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EXPERT REVIEW

Novel Treatments for Metastatic Cutaneous Melanoma and the Management of Emergent Toxicities

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Abstract: The last 12 months have seen the beginning of a new era in the treatment options available for patients with metastatic cutaneous melanoma, a disease previously characterised by its poor prognosis and limited treatment options. Two mechanistically diverse agents have now demonstrated an overall survival benefit in different patient subgroups and further clinical trials are ongoing with emerging single agents and novel combinations. The first agent to demonstrate an overall survival benefit was the CTLA-4 antibody, ipilimumab, illustrating the importance of the immune system and immunomodulation in melanoma tumorigenesis. The second group of agents to show a survival benefit were the selective BRAF inhibitors, vemurafenib and GSK2118436, in patients who are BRAF V600 mutation positive. In addition, in the same BRAF mutant patient population, MEK inhibitors also show promising results and are currently under investigation in later stage trials. Although ipilimumab, BRAF and MEK inhibitors are just passing through the clinical trials arena, their use will rapidly become more widespread. Along with their significant clinical benefits, there are also unique adverse events related to these agents. Although the majority are mild and can be managed with supportive treatment, some toxicities require special management strategies. We outline up-to-date clinical development and management guidelines for ipilimumab, as well as the BRAF and MEK inhibitors.

Keywords: BRAF-inhibitor, MEK-inhibitor, ipilimumab, cutaneous melanoma, ocular toxicity, squamous cell carcinoma, fever, diarrhea, skin rash, immune related adverse events

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Introduction

Malignant cutaneous melanoma is the sixth most common cancer in the UK with an annual incidence that is increasing more rapidly than any of the top ten cancers in males and females.[1] The age-standardised incidence rate in the UK is 9–12 per 100 000 population compared to 30–40 per 100 000 population in Australia/New Zealand. Although the least common skin cancer, cutaneous melanoma is the most life-threatening with metastases present in 10%–15% of patients at diagnosis.[2] Chemotherapy, usually with dacarbazine or temozolimide, has been the mainstay of firstline treatment despite the reported overall survival (OS) rates of only 6–10 months and limited clinical benefit.[3,4] It is only recently that advances in immunotherapy and agents targeting specific genetic aberrations in the mitogen-activated protein kinase (MAPK) pathway have dramatically improved outcomes in advanced cutaneous melanoma. While stimulating the immune system via T-cell activation to control tumour growth has demonstrated clinical benefit in an unselected group of melanoma patients,[5,6] so far the benefit of the BRAF and MEK targeted agents is confined to the BRAF V600 mutant population alone.[7,8] Furthermore, compared to standard chemotherapy, the adverse events require specialised management in a multidisciplinary team, particularly the immune related adverse events associated with ipilimumab. Increased awareness and cautious management is required to reduce the impact of these side-effects on the patient, increase quality of life and prevent treatment related mortality.

Ipilimumab

Ipilimumab is a fully human monoclonal antibody directed against cytotoxic T-lymphocyte antigen-4 (CTLA-4). CTLA-4 and CD28 are two proteins that are exported to the surface of T cells after immune cell activation and balance the stimulation and inhibition of T cell proliferation and activation. CTLA-4 exerts an inhibitory effect on further cell proliferation whereas CD28 is a costimulator of T cell proliferation and involved in IL-2 production.[9] By binding to the CTLA-4 T-cell receptor, ipilimumab blocks its inhibitory effect and potentiates cytotoxic T-cell activation.[10]

Pre-clinical studies demonstrated that injection of anti-CTLA-4 antibodies into mouse models could stimulate the rejection of murine tumours in colon, ovarian and fibrosarcoma models.[11,12] Early clinical studies demonstrated that the mean half-life was 14.7 days and that there was no apparent correlation between plasma concentrations or clearance with tumour response or toxicity. Clearance was not affected by renal or hepatic function.[13]

The early phase I/II studies in the metastatic melanoma setting demonstrated response rates in the order of 4.1%–15.7% with median OS between 8.5 months and 17.1 months.[14,15] Interestingly, some patients had sustained tumour responses over 25 months.[16] There also appeared to be a dose-response relationship demonstrated in some studies[14,16] but this relationship was by no means absolute, as some patients with prolonged disease control had no related toxicity, while other patients with toxicity did not demonstrate clinical benefit.

The pivotal phase 3 trial demonstrated an improvement in OS in human lymphocyte antigen (HLA-A*0201)-positive patients with previously treated metastatic melanoma randomised to either a vaccine gp100 versus gp100 in combination with ipilimumab (3 mg/kg at weeks 1, 4, 7, 10 with or without reinduction) versus ipilimumab alone.[5] Gp100 is a synthetic peptide cancer vaccine comprising HLA-A*0201-restricted peptides derived from the melanosomal protein glycoprotein 100, that appeared to improve the efficacy of high-dose interleukin-2 in metastatic melanoma and provided a rationale for its use to potentiate the efficacy of ipilimumab.[17] There was an improvement in median OS from 6.4 months with gp100 alone compared to 10.0 months with gp100 plus ipilimumab and 10.1 months with ipilimumab alone. Importantly the 1- and 2-year survival rates improved to 45.6% and 23.5% respectively with ipilimumab compared to 25.5% and 13.7% with gp100 alone. There was no additional effect of gp100 to ipilimumab.

