Blinded by the light: why the treatment of metastatic melanoma has created a new paradigm for the management of cancer

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***Abstract*:** Until recently, treatment for metastatic melanoma was characterised by a limited availability of treatment options that offer objective survival benefit. Cytotoxic agents fundamentally lack the ability to achieve disease control and cytokine therapy with interleukin-2 has an unacceptably high – for the use across all patient cohorts – rate of toxicities. The validation of *braf* as an oncogene driving melanoma tumorigenesis, as well as the discovery of the role of CTLA-4 receptor in the evasion of anticancer immune response by melanoma, has revolutionised our treatment options against a disease with dismal prognosis. Quick implementation of translational discoveries brought about BRAF/MEK inhibition in clinic, while at the same time, wider experience with CTLA-4 blockade enabled clinicians to manage previously fatal immune-related toxicities with greater confidence. The suitability for clinical use of other oncogenic drivers such as *NRAS* and *c-kit* is currently being tested whilst the PD-1/PD-L1/PD-L2 axis has emerged as a new immunotherapy target with exciting early phase results. The recent exponential progress in treatment of melanoma has set an example of translational medicine and the current review aims to explain why, as well as suggesting new goals for the future.

Keywords: BRAF/MEK inhibition, ipilimumab, metastatic melanoma, molecularly targeted treatment, PD-1/PD-L1/PD-L2 axis

Introduction

Melanoma is the most lethal form of skin cancer, accounting for the majority of skin cancer deaths. There are approximately 132,000 new cases globally each year, an incidence that has been steadily rising in the western world for the past few decades: this increase relates to both improved detection and an increase in the frequency of exposure to UV radiation [WHO, 2013; De Vries and Coeburgh, 2005]. Factors such as skin colour, hair colour and pre-existence of more than 20 nevi increase the risk of melanoma occurrence; fair-skinned people are the ones mostly suffering from the disease with the White race having a risk ratio of 5–10 *versus* Black or Asian race [Linette, 2012]. Approximately 20% of melanoma sufferers over the age of 65 years will present with metastases and therefore be incurable at diagnosis; the 5-year survival of patients with metastatic melanoma is 8% for males and 25% for females, with a median survival of 6 months [Cancer Research UK, 2013a]. Metastases to skin, subcutaneous tissue or lymph nodes confer the best prognosis in the metastatic setting whereas lung metastases have an intermediate prognosis. Patients with disease to any other visceral sites (liver, bone, brain) or any site combined with an elevated lactate dehydrogenase (LDH) carry the worst prognosis with a 33% 1-year survival rate [Balch *et al.* 2009].

Recent successes in the oncological treatment of melanoma have reminded us that sometimes the biggest disappointments can create great opportunities. This review aims to present the poor progress made with conventional cytotoxic therapies for metastatic melanoma, as well as offering some biological and translational insight on why we have over the last few years had a rapid progress with an explosion of potential treatments compared with other cancers.

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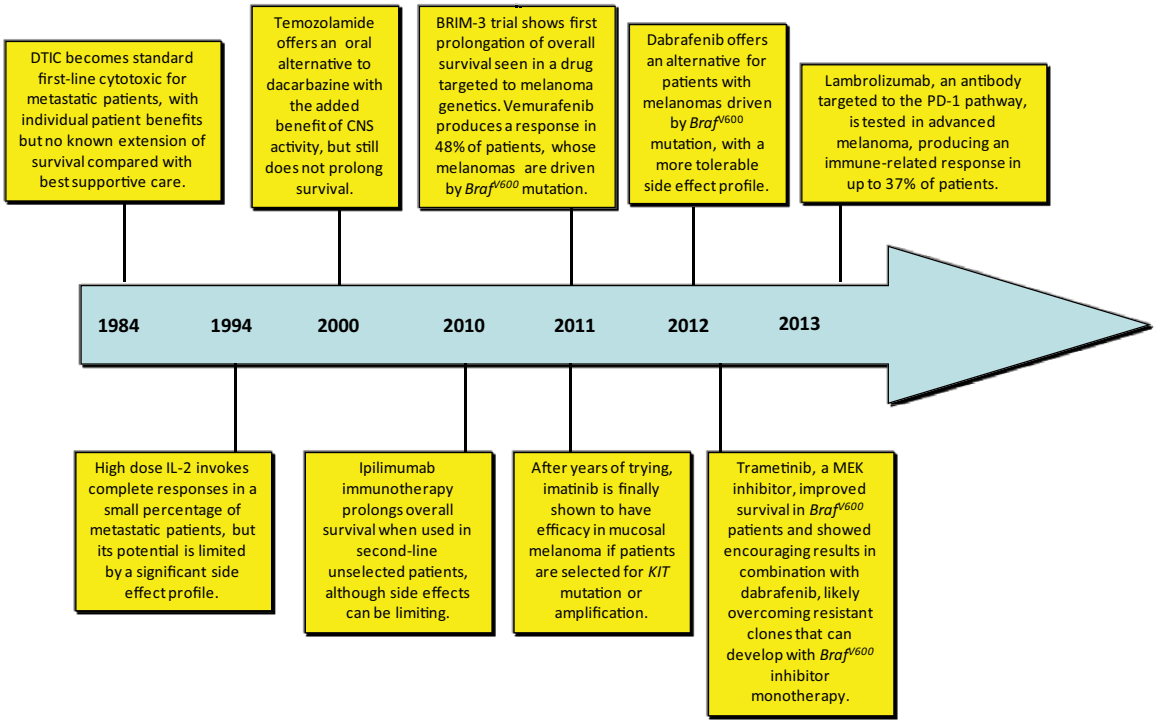

Figure 1. Timeline of key therapeutic developments in metastatic melanoma. Most of these advances have occurred in the last 3–4 years.
CNS, central nervous system; IL-2, interleukin-2; MEK, mitogen-activated protein kinase kinase.

