From genes to drugs: targeted strategies for melanoma

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Abstract | The past decade has revealed that melanoma is comprised of multiple subclasses that can be categorized on the basis of key features, including the clinical stage of disease, the oncogenic molecular 'drivers', the anatomical location or the behaviour of the primary lesion and the expression of specific biomarkers. Although exercises in subclassification are not new in oncology, progress in this area has produced both conceptual and clinical breakthroughs, which, for melanoma, are unprecedented in the modern history of the disease. This Review focuses on these recent striking advances in the strategy of molecularly targeted approaches to the therapy of melanoma in humans.

Driver mutations
Sequence alterations in a cancer cell that influence the corresponding proteins to result in stimulation of cancerous activity within a cell.

Melanocyte
Melanin pigment-producing cell, usually located within the epidermis; the neoplastic transformation of this cell type gives rise to a nevus (benign) or melanoma (malignant).

Targeted therapy
A treatment designed to block a specific molecular species that is known to be functionally important.

Lineage-restricted oncogenes
Oncogenes the expression of which is limited to certain cell types.

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Although melanoma is among the most notoriously aggressive and treatment-resistant of human cancers, there has been a veritable explosion of recent progress in understanding melanoma and in exploiting this information for clinical benefit. As described in this Review, recent years have yielded the identification of multiple melanoma oncogenes, several of which seem to have been successfully targeted with small molecules. Improved understanding of immune tolerance checkpoints has led to additional therapeutic opportunities for patients. This remarkable progress has been born from the collective efforts of basic scientists and clinicians, working in both academia and industry.

None of the oncogenes or tumour suppressor genes identified in melanoma is thought to be deregulated in stand-alone events, although some are mutually exclusive. TABLE 1 provides a list of melanoma oncogenes and tumour suppressors based on extensively studied genetic aberrations. NRAS, which activates RAF kinases in response to growth factor receptor activation, harbours activating mutations in 15–20% of melanomas[1,2]. As BRAF mutations confer RAS-independent activation of the MAPK pathway, it is not surprising that BRAF-V600E and NRAS mutants are rarely found concomitantly[3,4]. The loss of expression of the p16 (also known as INK4A) tumour suppressor, by mutation, deletion or transcriptional silencing of the *CDKN2A* locus, is a fairly frequent event in melanoma[5]. There is significant overlap between BRAF mutation and $CDKN2A^{p16}$ deletion or mutation in melanoma[6]. *PTEN* mutation and deletion have only been described in a minority of melanomas, and these events also seem to coincide with BRAF mutation[3,6,7]. Although PTEN is known to regulate numerous cellular processes, one of its best-described functions is the inactivation of the PI3K pathway. Thus, it has been deduced that the coincidence of BRAF mutation and PTEN loss reflects the importance of these two RAS-effector pathways in melanoma, both of which would presumably reside downstream of RAS mutations. Less common genetic alterations, such as cyclin D1 (*CCND1*) amplification and cyclin-dependent kinase 4 (*CDK4*) mutation have also been identified in association with BRAF mutation, and are mutually exclusive with p16 loss[7–9]. Other genomic aberrations, such as amplification of microphthalmia-associated transcription factor (*MITF*), also represent oncogenic events, and are discussed in terms of the therapeutic targeting strategies that are indicated by their activities.

Targeting causal alterations in melanoma

Oncogenic driver mutations in melanoma have been recognized to reside within key signalling or developmental pathways that are central to the survival or the proliferation of the melanocyte lineage[10,11]. The most commonly observed recurrent mutations reside within the MAPK pathway, although numerous validated mutations have also been identified within non-MAPK pathway genes. Both categories of mutant oncogenes are subject to clinical investigation through targeted therapy approaches (TABLE 2). There seems to be a lineage-specific element to certain pathways that are able to transform melanocytes and that contribute to the robust metastatic potential of melanoma[12]. Examples of lineage-restricted oncogenes include *KIT* and *MITF*; whereas germline melanocortin 1 receptor (*MC1R*) variant alleles (which cause red hair) significantly predispose to melanoma.

At a glance

- Oncogenic mutations in melanoma are increasingly well categorized and are not stand-alone events.
- Several highly recurrent oncogenic mutations in melanoma occur within known signalling pathways. The most common of these is BRAF-V600E, which occurs in approximately 50% of melanomas.
- Targeted therapies seek to inhibit functionally causative oncoproteins and have shown substantial promise in recent months.
- Successful targeting of the BRAF-V600E or mutant KIT kinases has produced significant clinical responses in patients with advanced melanoma harbouring those mutations.
- Targeted inhibition of the immune tolerance checkpoint with a blocking antibody approach has produced significant clinical responses in patients with advanced melanoma.
- Permanent control of advanced melanoma remains uncommon for suppression of signalling or immune checkpoint targets. Improved strategies focus both on the development of new targeted therapeutics and on the analysis of combinations of these treatments.

Activating mutations or gene amplifications have been described in some of these developmentally important factors (FIG. 1). The most common oncogenic events, such as mutation of BRAF, constitutively activate common portions of pathways that do not intrinsically contain lineage-specific components. From a therapeutic perspective, targeting these non-lineage-specific pathways raises the general concern that pharmacological inhibitors might have a low therapeutic index. Opposing arguments, however, rely on 'oncogene addiction', whereby a tumour may exhibit a particular requirement for the maintained activity of the mutant oncoprotein but normal cells are less dependent on the same factor for cellular survival[13]. However, as discussed below, a variety of approaches, as well as drug-associated features, exist to enhance the therapeutic index of targeted anticancer therapies.

Transcription factors. MITF has been defined as the master regulator of melanocyte development, differentiation and pigmentation[14] (FIG. 1). Loss-of-function mutations of MITF result in the complete absence of the melanocyte lineage. Amplification of *MITF* has been identified in approximately 15% of melanomas[15]. Activation of the MAPK pathway, which occurs through mutational events in a high proportion of melanomas, has been shown to enhance the recruitment of the MITF transcriptional co-activator and histone acetylatransferase p300 (REF. 16) and to simultaneously accelerate turnover of MITF[17], at least partly through the activation of MAPK-mediated proteasomal degradation of MITF[18]. A recurrent activating point mutation in MITF has recently been discovered in cases of familial melanoma, and it seems to ablate a SUMO modification on MITF[19,190]. Evidence of its gain-of-function activity is the presence of non-blue eye colour in affected individuals[19]. Suppression of MITF expression has been shown to be lethal to most melanoma cell lines *in vitro*[20,21]. Because MITF is a lineage survival factor for melanocytes[14], and because many melanomas retain expression and dependence on MITF as a survival factor, it is plausible that therapeutic targeting of MITF may be beneficial even for melanomas that lack *MITF* amplification. However, a subset of melanomas may lose or downregulate MITF expression and would presumably be refractory to MITF-targeted strategies.

A pharmacological approach that suppresses MITF expression is the use of broad-spectrum histone deacetylase inhibitors[22] (HDACis), which were shown to potently suppress the expression of *MITF*, probably through the inhibition of SRY-box 10 (SOX10) expression, which occurs before MITF suppression on exposure to HDACis[23]. *CDK2* has been shown to be a vital target of MITF in melanoma[21], which raises the possibility of treating the subset of melanomas with *MITF* amplification and BRAF mutation using a CDK2 inhibitor in conjunction with a BRAF inhibitor. Although none of these approaches directly targets MITF, the prospect of modulating MITF activity remains attractive because of its potential lineage dependence — both in *MITF*-amplified and in non-*MITF*-amplified melanomas (which rely on MITF as a lineage-specific survival factor).

KIT. *KIT*-activating mutations or amplifications have been reported in ~20–25% of melanomas arising in mucosal, acral or chronically sun-damaged skin (which is determined by the pathological presence of solar elastosis)[24]. The most common melanoma KIT mutation is L576P, which is found in approximately one-third of cases[25]. The activation of this tyrosine kinase results in the stimulation of the MAPK and PI3K–AKT pathways, producing both proliferation and survival advantages[26]. Melanoma cells harbouring a vulnerable KIT mutation when exposed to the tyrosine kinase inhibitor imatinib exhibit potent suppression of melanoma cell proliferation, apoptosis and inhibitory effects on MAPK, PI3K–AKT, janus kinase (JAK)–signal transducer and activator of transcription (STAT) and anti-apoptotic pathways[27].

The clinical testing of imatinib in patients with melanoma has been described in a series of case reports and in a Phase II multi-institutional trial[24,28,29]. So far, responses have been limited to a subset of patients harbouring certain mutations in KIT[24]. It is currently unclear whether these limitations reflect varied capacity of the drug for targeting specific mutant alleles or varied tumour dependency for specific mutant *KIT* alleles. Additional multicentre Phase II trials are currently underway (TABLE 2) to evaluate agents that target KIT, including imatinib, sunitinib, nilotinib and dasatinib. Initial evidence suggests that a ratio of the presence of *KIT* mutant to wild-type alleles within a tumour greater than one predicts improved response[24], which suggests that amplification of wild-type *KIT* is not predictive of a favourable response to imatinib. The current clinical trials will help to assess whether differential sensitivities among the various KIT-targeting agents exist or correlate with particular KIT mutations. Potential mechanisms of resistance are also being actively investigated. In gastrointestinal stromal tumours (GISTs), the acquisition of additional KIT mutations is a common mechanism of resistance to KIT-targeted drugs[30]. Whether this is

Oncogene addiction
Tumour cell dependency on the molecular activity of an oncogene.

Mucosal
Referring to the cellular lining along internal cavities such as the gastrointestinal, genitourinary, oral or respiratory tracts.

Acral
Refers to hairless skin regions, such as palms and soles.

Solar elastosis
Sun-induced chronic damage to elastin and other connective tissue components within the dermis, typically seen in older people following chronic sun exposure.

Table 1 | **Oncogenes and tumour suppressors that are thought to be drivers of melanomagenesis***

Gene	Alteration	Frequency	Clinical subtypes of melanoma that are affected	Pathways affected by alteration	Refs
Kinases or signalling factors					
BRAF	Point mutation	50%	All types, but particularly superficial spreading and nodular melanoma	MAPK	39,7
NRAS	Point mutation	20%	All types, but particularly superficial spreading and nodular melanoma	MAPK, PI3K and RALGDS	7
KIT	Point mutation	1% overall	Acral lentiginous (10%), mucosal (10%) and, less commonly, lentigo maligna	MAPK and PI3K	177
CDK4	Point mutation or amplification	5%	All types	Cell cycle	96
CCND1	Amplification	10%	All types	Cell cycle	7
ERBB4	Point mutation	15–20%	All types	PI3K	178
AKT1, *AKT2* and *AKT3*	Point mutation or amplification	<1% point mutation; 25% amplification (*AKT3*)	All types	PI3K	86,179
NEDD9	Amplification	50–60%	All types	Scaffold protein	180
GNAQ	Point mutation	<1% overall	Uveal melanoma (40%)	PKC pathway	81
GNA11	Point mutation	<1% overall	Uveal melanoma (40%)	PKC pathway	81
Transcription factors					
MITF	Amplification	20%	All types	Melanocyte lineage and cell cycle	15
MYC	Amplification	20%	All types	Cell cycle	181
ETV1	Amplification	15%	All types	MITF	182
Tumour suppressors					
CDKN2A^{p16}	Point mutation or deletion	30%	All types	Cell cycle	183
TP53	Point mutation	5%	All types	Cell cycle	184
BAP1	Point mutation	<1% overall	Uveal melanoma (80%)	BRCA1	185
PTEN	Point mutation or deletion	50–60% point mutation or hemizygous deletion; 10% homozygous deletion	All types	PI3K	186

BAP1, BRCA1-associated protein 1; *CCND1*, cyclin D1; *CDK4*, cyclin-dependent kinase 4; *ETV1*, ets variant 1; *GNAQ*, guanine nucleotide-binding protein; *MITF*, microphthalmia-associated transcription factor; PKC, protein kinase C; RALGDS, RAL guanine nucleotide dissociation stimulator. *Oncogenes that function dominantly (by gain-of-function alterations, primarily mutation or amplification) are categorized as kinases, signalling factors or transcription factors. Tumour suppressors are listed separately.

true for KIT-mutant melanomas, or whether additional mechanisms are involved, such as amplification or the activation of alternative signalling factors, needs to be determined. There remains great enthusiasm for targeting KIT in mucosal, acral and chronically sun-damaged melanoma subtypes, as *KIT* is a known oncogene with validated inhibitors.

