunotherapy, melanoma targeted therapy, melanoma combination treatments, combined immune therapy, MM treatments, MM therapy, CTLA-4, PD1, BRAF inhibitor, ipilimumab, vemurafenib, dabrafenib, nivolumab, cobimetinib, trametinib, pembrolizumab, MEDI4736, atezolizumab, pidilizumab, talimogene, TVEC, indoleamine 2,3-dioxygenase and melanoma, IDO inhibitor, CD40 and melanoma, CD137 and melanoma, OX40 and melanoma, glucocorticoid-induced tumor necrosis factor-related gene receptor (GITR) and melanoma, LAG-3 and melanoma, Tim-3 and melanoma, indoximod (D-1MT), epacadostat (INCB024360), NLG919, CP–870,893, SGN-40, HCD 122, dacetuzumab, urelumab, 9B12, MEDI6469, TRX518, INCAGN01876, IMP321, BMS-986016, LAG525, and MBG453.

2. FDA-Approved monotherapies

2.1. CTLA-4 inhibition

Ipilimumab gained fame as the first treatment to demonstrate increased overall survival in a randomized control trial of MM (Hodi et al., 2010). This therapy is a fully human IgG1 monoclonal antibody (mAb) against cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4). CTLA-4, an inhibitory molecule on T-cells, binds to B7 on antigen-presenting cells (APCs) and impedes co-stimulatory signaling required for T-cell activation (Buchbinder and Desai, 2016). Thus, CTLA-4:B7 interaction leads to T-cell inactivation. Blocking this interaction, as with ipilimumab, increases the priming and activation of effector T-cells and promotes differentiation. Ipilimumab has proven its efficacy in multiple clinical trials of both previously treated and previously untreated patients (Table 1). Table (Hodi et al., 2010; Robert et al., 2011; Maio et al., 2015; Schadendorf et al., 2015).

Because of its method of action, ipilimumab is associated with immune-related side effects, including severe and fatal autoimmune reactions. The most common adverse effects (AEs) include dermatitis, diarrhea, colitis, hepatitis, hypophysitis, thyroiditis, hypothyroidism, and adrenal insufficiency. Grade three or higher AEs are seen in approximately one-fourth of patients treated with ipilimumab (Hodi et al., 2010; Larkin et al., 2015a; Postow et al., 2015). In KEYNOTE-006 (see below), the most grade 3–5 AEs were colitis (7.0%), diarrhea (3%), hypophysitis (1.6%), and fatigue (1.2%) (Robert et al., 2015a; Schachter et al., 2017). In Checkmate-067 (see below), these included colitis (8%), diarrhea (6%), and increased lipase (4%) (Larkin et al., 2015b).

2.2. BRAF inhibition

Very important in the mutagenesis of melanoma is the RAS-RAF-MEK-ERK pathway, also known as mitogen-activated protein kinase (MAPK) pathway. Nearly half of MMs contain driver mutations in the serine-threonine protein kinase BRAF at V600 (Davies et al., 2002). Mutated BRAF stays constitutively activated (400 times more active than wild type), causing substantial extracellular-signal-regulated kinase (ERK) activation and subsequent melanoma cell proliferation and survival (Beeram et al., 2005; Garnett and Marais, 2004).

BRAF inhibitors, have shown significantly improved PFS and OS over chemotherapy (Table 1); (Sosman et al., 2012; McArthur et al., 2014; Larkin et al., 2014; Robert et al., 2015b; Long et al., 2015a). However, drug resistance is common and responses are often short-lived (Sosman et al., 2012; McArthur et al., 2014; Larkin et al., 2014; Robert et al., 2015b; Long et al., 2015a; Chapman et al., 2011; Hauschild et al., 2012). In one Phase III study, approximately 50% of patients receiving dabrafenib (versus dacarbazine) developed acquired resistance approximately 5 months after initiation of treatment (Hauschild et al., 2012).

The side effect profile of BRAF inhibitors includes arthralgia, fatigue, photosensitivity, and rash. Unfortunately, the development of secondary squamous cell carcinoma (SCC) is a relatively common AE of BRAF inhibitor monotherapy (~18–26% with vemurafenib, ~7–10% with dabrafenib) (Sosman et al., 2012; Chapman et al., 2011; Ascierto et al., 2013). Selective BRAF inhibition can lead to paradoxical activation of MAPK pathway in BRAF wild cells and lead to unregulated growth, especially in cells with preexisting RAS mutations (Su et al., 2012). For vemurafenib, grade three to four AEs also include keratoacanthoma (10% of patients), rash (9%), asymptomatic elevated transaminases (11%), and new primary melanomas (2.4%) (McArthur et al., 2014). For dabrafenib, grade three to four adverse events also include pyrexia (3%). (Hauschild et al., 2012).

2.3. MEK inhibition

Targeted inhibition of MEK is also important in melanoma therapy. The MEK inhibitor trametinib demonstrated significant superiority to chemotherapy and is approved as monotherapy (Table 1) (Flaherty et al., 2012). The most common grade three to four side effects in the largest trametinib phase III study (METRIC) were fatigue (3%), hypertension (3%), vomiting (2%), and diarrhea (2%) (Flaherty et al., 2012).

2.4. PD-1 inhibition

Programmed death 1 (PD-1), another immunomodulatory molecule on T-cells, binds to its ligands (PD-L1 and PD-L2) on APCs, leukocytes, and non-hematopoietic cells (Buchbinder and Desai, 2016). More importantly, PD-L1 is an inducible ligand on tumor cells. In fact, interferon-mediated signals initiated by tumor-infiltrating lymphocytes incite PD-L1 expression by tumor cells (Pardoll, 2012). Normally, PD-1/PD-L interaction leads to T-cell exhaustion. Nivolumab and pembrolizumab are monoclonal IgG4 antibodies against PD-1 immune-checkpoint–inhibitor; nivolumab is fully human while pembrolizumab is fully humanized (Topalian et al., 2014; Sullivan and Flaherty, 2015). Nivolumab and pembrolizumab block PD-1, impeding interactions with its ligand and thus allowing for anti-tumor effector responses, T-cell activation, proliferation, and survival.

Melanoma tumor cells liberally express PD-L1, and nivolumab and pembrolizumab have demonstrated significantly increased in OS in MM patients compared to both chemotherapy and ipilimumab (Table 1) (Schachter et al., 2017; Robert et al., 2015c). The original studies and use of PD1 inhibitors included patients with disease progression despite ipilimumab or BRAF inhibitor therapy. Their success has since lead to their use as first-line agents, especially in combination with ipilimumab or novel agents. In fact, the first major phase III study comparing pembrolizumab with ipilimumab (KEYNOTE-006) was stopped early, unblinded, and ipilimumab patients were offered pembrolizumab (Robert et al., 2015c). Although these agents have lower ORRs than those seen with BRAF inhibitor therapy, PD-1 inhibitors typically lead to long-term remissions. These agents are better tolerated compared to

Table 1
Summary of phase III trials of FDA approved agents for unresectable or metastatic melanoma.