Melanoma pathophysiology precludes any survival benefit from traditional cytotoxics

Until recently, no therapy administered to UK patients with metastatic melanoma could extend overall survival. Dacarbazine, a cytotoxic previously considered the standard of care, only offers limited benefit with improvement in symptoms of carefully selected patients (Figure 1) [Tarhini and Agarwala, 2006]. Response rates to dacarbazine (complete and partial response) have most recently been shown to be of approximately 10% and, as a consequence of its historical development, no randomized phase III studies exist to confirm its benefit over best supportive care [Robert *et al.* 2011]. Temozolamide, an imidazotetrazinone derivative with good brain tissue penetration and the advantage of oral administration, was a more recent hope for cytotoxic development in melanoma, especially in patients with brain metastatic disease [Stevens *et al.* 1987]. However, in a phase III randomized trial focusing on central nervous system (CNS) involvement, temozolamide affected neither the occurrence of CNS failure as first site of metastases nor the overall survival (OS) in these patients (Figure 1) [Chiarion-Sileni *et al.* 2011]. Some physicians elect to use the combination of carboplatin/paclitaxel in the second-line setting as it showed modest antitumour activity in a small study of pretreated patients [Rao *et al.* 2006]. Nevertheless, this treatment offers no survival benefit, similar to a number of other single agents or combination chemotherapeutic regimens that have been assessed [Jilavenau *et al.* 2009].

Melanocyte biologists might argue that they are unsurprised by such results since the pathogenesis of melanoma is characterised by two central physiological properties of cells from the melanocyte lineage.

1. **Adult melanocytes (MCs) are resistant to apoptosis**. Their major role is lifelong protection of the skin from ultraviolet radiation (UVR) through the production of melanin and the consequent tanning response [Boissy and Nordlund, 1997]. The capacity of MCs to resist apoptosis and survive the highly mutagenic skin environment is reflected in melanoma by its resistance to traditional cytotoxics [Terzian *et al.* 2010]. Melanoma cells have been shown to display resistance to drug-induced apoptosis *in vitro* and also show low levels of

spontaneous apoptosis *in vivo* [Soengas and Lowe, 2003]. Thus, melanoma cells can 'hijack' the molecular mechanisms of other cells in the MC lineage for their own devices.

2. **MC embryological precursors, melanoblasts (MBs), have a propensity to migrate**. The migratory propensity of MCs is indicated by their anatomical localisation in adults, where they classically reside in the skin, but also exist in the eyes, heart, inner ear and brain [Brito and Kos, 2008]. MCs arise from a uniquely motile embryonic precursor population, MBs. This lineage-specific predisposition of MBs to migrate is also present in early stage primary melanomas, where metastases can develop from primary lesions as small as less than 1 mm in size and cause devastating effects through involvement of multiple viscera [Gupta *et al.* 2005; Corsetti *et al.* 2000]. Not unusually, we see cases of metastatic melanoma in clinic where identification of the primary is impossible, either because it has regressed or it is too small to detect. Given its biological capacity for aggression, it seems likely that the relatively high 80% survival rate from melanoma accounts only for those early detected primary tumours on an easily visualised organ, the skin. Unlike many other cancers with developed cytotoxic treatment protocols, melanoma therefore offered an attractive niche for the development of innovative targeted treatments that were tailored to its underlying genetic aberrations. There was no 'standard of care' to compete with in the treatment of metastatic disease.

The discovery of Braf^{V600} mutations presents an opportunity

In 2002, a systemic screen of genetic alterations in proteins Ras, Raf, mitogen-activated protein kinase kinase (MEK) and extracellular signal regulated kinase (ERK) was reported in a number of different cancer cell lines [Davies *et al.* 2002]. Their key discovery was a missense mutation in the serine threonine kinase, BRAF, at codon 600: a single site for mutations which occur with high frequency in cutaneous melanomas. The mutation itself is an oncogenic gain-of-function mutation that renders BRAF constitutively active, thus u-regulating the mitogen-activated protein kinase (MAPK) pathway in the absence of extracellular growth signals. Subsequently, *Braf^{V600}* has been functionally validated as an oncogenic driver in both melanoma animal models and cell lines with a prevalence now estimated at 52% across patients with cutaneous melanoma [Gray-Schopfer *et al.* 2007; Dhomen *et al.* 2009]. This was the first identification of *Braf* as an oncogene, and compared with other cancers, its high prevalence in melanoma was unique. Moreover, the amino acid substitutions caused by *Braf^{V600}* mutations and their positioning in the BRAF protein residues offered an easy 'target' for the subsequent 'hit-to-lead' process.