NRAS. NRAS mutations are found in a substantial subset of melanomas[2], including those arising from intermittently sun-exposed skin, but not exclusively so. Activating mutations in NRAS occur at either codon 12 or codon 61 in melanoma[31,32]. NRAS mutations and BRAF-V600E are thought to be mutually exclusive in most cases, which highlights the importance of each in melanoma pathophysiology: either is sufficient to constitutively activate the MAPK pathway, whereas NRAS is thought to simultaneously activate the PI3K pathway. Selective pharmacological inhibition of NRAS remains technically challenging because its GTPase activity has so far precluded the successful design of specific small-molecule antagonists. Small interfering RNA (siRNA)-mediated depletion of NRAS in melanoma cell lines inhibits proliferation and renders cells much more sensitive to chemotherapy[33]. Farnesyltransferase inhibitors (FTIs) were hoped to inhibit RAS activation by blocking farnesylation, a key post-translational modification of RAS[34,35] that is also common in many membrane-localized proteins. One FTI, R115777 (also known as tipifarnib), was evaluated in a single-agent, single-arm Phase II trial in patients with metastatic melanoma[36], but the lack of responses among the first 14 patients led to the early closure of the trial. Although these patients were unselected with regard to the presence or absence of NRAS mutations in their tumours (anticipated to occur in ~15%) a paucity of efficacy has been observed for this approach in other RAS-mutated malignancies. In the absence of more specific RAS inhibitors, it seems to be rational to investigate concomitant inhibition of RAS effector pathways.

Table 2 | **Drugs in clinical development for melanoma**

Oncogene or pathway	Pathway	Drug	Phase of clinical trial in melanoma	Key trial findings (or NCT listing if trial is not completed)
Drugs targeting the MAPK pathway				
KIT	Growth factor receptor	Imatinib	Phase II	Completed; objective responses observed and correlated with type of KIT mutation[24,187]
		Nilotinib	Phase III first-line	NCT01028222
			Phase II second-line	NCT01099514
		Dasatinib	Phase II	NCT00700882
BRAF	MAPK	Sorafenib (non-mutant-selective inhibitor)	Phase III	Completed; failed to meet primary end point[55,67]
		XL-281 (non-mutant-selective inhibitor)	Phase I	Completed; efficacy data in melanoma not yet reported[57]
		RAF-265 (non-mutant-selective inhibitor)	Phase I	NCT00095693
		PLX4032 (mutant-selective inhibitor)	Phase III	Completed; met overall survival primary end point[62,188]
		GSK2118436 (mutant-selective inhibitor)	Phase III	Completed; data maturing, not yet presented[60]
MEK	MAPK	AZD6244	Phase II	Completed; failed to demonstrate higher response rate in comparison to temozolomide[79]
		PD0325901	Phase I	Completed; further clinical development terminated[77]
		GSK1120212	Phase III	Completed; data maturing, not yet presented[80]
Drugs targeting MAPK-independent oncogenes and pathways				
NRAS	RAS	R115777	Phase II	Completed[36]
PI3K	PI3K	GDC0941	Preclinical	No ongoing trials in melanoma
		XL147	Preclinical	No ongoing trials in melanoma
AKT	PI3K	MK-2206	Preclinical	NCT01510444, in combination with AZD6244 (MEK inhibitor)
		GSK690693	Preclinical	No ongoing trials in melanoma
mTOR	PI3K	Temsirolimus	Phase II	Completed[89]
CDK2 (MITF)	Cell cycle	SCH 727965	Phase II	NCT00937937
CDK4	Cell cycle	PD032991	Preclinical	No ongoing trials in melanoma
		LY2835219	Phase I expansion	NCT01394016
HSP90	Protein chaperone	17-AAG	Phase II	Completed[70]
HDAC	Histone acetylation (transcriptional repression)	MS 275	Phase II	Completed[189]
		LBH589	Phase II	NCT01065467
Notch (γ-secretase)	Developmental	RO4929097	Planned Phase II	NCT01120275, single agent in stage IV melanoma NCT01196416, in combination with cisplatin, vinblastine and temozolomide NCT01216787, in patients with resected stage III/IV melanoma

CDK, cyclin-dependent kinase; HDAC, histone deacetylase; HSP90, heat shock protein 90; MITF, microphthalmia-associated transcription factor.

BRAF. BRAF is most highly expressed in neuronal tissues and melanocytes, as well as in testis and haematopoietic cells. MEK is the only known substrate of BRAF[37]. By contrast, RAF1 (also known as CRAF) is activated on RAS activation and can participate in signalling events outside the MEK–MAPK pathway[38]. Activating mutations in BRAF have been identified in approximately 50% of all melanomas, with the vast majority found in melanomas that arise from intermittently sun-exposed skin[7,39]. The most common of the *BRAF* mutations is the T1796A point mutation that results in the V600E substitution, which causes the protein to be in the active conformation; this accounts for nearly 90% of all the BRAF mutations found in clinical pathology samples, including non-melanomas[39]. All other putative oncogenic *BRAF* mutations occur in exon 11 and exon 15 (where the T1796A mutation also resides), which encodes the kinase domain. Not all BRAF mutations have been characterized, but those that have fall into two categories: those that cause RAS-independent activation of MEK

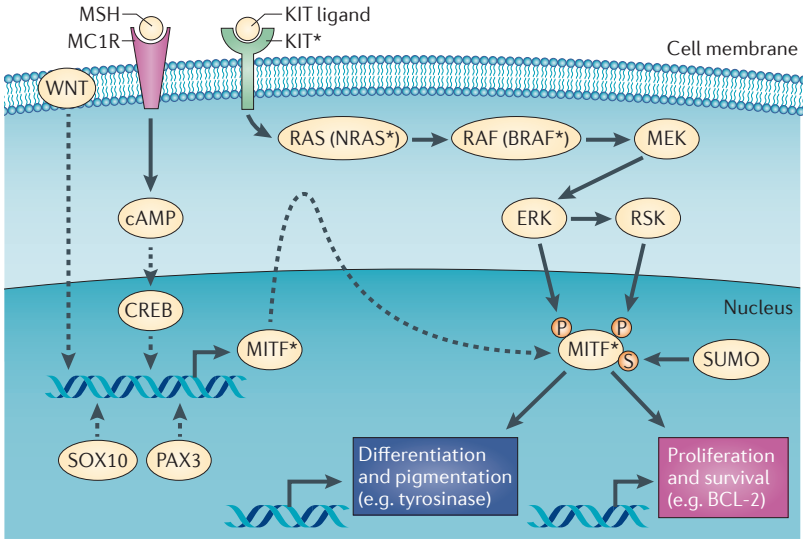

Figure 1 | **Melanocyte differentiation: the MITF axis.** The KIT ligand (also known as STEEL)–KIT receptor tyrosine kinase signalling pathway is essential for melanocyte development. Four genes encoding proteins in this pathway are known to be melanoma oncogenes: *KIT*, *NRAS*, *BRAF* and microphthalmia-associated transcription factor (*MITF*). The MITF transcription factor is phosphorylated (P) by MAPK–ERK signalling, as well as by ribosomal S6 kinases (RSKs). The expression of *MITF* is regulated by the melanocyte-stimulating hormone (MSH) pathway, the receptor of which is the G protein-coupled receptor melanocortin receptor 1 (MC1R; also known as MSHR) that is responsible for the red-hair variant phenotype associated with light pigmentation and elevated melanoma risk. Other pathways, such as WNT, contribute to MITF expression, and other post-translational modifications such as the addition of the SUMO peptide (S) are also known to regulate MITF activity. MITF-target genes seem to regulate both differentiation and pigmentation, and melanocyte proliferation and survival. Known mutated melanoma oncogenes are depicted with an asterisk (NRAS, BRAF and MITF). Dashed arrows indicate signalling pathways involving multiple proteins or subcellular translocation. cAMP, cyclic AMP; CREB, cAMP-responsive element-binding protein; PAX3, paired box 3; SOX10, SRY-box 10.

and ERK (including BRAF-V600E) and those that have a minimal ability to directly phosphorylate MEK, but that instead activate RAF1 directly[40]. Whereas the simultaneous occurrence of BRAF-V600E and NRAS mutations are exceptionally rare events in newly diagnosed melanomas, there is evidence for the co-occurrence of non-V600E BRAF mutations together with NRAS mutation[41]. The introduction through exogenous expression of BRAF-V600E into melanocytes induces nevus (which has features of senescence) formation, whereas concomitant mutation of p16 permits transformation *in vitro*, and concomitant deletion of *Pten* in mice or of *tp53* (which encodes p53) in zebrafish results in the formation of invasive and metastatic melanomas[42–45]. Concomitant induction of BRAF-V600E and deletion of the *Cdkn2a* locus, which encodes both p16 and ARF, also leads to the formation of invasive melanoma in mice[46]. Most preclinical studies investigating the potential therapeutic value of targeting BRAF have focused on BRAF-V600E. *In vitro*, the depletion of BRAF-V600E using siRNA decreases ERK activation, inhibits DNA synthesis and eventually induces some degree of apoptosis, whereas the depletion of *BRAF* mRNA in melanoma cell lines lacking a BRAF mutation had minimal effect[47]. Knockdown of RAF1 by siRNA lacked these effects, which is consistent with BRAF-V600E functioning as the dominant oncoprotein in melanoma[47–49]. Interestingly, in the setting of wild-type BRAF with upstream activation of the RAS pathway or NRAS mutation, inhibition of BRAF with selective inhibitors leads to increased RAF1 activity and downstream MAPK pathway activity[41,50,51]. This effect seems to involve the suppression of monomeric, catalytically active BRAF-V600E by the drug compared with its enhancement of wild-type RAF oligomerization in the context of upstream (RAS) pathway activation. *In vivo*, stably expressed BRAF siRNA in BRAF-V600E human melanoma cells transplanted into immunocompromised mice greatly slowed the growth of these xenografts, but did not completely abrogate tumour growth[49].

Sorafenib (also known as BAY 43-9006) was first selected for development as an inhibitor of RAF1 and is more than tenfold less potent against BRAF-V600E[52]. Sorafenib binds to RAF kinases in the inactive conformation[53]. *In vitro*, sorafenib inhibits BRAF-V600E at nanomolar concentrations, but it is only cytotoxic to melanoma cells that express BRAF-V600E at low micromolar concentrations, at which doses cells with wild-type BRAF are equally sensitive[48]. At the maximum tolerated dose, sorafenib was found to have significant single-agent activity in renal cell carcinoma but not in melanoma, presumably owing to the antagonism of vascular endothelial growth factor receptor (VEGFR) by this agent[54,55]. Mechanistic investigations suggest that sorafenib does not effectively inhibit ERK activation[56], thus more potent and selective RAF inhibitors have been developed for clinical applications in melanoma.