Drug	Phase III trial	Dose	Study Population	Number of Patients	Average OS (months)	Average ORR (%)	Average PFS (months)	References
Immunotherapy - Monotherapy								
CTLA-4 inhibitor								
Ipilimumab	MDX010-20	4 doses at 3 mg/kg	Previously treated	137	10.1	10.9	2.8	(Hodi et al., 2010)
Ipilimumab	CA184-024	10 mg/kg + dacarbazine	Treatment naive	250	11.2	–	2.8	(Robert et al., 2011)
Ipilimumab	CheckMate 067	4 doses at 3 mg/kg	Treatment naive	315	19.9	19	2.9	(Larkin, 2015a, 2015b, 2015c; Wolchok et al., 2017)
Ipilimumab	KEYNOTE-006	4 doses at 3 mg/kg	Previously treated and treatment-naive	278	16	13	2.8	(Schachter et al., 2017; Robert et al., 2015a, 2015b, 2015c, 2015d)
PD-1 inhibition								
Pembrolizumab	KEYNOTE-006	10 mg/kg every 2 weeks or every 3 weeks	Previously treated and treatment naive	279, 277	not reached	36, 37%	5.6, 4.1	(Schachter et al., 2017; Robert et al., 2015a, 2015b, 2015c, 2015d)
Nivolumab	CheckMate066	3 mg/kg every 2 weeks	Treatment naive	210	not reached	40	5.1	(Robert, 2015d)
Nivolumab	CheckMate037	3 mg/kg every 2 weeks	Previously treated with ipilimumab +/– BRAF inhibitor	272	16	27	3.1	(Webber 2015; Larkin et al., 2018)
Nivolumab	CheckMate 067	3 mg/kg every 2 weeks	Treatment naive	316	37.6	44	6.9	(Larkin, 2015a, 2015b, 2015c; Wolchok et al., 2017)
Targeted Monotherapy								
BRAF-inhibition								
Vemurafenib	BRIM-3	960 mg orally twice daily	Treatment naive	336	13.6	57	6.9	(Chapman et al., 2011; McArthur, 2014; Chapman, 2017)
Vemurafenib	coBRIM	960 mg twice daily	Treatment naive	247	17.4	45	7.2	(Larkin et al., 2014; Ascierto et al., 2016)
Vemurafenib	COMBI-V	960 mg twice daily	Treatment naive	352	18	51	7.3	(Robert, 2014)
Vemurafenib	COLUMBUS	960 mg twice daily	Previously treated and treatment naive	191	–	40	7.3	(Dummer et al., 2018)
Dabrafenib	BREAK-3	150 mg twice daily, orally	Treatment naive	187	18.2	50 (IRC), 53 (investigator)	6.9	(Hauschild et al., 2012; Hauschild et al., 2012)
Dabrafenib	COMBI-D	150 mg twice daily, orally	Treatment naive	212	18.7	53	8.8	(Long et al., 2015a, 2015b, 2015c)
Encorafenib	COLUMBUS	450 mg once daily	Previously treated and treatment naive	194	–	51	9.6	(Dummer et al., 2018)
MEK inhibitor								
Trametinib	METRIC	2 mg orally) once daily	Previously treated	214	15.6	22	4.8 months	(Flaherty et al., 2012)
Tumor vaccine - Monotherapy								
TVEC	OPTiM	10^6 pfu/mL at week zero; 10^8 pfu/mL at week 3 weeks and then every 2 weeks.	Previously treated and treatment naive	295	23.3	26	N/A	(Andtbacka et al., 2015)

both chemotherapy and ipilimumab. In KEYNOTE-006, pembrolizumab-related grade 3–5 adverse events occurred in 17% of patients, the most common being diarrhea (1–3%), colitis (1.4–2.5%), and hepatitis (1.1–1.8%) (Robert et al., 2015c). Rash, although not severe, is a common grade 1–2 AE of pembrolizumab (approximately 14% of patients) and often requires referral to dermatology. In comparison, ipilimumab-related grade 3–5 adverse events occurred in 20% of patients.

Similar results have been reported with nivolumab, which has been tested in phase III trials (Table 1) against dacarbazine (CheckMate 066), dacarbazine or paclitaxel plus carboplatin (CheckMate 037), and with or without ipilimumab (CheckMake 067) (Larkin et al., 2015b; Topalian et al., 2014; Robert et al., 2015d; Long et al., 2016a; Larkin et al., 2018). Grade three events of CheckMate037 occurred in 9% of nivolumab-treated patients (most commonly increased lipase [1%], increased alanine aminotransferase (ALT) [1%], fatigue [1%], and anemia [1%]) compared to 31% of the chemotherapy arm. (Weber et al., 2015) In Checkmate-067 (see below), these included increased lipase (4%), diarrhea (3%), and increase amylase (2%) (Larkin et al., 2015b).

Other PD-L1 monoclonal antibodies include atezolizumab. Phase I study results of atezolizumab across 45 advanced melanoma patients yielded an ORR of 29% and a 24 week PFS of 35% (Hamid et al., 2013).

Unfortunately, resistance mechanisms are also present in anti-PD-1 therapy and can lead to disease relapse (Gide et al., 2018).

2.5. Talimogene laherparepvec (TVEC)

TVEC is currently the only oncolytic virus approved for melanoma and is administered intralesionally. The therapy consists of herpes-simplex-1 (HSV1) genetically modified to stifle virulence and instead include cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) encoding sequences. TVEC multiplies within neoplastic cells, which leads to both local production of GM-CSF and tumor cell lysis (Kohlhapp and Kaufman, 2016). This subsequently causes the release of tumor-derived antigens, allows for priming of CD8+ cells by dendritic cells, and generates an immune response targeting melanoma cells. (Andtbacka et al., 2015)

In the phase III OPTiM trial of intralesional TVEC showed superiority over GM-CSF (Table 1) (Andtbacka et al., 2015) and a substantial (≥50%) decrease in 64% of injected lesions as well as 34% of non-injected, non-visceral and 15% of visceral lesions (Andtbacka et al., 2016). Overall 47% of injected, 22% of non-injected, non-visceral, and 9% of visceral lesions had a complete response. This suggests TVEC induces systemic immune effects against melanoma (Andtbacka et al., 2016). TVEC also possess a relatively low toxicity profile, with the most frequent AEs being inflammation and pain at injection sites.

3. Combination therapy

Acquired resistance is a major drawback to both targeted therapy and immunotherapy. Further, individual treatments have relatively low ORRs. Different combination therapies have been and are currently being considered to overcome these problems, and there is a general agreement in the melanoma field that the future is indeed for combination therapies. Nonetheless, this can come at the price of increased toxicity. Arrays of combinations have been tested preclinically and clinically to find the best complementary pairs and triplets with the highest increase in survival and lowest added AEs. Below, we outline established combination therapy regimens as well as current clinical trials. Table 2 provides a summary of completed phase III trials of FDA approved combination therapies in advanced melanoma.

3.1. Combined BRAF and MEK inhibition

The inevitable disease relapse for patients treated on BRAF inhibitor monotherapy is well known and secondary to drug resistance. The

Table 2
Summary of phase III trials of FDA approved combination therapies for unresectable or metastatic melanoma.