More recently, Study 024 compared firstline treatment with dacarbazine (850 mg/m2 every 3 weeks for 22 weeks) plus placebo versus dacarbazine plus ipilimumab (10 mg/kg at weeks 1, 4, 7, 10) in patients with unresectable stage III or stage IV metastatic melanoma.[6] Again, there was an improvement in median OS from 9.1 months to 11.2 months (HR 0.72, 95% CI 0.59–0.87, $P = 0.0009$) with an increase in the 1 year (36.3% vs. 47.3%), 2 years

(17.9% vs. 28.5%) and 3 years (12.2% vs. 20.8%) survival rate respectively. There was no clinically significant difference in median progression free survival, measuring 2.6 months and 2.8 months respectively (P = 0.006). Although the disease control rate was similar (30.2% vs. 33.2%), the duration of response was markedly improved, from 8.1 months to 19.3 months in patients who received ipilimumab.

A phase 3 trial is in development to compare ipilimumab at 3 mg/kg versus ipilimumab at 10 mg/kg, as well as ipilimumab in combination with other agents to help determine its optimal dose and placement in the treatment of metastatic melanoma.[18]

Significance of the MAPK Pathway

Improved understanding of the genetic heterogeneity in melanoma, the detection of oncogenic aberrations and the ability to target these changes, are factors that have further expanded the treatment options available for this disease.

The MAPK pathway is particularly important in melanoma tumorigenesis and regulation of cell growth, proliferation and differentiation. Activation of the Raf Sarcoma (RAS) family of GTPases by growth factors or by RAS mutation then drives activation of the RAF kinase family (ARAF, BRAF, CRAF) with subsequent phosphorylation and activation of MEK kinases (MEK 1 and 2) and extracellular signal-regulated kinases (ERK 1 and 2).[19] This leads to phosphorylation of the Erythroblast Transformation Specific (ETS) protein family, nuclear transcription factor activation and finally to cell-cycle progression and regulation of normal cellular functions, including apoptosis and survival. MAPK pathway activity is key for normal cell function but abnormal activation, through mutations and other aberrations have been implicated in a number of cancer sub-types, including melanoma, colorectal cancer and borderline ovarian cancer, among others.[19]

Genetic aberrations in the MAPK pathway are present in over 80% of cutaneous melanomas, involving abnormalities in RAS, RAF, MEK and ERK.[20] The most common mutation appears to be in the activating v-raf murine sarcoma viral oncogene homologue B1 (BRAF), occurring in 36%–59% of primary melanomas and 42%–66% of metastatic melanomas[21–23] and has been characterised as an oncogenic mutation.[19,24] The most common somatic mutation is found at V600E in exon 15 in 66%–90% of BRAF mutant melanomas.[23,25,26] This is a point mutation in DNA (1799T->A) resulting in a single amino-acid substitution at Valine 600 to Glutamic acid in the activating segment, which leads to elevated kinase activity compared with BRAF wild type, stimulated phosphorylation of downstream endogenous ERK and subsequent cellular proliferation and survival.[19,27]

The V600 K mutation has been reported in 7%–28.5% of patients with BRAF mutant metastatic melanoma[23,25,28,29] and involves two point mutations (GTG to AAG) with a lysine for valine substitution. Other non-V600E mutations have also been reported and will become increasingly relevant in interpretation of current and future clinical trials.

The presence of a BRAF mutation is a demonstrated poor prognostic factor with a strong association with inferior outcome in the metastatic setting.[21,30,31]

Selective BRAF Inhibitors

Pre-clinical data demonstrated that selective BRAF inhibition results in growth arrest and induction of apoptosis in cell lines and xenograft models.[32,33] The multiple tyrosine kinase inhibitor, sorafenib, was initially developed as a RAF inhibitor and was studied in some of the earlier clinical trials of RAF inhibition in metastatic melanoma. Despite encouraging phase 2 results reporting disease stabilisation in a few unselected advanced melanoma patients,[34] further phase II and III testing in the first-line and second-line setting respectively, failed to demonstrate clinically significant activity.[35,36]

The two agents that have demonstrated significant clinical benefit in melanoma are vemurafenib (PLX4032/RG 7204) and GSK2118436.

Vemurafenib is an orally available, highly potent, ATP competitive inhibitor of mutant BRAF. It is well absorbed after oral administration and is metabolized in the liver by CYP3A4. The half life is approximately 57 hours and it reaches steady state after 15–22 days.[37] It is highly protein bound and is excreted primarily via the faeces.

The phase I trial of vemurafenib included a dose escalation phase (from 160 mg twice daily to 1120 mg twice daily) and dose extension phase (at 960 mg twice daily) and demonstrated a response rate (RR) of 69% (11 from 16 patients) and 81% (26 from 32 patients) respectively. The estimated median progression-free

survival (PFS) was more than 7 months with duration of response ranging from 2 months to over 18 months.[38] The BRAF in Melanoma 2 (BRIM-2) phase II study enrolled 132 patients with previously treated BRAF V600E mutant stage IV melanoma and demonstrated a RR of 53%, stable disease in a further 29%, median PFS of 6.7 months and OS at 6 and 12 months of 77% and 58% respectively.[39] Additionally in early 2011, the phase III BRAF Inhibitor in Melanoma 3 (BRIM3) trial included 675 BRAF V600E mutation positive metastatic melanoma patients and reported a significant improvement in OS in patients who were treated with vemurafenib compared to the standard firstline chemotherapy dacarbazine.[7] The RR was 48% versus 5% with a significant prolonged median PFS of 5.3 months in the vemurafenib arm compared to 1.6 months on dacarbazine [HR 0.26 (95% CI 0.20–0.33) $P < 0.0001$]. At 6 months the overall survival was 84% for patients on vemurafenib compared to 64% for patients on dacarbazine with a 63% relative risk reduction for death.