RAF-265 and XL281 are examples of broad-spectrum kinase inhibitors that have a greater potency and modestly improved selectivity for BRAF compared with sorafenib[57,58]. It remains to be seen whether the potency of these agents for BRAF will be sufficient to provide effective BRAF inhibition in patients. Meanwhile, several more highly selective BRAF inhibitors are being rapidly developed[59–61]. It has recently been reported that PLX4032 (also known as vemurafenib) extended the survival of patients with BRAF-V600E metastatic melanoma compared with dacarbazine[62]. PLX4032 was developed with the crystal structure of BRAF-V600E as the template in order to generate an inhibitor of the active conformation[63,64]. In kinase assays, PLX4032 and related analogues inhibited BRAF-V600E, wild-type BRAF and RAF1 at similar concentrations[65]. However, through BRAF-V600E suppression, PLX4032 inhibits the MAPK pathway and inhibits proliferation in melanoma cells while simultaneously stimulating wild-type RAF activity in the context of upstream pathway activation or mutation[41,50,51]. In a Phase I trial of PLX4032, ~70% of patients who were BRAF-V600E-positive had at least 30% tumour shrinkage[61]; a subsequent trial demonstrated similar efficacy, with approximately 5% of patients achieving complete response[61]. The observed median time to progression of 7 months nearly doubled that conferred by the standard-of-care[66,67].

In a Phase I trial with GSK2118436 (also known as dabrafenib), which also selected patients according to the presence of BRAF mutations, ten of 16 patients

Nevus
Benign pigmented lesion with features of senescence that can exhibit varying degrees of growth irregularity that may be concerning for transformation to melanoma. Melanocytic nevus refers to a benign pigmented lesion composed of nests of melanocytes.

treated at the two highest dose levels that were evaluated experienced objective responses[60]. These early studies established BRAF-V600E as a validated therapeutic target in melanoma despite the presence of numerous concomitant genetic alterations that contribute to the formation and maintenance of these tumours. Importantly, the more recent demonstration that single-agent treatment with PLX4032 produced an actual survival advantage (rather than purely tumour shrinkage)[62] probably contributed to the US Food and Drug Administration (FDA) approval of this drug for the treatment of advanced or unresectable melanoma. Although much more investigation is needed to understand how to achieve durable and/or complete responses, these data indicate that matching the relevant oncogene with a potent and selective antagonist can alter the viability and natural history of metastatic melanoma. Of note, several patients developed keratoacanthoma, a low-grade variant of squamous cell carcinoma of the skin, early in the course of therapy with PLX4032 or GSK2118436. The growth of these lesions during therapy seems to be a consequence of a stimulatory effect of selective BRAF inhibitors on the activity of the MAPK pathway in cells lacking BRAF mutations, in which upstream activation of the MAPK pathway has occured[41,50]. In keratoacanthomas this frequently arises in the context of activating RAS mutations[191]. Notably, 20–30% of cutaneous squamous cell carcinomas have HRAS mutations[68], so it is plausible that similar mutations may predispose to keratoacanthomas in the context of selective BRAF inhibitor therapy, particularly because these drugs are capable of activating RAF1 in the presence of upstream activating signals, such as receptor tyrosine kinase signalling or activating RAS mutations[41,50,51].

There is evidence that mutated oncogenes, such as BRAF-V600E, may be particularly dependent on chaperone proteins that control their folding or trafficking, such as heat shock protein 90 (HSP90)[69]. A first-generation, geldanamycin-derivative HSP90 inhibitor, tanespimycin, has been evaluated in a Phase II trial in melanoma. Activity was not clearly evident among 15 patients (nine with BRAF mutations and six with wild-type BRAF)[70], although additional analogues are early in clinical development and would still be of interest in melanoma.

A crucial area of active investigation is the discovery of mechanisms conferring resistance to BRAF-targeted therapies within BRAF-mutant melanomas[71–75]. As described in BOX 1, multiple recent studies have revealed escape mechanisms that rescue melanoma cell survival in this setting. Ongoing high-priority research is focusing on the discovery of additional mechanisms of resistance, as well as rapid preclinical and clinical testing of strategies to overcome such resistance.

MEK. MEK kinases, which function immediately downstream of BRAF, have been considered as a potential point of intervention in the MAPK pathway in BRAF- and NRAS-mutant melanoma. PD0325901 and AZD6244 (also known as selumetinib) are potent and selective inhibitors of MEK1 and MEK2 (REFS 76,77). *In vitro*, both inhibit the proliferation of melanoma cells expressing BRAF-V600E, with some activity against NRAS-mutant cells but little effect on BRAF and NRAS wild-type cells[76,78]. Whether this is a matter of residual MEK activity despite high concentration of the drug or due to the fact that BRAF-V600E is able to circumvent MEK inhibition by signalling through another pathway is unknown. In a Phase I trial of PD0325901, which included extensive investigation of target inhibition in serial tumour biopsies, significant reduction in ERK phosphorylation was noted in patients with metastatic melanoma, but BRAF mutation status was generally unknown in these patients. One patient with a BRAF-V600E mutation and one with an NRAS mutation demonstrated objective responses. This observation helped to establish the proof-of-concept that MEK could be an efficacious point of intervention. In a Phase II trial of single-agent AZD6244, six of 100 genetically unselected patients with metastatic melanoma experienced objective responses[79]. Retrospectively, 45 of these patients were found to have BRAF mutations, five of which were the responders (~11% response rate). It is unclear why only a small subset of BRAF-mutant patients responded, but a possibility is that there was insufficient target inhibition at the maximum tolerated dose. The most recently evaluated and apparently most active MEK inhibitor, GSK1120212 (also known as trametinib), produced objective responses in eight (40%) of the 20 patients with BRAF-mutant melanoma treated in this Phase I trial[80]. There was no clear evidence of activity among a small cohort of patients with metastatic melanoma harbouring activating mutations in NRAS who were treated in the same trials. Although this level of therapeutic activity validates MEK inhibition as a strategy in BRAF-mutant melanoma, the activity of these agents seems to be lower than that observed with a potent, selective BRAF inhibitor. It is unclear whether this reflects the underlying dependence of BRAF-V600E melanoma on BRAF–MEK signalling or whether these inhibitors are unable to achieve adequate target inhibition at clinically tolerable doses with the schedule of administration used.

G protein-coupled receptor signalling. Recently, activating mutations in two highly related G protein-coupled receptor (GPCR) α-subunit signalling molecules, guanine nucleotide-binding protein Q (GNAQ) and GNA11, have been described[81]. These seem to be found exclusively in approximately 70% to 80% of cases of uveal melanoma. It was previously known that KIT, NRAS and BRAF mutations do not occur in uveal melanoma, so this genetic discovery fills a large gap in knowledge regarding oncogenes in this particular clinical subtype. Like NRAS, these GPCRs are not ATPases, and thus lack domains that are readily amenable to pharmacological inhibition with standard small-molecule inhibitor strategies. GPCRs are known to activate numerous signal transduction pathways, including those already known to be relevant in other subtypes of melanoma, such as the MAPK and PI3K pathways. In preclinical systems, MEK inhibition resulted in cell cycle arrest in a subpopulation of GNAQ-mutant cells. Given the availability of MEK inhibitors for

G protein-coupled receptor (GPCR). Family of cell surface transmembrane proteins that are regulated by extracellular ligands to modulate intracellular signalling via interactions with cofactors, the interaction of which is mediated by guanine nucleotide molecules.

Uveal melanoma
Melanoma arising in one of three anatomic locations within the eye: the iris, the choroid or the ciliary body.

Box 1 | **Resistance mechanisms to targeted BRAF-V600E inhibition**

The emergence of NRAS-mutant clones
Co-occurrence of an activating NRAS mutation together with BRAF mutation is thought to occur rarely, if ever. However, selection during drug treatment of tumour cells containing simultaneous BRAF plus NRAS mutation is predicted to induce resistance to BRAF-targeted therapy; mutation of NRAS and BRAF-V600E have not yet been reported in the literature[73].

The amplification of *MAP3K8* (also known as *COT1*)
The *MAP3K8* kinase is overexpressed or genomically amplified in a subset of melanomas, and is capable of rescuing ERK pathway activation despite BRAF suppression by BRAF-targeted drugs[72].

The overexpression of platelet-derived growth factor (PDGF)
The PDGF–PDGF receptor pathway can be overactivated through changes in expression, and may rescue BRAF inhibition via alternative signalling around BRAF to activate the MAPK and/or PI3K pathways[73].

Increased insulin-like growth factor 1 receptor (IGF1R) signalling
Activation of this pathway may rescue BRAF suppression by activating MAPK or PI3K signalling[75].

The loss of PTEN and BCL-2-interacting mediator of cell death (BIM)
Intrinsic resistance to apoptosis on BRAF-V600E suppression occurs on loss of the PI3K antagonist *PTEN*. The mechanism involves regulated expression of the pro-apoptotic factor BIM[74].

MEK mutation
The presence of a mutation in MEK1 (downstream of BRAF) restores MAPK pathway activation and confers resistance to BRAF-targeted drugs[71].

Alterations to *BRAF*
Selection for an alternatively spliced variant of BRAF-V600E[192] and amplification of the mutant *BRAF* allele[193] confers resistance to BRAF inhibitors.

clinical evaluation (as noted above) clinical trials using them as single agents in uveal melanoma have been proposed (NCT01143402).

PI3K pathway. The loss of PTEN in a subset of melanomas, particularly some of those with BRAF mutations, eliminates a mechanism of negative regulation of AKT and downstream components of the PI3K pathway[82]. Loss of PTEN seems to participate in the formation of a subset of invasive melanomas[83]. Mice conditionally expressing BRAF-V600E in melanocytes developed benign melanocytic lesions that resembled nevi[44]. However, induction of BRAF-V600E and homozygous deletion of *Pten* resulted in the extremely rapid and efficient formation of invasive and metastatic melanomas. The independent therapeutic value of inhibiting the PI3K pathway in melanoma has not been well established, but a substantial body of preclinical evidence supports targeting this pathway as an important adjunct to MAPK pathway-targeted therapy. Both direct PI3K and mTOR inhibitors have been suggested to produce synergistic responses in combination with sorafenib or MEK inhibitors, but similar evidence has not yet been generated in combination with potent and selective BRAF inhibitors[84,85].

The optimal point of therapeutic intervention in this pathway is unclear. Recent evidence suggests that either AKT or glycogen synthase kinase 3β (GSK3β) are potentially promising targets within this pathway for melanoma[86,87] because specific inhibitors of either may be less likely to be associated with the same systemic toxicity as agents that inhibit the PI3K pathway at more upstream steps (such as direct PI3K inhibitors). The lack of PI3K and AKT inhibitors currently available for clinical investigation in melanoma has turned attention to mTOR, for which numerous inhibitors are in clinical development. In favour of this approach, rapamycin (also known as sirolimus) has been reported to inhibit the proliferation of melanoma cell lines and was suggested to demonstrate synergy with sorafenib[84,88]. However, a Phase II clinical trial with single-agent temsirolimus (a rapamycin analogue) resulted in only one objective response among 33 patients with melanoma and in the early closure of the study[89]. A Phase II trial combining sorafenib and temsirolimus is currently enrolling patients. mTOR inhibition also seems to sensitize melanoma cells to cytotoxic chemotherapy[90], but such a combination has not been clinically evaluated in melanoma. Although the MAPK pathway might represent an essential point of intervention in the treatment of melanoma, it is crucial that the potential relevance of blocking the PI3K pathway in conjunction with other therapeutic interventions is not overlooked. It is also of great importance to understand the mutational range of tumours that do (or do not) respond to such targeted approaches.