Within 8 years of this discovery, the translational development of Braf^{V600} inhibition succeeded at near unparalleled levels and set a benchmark for other cancers to follow. A phase I trial of the small molecule Braf^{V600} inhibitor, vemurafenib, showed remarkable clinical efficacy with a tolerable side effect profile in a large subset of melanoma patients preselected for their *Braf^{V600}* status (Figure 1) [Flaherty *et al.* 2010]. In 2011, a phase III trial confirmed vemurafenib as the first agent targeted to melanoma genetics that could offer an extension in OS of metastatic patients. Median OS was significantly longer in the group treated with vemurafenib than in control group treated with dacarbazine (13.6 months [95% confidence interval (CI) 12.0–15.2] *versus* 9.7 months [95% CI 7.9–12.8; hazard ratio (HR) 0.70 (95% CI 0.57—0.87); $p = 0{\cdot}0008$], as was median progression-free survival (PFS) [6.9 months (95% CI 6.1–7.0) *versus* 1.6 months (95% CI 1.6–2.1); HR 0.38 (95% CI 0.32–0.46); $p < 0.0001$] respectively (Table 1) [Chapman *et al.* 2011]. Patient selection was limited to melanoma patients carrying the *Braf^{V600}* mutation and toxicity was generally tolerable.

Another Braf^{V600} inhibitor, dabrafenib, has also emerged into clinical use with striking clinical trial results (Figure 1, Table 1). Following encouraging phase I/II trial results, a phase III comparison of dabrafenib with dacarbazine in patients with Braf^{V600E} expressing melanoma showed a significant improvement in PFS with 6.9 months *versus* 2.7 months, respectively, and a preliminary median OS favouring dabrafenib (18.2 months *versus* 15.6 months) [Hauschild *et al.* 2013]. Dabrafenib has a generally favourable toxicity profile. Taking into consideration that comparisons between trial results are not always precise, a lower incidence of epithelial squamous cell lesions was noticed with dabrafenib when compared with

Table 1. Key details of recent breakthrough studies in metastatic melanoma.

	BRIM-3 [Chapman *et al.* 2011]	BREAK-3 [Hauschild *et al.* 2012]	METRIC [Flaherty *et al.* 2012b]	COMBI- v [Robert *et al.* 2014]	MEK162 [Ascierto *et al.* 2013]	Imatinib [Guo *et al.* 2011]	Ipilimumab [Hodi *et al.* 2010]	Ipilimumab/ dacarbazine [Robert *et al.* 2011]	Nivolumab [Topalian *et al.* 2014]
Phase	III	III	III	III	II	II	III	III	I/II
Line of treatment	1st	1st[t]	1st or 2nd	1st	>2nd	2ndor 3nd	2nd	1nd	> 2nd
Number of patients	675	250	322	704	71	43	676	502	107
Drug	Vemurafenib	Dabrafenib	Trametinib	Dabrafenib/ trametinib	MEK162	Imatinib	Ipilimumab	Ipilimumab/ dacarbazine	Nivolumab
Mechanism	$Braf^{V600}$ inhibitor	$Braf^{V600}$ inhibitor	MEK inhibitor	$Braf^{V600}$ inhibitor + MEK inhibitor	MEK inhibitor	KIT inhibitor	Anti-CTLA-4 antibody	Anti-CTLA-4 antibody + cytotoxic	Anti-PD-1 antibody
Patient selection	$Braf^{V600E}$ or ^{V600K} mutated	$Braf^{V600}$ mutated	$Braf^{V600}$ mutated	$Braf^{V600}$ mutated	$Braf^{V600}$ or NRAS-mutated	cKIT mutated and/or amplified	HLA-A*0201 positive	no	no
RR (%)	57	50	22	N/A	20*	22	10.9	15	31[¥]
Median PFS (months)	6.9*	6.9*	4.8*	11.4	4	3.5*	2.9	<3	3.7
Median OS (months)	13.6	18.2	Not mature	Not mature*	Not mature	Not mature	10.1*	11.2*	16.8

All trials were considered to have positive results. Last three columns detail clinical trials with immune checkpoint antibodies.
*Primary endpoint
[¥]Secondary endpoint
MEK, mitogen-activated protein kinase kinase; OS, overall survival; PFS, progression-free survival; RR, relative risk.

vemurafenib. There are speculations that dabrafenib's lower specificity for wildtype BRAF and CRAF could prevent undesirable activation of wildtype RAF dimers and therefore lead to less cutaneous adverse events [Hauschild *et al.* 2012].

The breakthroughs with vemurafenib/dabrafenib were engendered by our predictive selection of patients with melanomas expressing mutated $Braf^{V600}$, something that can be explored further on two quantifiable levels:

1. *Efficacy.* This is reflected by improved response rates and extension of survival with vemurafenib/dabrafenib and may well not have been the case had all patients (i.e. those without $Braf^{V600}$ mutation) been treated with the $Braf^{V600}$ inhibitor. In fact there is significant preclinical concern to suggest that patients with melanomas driven by other genetic signatures could respond adversely to drugs such as vemurafenib, with a paradoxical MAPK pathway upregulation and consequent deterioration/progression of their disease [Heidorn *et al.* 2010].
2. *Toxicity.* As vemurafenib is specifically targeted to melanoma cells with $Braf^{V600}$ mutation, rather than other cells with wildtype *Braf*, it was tempting to speculate that patients on treatment would be spared side effects due to a lack of effect on cells other than melanoma cells. This has turned out not to be the case and a number of idiosyncratic side-effects have been characterised with $Braf^{V600}$ inhibition, including an approximately 12% incidence of cutaneous squamous cell cancers [Chapman *et al.* 2011]. The reasons for this are still not fully elucidated, although it is clear that vemurafenib again invokes a paradoxical upregulation of the MAPK pathway in cells affected by mutated RAS [Su *et al.* 2012]. Toxicity is therefore not as minor as might have been hypothesised at the outset of clinical trials, but the side effect profile from $Braf^{V600}$ inhibitors is undoubtedly tolerable in most cases.