GSK2118436 is another ATP competitive, reversible inhibitor of mutant BRAF V600E, as well as V600D/K and V600G kinases. The phase I/II trial included 61 patients, 52 with BRAF mutant melanoma and V600E/D/K mutations. At the time of reporting the RR was 60% (18 from 30 patients) and PFS 8.3 months at the recommended phase-II dose level of 150 mg twice daily.[8] Pharmacokinetic studies indicated that peak plasma levels were reached after 2 hours and the half-life was 4–9 hours. At this phase II dose, the drug inhibited intratumoural phosphorylated ERK levels by >90%, indicating MAPK pathway suppression. In the dose expansion cohort of 20 melanoma patients, the overall RR was 77%. The majority of patients (77%) had a BRAF V600E mutation but 19% had a V600K mutation and the RR in this group was 44% (4 out of 9).

The dose-escalation phase also included a cohort of 10 patients with previously untreated brain metastases who also demonstrated a significant response to treatment. Brain metastases decreased in size in 9 out of 10 patients, correlating with extra-cranial response. This reduction ranged from a 20% to 100% decrease in size of metastases that were 3–15 mm in size prior to treatment.[40] Ongoing studies are assessing GSK2118436 versus dacarbazine in previously untreated patients with BRAF mutant advanced or metastatic melanoma, as well as a study of GSK2118436 in BRAF mutant metastatic melanoma to the brain.[18] Both these studies have completed recruitment.

MEK Inhibitors

In addition to inhibiting BRAF signalling with selective BRAF inhibitors, there is good pre-clinical evidence of anti-proliferative activity of MEK inhibitors in melanoma.[41] Several inhibitors of the downstream checkpoint MEK are currently in development and phase 1–3 clinical trials are underway.

The earliest MEK inhibitors in preclinical and clinical development demonstrated limited clinical activity and included PD98059, UO126 and CI-1040.[42] PD0325901, a structural analogue of CI-1040, was a second generation MEK inhibitor evaluated in phase I and II trials in metastatic cutaneous melanoma patients with some early evidence of response and disease stabilisation, but its further development was limited by toxicity, particularly ocular toxicity.[43] AZD6244 was another MEK inhibitor to show small numbers of response and disease stabilisation in melanoma patients;[44] however failed to show any improvement in PFS compared to temozolomide in a randomised phase II trial.[45] Single-agent clinical trials have not been pursued with this agent but phase 2 combination trials with dacarbazine, docetaxel and temsirolimus are currently underway in BRAF mutant metastatic melanoma in the first-line setting.[18]

In contrast, the phase I/II study of the MEK inhibitor GSK1120212showed good tolerability in the 162 enrolled patients, predominantly with melanoma and pancreatic cancer. It has a half life of approximately 4.5 days and reaches steady state by day 15. There were 20 evaluable patients with BRAF mutant melanoma and at the recommended phase-II dose of 2 mg once daily, RRs were 40% (8 from 20 patients) and a further 18% had stable disease (SD).[46] Further single agent activity is being assessed in an ongoing phase-III trial, randomising patients to GSK1120212 versus first or second line chemotherapy.[18]

There is also pre-clinical and early clinical evidence that the combination of a BRAF and MEK inhibitor (GSK2118436 and GSK1120212) shows clinical activity in BRAF V600 mutant melanoma not only with a potential reduction in drug resistance but

also with decreased toxicity.[47] Resistance to BRAF inhibition can be mediated by a number of different mechanisms that may occur upstream (eg, NRAS mutation), or downstream (eg, MEK mutation) of BRAF along the MAPK pathway, as well as via bypass signalling pathways through the PI3 K-AKT pathway.[48–51] Addition of a MEK inhibitor to a BRAF inhibitor may thus overcome resistance mediated by mechanisms downstream of BRAF. Preliminary results at doses of GSK2118436 150 mg twice daily and GSK1120212 2 mg daily in 19 patients have demonstrated a complete response rate of 11%, a total RR (CR + PR) of 74% and clinical benefit rate (CR + PR + SD) of 100%.[47]

Emerging New Toxicity Profiles and Recommended Management Strategies

The novel mechanisms of action of these agents confer clinically relevant toxicities requiring special management and screening strategies (Table 1). The corresponding Level of Evidence (Table 2) is also outlined.

Ipilimumab

The adverse effects mediated by ipilimumab are predominantly immune response related. The first phase III trial reported all grade drug-related events in 80.2% of patients including expected adverse events related to monoclonal antibodies such as anaphylaxis as well as immune related adverse events (irAEs) in the range of 60%.[5] These irAEs covered a broad spectrum of systems; including dermatologic (pruritus in 17.6% and rash in 17.6%), gastrointestinal (diarrhoea in 30.3% and colitis in 5.3%), hepatic (increase ALT in 0.8% and AST in 1.1%, autoimmune hepatitis in 0.5%) and endocrine (hypothyroidism, hypopituitarism, hypophysitis, adrenal insufficiency, increase in serum thyrotropin level and decrease in serum corticotrophin level). There were seven of fourteen deaths that were associated with irAEs; 1 with grade 3 colitis and septicaemia, 4 with bowel perforation/peritonitis, 1 with Guillain-Barre syndrome and 1 with liver failure. The higher grade 3 and 4 irAEs are outlined in Table 3.

The adverse events in the phase III trial comparing ipilimumab and dacarbazine with dacarbazine alone were consistent with these findings (Table 3) and were higher in the ipilimumab plus dacarbazine arm, with predominantly dermatologic (pruritus in 26.7% and rash in 22.3%), gastrointestinal (diarrhoea in 32.8% and colitis in 4.5%), hepatic (increase alanine aminotransferase in 29.1% and aspartate aminotransferase in 26.7%, autoimmune hepatitis in 1.6%) and endocrine events.[6] There was a lower rate of grade 3 or 4 diarrhoea and colitis but a higher rate of transaminitis in this study compared to earlier single agent studies. Importantly, no patient died as a result of complications of immune related hepatitis or enterocolitis.