Drugs	Phase III trial	Dose	Study Population	Number of Patients	Average OS (months)	Average ORR (%)	Average PFS (months)	References
Combination Therapies								
Immunotherapy - Combination								
Ipilimumab + Nivolumab	CheckMate 067	4 doses at 3 mg/kg + 3 mg/kg every 2 weeks	Treatment naive	314	not reached	58	11.5	(Larkin, 2015a, 2015b, 2015c; Wolchok et al., 2017)
Targeted Therapay - Combination								
Vemurafenib + Cobimetinib	coBRIM	960 mg twice daily + 60 mg once daily	Treatment naive	247	22.3	68	12.3	(Larkin et al., 2014; Ascierto et al., 2016)
Dabrafenib + Trametinib	COMBI-V	150 mg twice daily + 2 mg once daily	Treatment naive	352	25.6	64	11.4	(Robert et al., 2015a, 2015b, 2015c, 2015d)
Dabrafenib + Trametinib	COMBI-D	150 mg twice daily + 2 mg once daily	Treatment naive	211	25.1	69	11	(Long, 2015a, 2015b, 2015c)
Encorafenib + Binimetinib	COLUMBUS	450 mg once daily + 45 mg twice daily	Previously treated and treatment naive	192	–	63	14.9	(Dummer et al., 2018)

mechanisms for resistance are numerous and include alternate splicing of mutant BRAF, gene amplification of mutant BRAF, RAS mutations (most commonly NRAS), MEK mutations, overexpression of Cancer Osaka Thyroid (COT), and loss of Cyclin-Dependent Kinase Inhibitor 2A (CDKN2A) (Monsma et al., 2015; Shi et al., 2014; Trunzer et al., 2013).

As the majority of these mechanisms ultimately lead to activation of the MAPK pathway despite BRAF inhibitor therapy, MEK inhibition in conjunction with BRAF inhibitor therapy became a logical avenue of investigation. Now, due to the success proven by multiple clinical trials, the combinations of vemurafenib and cobimetinib, dabrafenib and trametinib or encorafenib binimetinib have become standard treatment for patients with BRAF V600-mutated MM.

The BRAF/MEK inhibitor combinations delay resistance and demonstrate increased ORR, PFS, and OS, as shown in the multiple phase III trials. The coBRIM trial randomized 495 BRAF mutated late-stage melanoma patients to either vemurafenib (960 mg twice daily) with cobimetinib (60 mg daily) or vemurafenib (960 mg twice daily) with placebo and reported an ORR of 68% in the combination group and 45% in the vemurafenib group ($P < 0.001$) (Larkin et al., 2014). The combination group median OS was 22.3 months (95% CI, 20.3–not reached), whereas the vemurafenib group median OS was 17.4 months (HR 0.70 [95% CI 0.55–0.90], $P = 0.005$) (Ascierto et al., 2016). The combination group also outperformed vemurafenib in median PFS at 12.3 months versus 7.2 months (HR 0.58 [95% CI 0.46–0.72], $P = 0 < 0.0001$) (Larkin et al., 2015c). Grade three AEs occurred at the same frequency (49%) in both study arms, but grade four events were higher in the combination group (13% versus 9%) (Larkin et al., 2014). In the combination group, grade three or higher events included increased gamma-glutamyl transferase (GGT) (15%), increased blood creatine phosphokinase (CPK) (12%), increased alanine transaminase (11%), increased pyrexia (2%), dehydration (2%) (Ascierto et al., 2016), Dabrafenib plus trametinib has demonstrated similar results in COMBI-V, a phase III trial of 704 metastatic and BRAF V600E-mutated melanoma, in which patients were randomized to either dabrafenib (150 mg twice daily) plus trametinib (2 mg once daily) combination or vemurafenib (960 mg twice daily) alone. The investigators reported an ORR of 64% in the combination group versus 51% in the vemurafenib group ($P < 0.001$) and a PFS of 11.4 months versus 7.3 months ($P < 0.001$) (Robert et al., 2015b). In this study, grade three to four AEs, of which the most common were pyrexia (4%) and decrease in ejection fraction (4%), occurred in 52% of patients (Robert et al., 2015b). Two year updated data showed an OS of 25.6 months versus 18 months for combination and vemurafenib alone, respectfully (Robert et al., 2018). Three years updated data demonstrated a median DOR as 13.8 months for combination and 7.6 months for vemurafenib alone (95% CI, 7.4–9.3) (Robert et al., 2016). In a pooled study of 617 dabrafenib plus trametinib combination patients across a median follow-up of 20 months, median PFS was 11.1 months and median OS was 25.6 months (Long et al., 2016b).

Although some AEs occur more often with combination therapy, the AE of squamous-cell carcinoma and keratoacanthomas paradoxically decrease, as adding a MEK inhibitor combats the mechanism outlined above. As reported in COMBI-V, cutaneous SCCs and keratoacanthomas occurred in 1% of patients treated with combination therapy and 18% of those with vemurafenib alone (Robert et al., 2015b).

Another BRAF/MEK inhibitor combination is encorafenib and binimetinib. In a phase Ib/II trial (CMEK162 × 2110) of 55 advanced BRAFV600-mutated melanoma BRAF inhibitor–naïve patients, encorafenib plus binimetinib showed an ORR of 74.5% and a median PFS of 11.3 months (95% CI, 7.4–14.6) with higher grade three to four toxicities occurring in the high-dose group (Sullivan et al., 2015). Phase III data of 577 patients randomized to encorafenib plus binimetinib, encorafenib alone or vemurafenib alone showed an ORR of 63%, 51%, and 40% and PFS of 14.9, 9.6, and 7.3 months, respectively (Dummer et al., 2018). Grade three to four AEs occurred less frequently in the combination group (58%) compared to encorafenib alone (66%) and vemurafenib alone (63%). The most common grade three or higher AEs included increased GGT (9%), increased CPK (7%), and hypertension (6%) for the combination group and palmoplantar erythrodysaesthesia syndrome (14%), myalgia (10%), and arthralgia (9%) for encorafenib alone (Dummer et al., 2018).

3.2. Combined immunotherapies

Although drug resistance is also common for immunotherapies, the mechanisms involved are not fully understood. Combination therapy is attractive in this arena to avoid resistance and to boost the relatively low ORR seen in this class.

Combined immunotherapy has thus far fulfilled the goals of expanding the number of patients who respond to cancer immunotherapy and strengthening clinical response. The most well studied and robust immunotherapy combination is ipilimumab with nivolumab. In the Phase III trial CheckMate-067, 945 treatment-naïve patients were randomized to receive ipilimumab alone, nivolumab alone, or ipilimumab and nivolumab combination. ORRs were 19% for ipilimumab alone, 44% for nivolumab alone, and 58% for combination (Larkin et al., 2015b; Wolchok et al., 2017). The PFSs were 2.9, 6.9, and 11.5 months, respectively. OS was 19.9 months for the ipilimumab alone group, 37.6 months for the nivolumab alone group and was not reached for the combination group (median follow-up time of 36 months) (Wolchok et al., 2017). The downside to this combination is the significantly increased toxicity. Grade three or higher toxicities occurred in 28% of the ipilimumab alone group, 21% of the nivolumab alone group, and 59% of the combination group (Wolchok et al., 2017). For the combination group, these included increased lipase (11%), diarrhea (9%), colitis (8%), and elevated ALT (9%) (Larkin et al., 2015b).

Median PFS for the ipilimumab, nivolumab, and combination were 4, 14, and 14 months, respectively, for PD-L1-positive; and 3, 5, and 11 months, respectively, for PD-L1-negative. Objective response rates for the ipilimumab, nivolumab, and combination were 21, 58, and 72 percent, respectively, for PD-L1-positive versus 18, 41, and 55 percent, respectively, for PD-L1-negative (Larkin et al., 2015b).