To finish, it is important to comment on a level of progress with $Braf^{V600}$ inhibition that is more difficult to quantify, something that we may not be able to fully assess for a number of years: our knowledge of the tumorigenic process in melanoma now has a preclinical and clinical platform from which we can measure all future advances. The process of development of $Braf^{V600}$ inhibitors in melanoma, from target identification to clinical implementation, happened with an exemplarily quick pace, proving that the historical 10–15 year duration of drug discovery for small molecule drugs can be achieved or even outreached. It created a paradigm from which cancer researchers can take confidence in their pursuit of successful molecularly targeted treatments for other cancers. One of the first questions oncologists must now ask themselves with all cases of metastatic cancer is: should we still resort to the traditional and molecularly uninformed use of cytotoxics when a trial of an oral drug that specifically targets the main driver mutation of a cancer might suffice?

The historical failure and lack of chemotherapy benefit in metastatic melanoma was in many senses the vehicle for this breakthrough, and the prior success of imatinib in chronic myeloid leukaemia (CML) and gastrointestinal stromal tumours (GIST) followed a similar path [Dematteo *et al.* 2009; Demetri *et al.* 2002; Joensuu *et al.* 2012; Blanke *et al.* 2008; O'Brien *et al.* 2003; Verweij *et al.* 2004]. More recently, the eventual success of erlotinib in nonsmall cell lung cancer (NSCLC) has proven that chemotherapy can be sidelined for less toxic targeted treatments tailored to tumour genetics [Zhou *et al.* 2011; Rosell *et al.* 2012; Fukuoka *et al.* 2011].

With all these advances, a need for innovative clinical trial designs has emerged, whereby patients are carefully selected with the use of validated biomarkers and 'matched' to the appropriate drug. These studies, such as *enrichment* and *adaptive-design* studies will help patients derive the maximum benefit from targeted treatments and clinical researchers extract a vast amount of translational information [Temple, 2005; Berry, 2011].

MEK inhibition: building on the knowledge gained from $Braf^{V600}$ inhibition

Shortly after its clinical success, several resistance mechanisms to $Braf^{V600}$ inhibition were reported in preclinical literature, offering important insights for oncologists to reiteratively dissect treatment of patients resistant to vemurafenib/dabrafenib with further rationally designed second line and combination trials [Heidorn *et al.* 2010; Poulikakos *et al.* 2011; Hatzivassiliou *et al.* 2010; Johannessen *et al.*

2010; Gopal *et al.* 2010; Nazarian *et al.* 2010; Shao and Aplin, 2011; Emery *et al.* 2009]. One key mechanism of resistance described was the mutation and upregulation of downstream MEK, a protein which has kinase activity in about 90% of untreated human melanomas [Gray-Schopfer *et al.* 2007; Emery *et al.* 2009]. A protein susceptible to treatment with kinase inhibitors had once again been uncovered as relevant to melanoma pathogenesis. Trametinib, an allosteric non-adenosine triphosphate (ATP) competitive molecule, is the first of many MEK inhibitors under development for treatment of metastatic melanoma and other malignancies (Figure 1, Table 1). In line with preclinical data which showed efficient inhibition of phosphorylated ERK 1/2, its activity in advanced $Braf^{V600}$-mutant melanoma was confirmed in phase I trials [Falchook *et al.* 2012a; Infante *et al.* 2012]. Clinical efficacy of trametinib was confirmed by the phase III METRIC trial, which showed significant improvements in PFS (4.8 *versus* 1.5 months) and rate of OS (81% *versus* 67% 6-month survival) for $Braf^{V600}$-mutant patients randomized to trametinib rather than chemotherapy (Table 1). Adverse events with trametinib were easily managed and most importantly, skin neoplasms were completely absent. Notably, trametinib-induced rash was papulopustular in nature, as opposed to the hyperkeratotic, maculopapural rash of the BRAF inhibition [Flaherty *et al.* 2012b]. Severe treatment toxicity was rare, a finding which next led to a feasibility study assessing the combination of trametinib with dabrafenib [Flaherty *et al.* 2012a]. The biological rationale here was that MEK inhibition could prevent both the resistance and toxicity caused by MAPK upregulation following $Braf^{V600}$ inhibition. Phase I/II data on 247 patients were reported showing significant improvements in PFS (9.4 *versus* 5.8 months) and a significant reduction in skin toxicity (5% *versus* 19% incidence of squamous cell carcinoma) with trametinib/dabrafenib compared with dabrafenib alone [ClinicalTrials.gov identifier: NCT01584648].