Dermatologic Toxicity

Dermatologic toxicity is the most common irAE reported (all grades up to 43.5% and grade 3/4 1%–3%) and generally appears after 2–4 weeks of treatment. Symptomatic management is recommended

Table 1. Management of immune related adverse events from ipilimumab.

	Skin toxicity	Diarrhoea and enterocolitis	Hepatitis
Supportive care	Sunscreen Emollients Topical steroid cream +/– antibiotics	Diet modification Hydration Loperamide	If abnormal, monitor LFTs every 3–7 days
Initiation of corticosteroids		Budesonide for prolonged G2 diarrhoea Methylprednisolone IV for grade 3/4 diarrhoea Slow wean of prednisone over 4–6 weeks	Methylprenisolone IV for ALT > 8 xULN and bilirubin > 5 xULN Prednisone for persistent Grade 2 changes in LFTs or Grade 3 changes (ALT > 5 xULN)
Other recommendations		Infliximab Consider TPN or surgical intervention	Mycophenolate mofetil Tacrolimus Infliximab Anti-thymocyte globulin

Table 2. NHMRC levels of evidence.

Level	Intervention
I	A systematic review of level II studies
II	A randomised controlled trial
III-1	A pseudorandomised controlled trial (ie, Alternate allocation or other method)
III-2	A comparative study with concurrent controls (non-randomised experimental trial, cohort study, case-control study, interrupted time series with a control group)
III-3	A comparative study without concurrent controls (nistorical control study, two or more single arm study, interrupted time series without a parallel control group)
IV	Case series with either post-test or pre-test/post-test outcomes)

with non-irritant moisturisers and body wash, low dose topical corticosteroids (betamethasone 0.1% or hydrocortisone 1%) or urea-based topical therapies with antipruritic agents.[52] Sun avoidance and the use of broad-spectrum sunscreen is recommended prophylactically. The use of cool compresses and oral anti-histamines may also assist with symptomatic relief of pruritus. Higher dose topical corticosteroids may be required for more severe rash (hydrocortisone 2%) and oral prednisone at 1 mg/kg/day should be initiated if there is no improvement or if the rash is complicated by dermal ulceration, necrotic, bullous or haemorrhagic

Table 3. Immune related adverse events from Ipilimumab in phase 2 and 3 trials.

Patient number (n =)	**Phase III[6] Ipi and DTIC (Ipi at 10 mg/kg) 250**	**Phase-III[5] Ipi monotherapy (3 mg/kg) 131**	**Phase-II[49] Ipi monotherapy different doses 217 (71 each at 3 mg/kg and 10 mg/kg)**
All grade irAE %	77.7	61.1	65 at 3 mg/kg [7]
[Grade 3, 4%]	[31.6, 10.1]	[12.2, 2.3]	70 at 10 mg/kg [25]
Dermatologic %		43.5	45 at 3 mg/kg [1.5]
[Grade 3, 4%]		[1.5, 0]	46 at 10 mg/kg [4.2]
Pruritus%	26.7 [2.0, 0]	24.4 [0, 0]	21.3 at 3 mg/kg [1.4]
			32.4 at 10 mg/kg [2.8]
Rash	22.3 [1.2, 0]	19.1 [0.8, 0]	23.9 at 3 mg/kg [1.4]
			22.5 at 10 mg/kg [0]
Vitiligo		2.3 [0, 0]	
Gastrointestinal %		29	32 at 3 mg/kg [2.8]
[Grade 3, 4%]		[7.6, 0]	39.4 at 10 mg/kg [15.5]
Diarrhoea %	32.8 [4.0, 0]	27.5 [4.6, 0]	25.3 at 3 mg/kg [1.4]
			39.4 at 10 mg/kg [14.0]
Colitis	4.5 [1.6, 0.4]	7.6 [5.3, 0]	5.6 at 3 mg/kg [1.4]
			5.6 at 10 mg/kg [2.8]
Endocrine %		7.6	5.6 at 3 mg/kg [2.8]
[Grade 3, 4%]		[2.3, 1.5]	4.2 at 10 mg/kg [1.5]
Hypothyroid %		1.5 [0, 0]	
Hypopituitarism		2.3 [0.8, 0.8]	
Hypophysitis		1.5 [1.5, 0]	
Adrenal insufficiency		1.5 [0, 0]	
Increase thyrotropin		0.8 [0, 0]	
Decrease corticotrophin		1.5 [0, 0.8]	
Hepatic %		3.8	0 at 3 mg/kg
[Grade 3, 4%]		[0, 0]	2.8 at 10 mg/kg [2.8]
Increase ALT %	29.1 [15.0, 5.7]	1.5 [0, 0]	
Increase AST	26.7 [13.8, 3.6]	0.8 [0, 0]	
Hepatitis	1.6 [1.2, 0]	0.8 [0, 0]	

Abbreviations: irAE, immune related adverse event; DTIC, dacarbazine; Ipi, ipilimumab; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

manifestations.[53] Scalp lesions can be particularly troublesome for patients and may benefit from use of low dose corticosteroid-containing shampoo or topical cold tar (Level of Evidence II).

Gastrointestinal Toxicity

Diarrhoea is the second most common toxicity, affecting up to 30% of patients, and the most common toxicity with grade 3 or 4 symptoms, reported in 7.6% with monotherapy at 3 mg/kg, 5.6% with DTIC and up to 15% of patients at higher doses of ipilimumab.[5,6,54] Interestingly and taking into account the difficulties with cross trial comparison and the greater experience with management, ipilimumab at 10 mg/kg in combination with dacarbazine in the phase 3 trial compared to monotherapy in phase 2 data seemed to be associated with less grade 3 and 4 diarrhoea and colitis.[6,55] Clinically, the average time of onset of diarrhoea was at 6–8 weeks, but can occur as early as 3 days post initiation of treatment with rapid progression to colitis,[56] such that early multidisciplinary management is crucial. Grade 1 and 2 diarrhoea may be managed symptomatically on an outpatient basis with immediate initiation of loperamide and close clinical follow-up. Modification of diet with small frequent meals, introduction of a BRAT diet (bananas, rice, apples, toast), ceasing lactose containing products and maintaining fluid intake with electrolyte replacement is recommended (Level of Evidence II).