Weber et al. tested nivolumab and ipilimumab in sequence. Both the ORR (41 versus 20%) and the 12-month OS rate (76 versus 54%) were increased in the nivolumab to ipilimumab group compared to ipilimumab then nivolumab. Grade three to five AEs were more pronounced in the nivolumab to ipilimumab sequence group, 50% compared to 43% (Flaherty et al., 2012; Weber et al., 2016). For the nivolumab to ipilimumab sequence group compared to the reverse sequence, colitis, increased lipase and diarrhea occurred in 15 vs 20%, 15 vs 17%, and 12 vs 7%, respectively.

The combination of ipilimumab with pembrolizumab has yielded similar results. Long et al. and Carlino et al. presented preliminary results of a phase I study (KEYNOTE-029) (Long et al., 2016c; Carlino et al., 2017). A total of 153 patients received pembrolizumab (2 mg/kg every three weeks) with ipilimumab (1 mg/kg for four doses), then pembrolizumab alone for up to two years. This trial demonstrated an ORR of 61% (15% experienced complete responses). Both median PFS and OS were not reached after a median follow-up of 17 months. Grade three to four AEs were seen in 45% of patients, most commonly elevated lipase (16%) and autoimmune hepatitis (6%) (Weber et al., 2016).

3.3. Immunotherapy with TVEC combination

Oncolytic viruses work synchronously with immunotherapy in melanoma treatment. TVEC can expose tumor-associated antigens, prime the antitumor immune response, and thus enhance immune checkpoint inhibitor activity. A phase Ib study of TVEC with ipilimumab (n = 19) demonstrated an ORR of 50%, PFS of 50% at 18 months, and OS of 67% at 18 months. Grade three to four toxicities were reported as 26.3% (15.8% attributed to T-VEC, and 21.1%

attributed to ipilimumab) and there were no dose-limiting toxicities (DLTs) (Puzanov et al., 2016). In a randomized phase II study of TVEC in combination with ipilimumab vs. ipilimumab alone, more responses were seen in the combination (39% vs. 18%) arm as compared to ipilimumab alone. Combination treatment also showed a greater decrease in visceral lesions (52% vs. 23%) and a higher incidence of grade three or higher toxicities (45% vs. 35%) as compared to ipilimumab alone (28981385).

The combination of TVEC and pembrolizumab has also been tested. In the MASTERKEY-265 trial, 21 stage IIIB-IV melanoma patients underwent pembrolizumab therapy with TVEC. The ORR was reported as 48%, and there were grade three to four AEs seen in 33% of patients (rash, headache, hyperglycemia, increased ALT). This response rate is relatively higher than that seen with pembrolizumab alone (Long et al., 2015b). Because of these results, a large randomized phase III trial (KEYNOTE-034) of TVEC and pembrolizumab was launched (NCT02263508).

3.4. Immunotherapy with granulocyte macrophage colony stimulating factor

Ipilimumab combined with sargramostim (a recombinant granulocyte macrophage colony-stimulating factor [GM-CSF]) has the potential to enhance ipilimumab while reducing toxicity (Hodi et al., 2014a). This combination yielded increased OS (17.5 for combination vs. 12.7 months for ipilimumab alone), but there was no difference in PFS (both 3.1 months) or ORR (both approximately 15%). Sargramostim displayed a protective effect, as the sargramostim with ipilimumab combination group experienced fewer grade three to five adverse events compared to the ipilimumab alone group (44.9% vs. 58.3%, $P = 0.04$) (Hodi et al., 2014a). For example, diarrhea and colitis developed in 12.7% and 5.9% of the combination group vs. 13.3% and 8.3% ipilimumab alone group, respectively. Combination therapy with GM-CSF and immunotherapy continue with the triple combination of GM-CSF, nivolumab, and ipilimumab in the clinical trial NCT02339571.

3.5. Immunotherapy with vascular endothelial growth factor antibody

A phase I study of ipilimumab plus bevacizumab (vascular endothelial growth factor-A mAb) in 46 advanced melanoma patients demonstrated a disease-control rate (DCR) of 67.4% and median survival of 25.1 months with approximately 35% grade three to four adverse events (fatigue, rash, headache, and cough) (Hodi et al., 2014b).

3.6. Combination targeted therapy and immunotherapy

Due to the individual success of targeted and immunotherapies and increased biochemical understanding, clinical trials are underway for combination arrays of BRAF, MEK, and PD1 or PD-L1 inhibitors. Preclinical studies have displayed increased PD-L1 expression in melanoma cell lines resistant to the BRAF inhibitors vemurafenib and dabrafenib (Jiang et al., 2013). Moreover, vemurafenib therapy may increase immune recognition and response to melanoma by enhancing CD8+ infiltration and clonality as well as increase melanoma-associated antigens and melanoma MHC receptors (Wilmott et al., 2012; Frederick et al., 2013). BRAF inhibitors have an increased overall response, while immunotherapies display more stability of response. Early studies of ipilimumab and vemurafenib were small and demonstrated asymptomatic and reversible hepatotoxicity in 50% of patients, which led to study termination. Since then, different combinations have been tested with more favorable side effect profiles. Ribas et al. presented a 50 patient phase I study examining combinations and sequential therapy of a PD-L1 inhibitor durvalumab (MEDI4736; at 3 or 10 mg/kg) with the MEK inhibitor trametinib or MEDI4736 with both trametinib and dabrafenib (Ribas et al., 2015a). Complete responses were reported as 100% in the lower dose triplet group, 67% in the higher dose triplet group, 21% in the doublet group, and 50% in the sequential group. DLTs occurred in the triple combination group (one patient). Grade three or higher AEs were reported as 17% in the 3 mg/kg triplet group, 39% in the 10 mg/kg triplet group, 40% in the doublet group, and 17% in the sequential group.

In KEYNOTE-022, a phase I/II study, pembrolizumab was tested in combination with dabrafenib and trametinib for 15 BRAF mutant advanced melanoma patients. Unconfirmed ORR was 60% (Ribas et al., 2016). Ten patients (67%) experienced grade 3–4 AEs. There were DLT in three patients (20%), neutropenia and elevated ALT, aspartate aminotransferase (AST), and GGT (NCT02130466).

Vemurafenib and cobimetinib have been tested in combination with a PD-L1 mAb, atezolizumab. Thirty-four patients were treated with this triple combination in a phase Ib study (NCT01656642). Unconfirmed ORR was reported as 85.3% with six complete and 23 partial responses. Grade three to four adverse events were seen in 44.1% of patients, with drug discontinuation in four patients (one for rash, three for elevated ALT or AST) (Sullivan et al., 2017; Hwu et al., 2016a). A phase III study of this combination is currently recruiting (NCT02908672).

4. Other mono- and combination therapies

Despite their established use in clinical practice, BRAF/MEK inhibition and CTLA-4/PD-1 blockade are not the only agents that have shown success in treating melanoma. Some agents can lead to inflammation of the tumor environment, which exposes tumor antigens and enhances the body's anti-tumor response. Several members of different immunomodulatory module "super families" are undergoing clinical trials. Costimulatory proteins belonging to the TNF receptor super family (TNFRSF) include CD40, CD137 (4-1BB), OX40 (CD134), and GITR. Agonist antibodies of these proteins can enhance antitumor lymphocyte function. Members of the immunoglobulin superfamily, including LAG-3, T-cell immunoglobulin and mucin containing protein-3 (Tim-3), and B7, impede antitumor immune response as co-inhibitory receptors. Indoleamine 2, 3-dioxygenase (IDO) is unique in that it leads to T-cell suppression and immune tolerance through tryptophan metabolism. Inhibitors of this molecule have proven success in treating melanoma. With these principles, as well as the synergistic effects of acting on different pathways, many combination regimens have coupled immunotherapy with established or novel therapeutics.