These results led to the large-scale phase III trial COMBI-v where the superiority of combination of BRAF/MEK blockade with dabrafenib/trametinib was tested against monotherapy with vemurafenib in patients with unresectable or metastatic melanoma. Median PFS was 11.4 *versus* 7.3 months in favour of dabrafenib/trametinib arm (HR 0.56, $p < 0.001$) whilst median OS has not been reached as yet [Robert *et al.* 2014]. Even more extended PFS was reported in a phase Ib trial with vemurafenib and a novel MEK inhibitor, cobimetinib; the primary endpoint was safety of the combination treatment but nonetheless a PFS of 9.9 months (hazard ratio for death or disease progression, 0.51; 95% CI 0.39–0.68; $p < 0.001$) against 6.2 months with vemurafenib monotherapy was demonstrated [Larkin *et al.* 2014].

One key remaining question for MEK inhibition is whether it may also be efficacious in the 15% of cutaneous melanoma patients with an NRAS (rather than BRAF) mutation. Melanoma activating mutations of BRAF and NRAS are generally mutually exclusive, a finding which suggests they stimulate the same linear pathway involving MAPK deregulation [Davies *et al.* 2002; Goel *et al.* 2006; Rajagopalan *et al.* 2002]. Mutation of NRAS drives the majority of cutaneous melanomas unaccounted for by $Braf^{V600}$ mutation [Cohen *et al.* 2002]. Despite their mutual stimulation of the MAPK pathway, there is preclinical evidence to suggest that $Braf^{V600}$ inhibition will have no effect in patients with melanomas driven by mutation of NRAS, and in fact this may be a counterproductive strategy [Nazarian *et al.* 2010].

Targeting Ras, a GTPase rather than a kinase, therefore remains an elusive 'holy grail' of melanoma, as it is in other cancers. No direct inhibitors of Ras are currently being assessed in clinical trials, although a logical next step would be to consider MEK inhibition in Ras-mutated melanoma given its downstream upregulation of the MAPK pathway. A small phase II study of another MEK inhibitor, MEK-162, suggested that this may well be biologically and clinically plausible as 20% of patients with NRAS mutated advanced melanoma achieved an initial partial response to treatment (Table 1) [Ascierto *et al.* 2013]. Given the frequent cell cycle checkpoint dysregulation in NRAS-mutant melanoma, MEK-162 was combined with the selective CDK4/6 inhibitor, LEE011, in a phase Ib/II study which saw a 43% rate of partial responses; further results of the phase II part of the study are awaited with great interest for this subtype of melanoma with particularly poor prognostic profile [Sosman *et al.* 2014].

Thus it is quite likely that, in the near future, a vast majority of patients with advanced melanoma will have the option of a molecularly targeted agent, depending on the particular genetic

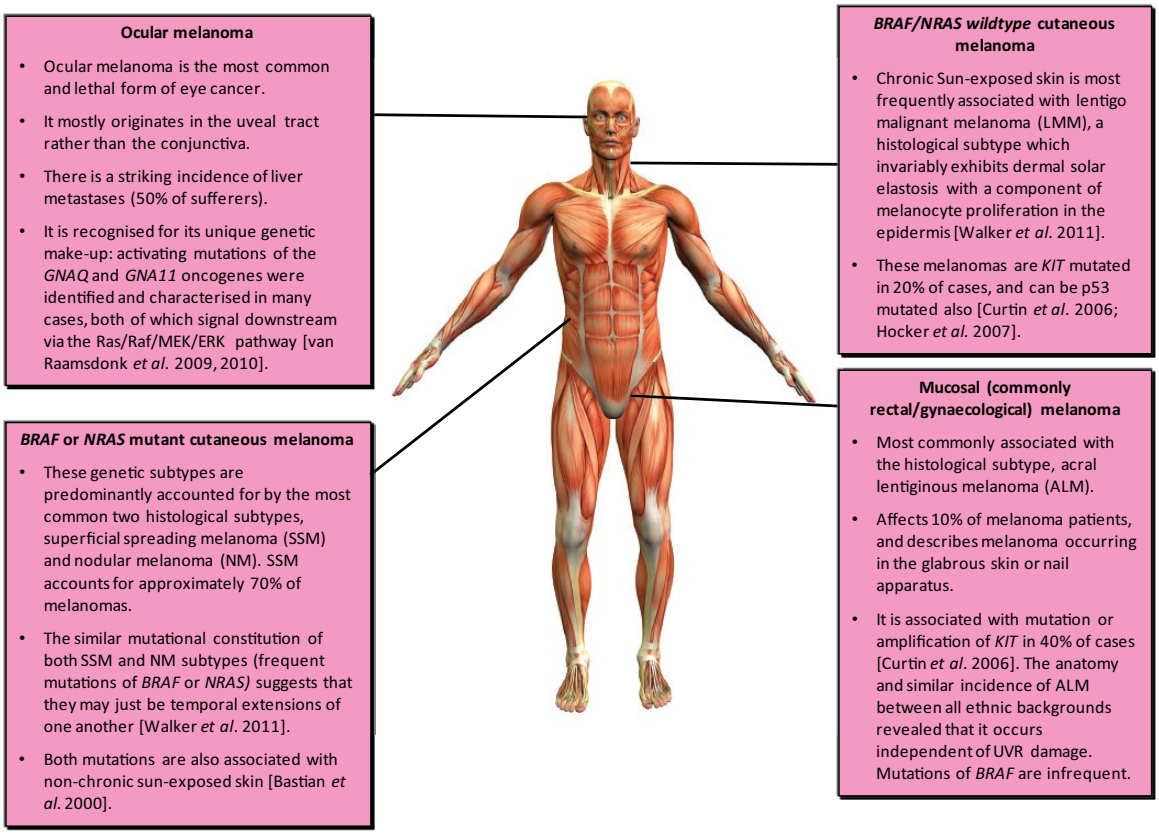

Figure 2. The emerging pathological landscape of melanoma: how traditional histological subtypes are molecularly characterised. More than in any other cancers, this genetic constitution has shaped melanoma treatment and may ultimately create pressure for an altered taxonomy by which it can be defined.
Picture of human body obtained from https://washhouseanatomy.wikispaces.com/The+Wonders+Of+The+Human+Body (accessed 26 July 2014).