For prolonged grade 1–2 and higher grade diarrhoea, oral and/or IV corticosteroids are essential (Level of Evidence II). For grade 1–2 diarrhoea lasting over 5 days, oral budesonide should be commenced starting at 9 mg daily (as a single dose or in 3 divided doses) for up to 8 weeks, with a tapering dose over the final 2–4 weeks of treatment.[56] Alternatively, oral prednisone can be used at 1 mg/kg/day and similarly, have a slow taper. Endoscopy should be considered at this stage and clinicians should be aware there can be rapid progression from grade 1 diarrhoea to severe colitis, requiring urgent hospital admission and management. Persistent grade 1–2 diarrhoea after oral corticosteroids or the development of grade 3–4 diarrhoea, should prompt urgent endoscopy, and initiation of high dose intravenous (IV) steroids.[56] Further treatment with ipilimumab should be with-held (Level of Evidence IV).

In a recent study of 198 patients treated with ipilimumab, 41 patients developed grade 3/4 diarrhoea of which 39 patients were endoscopically diagnosed with enterocolitis and histopathological findings were of neutrophilic inflammation with cryptitis in 46%, lymphocytic inflammation in 15% or combined neutrophilic and lymphocytic inflammation in 38%.[57]

In this scenario, recommended treatment is high dose IV methylpredisolone (2 mg/kg for 1–2 weeks) followed by a 30 to 45-day taper of prednisone starting at 60 mg per day. A rapid reduction in steroid dose should be avoided and may risk the development of recurrent symptoms and the need for escalation of care.[56] An alternative steroid regimen is with intravenous dexamethasone 4 mg every 6 hours, initially over 7 days.[57] At this stage, a more rapid dose reduction over 17 days was also reviewed. Out of 34 patients treated with this regimen, 12 patients had refractory enterocolitis with 5 patients not responding within 7 days and 7 patients relapsed after tapering the steroid dose. Four of these were treated with infliximab with a rapid and durable response (Level of Evidence IV).

Infliximab (5 mg/kg IV) is recommended if there is no response within one week of steroid initiation, or if there is relapse following steroid reduction, unless there are other contraindications such as bowel perforation or sepsis. An additional dose at a 2 week interval may be required (Level of Evidence II).

Small numbers of patients have been treated with an alternative regimen including the initiation of infliximab at the onset of grade 2 diarrhoea, with a second dose 2 weeks later, and given in combination with mesalamine, loperamide and hydrocortisone enemas.[58] 4 out of 6 patients were treated successfully with this regimen, avoiding the use of systemic steroids.

Good supportive care during this initial 1–2 week period is essential with view to early initiation of total parenteral nutrition (TPN) if necessary and surgical review if diarrhoea is not settling. The need for surgical intervention with formation of a diverting ileostomy or a partial/complete colectomy has been reported in some cases with the potential for clinical benefit if medical management fails.[59]

In all symptomatic cases, infective aetiology should be excluded by means of stool culture, microscopy for cells, ova, cyst and parasites and investigation for

clostridium difficile toxin. Polymerase chain reaction for noravirus or other pathogens may also be necessary depending on suspected exposure.

Prophylactic budesonide for the prevention of gastrointestinal toxicity has been studied but did not reduce the incidence of grade 2–4 diarrhoea and is not recommended[60] (Level of Evidence II).

Importantly, early initiation of diarrhoea treatment guidelines (DTGs) has been shown to reduce bowel perforation and colectomy rates, drug-related diarrhoea and serious gastrointestinal irAEs by up to 50%[61] (Level of Evidence III-2).

Hepatic Toxicity

Hepatotoxicity occurs in up to 29% of patients with grade 3 and 4 toxicity reported in up to 15% with the combination of ipilimumab and dacarbazine and 0.8% with ipilimumab alone (at 3 mg/kg dose), usually between 6–8 weeks after commencing ipilimumab.[5,6] Progressive liver disease, viral hepatitis and alternative drug toxicity should be excluded. Investigation of immune and non-immune hepatotoxicity should included hepatitis serology, CMV and EBV serology, anti-nuclear antibodies (ANA), antimitochondrial antibodies, liver-pancreas-specific antigen, liver-kidney microsomes and smooth muscle antigen (SMA) as well as consultation with a hepatologist[52] (Level of Evidence II).

For grade 2 changes in liver function tests (LFTs, Table 4), ipilimumab should be with-held and LFTs measured daily for 3 days, with the initiation of oral steroid therapy (prednisone 1 mg/kg/day) if there is no improvement after 48–72 hours.[52] This should be continued for a minimum of 30 days and LFTS monitored closely until they normalise. Grade 3 changes should also prompt the initiation of a course of oral corticosteroids and ipilimumab should be discontinued[62] (Level of Evidence II).

If the ALT rises $>$ 8 xULN and bilirubin $>$ 5 xULN, high dose steroid therapy is recommended with intravenous methylprednisonlone 2 mg/kg daily. If there is no improvement in transaminases after 48 hours, the addition of mycophenolate mofetil 1 g twice daily is recommended.[56] After a further 5–7 days, tacrolimus 0.10–0.15 mg/kg/day is recommended with measurement of target trough levels. Treatment with infliximab 5 mg/kg as a single dose may also be required in refractory and severe cases (Level of Evidence II).