Cell cycle checkpoints. Deregulation of cell cycle checkpoints has been well described in melanoma[91]. Unlike other common solid tumours, genetic alterations of *TP53* (which encodes p53) and retinoblastoma (*RB1*) are infrequently observed in melanoma[92]. However, the apoptotic function of p53 is deficient in melanoma[5]. *CDKN2A*^{p16} deletions, mutations or silencing occur (collectively) at significant frequency, with consequent upregulation of CDK4 in both familial and sporadic melanoma[93]. Restoration of p16 expression in melanoma cell lines results in the phosphorylation of RB and decreased proliferation[94]. Pharmacological inhibition of cell cycle regulatory kinases, such as CDK4, which are deregulated when *CDKN2A*^{p16} is deleted, are thus being explored as possible therapeutic strategies[95].

The lack of p53-dependent apoptotic activity in melanoma has been suggested to arise from the loss of ARF, which is also encoded by the *CDKN2A* locus[5]. ARF suppresses the activity of MDM2, an antagonist of p53, and *MDM2* is also sometimes amplified in melanoma[96]. The development of agents that restore p53 activity is of great interest in melanoma. One strategy currently under exploration is the development of inhibitors that disrupt the interaction of p53 with MDM2 (REF. 97). Signal transduction inhibitors that modulate pathways that result in the downregulation of MDM2 expression may indirectly produce the same effect[87].

Angiogenesis. Melanomas are highly vascular tumours that frequently overexpress angiogenic factors, suggesting the plausibility of targeting angiogenic pathways clinically. VEGFA, platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF; also known as FGF2) and interleukin-8 (IL-8) have been linked to melanoma progression, although their precise association with prognosis is not fully understood[98–105]. As single agents, bevacizumab (an antibody that targets

Cell cycle checkpoints
Nodal points in the cell cycle that regulate the ability of the cyclin-dependent kinases to induce the progression through the phases of the cell cycle.

VEGFA) and the VEGFR inhibitors, sorafenib and axitinib, are associated with modest clinical activity in metastatic melanoma[55,106,107]. The most promising clinical results to date with single-agent anti-angiogenic therapies have been seen with axitinib and E7080 (also known as lenvatinib) — agents that are distinguished from other VEGFA and PDGF inhibitors by their potent inhibitory activity towards FGF receptor[107].

Targeting the immune system

Melanoma has been a major focus for the study of cancer immunotherapies owing to the occurrence of spontaneous regression in primary tumours, the association of tumour-infiltrating lymphocytes, and the detection of antigen-specific cytotoxic T cells and antibodies in patients with melanoma[108,109]. Several families of melanoma antigens have been identified as targets of cellular immune responses including, melanocyte differentiation antigens, such as melanoma antigen recognized by T cells 1 (MART1; also known as melan-A), gp100 (also known as SILV and PMEL), tyrosinase (TYR), tyrosinase-related protein 1 (TYRP1; also known as DHICA oxidase) and TYRP2 (also known as DCT); cancer-testis antigens that include the melanoma antigen (MAGE), B melanoma antigen (BAGE) and G antigen (GAGE) gene families, as well as NY-ESO-1 (also known as CTAG1); and mutated or overexpressed antigens with broad tissue distribution such as β-catenin, inhibitor of apoptosis (IAP) proteins and CDK4 (REFS 110–123). There are also hundreds of melanoma antigens that have been identified as the targets of humoral immune responses.

Enhancing melanoma immune responsiveness. Several antigen-specific vaccination strategies have been tested that include the use of peptide epitopes with strong affinity for particular human leukocyte antigens (HLAs; molecules that modulate immune responsiveness)[124], as well as the use of dendritic cells that are pretreated to take up antigenic peptides or that are engineered by gene transfer techniques to express and subsequently process antigen for presentation on their surface, which thereby enhances T cell activation against melanoma cells[125]. Dendritic cell vaccination can be divided into *ex vivo* and *in vivo* approaches[126]. The *ex vivo* approach is possible owing to the ability to manufacture large numbers of clinical-grade dendritic cells from haematopoietic stem and progenitor cells or from precursor cells that are extracted from human donors by pheresis; these cells are grown in the presence of granulocyte–macrophage colony stimulating factor (GM-CSF; also known as CSF2) for their expansion into dendritic cells[125,127–132]. For example, a Phase I trial that tested dendritic cells that were engineered to express peptides representing the antigens MART1, TYR, MAGE3 and gp100 included the immune adjuvants influenza matrix peptide and keyhole limpet haemocyanin in the therapy, and this was reported to be safe in 18 patients with metastatic melanoma. These patients had a median overall survival of 20 months, and four patients survived for 63 months or longer (although the precise relevance of these numbers is unclear owing to the lack of a control group in Phase I studies)[133]. Interestingly, patients who developed immune responses to more than one peptide epitope expressed on the dendritic cells experienced improved median survival (36 months versus 8 months; hazard ratio 6.3; $p<0.0001$). Additional methods to augment dendritic cell function in patients include the co-administration of CpG oligodeoxynucleotides (which are Toll-like receptor 9 (TLR9) agonists) or imiquimod (a TLR7 agonist), α-galactosylceramide (which promotes dendritic cell and natural killer T (NKT) cell interactions with tumour cells), fms-related tyrosine kinase 3 (FLT3) ligand or GM-CSF[134–141].

Using whole cells in vaccination strategies offers the ability to target immune responses for antigens that are either shared between patients or unique to each patient[142]. One approach uses genetically modified cells that are engineered to secrete GM-CSF[143], the action of which is dependent on coordinated antitumour immune responses mediated by $CD4^+$ T cells and $CD8^+$ T cells, CD1d-specific NKT cells, and tumour-specific antibodies[143–147]. Phase I trials of vaccination with irradiated, autologous melanoma cells that were engineered to secrete GM-CSF by retroviral- and adenoviral-mediated gene transfer in patients with metastatic melanoma demonstrated T cell and plasma cell infiltrates effecting tumour destruction in the majority of patients (determined by biopsies of pre-existing tumours)[148,149]. Approximately 30% of patients remain alive with a minimum follow-up of 40 months, and 10% of the treated patients have no evidence of disease following tumour harvest and completion of the vaccination regimen. To build on the extensive experience with vaccination strategies, combinatorial approaches should now be considered.

Immune checkpoint blockade. Cytotoxic T lymphocyte-associated antigen 4 (CTLA4) is essential for the natural development of regulatory T cells[150]. CTLA4 is a member of the immunoglobulin receptor superfamily and, when activated by ligand expressed on antigen-presenting cells, transmits an inhibitory signal, which results in diminished immune responsiveness (for example, suppression of autoimmune responsiveness). Antibodies that bind to the extracellular domains of CTLA4 and that block its inhibitory signalling can 'revive' antitumour immune responsiveness and can eliminate tumours in a variety of immunogenic tumour models[151]. The combination of CTLA4 blockade and vaccination with irradiated tumour cells that were engineered to overexpress GM-CSF resulted in improved efficacy against B16 melanoma and SM1 breast carcinoma xenograft tumours compared with either CTLA4 blockade or the tumour vaccine alone. The combination treatment was capable of eliminating small pre-existing tumours while also protecting against subsequent tumour engraftment[152]. This impressive antitumour immunity, however, was associated with a loss of tolerance to normal melanocytes, with approximately 50% of mice developing progressive fur depigmentation (vitiligo).

CTLA4 antibody-mediated blockade has been tested in a number of clinical trials (TABLE 3). In a study of 14 HLA-A*0201 patients with stage IV melanoma, the MDX-010 antibody (also known as ipilimumab)

Humoral immune responses
Immune responses mediated by antibodies.

Pheresis
Removal of a blood component, as in removal of autologous dendritic cells (antigen-presenting cells), which may be used for adoptive transfer.

Hazard ratio
The effect of a variable on the hazard (or risk) of an event occurring.

Autologous
Pertaining to the host.

Table 3 | **Immunotherapy drugs in clinical development for melanoma**

Target	Drug	Phase of clinical trial in melanoma	Key trial findings (or NCT listing if trial is not completed)
CTLA4	• Ipilimumab • Tremelimumab	Phase III	Completed; improved survival compared with peptide vaccine as second line therapy; ipilimumab combined with dacarbazine chemotherapy improved survival compared with dacarbazine alone in previously untreated patients; tremelimumab did not confer a survival difference compared with chemotherapy[161,162]
PD1	• BMS 936558 (MDX-1106; ONO-4538) • MK-3475 (SCH900475) • CT-011	• Phase I/II • Phase II in selected cancers	Ongoing: activity seen, data maturing; Phase II in development (NCT00730639, NCT01295827 and NCT01435369)
PDL1	BMS-936559 (MDX-1105-01)	Phase I	Ongoing; not yet presented (NCT00729664)
4-1BB activation	BMS-663513	Phase II	Completed Phase II; development on hold per company
GITR activation	TRX518	Phase I	Development on hold
CD40 activation	CP-870,893	Phase I/II	Phase I completed[174]
OX40	OX40 agonsist (Portland Providence Medical Center)	Phase I	Ongoing (NCT01303705 and NCT01416844)

CTLA4, cytotoxic T lymphocyte-associated antigen 4; GITR, glucocorticoid-induced TNF receptor-related gene; PD1, programmed cell death protein 1; PDL1, PD1 ligand 1.

— which targets CTLA4 — was administered with vaccination of modified gp100 peptides emulsified in incomplete Freund's adjuvant[153]. Two patients experienced tumour shrinkage to an undetectable degree, and one patient experienced partial shrinkage. However, six patients experienced significant autoimmune toxicities that included inflammation of the large intestine, pituitary gland, liver and skin. Interestingly, all of the patients who experienced disease regression also experienced significant autoimmune toxicities. Similar autoimmune events have subsequently been reported in several clinical studies[154,155] in addition to autoimmune complications of the liver, skin and kidney. Gastrointestinal inflammation is the predominant autoimmune event, with an incidence of approximately 21%[156]. As a result, it has been suggested that an association exists between clinically significant autoimmunity and antitumour immune responses, both of which are reversible when treatment stops and which sometimes require therapeutic intervention, such as the use of steroids. Serious adverse events typically limit continued treatment but, despite side effects that necessitate stopping treatment, patients may still receive a benefit in treating their cancer.

Phase I, II and III clinical trials have been completed with human monoclonal antibodies to CTLA4 (ipilimumab and tremelimumab). Currently, published reports demonstrate overall response rates (partial or complete) of 8–18% using standard clinical staging criteria. Responses can be delayed compared with those observed with traditional cytotoxic therapies, but are frequently durable[156–160].

Building on these earlier clinical observations, a large Phase III study of ipilimumab administered to previously treated patients with metastatic melanoma was recently reported[161]. Patients were randomized to receive ipilimumab plus a gp100 peptide vaccine, ipilimumab alone or the gp100 vaccine alone. Ipilimumab, either alone or with the vaccine, improved overall survival to a median of approximately 10 months, compared with 6.4 months in the gp100 vaccine-alone group. The majority of patients experienced an adverse event, with 10% to 15% experiencing major immune-related adverse events that were dominated by rash and diarrhoea. An additional Phase III study recently compared the effectiveness of CTLA4 blockade in combination with the DNA-damaging chemotherapy drug dacarbazine[162]. The combination improved survival with a similar adverse event profile to that observed in previous studies. Interestingly, the incidence of elevated liver function tests was higher but gastrointestinal events were less frequent than had been previously reported[162]. Additional combination trials with ipilimumab should be forthcoming, including combination with BRAF-targeted therapy. Ipilimumab has recently been approved by the FDA for the treatment of metastatic melanoma.