aberrations of their disease. Key driver mutations of nearly all histological subtypes have been identified, and are readily being assessed for targeted therapy (Figure 2). It is clear that we are already beginning to realise the opportunities made available through the knowledge gained with genetic testing both before and after $Braf^{V600}$ inhibition. The story of developing MEK inhibition strategies after observing drug resistance mechanisms against BRAF inhibition reiterates the importance of understanding tumour biology prior to instigating the drug development of targeted agents.

Perhaps the most disappointing targeted therapeutic applied in melanoma trials is sorafenib, employed on the basis of its anti-VEGFR and anti-Raf activity: poor efficacy was seen in both monotherapy and combination trials [Hauschild *et al.* 2009; Ott *et al.* 2010; O'Brien *et al.* 2003; Eisen *et al.* 2006]. Initially, the use of imatinib in metastatic melanoma was also an example of poorly considered translational research, with a protracted length of development comparable with that of epidermal growth factor receptor (EGFR) inhibitors in lung cancer [Zhou *et al.* 2004]. Imatinib is a tyrosine kinase inhibitor which selectively targets BCR-Abl and KIT tyrosine kinases. It is best known for the excellent disease control it offers in treatment of advanced CML, where it targets the BCR-Abl, and GIST, where it targets KIT [Demetri *et al.* 2002; Blanke *et al.* 2008; Dematteo *et al.* 2009]. More recently, its role in the adjuvant treatment of GIST has also been confirmed [Joensuu *et al.* 2012, Curtin *et al.* 2006]. KIT is mutated or amplified in 20% of lentigo malignant melanoma and 40% of acral lentiginous melanoma, two subtypes which represent a significant minority of patients (Figure 2) [Wyman *et al.* 2006]. Three phase II trials of imatinib were initially performed in ungenotyped metastatic melanoma patients, with only 1 of 64 showing a partial treatment response [Ugurel *et al.* 2005; Kim *et al.* 2008; Guo *et al.* 2011]. After a

delay of 6 years, a fourth phase II trial assessed its use in metastatic melanoma patients whose cancers were predicatively selected for KIT mutation or amplification, showing a 23% response rate and 30% rate of disease stability (Figure 1, Table 1). The mutations and amplification state of patients resistant to imatinib were also clarified in this trial, offering further opportunities for re-iterative translational research [Wolchok, 2012].

Thus, our biological understanding of simple melanoma genetics was eventually acknowledged in imatinib clinical trial design, leading to its significant success. The portfolio of molecularly targeted therapies available for control of mucosal, as well as cutaneous, melanomas was also further expanded (Figure 2).

What is the treatment niche for immunotherapy?

Immunotherapy is another facet of recent clinical progress in metastatic melanoma, having been responsible for some of the more historical treatment approaches with a degree of early success too. This section is designed to give an overview of the key developments with immunotherapy use in advanced melanoma. There have been a number of trials over the past two decades, some of which we do not expect to comprehensively cover, but more detail is reviewed elsewhere [Rosenberg *et al.* 1993].

Clinical trials assessing the use of high dose interleukin-2 (IL-2) in advanced melanoma were reported in the 1990s (Figure 1) [Atkins *et al.* 1999; Hodi *et al.* 2010]. In general these trials showed that melanoma had a low response rate to treatment (16% objectively), but that this response was durable in a significant percentage of this minority: at follow up of 6 years, 44% of patients with a treatment response were still alive. Unfortunately many clinicians were forced to limit their use of high dose IL-2 due to concern over potentially serious pro-inflammatory side effects, which included problems such as hypotension, arrhythmia, pulmonary oedema and sepsis. Ultimately, with no clear predictive markers for treatment response forthcoming, it was often difficult to justify the risk of these toxicities to patients when they had a less than 1 in 5 chance of benefit.

Approximately 12 years after the approval of IL-2 by the US Food and Drug Administration (US FDA) in 1998, results from the use of ipilimumab in pretreated patients with advanced melanoma were reported [Hodi *et al.* 2010]. Ipilimumab is a monoclonal antibody which targets cytotoxic T-lymphocyte antigen-4 (CTLA-4), a negative regulator of the activated immune system which would normally prevent a T-cell response against melanoma. In a vaccine-controlled phase III trial of patients with metastatic melanoma, second-line administration of ipilimumab significantly extended median OS from 6.4 months to 10 months. Like IL-2, RR in the ipilimumab monotherapy cohort was relatively low at 10.9%, but 60% of these responders had persisting disease control at 2 years. Grade 3 and 4 immune-related adverse events secondary to ipilimumab (such as dermatitis, colitis) were as high as 10–15% and, more notably, there was a 2.1% rate of drug-related deaths.