4.1. IDO inhibitors

Tryptophan is a potent regulator of immunity, and tryptophan breakdown facilitates immune tolerance. IDO1, an enzyme that catalyzes the rate-limiting step in tryptophan catabolism to kynurenine, plays an important role in peripheral immune tolerance and remains a critical player in local immunosuppression (van Baren and Van den Eynde, 2015). Tumor cells can overexpress IDO as well as indirectly induce IDO expression. Both of these tumor-initiated mechanisms of increased IDO serve as an instrument of immune escape from antitumor immunity. Recent data of IDO1 inhibitors demonstrate that these agents work especially well in combination with immune checkpoint inhibitors (Mautino et al., 2014). Multiple agents targeting tryptophan catabolism via inhibition of IDO1, IDO2, tryptophan 2,3-dioxygenase (TDO), or downstream signaling pathways are currently in development (PMID 29247038). Two of these agents, indoximod (NLG-8186) and epacadostat (INCB024360), are currently in phase III clinical trials (Prendergast et al., 2017). Other IDO1 inhibitors in earlier-phase clinical trials include Navoximod (NLG-919) and BMS-986205.

As opposed to other direct IDO enzymatic inhibitors, indoximod, a "tryptophan memetic," acts directly on immune cells and creates an artificial tryptophan-sufficiency signal to reverse IDO pathway–mediated suppression. It appears to indirectly target IDO1 signaling by reversal of mTORC1 suppression in T cells (Prendergast et al., 2017). It also disrupts aryl-hydrocarbon receptor (AHR) transcription factor

activation by kynurinin and other tryptophan catabolites, resulting in an augmented antitumor immune response (Brincks et al., 2018). Indoximod has demonstrated success and a good safety profile in multiple phase I and II studies of advanced solid tumors and various cancers, especially in combination with chemotherapy (NCT01792050 [breast cancer], NCT0277881 [pancreatic cancer], NCT02052648 [brain tumors]) (Soliman et al., 2012; Jackson et al., 2013; Soliman et al., 2014; Zakharia et al., 2015; Bahary et al., 2016 and immune therapy (NCT01042535 [breast cancer], NCT01560923 [prostate cancer]) (Soliman et al., 2013).

In a phase II trial, patients with MM were treated with indoximod 1200 mg daily with provider choice of pembrolizumab, nivolumab, or ipilimumab (NCT02073123) until disease progression or toxicity (Zakharia et al., 2016). The interim analysis of 51 patients treated with indoximod and pembrolizumab combination therapy yielded an ORR of 61% (complete response in 20% and partial response in 41%) with a DCR of 80% and median PFS of 12.9 months. The combination of pembrolizumab and indoximod was well tolerated and comparable to what would be expected with a single agent PD-1 inhibitor with the most common AEs being fatigue, diarrhea, nausea, arthralgia, headache, cough, rash, pruritus, and hypertension (Zakharia et al., 2017a; Zakharia, 2017). A further study is currently underway to evaluate the combination of indoximod with anti-PD-1 inhibitors (NCT03301636).

Epacadostat (INCB024360) is another orally administered and well-studied direct IDO1 enzymatic inhibitor (Jochems et al., 2016). It has shown encouraging data when used in combination with pembrolizumab or nivolumab in advanced MM patients.

In a recent update of ECHO-202/KEYNOTE-037 phase I/II trial among 54 evaluable patients with advanced MM treated with pembrolizumab (2 mg/kg or 200 mg/kg IV every 3 weeks) with epacadostat (25, 50, 100, or 300 mg oral twice daily) the ORR was 56% and DCR was 78%. Median PFS was 12.4 months. Most common treatment-related toxicities (all grades, > 15%) were fatigue (39.1%), rash (32.8%), pruritus (26.6%), and arthralgia (15.6%) (Lara et al., 2017). Despite these encouraging results, the double-blind phase III study of approximately 706 patients, KEYNOTE-252/ECHO-301, showed no change in PFS of combination therapy compared to pembrolizumab monotherapy (primary endpoint), and the OS was the same (74%) in both groups (NCT02752074) (Anon, 2018).

4.2. Immunotherapy with toll-like receptor agonists

Activation of the cytosine guanine dinucleotide (CpG)- Toll- like receptor (TLR) 9 pathway can upregulate proinflammatory genes and lead to antitumor effects (Adamus and Kortylewski, 2018). Various classes of CpG oligodeoxyneucelotides (ODN) are currently in clinical development. These include CpG class A which stimulate plamacytoid dendritic cells (pDC) maturation and secretion of interferon-α, CpG class B which activates B cells, induce maturation of pDC and TNF-α production and CpG class C which appears to combine effects of CpG class A and B (Adamus and Kortylewski, 2018). CMP-001 is a CpG-A ODN which is packaged in a virus-like particle and is injected intratumorally. The particle is to protect the CpG-A DNA from degradation. In a phase 1b study of intratumoral CMP-001 with pembrolizumab in patients with PD-1 resistant advanced melanomas, the ORR was 22% at weekly dosing and 7.7% at once every three week dosing (Milhem et al., 2018). The combination showed abscopal effect. The main grade three to four toxicity consisted of hypotension, which occurred in 13% of patients (Milhem et al., 2018).

SD-101, is a TLR9 agonist CpG-C class ODN (Adamus and Kortylewski, 2018) which is undergoing evaluation in combination with pembrolizumab in patients with advanced or metastatic melanoma. Available results showed an ORR of 66.7% in PD-1 naïve patients and 7.7% in patients with prior PD-1 inhibitor treatments. Most commonly observed grade three to four toxicities consisted of myalgia (13.6%) and injection site pain (13.6%) (Leung et al., 2017). IMO-2125 is another TLR9 agonist which is currently under investigation in melanoma in combination with ipilimumab (NCT02644967 and NCT03445533) and pembrolizumab (NCT02644967).

4.3. CD40

CD40 plays a critical role in immune activation. This member of the TNFR superfamily is expressed on activated B-cells and constitutively on APCs. Interaction of CD40 with its ligand CD40 L (CD154) leads to increased costimulatory molecules, APC functional maturation and survival, enhanced antigen presentation, and ultimately a maintained expansion of activated antigen-specific T cells. Similarly, this interaction and cascade via activated B-cell is vital to the humoral immune response (Elgueta et al., 2009).