Strategies to further improve antitumour immune responses focus on the enhancement of tumour antigen presentation, the inhibition of immune regulation and the amplification of T cell effector functions. The studies of targeting dendritic cells and immunomodulation with CTLA4 blockade will soon be expanded to include other manipulations of the immune system that target negative regulation (TABLE 3). Examples include antibodies to programmed cell death protein 1 (PD1) and one of its ligands PDL1 (REFS 163–165), glucocorticoid-induced TNFR-related protein (GITR; also known as TNFRSF18)[166], inducible T cell co-stimulator (ICOS)[167] and CD25 (also known as IL-2Rα)[168] (FIG. 2). PD1 has been shown to be an important modulator of tumour immune responses in preclinical models, and is another member of the immune checkpoint. PDL1 is highly expressed in tumour cells from several cancer types, including melanoma and renal cell carcinoma. This is important for the immune regulation that occurs within the tumour microenvironment and is an area of current clinical investigation. CD25 is one marker of regulatory immune cells. GITR and ICOS are additional signalling members that influence T cell potentiation and are of interest for future clinical development, as well as strategies to counteract

Freund's adjuvant
A water-oil emulsion (to which *Mycobacterium Tuberculosis* is sometimes added, complete Freund's adjuvant), which may potentiate immune responses when incorporated into a vaccine.

Immune checkpoint
Nodal point within signalling pathways that modulates the ability of the immune system to mount a robust response against a specific antigen or group of antigens.

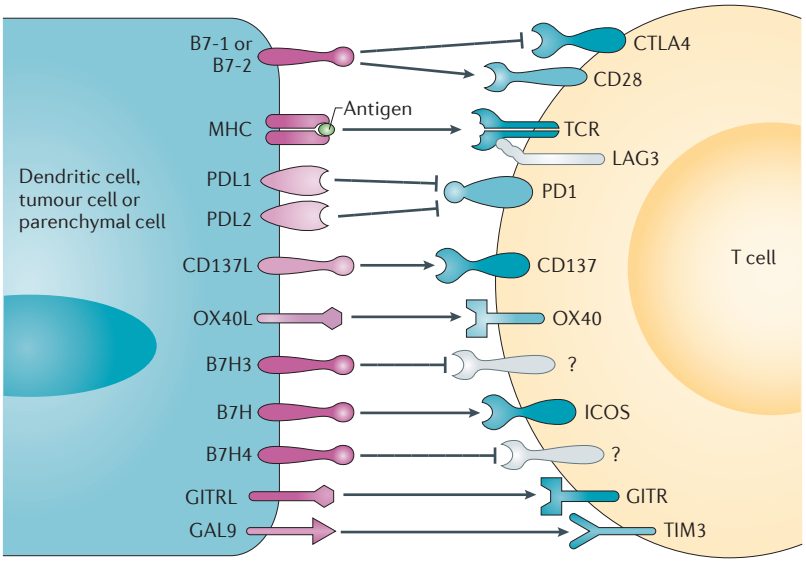

Figure 2 | **Immunomodulatory signalling.** Cell surface molecular interactions provide numerous possibilities for influencing the activity of T cells against tumour cells. Key defined regulatory interactions are shown. Many of these are currently being targeted or are planned for clinical development with therapeutic intent. CTLA4, cytotoxic T lymphocyte-associated antigen 4; GAL9, galectin 9; GITR, glucocorticoid-induced TNFR-related protein; GITRL, GITR ligand; ICOS, inducible T cell co-stimulator; LAG3, lymphocyte activation gene 3; MHC, major histocompatibility complex; PD1, programmed cell death protein 1; PDL, PD1 ligand; TCR, T cell receptor; TIM3, T cell membrane protein 3.

tumour-associated myeloid suppressor cells through the manipulation of reactive oxygen species (ROS). ROS promote the formation of myeloid suppressor cells that suppress antitumour immune responses in the tumour microenvironment[169]. Furthermore, strategies to potentiate previously described immune modulation include NKT cell agonists such as α-galactosylceramide[170], agonist antibodies to co-stimulatory molecules such as 4-1BB (also known as CD137 and TNFRSF9)[171,172], CD40 (also known as TNFRSF5)[173,174], CD28 (REF. 175) and OX40 (also known as TNFRSF4)[176]. Along with the experience of infusing *ex vivo* manufactured autologous antigen-specific T cells in patients through adoptive transfer protocols, the ability to rationally manipulate a multitude of immune modulatory mechanisms will have great clinical potential.

Myeloid suppressor cells
Cells of the myeloid (granulocytic) lineage that inhibit immune responsiveness and may limit antitumour immunity.

Adoptive transfer
A therapeutic strategy consisting of the removal of cells (typically immune cells), *ex vivo* modulation (such as population expansion) and the re-infusion of cells.

Conclusions

Although advanced melanoma remains a devastating disease, substantial progress has been made in identifying the contributing oncogenes and targeted small-molecule inhibitors. Targeted therapies directed against BRAF-V600E and mutant KIT have, in particular, produced major clinical responses in a fairly predictable manner. However, these responses are not typically complete or durable. Thus, major effort is needed to identify the mechanisms of resistance and to further target them. Combination approaches are also needed, and the choices of drug combinations are vast. Combinations that include the PI3K pathway or MEK are already underway or are in advanced stages of preparation. Other combinations will include targeted agents plus immune checkpoint blockade or the addition of anti-angiogenic factors. Other attractive combination components will include anti-apoptotic factors or molecules that target lineage-specific survival and signalling pathways. Agents that exhibit significant activity should be tested in earlier stage patients in order to prevent, rather than treat, metastatic disease. Mechanisms of resistance to immunotherapies need to be better understood and overcome. Despite the significant activity observed with targeted agents in melanoma, converting these transient and exciting — but incomplete — clinical responses into more predictable cures remains a major challenge. Perhaps targeted agents directed against previously 'undruggable' targets such as transcription factors may serve as attractive combination partners. Although the conversion of transient remissions to stable cures remains a primary goal for melanoma clinical investigators, the newly observed efficacy of these targeted approaches represents unprecedented progress and suggests that a new era has started for the treatment of melanoma.

1. Padua, R. A., Barrass, N. & Currie, G. A. A novel transforming gene in a human malignant melanoma cell line. *Nature* **311**, 671–673 (1984).
2. van 't Veer, L. J. *et al.* N.-ras mutations in human cutaneous melanoma from sun-exposed body sites. *Mol. Cell. Biol.* **9**, 3114–3116 (1989).
3. Tsao, H., Goel, V., Wu, H., Yang, G. & Haluska, F. G. Genetic interaction between NRAS and BRAF mutations and PTEN/MMAC1 inactivation in melanoma. *J. Invest. Dermatol.* **122**, 337–341 (2004).
4. Goel, V. K., Lazar, A. J., Warneke, C. L., Redston, M. S. & Haluska, F. G. Examination of mutations in BRAF, NRAS, and PTEN in primary cutaneous melanoma. *J. Invest. Dermatol.* **126**, 154–160 (2006).
5. Yang, G., Rajadurai, A. & Tsao, H. Recurrent patterns of dual RB and p53 pathway inactivation in melanoma. *J. Invest. Dermatol.* **125**, 1242–1251 (2005).
6. Daniotti, M. *et al.* BRAF alterations are associated with complex mutational profiles in malignant melanoma. *Oncogene* **23**, 5968–5977 (2004).
7. Curtin, J. A. *et al.* Distinct sets of genetic alterations in melanoma. *N. Engl. J. Med.* **353**, 2135–2147 (2005). **This manuscript helped to establish the presence of mutually exclusive, anatomically distinct subsets of melanomas that are driven by different oncogenes.**
8. Smalley, K. S. *et al.* Increased cyclin D1 expression can mediate BRAF inhibitor resistance in BRAF V600E-mutated melanomas. *Mol. Cancer Ther.* **7**, 2876–2883 (2008).
9. Smalley, K. S. *et al.* Identification of a novel subgroup of melanomas with KIT/cyclin-dependent kinase-4 overexpression. *Cancer Res.* **68**, 5743–5752 (2008).
10. Topczewska, J. M. *et al.* Embryonic and tumorigenic pathways converge via Nodal signaling: role in melanoma aggressiveness. *Nature Med.* **12**, 925–932 (2006).
11. Care, A. *et al.* HOXB7 constitutively activates basic fibroblast growth factor in melanomas. *Mol. Cell. Biol.* **16**, 4842–4851 (1996).
12. Gupta, P. B. *et al.* The melanocyte differentiation program predisposes to metastasis after neoplastic transformation. *Nature Genet.* **37**, 1047–1054 (2005).
13. Weinstein, I. B. & Joe, A. K. Mechanisms of disease: oncogene addiction—a rationale for molecular targeting in cancer therapy. *Nature Clin. Pract Oncol.* **3**, 448–457 (2006).
14. Levy, C., Khaled, M. & Fisher, D. E. MITF: master regulator of melanocyte development and melanoma oncogene. *Trends Mol. Med.* **12**, 406–414 (2006).
15. Garraway, L. A. *et al.* Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. *Nature* **436**, 117–122 (2005). **This study identified genomic amplifications of *MITF*, a lineage-specific regulator of melanocyte development, as an oncogenic event in a subset of melanomas.**
16. Price, E. R. *et al.* Lineage-specific signaling in melanocytes. C-kit stimulation recruits p300/CBP to microphthalmia. *J. Biol. Chem.* **273**, 17983–17986 (1998).
17. Wellbrock, C. *et al.* Oncogenic BRAF regulates melanoma proliferation through the lineage specific factor MITF. *PLoS ONE* **3**, e2734 (2008).
18. Wu, M. *et al.* c-Kit triggers dual phosphorylations, which couple activation and degradation of the essential melanocyte factor Mi. *Genes Dev.* **14**, 301–312 (2000).
19. Yokoyama, S. *et al.* A novel recurrent mutation in *MITF* predisposes to familial and sporadic melanoma. *Nature* **480**, 99–103 (2011).
20. McGill, G. G. *et al.* Bcl2 regulation by the melanocyte master regulator Mitf modulates lineage survival and melanoma cell viability. *Cell* **109**, 707–718 (2002).
21. Du, J. *et al.* Critical role of CDK2 for melanoma growth linked to its melanocyte-specific transcriptional regulation by MITF. *Cancer Cell* **6**, 565–576 (2004).