First-line administration of ipilimumab with dacarbazine was also reported in a later phase III trial of patients with advanced melanoma (Table 1). The combination treatment led to a statistically significant median survival benefit of 2 months compared with dacarbazine monotherapy, albeit at the cost of significant toxicity with a 56% incidence of grade 3/4 toxicities, commonly in the form of hepatotoxicity. With a better understanding of the immune-related reactions and well-designed algorithms to manage them, drug-related deaths were avoided completely and a 4-year survival of 21.2% was achieved (95% CI 16.1–26.5) *versus* 12.1% with dacarbazine monotherapy (95% CI 8.1–16.3) [Schadendorf *et al.* 2013]. An overlap of the PFS curves was observed until week 12 of treatment, confirming once again that the benefit derived by immunotherapy occurs later than the one observed with conventional cytotoxic agents.

More recently, pooled analysis of survival among patients with advanced melanoma treated with ipilimumab monotherapy in either phase II or phase III trials demonstrated a 3-year survival rate of 22% which is further stratified as 20% for pretreated patients and 26% for treatment-naïve patients; OS with ipilimumab seems to reach a plateau at 3 years which extends to ten years [Schadendorf *et al.* 2013].

The main focus of immunotherapy for melanoma has now shifted to a molecule called programmed cell death 1 (PD-1). PD-1 is an inhibitory cell receptor protein that negatively regulates T lymphocyte

activation and their effector mechanisms, consequently inhibiting the immune response against cancer cells [Blank *et al.* 2004; Freeman *et al.* 2000]. Its ligands, PD-L1 and PD-L2, are not only expressed on the cell surface of antigen-presenting cells but on the surface of cancer cells too [Latchman *et al.* 2001; Topalian *et al.* 2014]. Targeting the PD-1/PD-L1/PD-L2 axis with antibodies against PD-1 such as nivolumab (MDX-1106; BMS 936558; ONO-4538) or lambrolizumab (MK-3475) has offered results that are more promising than anything observed with melanoma immunotherapy in early phase clinical trials before (Figure 1, Table 1). A 31% objective response rate was reported in advanced melanoma patients treated in a phase I/II trial of nivolumab, with an estimated median response duration of 2 years [Topalian *et al.* 2012]. A 22% incidence of grade 3/4 adverse events occurred amongst 107 patients with melanoma whereas interestingly all the drug-related mortalities (1%) were observed in the nonmelanoma cohorts of the wider trial; two NSCLC patients and one colorectal cancer patient – all attributed to immune-related pneumonitis [ClinicalTrials.gov identifier: NCT01721772]. A phase III study with nivolumab *versus* dacarbazine as first-line treatment in melanoma is underway [Hamid *et al.* 2013]. Similarly, early phase results with lambrolizumab with varying dosing schedules in 135 patients with advanced melanoma, including patients pretreated with ipilimumab, showed RR as high as 52% [Brahme *et al.* 2012]. This striking result has led the US FDA to designate it as a 'breakthrough therapy' prioritised for expedited development.

Moreover, PD-L1 blockade with BMS-936559, a PD-L1 specific, immunoglobulin G4 (IgG40 monoclonal antibody achieved up to 29% response rates and disease stability at 24 weeks for 27% of 52 patients with advanced melanoma participating in a phase I trial. At the same time, severe immune-related events frequently noted with CTLA-4 inhibition was relatively infrequent with anti-PD-L1 blockade [Wolchok *et al.* 2013].

Combination of CTLA-4 and PD-1 blockade was tested in a phase I study where ipilimumab and nivolumab were administered either concurrently or sequentially. Both regimens showed promising clinical activity, but more interestingly, the concurrent treatment achieved deep tumour regressions of more than 80% in 53% of patients who received the highest acceptable dose [Pardoll, 2012].

CTLA-4 and PD-1 are undoubtedly the 'godfathers' of immune checkpoints but a plethora of costimulatory (ICOS, CD137, OX-40) and co-inhibitory (BTLA, LAG3, TIM3) molecules have now been identified. Some of them are still in preclinical development, whereas others have already entered early phase clinical trials in cancer immunotherapy [Ribas *et al.* 2013]. Nevertheless, the activity of PD-1/PD-L1 blockade across a variety of tumour types, previously thought to be nonimmunogenic, will most likely usher in a new paradigm in cancer treatment altogether.

How immunotherapy fits in to the treatment plan for a new patient with metastatic melanoma remains open to question. Currently, second-line ipilimumab represents the main immunotherapy option in Europe available to all patients in the clinic, a context which allows for first-line administration of molecularly targeted treatment (e.g. vemurafenib) in $Braf^{V600}$ positive tumours, or chemotherapy in $Braf^{V600}$ negative tumours. The safety of combining immunotherapy with molecularly targeted agents is still being tested in early phase clinical trials, although concerns about high occurrence of grade 3 transaminitis have already been raised. Ribas and colleagues recorded high rates of grade 3 transaminase elevation even in patients who were treated with a 'lead-in' period of vemurafenib before the administration of ipilimumab [Ribas *et al.* 2013]; when the drugs were given concurrently, transaminitis could occur as fast as within 2 weeks of initiation of drugs [Pozanov *et al.* 2014]. This phenomenon of hepatotoxicity was not observed when vemurafenib was substituted by dabrafenib, even with the addition of tramenitib (ipilimumab + dabrafenib = trametinib), according to early data reported by Puzanov and colleagues, suggesting that a different class of BRAF inhibitors might be better tolerated in combination with ipilimumab [Ascierto *et al.* 2012].