CD40 agonistic agents, therefore, have been investigated as cancer therapeutics including multiple clinical trials for solid tumors, recently MM. CP–870,893, SGN-40, and HCD 122 are fully human agonistic CD40 mAbs. Other agents include APX005 M, ADC-1013, Chi Lob 7/4 (all mAbs against CD40 (Dempke et al., 2017) and AdCD40 L, an adenovirus carrying the gene for the CD40 ligand (Loskog et al., 2016)). However, only modest benefits ensue when these agents are used as a single agent. In a phase I study analyzing CP-870,893 as monotherapy across 29 patients (15 with melanoma), four partial responses were documented (all in melanoma patients). Grade three AEs occurred in three patients (no grade four to five AEs), and the maximum tolerated dose (MTD) was estimated as 0.2 mg/kg (Vonderheide et al., 2007). A subsequent phase I study that included 11 melanoma patients yielded similar results of MTD and AEs and reported no partial or complete responses (Ruter et al., 2010). The most common AE of CP-870,893 is cytokine release syndrome (CRS), which is predominately grade one to two and controlled with standard supportive care.

CP-870,893 shows more promise in combination therapy. A combination therapy trial of CP-870,893 and the CTLA-4 inhibitor tremelimumab across 24 MM patients showed the following data: ORR of 27.3%, median PFS of 2.5 months, and a median OS of 26.1 months. DLT occurred in three patients (colitis, hypophysitis, and uveitis), and CRS occurred in 79% of patients (Bajor et al., 2015).

4.4. CD137 (4-1BB)

CD137 (also known as 4-1BB), an activation-induced co-stimulator and member of the TNFR superfamily, is integral in the activation and survival of CD4 +, CD8 +, and natural killer (NK) cells. Agonist antibodies to CD137 increase IFNγ production and cytolytic activity. Urelumab is a fully human anti-CD137 IgG4 mAb, and PF-05082566 is a fully human anti-CD137 IgG4 mAb (Fisher et al., 2012). Urelumab has been tested clinically as monotherapy and in combination.

During early urelumab studies, two patients died due to drug-related hepatic failure (Segal et al., 2017). Subsequently, urelumab trial enrollment ceased and a detailed analysis was completed. This analysis revealed dose as the single most important factor of severe transaminitis development (particularly at or above 1 mg/kg). Across these early studies (a total of 347 patients), grade three to four events occurred in 4.9% of patients at 0.1 mg/kg compared to 56.1% at ≥1 mg/kg. Treatment-related AEs lead to patient discontinuation in 37 of 229 patients (16%) at ≥1 mg/kg and seven of 61 patients (11%) at 0.1 mg/kg. Four years later, trials were reinitiated at lower doses (Melero et al., 2013).

Combined analysis of urelumab monotherapy clinical trials demonstrated a MTD of 0.1 mg/kg every 3 weeks, with fatigue (16%) and nausea (13%) being the most common treatment-related AEs at this dose (Segal et al., 2017).

Clinical trials of combination treatment regimens are underway with urelumab for non-melanoma cancers and melanoma (with nivolumab; NCT02253992). In one study, 40 melanoma patients were included in a total of 104 advanced cancer patients treated with urelumab

and nivolumab combination. The most frequent grade three to four treatment-related adverse events were elevated ALT and AST (3% each). There were no treatment-related deaths reported. Seven percent of patients discontinued treatment secondary to AEs (Massarelli et al., 2016). For melanoma patients only, an ORR of 50% (23/46 with 18 confirmed and 5 unconfirmed) and a DCR of 70% (ASCO Post, 2016) was recently described.

Other urelumab clinical trials include NCT02534506 and NCT00803374, albeit the second study was withdrawn prior to enrollment.

4.5. OX40

Agonist antibodies for OX40, a costimulatory CD 134 TNF receptor transiently expressed on activated CD4 and CD8 cells, have utility in MM treatment. Binding of OX40 to its receptor, OX40 L (CD252, which is present on APCs including melanoma tumor-infiltrating leukocytes) increases proliferation, migration, function, and survival of effector T cells, as well as inhibiting regulatory T cells. OX40 is found on CD4 and CD8 T cells, NK cells, and neutrophils (Croft et al., 2009). Patients with increased OX40 have longer survival (Ladanyi et al., 2004). Because of supporting preclinical studies, the safety and efficacy of OX40 is being established clinically in many cancers including melanoma. In one phase I study, thirty advanced solid tumor patients (23% with melanoma) received the anti-OX40 mAb, 9B12. Twelve patients (40%) experienced regression of at least one metastatic lesion. The only grade three to four event was lymphopenia, which occurred in 23% of patients and was transient. In this study, no MTD was reached (Curti et al., 2013).

Multiple clinical trials of anti-OX40 mAb in different advanced solid tumors are recruiting subjects.

4.6. GITR

GITR, a co-stimulatory receptor on T cells, also belongs to the TNFR superfamily. It has a minimal expression on naïve cells but is upregulated once the T cell is activated (Schaer et al., 2012). After engagement with its ligand GITRL (which is largely expressed on activated APCs), GITR leads to CD4+ and CD8+ activation, proliferation, cytokine production, and increased survival. Further, GITR blocks the inhibitory effects of regulatory T-cells (Cohen et al., 2010; Shimizu et al., 2002).

The humanized agonist anti-GITR mAbs, TRX518 (NCT01239134) and INCAGN01876 (NCT02697591, NCT03126110), are currently undergoing phase I and I/II clinical trials in solid tumors including advanced melanoma.

4.7. Anti-LAG-3 antibodies

Lymphocyte activation gene-3 (LAG-3) or CD223 is an inhibitory transmembrane protein and a part of the Ig superfamily. This molecule normally increases the susceptibility of activated T cells, B cells, and NK cells to suppression by regulatory T cells and serves as a checkpoint to prevent autoimmunity (Grosso et al., 2007; Durham et al., 2014). However, tumor environments possess persistent LAG-3 expression, hindering anti-tumor immune responses. Therapies antagonistic to LAG-3 are attractive potential cancer therapeutics, with four clinical trials currently undergoing.

There have been several phase I and II clinical trials of IMP321, a soluble LAG3 Ig recombinant fusion protein, for advanced renal cell carcinoma (Brignone et al., 2009), metastatic breast cancer (Brignone et al., 2010), and pancreatic adenocarcinoma (Wang-Gillam et al., 2013), in which IMP321 demonstrated good safety profiles. A phase I study of stage IV, previously treated melanoma patients was conducted in which the vaccine MART-1 peptide was combined with IMP321 in six patients and placebo in six control patients. Both groups also received lympho-depleting chemotherapy and adoptive transfer of autologous PBMCs; the only response (partial response) was seen in the IMP321 group. The IMP321 group showed significant CD8 MART-1-specific T cell expansion and reduction of regulatory T cells ($P < 0.04$) (Romano et al., 2014). Further, LAG-3 has been formulated as a vaccine with montanide ISA-51 (mannide monooleate surfactant and mineral oil T-cell vaccine adjuvant) and five synthetic peptides of melanoma-associated antigens and investigated in a phase I/IIa clinical trial. Across 16 melanoma study patients, there were no grade three or higher treatment-related systemic AEs; the majority of AEs were mild and localized to the injection site (Legat et al., 2016).

BMS-986016, a fully human IgG4 mAb against LAG-3, has been tested in combination with nivolumab. A phase I/II study of BMS-986016 alone and with nivolumab showed tolerability and preliminary clinical activity. Across all solid tumor patients, grade three to four events were seen in 9.1% of BMS-986016 and 17.2% of combination patients. There were 3% and 14% discontinuations secondary to treated-related AEs in BMS-986016 and combination groups, respectively, and no treatment-related deaths in either group. The MTD was not reached (NCT01968109) (Lipson et al., 2016). A combination study of BMS-986016 with nivolumab across 31 evaluable previously treated (prior anti–PD-1/PD-L1 ± anti–CTLA-4 or BRAF/MEK inhibitors) melanoma patients demonstrated an ORR of 16% and a DCR of 45% (Ascierto et al., 2017). These results are encouraging, as they demonstrate benefit despite anti–PD-1 therapy failure.