22. Yokoyama, S. *et al.* Pharmacologic suppression of MITF expression via HDAC inhibitors in the melanocyte lineage. *Pigment Cell Melanoma Res.* **21**, 457–463 (2008).
23. Yokoyama, S., Salma, N. & Fisher, D. E. MITF pathway mutations in melanoma. *Pigment Cell Melanoma Res.* **22**, 376–377 (2009).
24. Carvajal, R. D. *et al.* KIT as a therapeutic target in metastatic melanoma. *JAMA* **305**, 2327–2334 (2011).
25. Beadling, C. *et al.* KIT gene mutations and copy number in melanoma subtypes. *Clin. Cancer Res.* **14**, 6821–6828 (2008).
26. Webster, J. D., Kiupel, M. & Yuzbasiyan-Gurkan, V. Evaluation of the kinase domain of c-KIT in canine cutaneous mast cell tumors. *BMC Cancer* **6**, 85 (2006).
27. Jiang, X. *et al.* Imatinib targeting of KIT-mutant oncoprotein in melanoma. *Clin. Cancer Res.* **14**, 7726–7732 (2008).
28. Hodi, F. S. *et al.* Major response to imatinib mesylate in KIT-mutated melanoma. *J. Clin. Oncol.* **26**, 2046–2051 (2008).
29. Lutzky, J., Bauer, J. & Bastian, B. C. Dose-dependent, complete response to imatinib of a metastatic mucosal melanoma with a K642E KIT mutation. *Pigment Cell Melanoma Res.* **21**, 492–493 (2008).
30. Antonescu, C. R. The GIST paradigm: lessons for other kinase-driven cancers. *J. Pathol.* **223**, 251–261 (2011).
31. Stevens, C. W., Manoharan, T. H. & Fahl, W. E. Characterization of mutagen-activated cellular oncogenes that confer anchorage independence to human fibroblasts and tumorigenicity to NIH 3T3 cells: sequence analysis of an enzymatically amplified mutant HRAS allele. *Proc. Natl Acad. Sci. USA* **85**, 3875–3879 (1988).
32. Yuasa, Y. *et al.* Mechanism of activation of an N-ras oncogene of SW-1271 human lung carcinoma cells. *Proc. Natl Acad. Sci. USA* **81**, 3670–3674 (1984).
33. Eskandarpour, M. *et al.* Suppression of oncogenic NRAS by RNA interference induces apoptosis of human melanoma cells. *Int. J. Cancer* **115**, 65–73 (2005).
34. Yao, K. *et al. In vitro* hypoxia-conditioned colon cancer cell lines derived from HCT116 and HT29 exhibit altered apoptosis susceptibility and a more angiogenic profile *in vivo*. *Br. J. Cancer* **93**, 1356–1363 (2005).
35. Sebti, S. M. Protein farnesylation: implications for normal physiology, malignant transformation, and cancer therapy. *Cancer Cell* **7**, 297–300 (2005).
36. Gajewski, T. K. *et al.* Phase II study of the farnesyltransferase inhibitor R115777 in advanced melanoma: CALGB 500104. *J. Clin. Oncol. Abstr.* **24**, 8014 (2006).
37. Dhomen, N. & Marais, R. New insight into BRAF mutations in cancer. *Curr. Opin. Genet. Dev.* **17**, 31–39 (2007).
38. Alavi, A., Hood, J. D., Frausto, R., Stupack, D. G. & Cheresh, D. A. Role of Raf in vascular protection from distinct apoptotic stimuli. *Science* **301**, 94–96 (2003).
39. Davies, H. *et al.* Mutations of the BRAF gene in human cancer. *Nature* **417**, 949–954 (2002).
This publication first identified the occurrence of activating mutations in BRAF in melanomas (and in other malignancies).
40. Smalley, K. S. *et al.* CRAF inhibition induces apoptosis in melanoma cells with non-V600E BRAF mutations. *Oncogene* **28**, 685–694 (2009).
41. Heidorn, S. J. *et al.* Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. *Cell* **140**, 209–221 (2010).
42. Michaloglou, C. *et al.* BRAFE600-associated senescence-like cell cycle arrest of human naevi. *Nature* **436**, 720–724 (2005).
43. Wellbrock, C. *et al.* V599EB-RAF is an oncogene in melanocytes. *Cancer Res.* **64**, 2338–2342 (2004).
44. Dankort, D. *et al.* Braf(V600E) cooperates with Pten loss to induce metastatic melanoma. *Nature Genet.* **41**, 544–552 (2009).
45. Patton, E. E. *et al.* BRAF mutations are sufficient to promote nevi formation and cooperate with p53 in the genesis of melanoma. *Curr. Biol.* **15**, 249–254 (2005).
46. Dhomen, N. *et al.* Oncogenic Braf induces melanocyte senescence and melanoma in mice. *Cancer Cell* **15**, 294–303 (2009).
47. Hingorani, S. R., Jacobetz, M. A., Robertson, G. P., Herlyn, M. & Tuveson, D. A. Suppression of BRAF(V599E) in human melanoma abrogates transformation. *Cancer Res.* **63**, 5198–5202 (2003).
48. Karasarides, M. *et al.* B-RAF is a therapeutic target in melanoma. *Oncogene* **23**, 6292–6298 (2004).
49. Sumimoto, H. *et al.* Inhibition of growth and invasive ability of melanoma by inactivation of mutated BRAF with lentivirus-mediated RNA interference. *Oncogene* **23**, 6031–6039 (2004).
50. Poulikakos, P. I., Zhang, C., Bollag, G., Shokat, K. M. & Rosen, N. RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. *Nature* **464**, 427–430 (2011).
51. Hatzivassiliou, G. *et al.* RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. *Nature* **464**, 431–435 (2010).
52. Wilhelm, S. M. *et al.* BAY 43–9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res.* **64**, 7099–7109 (2004).
53. Wilhelm, S. *et al.* Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. *Nature Rev. Drug Discov.* **5**, 835–844 (2006).
54. Ratain, M. *et al.* Preliminary antitumor activity of BAY 43–9006 in metastatic renal cell carcinoma and other advanced refractory solid tumors in a phase II Randomized Discontinuation Trial (RDT). *J.Clin. Oncol. Abstr.* **22**, 4501 (2004).
55. Eisen, T. *et al.* Sorafenib in advanced melanoma: a Phase II randomised discontinuation trial analysis. *Br. J. Cancer* **95**, 581–586 (2006).
56. Flaherty, K. T. *et al.* Phase I/II, pharmacokinetic and pharmacodynamic trial of BAY 43–9006 alone in patients with metastatic melanoma. *J. Clin. Oncol. Abstr.* ***2005***, 3037 (2005).
57. Schwartz, G. K. *et al.* A phase I study of XL281, a selective oral RAF kinase inhibitor, in patients (Pts) with advanced solid tumors. *J. Clin. Oncol. Abstr.* **27**, 3513 (2009).
58. Venetsanakos, E. *et al.* CHIR-265, a novel inhibitor that targets B-Raf and VEGFR, shows efficacy in a broad range of preclinical models. *Proc. Am. Assoc. Cancer Res. Abstr.* **47**, 4854 (2006).
59. Sala, E. *et al.* BRAF silencing by short hairpin RNA or chemical blockade by PLX4032 leads to different responses in melanoma and thyroid carcinoma cells. *Mol. Cancer Res.* **6**, 751–759 (2008).
60. Kefford, R. *et al.* Phase I/II study of GSK2118436, a selective inhibitor of oncogenic mutant BRAF kinase, in patients with metastatic melanoma and other solid tumors. *J. Clin. Oncol. Abstr.* **28**, 8503 (2010).
61. Flaherty, K. T. *et al.* Inhibition of mutated, activated BRAF in metastatic melanoma. *N. Engl. J. Med.* **363**, 809–819 (2010).
This study demonstrated major clinical activity for PLX4032 in its first evaluation in humans.
62. Chapman, P. B. *et al.* Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N. Engl. J. Med.* **364**, 2507–2516 (2011).
This clinical study demonstrated enhanced survival features conferred by PLX4032 compared with dacarbazine in patients with advanced melanoma.
63. Xie, P. *et al.* The crystal structure of BRAF in complex with an organoruthenium inhibitor reveals a mechanism for inhibition of an active form of BRAF kinase. *Biochemistry* **48**, 5187–5198 (2009).
64. King, A. J. *et al.* Demonstration of a genetic therapeutic index for tumors expressing oncogenic BRAF by the kinase inhibitor SB-590885. *Cancer Res.* **66**, 11100–11105 (2006).
65. Tsai, J. *et al.* Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. *Proc. Natl Acad. Sci. USA* **105**, 3041–3046 (2008).
66. Middleton, M. R. *et al.* Randomized phase III study of temozolomide versus dacarbazine in the treatment of patients with advanced metastatic malignant melanoma. *J. Clin. Oncol.* **18**, 158–166 (2000).
67. Hauschild, A. *et al.* Results of a phase III, randomized, placebo-controlled study of sorafenib in combination with carboplatin and paclitaxel as second-line treatment in patients with unresectable stage III or stage IV melanoma. *J. Clin. Oncol.* **27**, 2823–2830 (2009).
68. Corominas, M., Kamino, H., Leon, J. & Pellicer, A. Oncogene activation in human benign tumors of the skin (keratoacanthomas): is HRAS involved in differentiation as well as proliferation? *Proc. Natl Acad. Sci. USA* **86**, 6372–6376 (1989).
69. da Rocha Dias, S. *et al.* Activated B-RAF is an Hsp90 client protein that is targeted by the anticancer drug 17-allylamino-17-demethoxygeldanamycin. *Cancer Res.* **65**, 10686–10691 (2005).
70. Solit, D. B. *et al.* Phase II trial of 17-allylamino-17-demethoxygeldanamycin in patients with metastatic melanoma. *Clin. Cancer Res.* **14**, 8302–8307 (2008).
71. Emery, C. M. *et al.* MEK1 mutations confer resistance to MEK and B-RAF inhibition. *Proc. Natl Acad. Sci. USA* **106**, 20411–20416 (2009).
This paper demonstrates the ability of MEK1 mutations to rescue BRAF-mutant melanomas from small-molecule BRAF inhibitors.
72. Johannessen, C. M. *et al.* COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. *Nature* **468**, 968–972 (2010).
This study identified the activation of *COT1* as a means of overcoming BRAF-V600E inhibition in melanoma.
73. Nazarian, R. *et al.* Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. *Nature* **468**, 973–977 (2010).
This publication identified the acquisition of resistance to BRAF-targeted therapy via overactivity of the PDGFR signalling pathway or NRAS activation.
74. Paraiso, K. H. *et al.* PTEN loss confers BRAF inhibitor resistance to melanoma cells through the suppression of BIM expression. *Cancer Res.* **71**, 2750–2760 (2011).
This study demonstrates the importance of PTEN loss, altered PI3K pathway activation and BIM expression in modulating the sensitivity to BRAF inhibition of BRAF-V600E-mutated melanomas.
75. Villanueva, J. *et al.* Acquired resistance to BRAF inhibitors mediated by a RAF kinase switch in melanoma can be overcome by cotargeting MEK and IGF-1R/PI3K. *Cancer Cell* **18**, 683–695 (2010).
This study demonstrates the functionally important roles of MEK and the IGF1R pathway in conferring resistance to BRAF-V600E-targeted melanoma therapy.
76. Davies, B. R. *et al.* AZD6244 (ARRY-142886), a potent inhibitor of mitogen-activated protein kinase/extracellular signal-regulated kinase kinase 1/2 kinases: mechanism of action *in vivo*, pharmacokinetic/pharmacodynamic relationship, and potential for combination in preclinical models. *Mol. Cancer Ther.* **6**, 2209–2219 (2007).
77. Lorusso, P. *et al.* A phase 1–2 clinical study of a second generation oral MEK inhibitor, PD 0325901 in patients with advanced cancer. *J. Clin. Oncol Abstr.* **23**, 3011 (2005).
78. Solit, D. B. *et al.* BRAF mutation predicts sensitivity to MEK inhibition. *Nature* **439**, 358–362 (2006).
79. Dummer, R. *et al.* AZD6244 (ARRY-142886) vs temozolomide (TMZ) in patients (pts) with advanced melanoma: an open-label, randomized, multicenter, phase II study. *J. Clin. Oncol.* **26**, 9033 (2008).
80. Infante, J. R. *et al.* Safety and efficacy results from the first-in-human study of the oral MEK 1/2 inhibitor GSK1120212. *J. Clin. Oncol. Abstr.* **28**, 2503 (2010).
81. Van Raamsdonk, C. D. *et al.* Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. *Nature* **457**, 599–602 (2009).
82. Guldberg, P. *et al.* Disruption of the MMAC1/PTEN gene by deletion or mutation is a frequent event in malignant melanoma. *Cancer Res.* **57**, 3660–3663 (1997).
83. Wu, H., Goel, V. & Haluska, F. G. PTEN signaling pathways in melanoma. *Oncogene* **22**, 3113–3122 (2003).
84. Molhoek, K. R., Brautigan, D. L. & Slingluff, C. L. Jr. Synergistic inhibition of human melanoma proliferation by combination treatment with B-Raf inhibitor BAY43–9006 and mTOR inhibitor Rapamycin. *J. Transl. Med.* **3**, 39 (2005).
85. Meier, F. *et al.* The RAS/RAF/MEK/ERK and PI3K/AKT signaling pathways present molecular targets for the effective treatment of advanced melanoma. *Front. Biosci.* **10**, 2986–3001 (2005).
86. Stahl, J. M. *et al.* Deregulated Akt3 activity promotes development of malignant melanoma. *Cancer Res.* **64**, 7002–7010 (2004).
87. Smalley, K. S. *et al.* An organometallic protein kinase inhibitor pharmacologically activates p53 and induces apoptosis in human melanoma cells. *Cancer Res.* **67**, 209–217 (2007).
88. Romano, M. F. *et al.* Rapamycin inhibits doxorubicin-induced NF-κB/Rel nuclear activity and enhances the apoptosis of melanoma cells. *Eur. J. Cancer* **40**, 2829–2836 (2004).
89. Margolin, K. *et al.* CCI-779 in metastatic melanoma: a phase II trial of the California Cancer Consortium. *Cancer* **104**, 1045–1048 (2005).