How clinicians might choose between these two approaches will of course depend on the data produced, although it seems logical to conclude that for patients with mutated $Braf^{V600}$ melanoma, treatment could be dictated by the disease burden. Patients who are asymptomatic, with low volume or indolent disease and absent poor prognostic factors (such as elevated LDH), could possible derive more benefit from receiving upfront immunotherapy which characteristically offers a late onset and more durable response, with BRAF inhibition reserved in case of clinical/radiological

progression. On the other hand, patients with bulky or aggressive disease would be better served by the rapidly induced effects of a BRAF inhibitor as first-line treatment [Leyvraz and Keilholz, 2012].

What does the future hold?

A number of important topics are likely to shape the management of melanoma (and, as a consequence, other cancers) in the years to come. Anticipated advances with immunotherapy and *NRAS* mutant melanoma have already been described in the sections above.

Uveal melanoma (Figure 2)

Melanoma is the most common type of ocular cancer and is associated with high rates of liver metastases [van Raamsdonk *et al.* 2009]. This cancer is often driven through upregulation of the MAPK pathway although, unlike cutaneous melanoma, this process is almost never triggered by mutations of BRAF or NRAS. Pioneering work by Bastian and colleagues has now shown that mutations of two G proteins, GNAQ and GNA11, will drive the MAPK pathway in the majority of ocular melanoma cases [van Raamsdonk *et al.* 2010; McWilliams *et al.* 2008]. This adds to the detail we already know (described above and in Figure 2) on the various driver mutations implicated in cutaneous and mucosal melanoma. As is the case with NrasQ61-driven tumours, one would anticipate that the new generation of MEK inhibitors may offer a rational, biologically considered, treatment option in ocular melanoma. In the longer term, it would be surprising if a novel generation of 'orphan' drugs targeting mutant Gnaq/Gna11 were not developed.

Targeting brain metastases

Brain metastases are common in end-stage melanoma, a problem which is associated with aggressive disease and which confers a life expectancy measured in months. As is the case with chemotherapy, melanoma is mostly resistant to radiotherapy, traditionally the main treatment modality offered for this problem [Falchook *et al.* 2012b]. Patients with melanoma brain metastases have consequently been almost universally excluded from clinical trial eligibility, or at least been heavily restricted in their access (e.g. stable disease and stable dose of steroids for a period of time). However, an emerging picture of small molecule and immunotherapy efficacy against melanoma brain metastases suggests that this position is becoming untenable [Di Giacomo *et al.* 2012; Long *et al.* 2012; Margolin *et al.* 2012; Cancer Research UK, 2013b]. More brain metastasis specific clinical trials are necessary if the eligibility criteria for these patients are not to be relaxed.

Approaching the new patient

Molecular advances such as the ones described above have altered the nature of new patient consultations in the clinic. Previously a new patient could begin empirical chemotherapy almost immediately, whereas now they are often asked to 'sit tight' and wait for their genetic test results to come back. In this circumstance, of course there is no guarantee that the result will be positive, or that they won't end up on chemotherapy a few weeks later than they might have initially. This can be a difficult and anxious wait for patients who will often want to begin treatment as soon as possible. It could be a justifiable wait given the relative merits of novel small molecules inhibitors compared with chemotherapy. Wherever possible, although there is no positive adjuvant data with small molecule inhibitors in melanoma as yet, it seems sensible to aim for testing of relevant mutations (e.g. BRAF, NRAS, KIT) after primary melanomas have been curatively excised. This will save time later on for the unfortunate few who develop recurrent metastatic disease. An example of such an initiative is the Cancer Research UK Stratified Medicine Programme [Cancer Research UK, 2013b]. Routine postresection computerized tomography (CT) scans in early stage melanoma patients may also become important given the developing portfolio of metastatic treatment options: often there is concern that rapid clinical deterioration of patients, when metastases are left to be diagnosed by clinical presentation alone, may mean that the treatment window is missed.

Lessons for other cancers

Progress with melanoma has created a fresh impetus for other cancers to re-focus their efforts in generating rational molecularly driven trials. A clear example of progress in other cancers is with metastatic NSCLC, where EGFR inhibition has replaced first-line chemotherapy when patients are predictively selected for the presence of an EGFR mutation [Zhou *et al.* 2011; Rosell *et al.* 2012; Thatcher *et al.* 2005]. This treatment was transformed as a consequence, having spent 10

years considered as a second-line treatment with modest benefits in unselected NSCLC patients [Shepherd *et al.* 2005]. Compared with most other cancers, an unexpected advantage from the outset with molecularly targeted treatments in melanoma was that there was no significant standard of care to replace. The example of NSCLC suggests the inertia that can be involved in replacing historical chemotherapy with successful (and often expensive) biologically targeted drugs.

In most incidences of cancer, opportunities with these novel drugs are still all too rare, but a key and unavoidable challenge for the future will be developing bold clinical trials where new targeted drugs are compared with the traditional option of empirical chemotherapy on carefully selected cohort of patients using validated molecular biomarkers. This is a 'leap' that may sometimes be difficult to square when a patient is sitting in front of their oncologist in the clinic, but the progress seen in melanoma so far would suggest that the long-term gains from such an approach could be exponential.

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