Successful therapy of fulminant hepatitis has also been reported with antithymocyte globulin (1.5 mg/kg given at 4 intervals over 2 weeks) rather than infliximab. This case demonstrated rapid improvement in hepatic transaminases and synthetic function in a patient refractory to corticosteroids and mycophenolate[63] (Level of Evidence IV).

Endocrinopathies

Although uncommon, the endocrinopathies are another emergent toxicity induced by ipilimumab

Table 4. CTCAE grading for diarrhoea, changes in liver function tests and visual changes.

	Grade 1	Grade 2	Grade 3	Grade 4
Diarrhoea	Increase of $<$4 stools per day over baseline; mild increase in ostomy output compared to baseline	Increase of 4–6 stools per day over baseline; moderate increase in ostomy output compared to baseline	Increase of $\geq$7 stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self care ADL	Life-threatening consequences; urgent intervention indicated
Liver function tests				
Bilirubin	ULN – 1.5 xULN	1.5 xULN – 3.0 xULN	3.0 xULN – 10 xULN	$>$10 xULN
AST/ALT	ULN – 3.0 xULN	3.0 xULN – 5.0 xULN	5.0 xULN – 20 xULN	$>$20.0 xULN
Visual changes	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL	Severe or medically significant but not immediately sight-threatening; Hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADLs	Sight-threatening consequences; urgent intervention indicated; blindness (20/200 or worse) in the affected eye

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase.

and may include hypopituitarism, hypophysitis, hypothyroidism and adrenal insufficiency.[5]

Physicians should be alerted by symptoms of headache, visual field changes, fatigue, nausea, vomiting, fever, hypotension, arrhythmias and electrolyte abnormalities. As these symptoms are non-specific, other potential causes including disease progression, syndrome of inappropriate ADH secretion (SIADH) and sepsis need to be excluded. Examination of the hypothalamic-pituitary axis should be assessed including thyroid stimulating hormone, free T4/T3, adrenocorticotropic hormone, serum cortisol +/− short synachten test, luteinizing hormone, follicle-stimulating hormone, testosterone and prolactin.[15] MRI of the head with the hypophysis and visual field testing should be performed and early endocrinologist involvement is recommended. Symptoms of hypophysitis usually develop after 6 weeks of treatment and MRI may show thickening of the hypophyseal stalk[52,64] (Level of Evidence II)

If suspicious of an adrenal crisis, stress dose IV steroids (dexamethasone 4 mg every 6 hours) should be administered as well as gonadal and thyroid hormone replacement as required and with the consultation of an endocrinologist. Longer term management will involve tapering the steroids over a minimum of 4 weeks; however many patients will require steroid replacement for longer periods or life-long if there is inadequate recovery of hypophyseal function. Ongoing monitoring of hormone substitution and assessment of laboratory endocrine studies is required. Ipilimumab may be re-instituted after resolution of grade 1–2 endocrinopathies but as the risk of further complications is unknown, it is not recommended in more severe cases (Level of Evidence II). Recently however, patients with hypophysitis with the need for hormone replacement have been retreated with ipilimumab without worsening side effects[15] (Level of Evidence IV).

Others

Other immune related complications have been described. A recent case report described a case of anti-CTLA-4 antibody-induced lupus nephritis, confirmed on renal biopsy and electron microscopy.[65] Antibodies to double-stranded DNA were also detected and regressed after ipilimumab was withdrawn and the patient was treated with predisone (1 mg/kg daily).

Ophthalmological side effects have been reported in less than 1% of treated patients, in some studies, in association with diarrhoea and colitis. These events usually resolve within a week but treatment with steroid eye drops may be required and systemic corticosteroids in more severe cases.[16]

Neurological toxicity such as Guillain-Barre syndrome, sensory or motor neuropathy or myasthenia gravis have been reported in less than 1% of patients.[52] Other non-inflammatory causes need to be assessed and neurological consultation may assist with consideration of EMG and/or nerve conduction studies. For grade 3 and 4 symptoms, hospitalisation, IV steroids, IV immunoglobulin or other immunosuppressants may be required and ipilimumab should be ceased.

Selective BRAF Inhibitors

The selective BRAF inhibitors are generally well tolerated with low rates of grade 3 and 4 toxicities

The most common adverse events with vemurafenib recently described in BRIM-3 included grade 2 and 3 arthralgias (18% and 3%), rash (10% and 8%), photosensitivity (12% grade 2 or 3), fatigue (11% and 2%), cutaneous squamous-cell carcinoma (SCC, 12%), keratoacanthoma (2% and 6%), nausea (7% and 1%) and diarrhoea (5% and $<$1%).[7] Dose interruption and modification were required in 38% of patients.

GSK2118436 is also very well tolerated with similar side effects reported in the phase I/II trial including skin changes (all grades 37%; grade 3 (G3):1 patient), low grade cutaneous SCC (2 pts/3%), headache (all grades 19%, G3:1 pt), nausea (18% G1), fatigue (15% G1) and vomiting (all grades 13%, G2:4).[8] Although the majority of side effects are similar between the two BRAF inhibitors, vemurafenib was associated with photosensitivity in up to 30% of patients (12% grade 2 or 3) while pyrexia is reported in 15% (2% grade 3) on GSK2118436.

Interestingly, the BRAF and MEK inhibitor combination (GSK2118436 and GSK1120212) not only demonstrated a potential reduction in drug resistance but also a lower incidence of rash, BRAF-induced hyperproliferative skin lesions and SCC.[47] There was a lower incidence of skin rash (all grade 25%, grade $\geq$ 3 2%) compared to single agent toxicities as well as a lower incidence of hyperproliferative skin lesions (1 from 109 patients with cutaneous SCC).