LAG525, a humanized mAb directed against LAG-3, is currently undergoing a phase I/II study of non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), melanoma patients as monotherapy or in combination with the anti-PD1 inhibitor PDR001 (NCT02460224).

4.8. TIM3

TIM3, an inhibitory molecule that leads to apoptosis of CD4 and CD8 T cells, is found on CD4, CD8, regulatory T cells, macrophages, monocytes, and dendritic cells. Blocking TIM3 binding to its ligand Gal-9 leads to CD4+ and CD8+ proliferation and enhanced function (cytokine production and cytotoxicity, respectively) (Zhu et al., 2011). TIM3 inhibitors have shown anti-tumor activity in preclinical melanoma models, especially in combination with PD1/PDL1 inhibitors (Sakuishi et al., 2010). Two anti-TIM3 antibodies are currently being studied in early clinical trials. NCT02817633 is a phase 1 study evaluating TSR-022 as a monotherapy and in combination with an anti-PD-1 antibody in patients with advanced solid tumors. NCT02608268 is a phase I–Ib/II study evaluating MBG453 as a monotherapy and in combination with an anti-PD-1 antibody in patients with advanced malignancies.

4.9. Other agents

NKTR-214, a CD-122-biased agonist, is being investigated in combination with nivolumab in patients with advanced/metastatic solid tumors. In a phase I/II study of the eight evaluable melanoma patients, one complete and four partial responses were seen. Correlative analysis showed immune activation in peripheral blood and tumor microenvironment (Diab, 2017).

Anti CD27 agents like varlilumab in combination with glembatumumab vedotin (NCT02302339), enoblituzumab (mAb targeting B7-H3) as a single agent (NCT01391143) and in combination with PD-1 (NCT02475213) and CTLA4 (NCT02381314)-based therapies are undergoing phase I development in tumors including melanoma. Bavituximab, a human-mouse chimeric mAb against phosphatidylserine, was undergoing a phase Ib trial in combination with ipilimumab in melanoma but was terminated due to change in standard of care (NCT01984255). × 4P-001, an oral C-X-C chemokine receptor type 4 (CXCR4) inhibitor is being evaluated in patients with advanced melanoma (NCT02823405).

Table 3
Analysis of Selected Agents and combinations.

Class	Agents of interest	Advantages	Limitations and comments	Reference
CTLA-4 inhibitor	Ipilimumab	Responses are durable	Has low ORR (~15%) PFS (~2.8 months) with high rate of grade 3/4 toxicities (18–34%).	(Hodi et al., 2010; Robert et al., 2011; Maio et al., 2015; Schadendorf et al., 2015
BRAF inhibitors	Vemurafenib, dabrafenib	Impressive response rate (~50%) even as single agent.	Effective only in BRAF V600E and V600 K mutations. As a single agent have low PFS (~5 months) and OS (13.6 months). Rapid development of resistance and toxicities, especially squamous cell carcinoma is a concern.	(Sosman et al., 2012; McArthur et al., 2014)
PD-1 inhibitors	Pembrolizumab, nivolumab	Will tolerated with durable responses	Low ORR due to primary resistance and development of acquired resistance remains a concern. Many patients do not benefit from therapy and median PFS is low (~5 months).	(Robert et al., 2015a; Topalian et al., 2014; Robert et al., 2015d; Long et al., 2016a; Ribas et al., 2015b; Robert et al., 2017)
Genetically modified oncolytic virus	TVEC	Low toxicities.	Response rate is low (~26%). Needs injectable lesion.	(Andtbacka et al., 2015, 2016; Long et al., 2015b)
BRAF with MEK inhibitors	vemurafenib with cobimetinib, dabrafenib with trametinib, encorafenib with binimetinib	Exceptional response rate (64–70%) Lower incidence of SCCs	Effective only in BRAF V600E and V600 K mutations. Responses are not durable (13–14 months). Median PFS (11.4–15 months) and OS (22.3–26.1 months) remains concerning. Higher overall toxicities as compared to single agent BRAF inhibitors and immunotherapies.	(Larkin et al., 2014; Robert et al., 2015b; Long et al., 2015a, (Ascierto et al., 2016)
CTLA-4 with PD-1 inhibitors	Ipilimumab with nivolumab, ipilimumab with pembrolizumab	A high response rate (~60%) with durability and better survival	A very high rate of grade 3/4 toxicities (50–60%) as compared to single agent anti PD-1 therapies is concerning.	(Wolchok et al., 2017; Weber et al., 2016; Long et al., 2017; Carlino and Long, 2016; Long et al., 2016d
Immunotherapy with TVEC	TVEC with ipilimumab, TVEC with pembrolizumab	Higher response rate (40–50%) compared to TVEC monotherapy.	Needs injectable lesion. Higher toxicities are seen. Phase III studies to determine benefit are ongoing.	(Puzanov et al., 2016; Long et al., 2015c)
Immunotherapy with TLR9 agonists	CMP-001, SD-101, IMO-2125 with pembrolizumab and ipilimumab	Interesting activity in PD-1 resistant tumors.	Needs injectable lesion. Phase III studies are ongoing.	(Milhem et al., 2018; Leung et al., 2017; Diab et al., 2017)
Immunotherapy with BRAF and MEK inhibitors	Durvalumab with dabrafenib and trametinib, pembrolizumab with dabrafenib and trametinib, tenolizumab with vemurafenib and cobimetinib	Higher response rates (~85%) has been seen	This strategy is effective only in BRAF-mutated melanomas. There is increased grade 3/4 toxicities. Results need to be confirmed in phase III studies to determine the risk-benefit ratio.	(Ribas et al., 2015a, 2016; Sullivan et al., 2017, (Hwu et al., 2016b)
IDO inhibitors with immunotherapy	Indoximod or epacadostat in combination with PD-1/PD-L1 or CTLA-4 based therapies	High response rate (~60%) with no appreciable increase in toxicities as compared to checkpoint inhibition.	Phase III study of epacadostat did not improve progression-free survival.	(Zakharia et al., 2016, 2017b; Long et al., 2017; Gangadhar et al., 2016; Zakharia et al., 2018)
CD40 agonists	Multiple monoclonal antibodies—CP-870,893, APX005 M, ADC-1013, lucatumumab, Chi Lob 7/4, dacetuzumab	NA	Serious toxicities including cytokine release syndrome are concerning. It showswide heterogeneity in action including agonism (CP-870,873) to antagonism (lucatumumab). The mechanism needs further elucidation for optimal combination trial designs.	(Dempke et al., 2017)
CD137 agonists	Urelumab with nivolumab	Appreciable response rate (50%)	Toxicities, especially transaminitis are concerning.	(Segal et al., 2017; Melero et al., 2013; Massarelli et al., 2016
OX40 agonist	Multiple monoclonal antibodies	NA	Human trial data in melanoma is awaited	(Curti et al., 2013)
GITR agonists	Multiple monoclonal antibodies	NA	Human trial data in melanoma is awaited,	(Schaer et al., 2012)
LAG 3 antagonists	IMP321, BMS-986016, LAG525	As single agents and in combination trials with immunotherapy showed acceptable toxicities.	Larger studies are needed to evaluate efficacy.	(Legat et al., 2016; Lipson et al., 2016; Ascierto et al., 2017)
TIM 3 antagonists	TSR-022, MBG453	NA	Human trial results in melanoma are awaited.	(Sakuishi et al., 2010)
Epigenetic modulation	Decitabine with vemurafenib and cobimetinib, histone deacetylase inhibitors with or without immunotherapies	Preliminary studies show potential for future development	Larger studies in melanoma are awaited.	(Zakharia et al., 2017b; Xia et al., 2014)
Chemotherapy and radiotherapy	Various agents in combination with immunotherapies	NA	Sequence and doses are very important to module immunity and tumor microenvironment. Current understanding in this regard is lacking.	(Chen et al., 2017; Patel et al., 2017; Wargo et al., 2015; Williams et al., 2017)
Vaccines	Vaccines targeting melanoma cells (4-1BBL), dendritic cell–based, peptide-based, vector -based and DNA-based vaccines	Gp100 in combination with IL-2 has shown a survival advantage.	Most are in preclinical development. Rational combinations with other agents need to be tested.	(Rodriguez-Cerdeira et al., 2017)