90. Thallinger, C. *et al.* Comparison of a treatment strategy combining CCI-779 plus DTIC versus DTIC monotreatment in human melanoma in SCID mice. *J. Invest. Dermatol.* **127**, 2411–2417 (2007).
91. Wang, Y. & Becker, D. Differential expression of the cyclin-dependent kinase inhibitors p16 and p21 in the human melanocytic system. *Oncogene* **12**, 1069–1075 (1996).
92. Jonsson, G. *et al.* Genomic profiling of malignant melanoma using tiling-resolution arrayCGH. *Oncogene* **26**, 4738–4748 (2007).
93. Kamb, A. *et al.* A cell cycle regulator potentially involved in genesis of many tumor types. *Science* **264**, 436–440 (1994).
94. Rotolo, S. *et al.* Effects on proliferation and melanogenesis by inhibition of mutant BRAF and expression of wild-type INK4A in melanoma cells. *Int. J. Cancer* **115**, 164–169 (2005).
95. O'Dwyer P. J. *et al. A* phase I dose escalation trial of a daily oral CDK 4/6 inhibitor PD-0332991. *J. Clin. Oncol. Abstr.* **25**, 3550 (2007).
96. Muthusamy, V. *et al.* Amplification of CDK4 and MDM2 in malignant melanoma. *Genes Chromosomes Cancer* **45**, 447–454 (2006).
97. Shangary, S. *et al.* Reactivation of p53 by a specific MDM2 antagonist (MI-43) leads to p21-mediated cell cycle arrest and selective cell death in colon cancer. *Mol. Cancer Ther.* **7**, 1533–1542 (2008).
98. Potti, A. *et al.* Immunohistochemical determination of vascular endothelial growth factor (VEGF) overexpression in malignant melanoma. *Anticancer Res.* **23**, 4023–4026 (2003).
99. Reed, J. A., McNutt, N. S., Prieto, V. G. & Albino, A. P. Expression of transforming growth factor-β2 in malignant melanoma correlates with the depth of tumor invasion. Implications for tumor progression. *Am. J. Pathol.* **145**, 97–104 (1994).
100. Straume, O. & Akslen, L. A. Importance of vascular phenotype by basic fibroblast growth factor, and influence of the angiogenic factors basic fibroblast growth factor/fibroblast growth factor receptor-1 and ephrin-A1/EphA2 on melanoma progression. *Am. J. Pathol.* **160**, 1009–1019 (2002).
101. Barnhill, R. L., Xiao, M., Graves, D. & Antoniades, H. N. Expression of platelet-derived growth factor (PDGF)-A, PDGF-B and the PDGF-α receptor, but not the PDGF-β receptor, in human malignant melanoma *in vivo*. *Br. J. Dermatol.* **135**, 898–904 (1996).
102. Rofstad, E. K. & Halsor, E. F. Vascular endothelial growth factor, interleukin 8, platelet-derived endothelial cell growth factor, and basic fibroblast growth factor promote angiogenesis and metastasis in human melanoma xenografts. *Cancer Res.* **60**, 4932–4938 (2000).
103. Forsberg, K., Valyi-Nagy, I., Heldin, C. H., Herlyn, M. & Westermark, B. Platelet-derived growth factor (PDGF) in oncogenesis: development of a vascular connective tissue stroma in xenotransplanted human melanoma producing PDGF-BB. *Proc. Natl Acad. Sci. USA* **90**, 393–397 (1993).
104. Nurnberg, W., Tobias, D., Otto, F., Henz, B. M. & Schadendorf, D. Expression of interleukin-8 detected by *in situ* hybridization correlates with worse prognosis in primary cutaneous melanoma. *J. Pathol.* **189**, 546–551 (1999).
105. Ugurel, S., Rappl, G., Tilgen, W. & Reinhold, U. Increased serum concentration of angiogenic factors in malignant melanoma patients correlates with tumor progression and survival. *J. Clin. Oncol.* **19**, 577–583 (2001).
106. Varker, K. A. *et al.* A randomized phase 2 trial of bevacizumab with or without daily low-dose interferon alfa-2b in metastatic malignant melanoma. *Ann. Surg. Oncol.* **14**, 2367–2376 (2007).
107. Fruehauf, J. P. *et al.* Axitinib (AG-013736) in patients with metastatic melanoma: a phase II study. *J. Clin. Oncol. Abstr.* **26**, 9006 (2008).
108. Clark, W. H. Jr. *et al.* Model predicting survival in stage I melanoma based on tumor progression. *J. Natl Cancer Inst.* **81**, 1893–1904 (1989).
109. Ferradini, L. *et al.* Analysis of T cell receptor variability in tumor-infiltrating lymphocytes from a human regressive melanoma. Evidence for *in situ* T cell clonal expansion. *J. Clin. Invest.* **91**, 1183–1190 (1993).
110. Van den Eynde, B. & Brichard, V. G. New tumor antigens recognized by T cells. *Curr. Opin. Immunol.* **7**, 674–681 (1995).
111. Van den Eynde, B. *et al.* Presence on a human melanoma of multiple antigens recognized by autologous CTL. *Int. J. Cancer* **44**, 634–640 (1989).
112. Van den Eynde, B. *et al.* A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. *J. Exp. Med.* **182**, 689–698 (1995).
113. van der Bruggen, P. *et al.* A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science* **254**, 1643–1647 (1991).
114. Van Der Bruggen, P. *et al.* Tumor-specific shared antigenic peptides recognized by human T cells. *Immunol. Rev.* **188**, 51–64 (2002).
115. Brichard, V. *et al.* The tyrosinase gene codes for an antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. *J. Exp. Med.* **178**, 489–495 (1993).
116. Coulie, P. G. *et al.* A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. *J. Exp. Med.* **180**, 35–42 (1994).
117. Kabbinavar, F. *et al.* Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/ leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. *J. Clin. Oncol.* **21**, 60–65 (2003).
118. Kawakami, Y. *et al.* Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. *Proc. Natl Acad. Sci. USA* **91**, 3515–3519 (1994).
119. Kawakami, Y. *et al.* Identification of a human melanoma antigen recognized by tumor-infiltrating lymphocytes associated with *in vivo* tumor rejection. *Proc. Natl Acad. Sci. USA* **91**, 6458–6462 (1994).
120. Kawakami, Y. *et al.* Identification of the immunodominant peptides of the MART-1 human melanoma antigen recognized by the majority of HLA-A2-restricted tumor infiltrating lymphocytes. *J. Exp. Med.* **180**, 347–352 (1994).
121. Kawakami, Y., Sumimoto, H., Fujita, T. & Matsuzaki, Y. Immunological detection of altered signaling molecules involved in melanoma development. *Cancer Metastasis Rev.* **24**, 357–366 (2005).
122. Robbins, P. F. *et al.* A mutated β-catenin gene encodes a melanoma-specific antigen recognized by tumor infiltrating lymphocytes. *J. Exp. Med.* **183**, 1185–1192 (1996).
123. Wolfel, T. *et al.* A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. *Science* **269**, 1281–1284 (1995).
124. Machiels, J. P., van Baren, N. & Marchand, M. Peptide-based cancer vaccines. *Semin. Oncol.* **29**, 494–502 (2002).
125. Young, J. W. & Inaba, K. Dendritic cells as adjuvants for class I major histocompatibility complex-restricted antitumor immunity. *J. Exp. Med.* **183**, 7–11 (1996).
126. Zhang, S., Wang, Q. & Miao, B. Review: dendritic cell-based vaccine in the treatment of patients with advanced melanoma. *Cancer Biother. Radiopharm.* **22**, 501–507 (2007).
127. Cerundolo, V., Hermans, I. F. & Salio, M. Dendritic cells: a journey from laboratory to clinic. *Nature Immunol.* **5**, 7–10 (2004).
128. Banchereau, J. *et al.* Immune and clinical responses in patients with metastatic melanoma to CD34$^+$ progenitor-derived dendritic cell vaccine. *Cancer Res.* **61**, 6451–6458 (2001).
129. Su, Z. *et al.* Immunological and clinical responses in metastatic renal cancer patients vaccinated with tumor RNA-transfected dendritic cells. *Cancer Res.* **63**, 2127–2133 (2003).
130. Schuler-Thurner, B. *et al.* Rapid induction of tumor-specific type 1 T helper cells in metastatic melanoma patients by vaccination with mature, cryopreserved, peptide-loaded monocyte-derived dendritic cells. *J. Exp. Med.* **195**, 1279–1288 (2002).
131. Mullins, D. W. *et al.* Route of immunization with peptide-pulsed dendritic cells controls the distribution of memory and effector T cells in lymphoid tissues and determines the pattern of regional tumor control. *J. Exp. Med.* **198**, 1023–1034 (2003).
132. Dhodapkar, M. V., Steinman, R. M., Krasovsky, J., Munz, C. & Bhardwaj, N. Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. *J. Exp. Med.* **193**, 233–238 (2001).
133. Fay, J. W. *et al.* Long-term outcomes in patients with metastatic melanoma vaccinated with melanoma peptide-pulsed CD34$^+$ progenitor-derived dendritic cells. *Cancer Immunol. Immunother.* **55**, 1209–1218 (2006).
134. Slingluff, C. L. Jr. *et al.* Clinical and immunologic results of a randomized phase II trial of vaccination using four melanoma peptides either administered in granulocyte-macrophage colony-stimulating factor in adjuvant or pulsed on dendritic cells. *J. Clin. Oncol.* **21**, 4016–4026 (2003).
135. Fong, L. *et al.* Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy. *Proc. Natl Acad. Sci. USA* **98**, 8809–8814 (2001).
136. Krieg, A. M. CpG motifs: the active ingredient in bacterial extracts? *Nature Med.* **9**, 831–835 (2003).
137. Srivastava, P. K. Peptide-binding heat shock proteins in the endoplasmic reticulum: role in immune response to cancer and in antigen presentation. *Adv. Cancer Res.* **62**, 153–177 (1993).
138. Smyth, M. J. *et al.* NKT cells - conductors of tumor immunity? *Curr. Opin. Immunol.* **14**, 165–171 (2002).
139. Rosenberg, S. A. *et al.* Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. *Nature Med.* **4**, 321–327 (1998).
140. Coulie, P. G. *et al.* Cytolytic T-cell responses of cancer patients vaccinated with a MAGE antigen. *Immunol. Rev.* **188**, 33–42 (2002).
141. Bendandi, M. *et al.* Complete molecular remissions induced by patient-specific vaccination plus granulocyte-monocyte colony-stimulating factor against lymphoma. *Nature Med.* **5**, 1171–1177 (1999).
142. Hodi, F. S. & Dranoff, G. Genetically modified tumor cell vaccines. *Surg. Oncol. Clin. N. Am.* **7**, 471–485 (1998).
143. Dranoff, G. *et al.* Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc. Natl Acad. Sci. USA* **90**, 3539–3543 (1993).
144. Mach, N. *et al.* Differences in dendritic cells stimulated *in vivo* by tumors engineered to secrete granulocyte-macrophage colony-stimulating factor or Flt3-ligand. *Cancer Res.* **60**, 3239–3246 (2000).
145. Huang, A. Y. *et al.* Role of bone marrow-derived cells in presenting MHC class I-restricted tumor antigens. *Science* **264**, 961–965 (1994).
146. Shen, Z., Reznikoff, G., Dranoff, G. & Rock, K. L. Cloned dendritic cells can present exogenous antigens on both MHC class I and class II molecules. *J. Immunol.* **158**, 2723–2730 (1997).
147. Hung., K. *et al.* The central role of CD4$^+$ T cells in the antitumor immune response. *J. Exp. Med.* **188**, 2357–2368 (1998).
148. Soiffer, R. *et al.* Vaccination with irradiated autologous melanoma cells engineered to secrete human granulocyte-macrophage colony-stimulating factor generates potent antitumor immunity in patients with metastatic melanoma. *Proc. Natl Acad. Sci. USA* **95**, 13141–13146 (1998).
149. Soiffer, R. *et al.* Vaccination with irradiated, autologous melanoma cells engineered to secrete granulocyte-macrophage colony-stimulating factor by adenoviral-mediated gene transfer augments antitumor immunity in patients with metastatic melanoma. *J. Clin. Oncol.* **21**, 3343–3350 (2003).
150. Chambers, C. A., Sullivan, T. J. & Allison, J. P. Lymphoproliferation in CTLA-4-deficient mice is mediated by costimulation-dependent activation of CD4$^+$ T cells. *Immunity* **7**, 885–895 (1997).
151. Leach, D. R., Krummel, M. F. & Allison, J. P. Enhancement of antitumor immunity by CTLA-4 blockade. *Science* **271**, 1734–1736 (1996).
152. van Elsas, A. *et al.* Elucidating the autoimmune and antitumor effector mechanisms of a treatment based on cytotoxic T lymphocyte antigen-4 blockade in combination with a B16 melanoma vaccine: comparison of prophylaxis and therapy. *J. Exp. Med.* **194**, 481–489 (2001).
153. Phan, G. Q. *et al.* Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. *Proc. Natl Acad. Sci. USA* **100**, 8372–8377 (2003).
154. O'Day, S. J. *et al.* Efficacy and safety of ipilimumab monotherapy in patients with pretreated advanced melanoma: a multicenter single-arm phase II study. *Ann. Oncol.* **21**, 1712–1717 (2010).
155. Wolchok, J. D. *et al.* Ipilimumab monotherapy in patients with pretreated advanced melanoma: a randomised, double-blind, multicentre, phase 2, dose-ranging study. *Lancet Oncol.* **11**, 155–164 (2010).