Recommendations for the skin rash, photosensitivity and diarrhoea are similar to that described for the MEK inhibitors below. Management of fever and SCC is discussed in detail.

Fever

Fever secondary to the BRAF inhibitors, primarily GSK2118436, can usually be managed with supportive care but thorough assessment is recommended to exclude a source of sepsis. For fever up to 39 degrees (grade 1), treatment with paracetamol every 8 hours is recommended, alternating with ibuprofen every 8 hours if needed. For fever between 39–40 degrees (grade 2), the BRAF inhibitor should be ceased, investigation to exclude sepsis should be performed and hospitalisation may be required. In case of temperature over 40 degrees (grade 3), hospitalisation and rehydration are recommended with further investigation for a source of sepsis. Care should be taken with choice of antibiotics to avoid drug interactions (Level of Evidence II).

Squamous Cell Carcinoma

As evidenced with both selective BRAF inhibitors though less commonly with GSK2118436,[66] there is an increased incidence of cutaneous SCC. This usually develops between weeks 2 to 14 and is hypothesised to be due to upstream RAS mutations in pre-existing SCC skin lesions, which may occur in approximately 15% of patients. Selective inhibition of downstream BRAF can lead to CRAF signalling by this mutant RAS with subsequent development of SCCs.[67,68] The majority of these SCCs are keratoacanthoma type, well-differentiated with no metastatic potential and can be treated with surgical excision.[38] Patients should have a thorough skin examination at baseline and be monitored regularly during treatment. At the development of any new skin lesions, a dermatologist should be consulted and excision arranged (Level of Evidence II).

MEK Inhibitors

Early results from the phase 1/2 study of the MEK inhibitor, GSK1120212, demonstrated that the most common adverse events were an acneiform rash (all grade 85%; ≥grade 3: 2%), usually on the face, torso and arms; diarrhoea (all grade 48%; >grade 3: 2%), fatigue (all grade 37%; ≥grade 3 7%), nausea (all grade 20%; ≥grade 3: 0), vomiting (all grade 24%, ≥grade 3: 2%). Less common events included left ventricular systolic dysfunction (9 from 162 patients), central serous retinopathy (CSR: 3 from 162 patients, at dose levels higher than 2 mg daily) and retinal vein occlusion (RVO: 1 from 162 at 2 mg daily).[46] Among the cases of left ventricular systolic dysfunction, few were considered related to GSK1120212 and it was rarely symptomatic. All 3 cases of CSR resolved upon drug interruption. RVO was not reversible, however the patient experienced significant improvement in visual acuity after intraocular bevacizumab treatment.[69]

A number of other early MEK inhibitor trials also demonstrated ocular toxicity with reports of visual disturbance, including halos, spots and decreased acuity. This is discussed in detail below.

Skin Rash

Prophylactic treatment to reduce the incidence of grade 3–4 skin rash is recommended. Patients should avoid excess exposure to sunlight and use broad-spectrum sunscreen (containing titanium dioxide or zinc oxide) with a skin protection factor SPF ≥ 15. Alcohol-free emollient cream should be used on dry skin two to three times daily with a mild strength topical steroid (hydrocortisone cream 1%) once daily. Oral doxycycline or minocycline is also recommended on the first day of treatment as part of the prophylactic regimen or topical antibiotics can be used if oral antibiotics cannot be tolerated (Level of Evidence II).

For moderate skin rash with pruritus or tenderness, topical steroids can be increased to hydrocortisone cream 2.5% or triamcinolone cream 0.1% if the prophylactic measures are unsuccessful. In case of higher grade rash despite topical steroid treatment a dose reduction should be considered. For more severe symptoms with a significant impact on activities of daily living (ADL) and risk of super-infection, the drug should be ceased until the rash improves and a dermatologist should be consulted with consideration of oral steroids.

Other symptomatic measures that may assist with pruritus are the use of cool compresses and oral antihistamines. Paronychia should be treated with antiseptic baths, local topical potent corticosteroids and oral antibiotics with referral to a dermatologist or surgeon if no improvement is seen. Infected lesions

should be assessed with bacterial and fungal cultures and treated with systemic agents as appropriate (Level of Evidence II).

Diarrhoea

For grade 1 and 2 (Table 4) diarrhoea, both non-pharmacological and pharmacological measures are recommended as discussed previously. For grade 2 diarrhoea, the drug should be with-held until symptoms have resolved to baseline or grade 1, at which point the agent may be reintroduced. If grade 1 or 2 diarrhoea persists for 48 hours despite dietary modification and the introduction of loperamide, second line agents including octreotide, budesonide or codeine-based treatment may be required.

If there is clinical deterioration or grade 3 or 4 diarrhoea, the MEK inhibitor should be ceased and if restarted, will require a dose reduction. Hospitalization, IV rehydration and regular loperamide are recommended. Severe dehydration should prompt the addition of octreotide. Colonoscopy should be performed to further assess for colitis and exclude an infective cause. Antibiotic cover including fluoroquinolones is recommended if grade 3 or 4 diarrhoea persists beyond 24 hours, if there is fever or grade 3 or 4 neutropaenia (Level of Evidence II).