4.10. Epigenetic modulation

Preclinical studies have shown that melanomagenesis is influenced by epigenetic modifications via down-regulation of tumor suppressor genes, immune recognition factors, DNA repair enzymes, and apoptotic mediators that contribute to cell survival and proliferation (Alcazar et al., 2012). $BRAF^{V600E}$ has been associated with hypermethylation of various genes including microtubule-associated protein (MAP2) protein, which might be mediated by upregulation of DNA methyltransferase 1 (Hou et al., 2012). The forced MAP2 expression has shown to result in apoptosis and inhibition of cell growth (Hou et al., 2012; Maddodi et al., 2010). We recently published results of a phase I study combining decitabine, a DNA hypomethylating agent, with vemurafenib in patients with $BRAF^{V600E}$-positive MM (Zakharia et al., 2017b). The ORR was 43% with a clinical benefit rate of 79%. A phase I/II study evaluating decitabine in combination with vemurafenib and cobimetinib is ongoing (Zakharia et al., 2017b).

A phase I trial has investigated decitabine with panobinostat, a panhistone deacetylase inhibitor, in combination with temozolomide in melanoma. Among eight evaluable patients, one complete response in mucosal melanoma was seen (Xia et al., 2014). Similarly, in a phase II trial vorinostat (400 mg oral daily), a class I and II histone deacetylase inhibitor, was investigated in patients with melanoma. Overall, 32 patients were enrolled, only two responses were seen, and the trial did not meet its primary endpoint (Haas et al., 2014). Currently, vorinostat as a single agent (NCT02836548) and entinostat (NCT02697630, NCT02437136) in combination with pembrolizumab are being investigated in patients with melanoma.

4.11. Chemotherapy

Chemotherapy have shown to modulate tumor microenvironment. Chemotherapy can make the tumor microenvironment less immunosuppressive by various mechanisms including decreasing T regulatory cells and function, decreasing myeloid-derived suppressor cells, and promoting maturation of dendritic cells and enhancing their function (Chen et al., 2017). It can prime tumor-specific T cells by promoting tumor antigen presentation after cancer death (Chen et al., 2017). Trials are currently underway to explore these alternatives in combination with immunotherapies at various dosage and schedules. Ipilimumab has been investigated in combination with temozolomide. In one study, the ORR was 31.2% and median OS was 24.5 with the main toxicities being nausea and constipation (Patel et al., 2017). Another study is investigating pembrolizumab with carboplatin and paclitaxel in patients with MM (NCT02617849). Pelareorep, a live, replication-competent, Reovirus Type 3 was investigated in a phase II, single-arm trial in combination with carboplatin and paclitaxel in patients with advanced melanoma. Across the 14 patients were enrolled in the study, no grade 4 toxicities were noted. Manageable grade-3 toxicities due to pelarerep included pyrexia, chills, myalgia, pain, fatigue, and nausea. The study met its efficacy and treatment goals for the first stage with three partial responses. The median PFS was 5.2 months and OS was 10.9 months (Mahalingam et al., 2017).

4.12. Radiotherapy

Radiation has shown to enhance anti-tumor response by releasing damage-associated molecular patterns, toll-like receptors, and increased expression of MHC class I antigen and tumor-associated antigens. It shows an abscopal effect as well as synergy with immunotherapy (Wargo et al., 2015). Ipilimumab has been investigated in combination with whole brain radiation therapy and stereotactic radiosurgery. No grade 4 or DLTs were seen (Williams et al., 2017). Multiple studies are currently evaluating radiation therapy in combination with PD-1 inhibitors (NCT02799901, NCT02716948, NCT02858869, NCT02407171), IL-2 (NCT01884961), CTLA-4 inhibitor (NCT01970527), PD-1 and CTLA-4 combination (NCT02913417), and with TVEC (NCT02819843).

5. Conclusion

Table 3 gives an analysis of selected agents and combinations.

Incredible progress has been made in melanoma treatment. The impressive survival rates seen with immuno and targeted therapies have fueled scientific exploration. Multiple targets for drug development have been identified and are currently undergoing development. In general, over the past ten years, the community has discovered the benefit of combination therapy. This advantage is most striking for targeted therapy. Although combination treatment in immunotherapy is apparent, it is not as pronounced, especially in PD-1 inhibitors. In particular, patients positive for PDL-1 experience the least benefit from combination therapy (Larkin et al., 2015b). Nonetheless, multiple novel agents are being tested in combination with PD-1 inhibitors that may change this current observation.

The targets for drug development include targeting immunological pathways (CTLA4, PD1, TNF, and TNFR suerpfamilies, immunoglobulin superfamily, B7, CD28, TLR-9, TLR-3, NK cells, suppressive myeloid cells and other mediators like IDO, TGFβ, CXCR4, adenosine pathway, VEGF, neuropilin, phosphatidylserine, CD47 and their ligands) (Mahoney et al., 2015; Vasquez et al., 2017), driver mutations (BRAF, NRAS, ckit and corresponding signaling pathways) (Cosgarea et al., 2017), genetically engineered viruses (e.g., TVEC) and vaccines (e.g., GP100, Vitespen, NEO-PV-01, NeoVax etc.) (Vasquez et al., 2017). On top of these, various ways to enhance their therapeutic effects by modulating epigenetics (hypomethylating agents and HDAC inhibitors) and combining chemotherapy and radiation are being explored. The possible combinations of these agents far exceed the number of available patients and current resources. Moreover, how to sequence these agents and combinations, which subset of patients benefit most from each of these combinations, and how long to continue them are still matter of research. Therefore, extensive preclinical testing of these agents and combinations in animal models is needed prior to initiating human trials so that only highly effective and rational drugs are investigated for maximum benefit and judicious use of resources. Future directions include investigating non-cutaneous melanomas, which includes uveal, mucosal, and acral melanomas, which are less responsive to immunotherapies and usually do not have BRAF mutations (Rozeman et al., 2017). The need is to make these rare melanomas more immunogenic or develop novel targeted agents against specific mutations therapies like GNAQ or GNA11.

Conflicts of interest

The authors have no conflicts of interest to declare.

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