156. Wolchok, J. D. & Saenger, Y. The mechanism of anti-CTLA-4 activity and the negative regulation of T-cell activation. *Oncologist* **13**, 2–9 (2008).
157. Lens, M., Ferrucci, P. F. & Testori, A. Anti-CTLA4 monoclonal antibody Ipilimumab in the treatment of metastatic melanoma: recent findings. *Recent Pat. Anticancer Drug Discov.* **3**, 105–113 (2008).
158. Fong, L. & Small, E. J. Anti-cytotoxic T-lymphocyte antigen-4 antibody: the first in an emerging class of immunomodulatory antibodies for cancer treatment. *J. Clin. Oncol.* **26**, 5275–5283 (2008).
159. O'Day, S. J., Hamid, O. & Urba, W. J. Targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4): a novel strategy for the treatment of melanoma and other malignancies. *Cancer* **110**, 2614–2627 (2007).
160. Keilholz, U. CTLA-4: negative regulator of the immune response and a target for cancer therapy. *J. Immunother.* **31**, 431–439 (2008).
161. Hodi, F. S. *et al.* Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* **363**, 711–723 (2010).
This study reports a Phase III trial demonstrating a survival advantage with the use of anti-CTLA4 (immune checkpoint blockade) in patients with metastatic melanoma.
162. Robert, C. *et al.* Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N. Engl. J. Med.* **364**, 2517–2526 (2011).
This report demonstrates improved efficacy for combination therapy using anti-CTLA4 (ipilimumab) plus dacarbazine in patients with metastatic melanoma.
163. Iwai, Y. *et al.* Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proc. Natl Acad. Sci. USA* **99**, 12293–12297 (2002).
164. Cai, G. *et al.* PD-1 ligands, negative regulators for activation of naive, memory, and recently activated human CD4$^+$ T cells. *Cell. Immunol.* **230**, 89–98 (2004).
165. Freeman, G. J. *et al.* Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J. Exp. Med.* **192**, 1027–1034 (2000).
166. Watts, T. H. TNF/TNFR family members in costimulation of T cell responses. *Annu. Rev. Immunol.* **23**, 23–68 (2005).
167. Herman, A. E., Freeman, G. J., Mathis, D. & Benoist, C. CD4$^+$CD25$^+$ T regulatory cells dependent on ICOS promote regulation of effector cells in the prediabetic lesion. *J. Exp. Med.* **199**, 1479–1489 (2004).
168. Shimizu, J., Yamazaki, S. & Sakaguchi, S. Induction of tumor immunity by removing CD25$^+$CD4$^+$ T cells: a common basis between tumor immunity and autoimmunity. *J. Immunol.* **163**, 5211–5218 (1999).
169. Kusmartsev, S. & Gabrilovich, D. I. Inhibition of myeloid cell differentiation in cancer: the role of reactive oxygen species. *J. Leukoc. Biol.* **74**, 186–196 (2003).
170. Chang, D. H. *et al.* Sustained expansion of NKT cells and antigen-specific T cells after injection of α-galactosyl-ceramide loaded mature dendritic cells in cancer patients. *J. Exp. Med.* **201**, 1503–1517 (2005).
171. Kwon, B., Lee, H. W. & Kwon, B. S. New insights into the role of 4–1BB in immune responses: beyond CD8$^+$ T cells. *Trends Immunol.* **23**, 378–380 (2002).
172. Kwon, B. S. *et al.* Immune responses in 4–1BB (CD137)-deficient mice. *J. Immunol.* **168**, 5483–5490 (2002).
173. Schultze, J. L., Grabbe, S. & von Bergwelt-Baildon, M. S. DCs and CD40-activated B cells: current and future avenues to cellular cancer immunotherapy. *Trends Immunol.* **25**, 659–664 (2004).
174. Vonderheide, R. *et al.* Clinical activity and immune modulation in cancer patients treated with CP-870,893, a novel CD40 agonist monoclonal antibody. *J. Clin. Oncol.*. **25**, 876–883 (2007).
175. Vonderheide, R. H. & June, C. H. A translational bridge to cancer immunotherapy: exploiting costimulation and target antigens for active and passive T cell immunotherapy. *Immunol. Res.* **27**, 341–56 (2003).
176. Dannull, J. *et al.* Enhancing the immunostimulatory function of dendritic cells by transfection with mRNA encoding OX40 ligand. *Blood* **105**, 3206–3213 (2005).
177. Curtin, J. A., Busam, K., Pinkel, D. & Bastian, B. C. Somatic activation of KIT in distinct subtypes of melanoma. *J. Clin. Oncol.* **24**, 4340–4346 (2006).
178. Prickett, T. D. *et al.* Analysis of the tyrosine kinome in melanoma reveals recurrent mutations in ERBB4. *Nature Genet.* **41**, 1127–1132 (2009).
179. Bastian, B. C., LeBoit, P. E., Hamm, H., Brocker, E. B. & Pinkel, D. Chromosomal gains and losses in primary cutaneous melanomas detected by comparative genomic hybridization. *Cancer Res.* **58**, 2170–2175 (1998).
180. Moore, S. R. *et al.* Detection of copy number alterations in metastatic melanoma by a DNA fluorescence *in situ* hybridization probe panel and array comparative genomic hybridization: a southwest oncology group study (S9431). *Clin. Cancer Res.* **14**, 2927–2935 (2008).
181. Kraehn, G. M. *et al.* Extra c-myc oncogene copies in high risk cutaneous malignant melanoma and melanoma metastases. *Br. J. Cancer* **84**, 72–79 (2001).
182. Jane-Valbuena, J. *et al.* An oncogenic role for ETV1 in melanoma. *Cancer Res.* **70**, 2075–2084 (2010).
183. Freedberg, D. E. *et al.* Frequent p16-independent inactivation of p14ARF in human melanoma. *J. Natl Cancer Inst.* **100**, 784–95 (2008).
184. Soussi, T., Kato, S., Levy, P. P. & Ishioka, C. Reassessment of the TP53 mutation database in human disease by data mining with a library of TP53 missense mutations. *Hum. Mutat.* **25**, 6–17 (2005).
185. Harbour, J. W. *et al.* Frequent mutation of BAP1 in metastasizing uveal melanomas. *Science* **330**, 1410–1413 (2010).
186. Stark, M. & Hayward, N. Genome-wide loss of heterozygosity and copy number analysis in melanoma using high-density single-nucleotide polymorphism arrays. *Cancer Res.* **67**, 2632–2642 (2007).
187. Guo, J. Phase II, open-label, single-arm trial of imatinib mesylate in patients with metastatic melanoma harboring c-Kit mutation or amplification. *J. Clin. Oncol.* **29**, 2904–2909 (2011).
188. Flaherty, K. T. *et al.* Inhibition of mutated, activated BRAF in metastatic melanoma. *N. Engl. J. Med.* **363**, 809–819 (2010).
189. Hauschild, A. *et al.* Multicenter phase II trial of the histone deacetylase inhibitor pyridylmethyl-N-{4-[(2-aminophenyl)-carbamoyl]-benzyl}-carbamate in pretreated metastatic melanoma. *Melanoma Res.* **18**, 274–278 (2008).
190. Bertolotto *et al*. A SUMOylation-defective MITF germline mutation predisposes to melanoma and renal carcinoma. *Nature* **480**, 94–98 (2011).
191. Su, F. *et al*. RAS mutations in cutaneous squamous-cell carcinomas in patients treated with BRAF inhibitors. *N. Eng. J. Med* **366**, 207–215 (2012).
192. Poulikos, I. *et al*. RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). *Nature* **480**, 387–390 (2011).
193. Shi, H. *et al*. Melanoma whole-exome sequencing identifies V600EB-RAF amplification-mediated acquired B-RAF inhibitor resistance. *Nature Comm.* **3**, 724 (2012).

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Competing interests statement
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DATABASES
ClinicalTrials.gov: http://clinicaltrials.gov/NCT01143402
National Cancer Institute Drug Dictionary: http://www.cancer.gov/drugdictionary
axitinib | AZD6244 | bevacizumab | dacarbazine | dasatinib | E7080 | GSK1120212 | GSK2118436 | imatinib | imiquimod | MDX-010 | nilotinib | PD0325901 | PLX4032 | R115777 | rapamycin | sorafenib | sunitinib | tanespimycin | temsirolimus | tremelimumab | XL281

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