Visual Changes

Ocular toxicity, particularly RVO and CSR, have been reported with a number of MEK inhibitors in development. The phase I study with PD0325901 demonstrated RVO in 3 from 66 patients, particularly with the continuous schedule, and this limited further trials beyond phase II.[70] After the first two cases of RVO, there was a protocol amendment to exclude patients with glaucoma, intraocular pressure >21 mmHg or any other significant abnormality on ophthalmic examination by an ophthalmologist. An ophthalmic examination was required every cycle for visual acuity, visual field examination, intraocular pressure, external eye exam and dilated fundoscopy at baseline and before each cycle. On retrospective analysis, predisposing factors for retinopathy included hypertension, diabetes, hypercholesterolemia and glaucoma. The occurrence of RVO on the continuous schedule raised the possibility that the ocular toxicity was associated with prolonged and/or high levels of pERK suppression. The phase II study with this agent on an intermittent schedule had similar exclusion criteira and although there was grade I/II visual disturbance with blurred vision, halo vision and diplopia, there were no cases of RVO.[71]

The phase I study of AZD6244 demonstrated transient and reversible blurred vision in 7 from 57 patients (12%) but no evidence of CSR nor RVO on ophthalmologic examination.[44] AZD6244 is ten-fold less potent than PD0325901 and it was hypothesised that the ocular side-effects may correlate with the extent of MEK inhibition.[72]

In the pre-clinical and phase I/II studies with GSK1120212, there have also been reports of RVO and CSR. RVO was reported in rabbit models and in one case in man;[47] however it was unclear whether this case was an adverse drug reaction or an adverse event due to a prothrombotic malignant state. CSR has been reported in 3 cases from 162 patients treated with GSK1120212 and changes were reversible on drug cessation in all cases.

The phase III study comparing GSK1120212 to chemotherapy in subjects with advanced or metastatic BRAF mutant melanoma excludes patients with a history of RVO or CSR and patients with predisposing factors such as uncontrolled glaucoma or ocular hypertension, uncontrolled hypertension, uncontrolled diabetes mellitus, or history of hyperviscosity or hypercoagulability syndromes.[73] Ophthalmologic examination is mandated at study entry and includes indirect and direct fundoscopy as well as tonometry. Patients with any visible retinal pathology considered a risk factor for RVO or CSR are also excluded, including evidence of new optic disc cupping or intraocular pressure >21 mmHg.

Knowledge of the assessment required prior to administration of the MEK inhibitors will be essential as the use of these agents increases.

Current recommendations outline that patients who are asymptomatic or with mild symptoms and clinical changes should have ophthalmology review to determine if there are changes consistent with RVO, in which case the drug should be discontinued. If there is CSR, the agent should be ceased until signs and symptoms have resolved and then can restart at a lower dose. If ophthalmologic examination is normal, the agent should be continued.

For grade 2 or 3 visual changes (Table 4), with moderate to severe symptoms and limiting instrumental or

self care activities of daily living, the agent should be ceased and urgent ophthalmology review obtained. If there is no RVO nor CSR, the agent should be ceased until symptoms have resolved to grade 1 or less, and can be restarted at a lower dose. For grade 4 visual changes that are sight-threatening, the agent should be permanently discontinued. CSR is reversible on drug discontinuation. RVO may require novel therapy with intraocular anti-VEGF therapy with the guidance of an ophthalmologist[47] (Level of Evidence II).

Place in Therapy

The treatment paradigm for metastatic melanoma is changing with a number of active agents now available for treatment of this disease. Challenges exist not only in the optimal choice of agents for these patients but also in the timely and aggressive management of associated toxicities. Particularly with the BRAF and MEK inhibitors, the duration of benefit is often short-lived as resistance develops. Understanding of both primary and secondary resistance mechanisms is key to novel drug development and future clinical trials both of single agent and combination therapies.[48] As discussed, understanding of the importance of the MAPK pathway in melanoma tumorigenesis and resistance pathways has already led to novel combination strategies and further such trials are underway and in development.[18]

There are also ongoing early clinical trials of vemurafenib in combination with ipilimumab, particularly as understanding increases as to how BRAF inhibition effects immune response and T-cell function. BRAF inhibition has been shown to improve recognition by antigen-specific T cells and increase intratumoural and peritumoural lymphocytes shortly after drug initiation. Interestingly, patients who develop resistance to BRAF inhibition demonstrate a decrease in intratumoural and peritumoural lymphocytes.[74] Compared to MEK inhibitors, BRAF inhibitors also seem to preserve T-cell function.[75] Together, this early data provides a rationale for the combination of ipilimumab and BRAF inhibition.

Importantly, there are a number of overlapping toxicities with these agents, particularly diarrhoea and skin rash, and optimal management of these will need to be developed concurrent with testing clinical efficacy.

Thus, there remain many challenges in the treatment of patients with metastatic melanoma – how to best treat patients without a BRAF mutation, how to overcome resistance mechanisms that develop to BRAF and MEK inhibition and how to best combine immune and targeted therapy to optimise patient outcome.

Conclusion

The availability of novel agents targeting immune system modulation and specific genetic aberrations in metastatic melanoma has given hope to those patients, who up to recent times, had limited treatment options. As outlined above, there are a number of class specific emergent toxicities calling for heightened vigilance and prompt initiation of therapy. Multidisciplinary management, education and awareness of these toxicities across disciplines is essential. Due to the severity and rapid progression of these toxicities, some recommend that these agents should be delivered in specialist centres by oncologists familiar with their use.[76] As the development of new oncological agents accelerates, physicians need to familiarise themselves with the toxicities and their management and the availability of evidence-based guidelines is key. These and other guidelines provide an outline of the expected toxicities and management for the most common and potentially severe adverse events from these agents.

Disclosures

Author(s) have provided signed confirmations to the publisher of their compliance with all applicable legal and ethical obligations in respect to declaration of conflicts of interest, funding, authorship and contributorship, and compliance with ethical requirements in respect to treatment of human and animal test subjects. If this article contains identifiable human subject(s) author(s) were required to supply signed patient consent prior to publication. Author(s) have confirmed that the published article is unique and not under consideration nor published by any other publication and that they have consent to reproduce any copyrighted material. The peer reviewers declared no conflicts of interest.

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