New treatment approaches in melanoma: current research and clinical prospects

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Abstract: Ipilimumab and vemurafenib have changed the clinical landscape in melanoma. Both drugs offer effective treatment for metastatic melanoma, but with limitations. Ipilimumab benefits only a minority of those treated, with no means to identify them prospectively. The efficacy of vemurafenib is tied to the presence of an activating mutation in BRAF, and so is more predictable. However, acquired resistance develops within months. As we understand these, and similar, agents better, the means to select patients for treatment, to increase the duration of response and to identify the best stage at which to intervene will lead to improved outcomes for patients. Several trials are already under way or being developed to build upon these exciting discoveries.

Keywords: clinical management, immunotherapy, melanoma, targeted therapy

Introduction

The incidence of melanoma continues to rise in the UK, with over 12,800 new cases being diagnosed in 2010 (Cancer Research UK, http://publications.cancerresearchuk.org/cancerstats/statsskin/keyfactsskin.html). This equates to a lifetime risk of developing melanoma of approximately 1 in 60. It is now the sixth most common cancer in the UK and represents the second most common cancer in young adults, aged 15–34 years old.

Surgery continues to provide a cure for localized and regional disease. However, once beyond surgery, metastatic melanoma has until recently been notoriously resistant to drug therapies and radiation. In the last decade there have been considerable advances in our understanding of melanoma biology, which have emphasized the heterogeneity of the disease. We now have a better understanding of which groups are at high-risk of recurrence after surgery, and of some of the different genetic drivers behind melanoma. Recently, exciting new treatments have emerged, built upon this understanding, that for the first time offer the prospect of improved outcomes for patients with metastatic disease. In this article, we consider the key clinical studies and highlight their impact on the future management of metastatic melanoma.

Cytotoxic chemotherapy

Dacarbazine has long been held as the standard of care for metastatic disease, despite a lack of evidence for any improvement in survival over supportive care. That being said, a small proportion of patients, between 5% and 10%, undoubtedly benefit from treatment, with durable responses to treatment recorded in 1–2%. Trials of polychemotherapy and combinations with cytokines, in the 1980s and 1990s, yielded better response rates for multi-agent regimens, but no improvement in overall survival. A wide range of agents have been tested in melanoma, including taxanes, vinca alkaloids and platinums, all yielding response rates (10–15%) in small studies similar to that of dacarbazine. The methylating agent has remained the reference agent of choice in large clinical trials [Atkins *et al*. 2009].

Cytokines

Melanoma has long been viewed as a tumour likely to be susceptible to immunotherapy. This view has persisted, despite decades of failed studies, only to be vindicated in the last 2 years. The exceptions have been the pleiotropic cytokines interleukin-2 and interferon-alpha. Both have been evaluated extensively, and the latter has gained relatively wide use in the adjuvant setting. There is good evidence for interferon increasing relapse-free survival and for a modest effect upon overall survival, giving a relative reduction in mortality of approximately 10% [Mocellin *et al*. 2010]. These data come from a meta-analysis of over a dozen trials, so that there remains considerable

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uncertainty over the correct dose, duration and route of administration for interferon. In addition, pegylated interferon-alpha has recently been shown improve relapse free and distant metastasis-free survival when given once weekly over 5 years [Eggermont *et al*. 2008]. No difference was observed in overall survival. In the United States the high-dose regimen pioneered by the Eastern Co-operative Oncology Group is used and pegylated interferon is also licensed, but in Europe lower doses delivered subcutaneously hold sway. Recent retrospective analyses have suggested that tumour ulceration and/or microscopic nodal involvement may provide the means for selecting patients for treatment. This is now being assessed in a prospective study, and cannot yet be considered as the basis for deciding who should be treated with interferon.

High-dose intravenous interleukin-2 treatment is licensed by the United States Food and Drug Administration (FDA), but relatively little used outside of North America. Durable responses lasting several years have been reported in a subset of patients, but no large-scale direct comparison with supportive care or chemotherapy has been undertaken [Atkins *et al*. 2000].

Emerging therapies

Forty years of failed clinical trials in melanoma have been turned on their head in the last 2 years. Two classes of treatment have shown survival benefits in metastatic disease, and indicated several means to improve upon results obtained to date.

Checkpoint inhibitors

The failure of previous attempts to invoke the immune system in treating melanoma has improved our understanding of the means by which the tumour evades detection and attack. This has led to the targeting of inhibitory T-cell signalling pathways, initially cytotoxic T-lymphocyte antigen 4 (CTLA-4) and more recently programmed death 1 (PD-1) and its ligand PD-L1.

CTLA-4 is expressed on the surface of activated CD4+ and CD8+ T-cells and binds B7 molecules on antigen-presenting cells, which represses T-cell activation. Ipilimumab is a fully human immunoglobulin specific for CTLA-4 that, in binding, inhibits this negative feedback, potentiating the T-cell-mediated immune response. Two phase III trials with dacarbazine or peptide vaccine comparators have established that melanoma patients treated with ipilimumab have improved overall survival.

In the first study to report the effect of ipilimumab, 3 mg/kg IV was evaluated in HLA-A2 positive patients who had progressed through at least one line of treatment for their metastatic melanoma [Hodi *et al*. 2010]. Patients received ipilimumab alone for four 3-week cycles, a gp100 peptide vaccine or both agents. The CTLA-4 antibody reduced the risk of death by 32–34% compared with the peptide vaccine, with similar results for the single-agent and combination arms. Median survival was increased from 6.4 to 10 months, but more importantly the proportion of patients surviving for 2 years increased from 14% to 22%.

In a trial involving 502 patients, first-line treatment with ipilimumab 10 mg/kg IV and dacarbazine produced moderately better median survival than dacarbazine alone (11.2 *versus* 9.1 months, hazard ratio 0.72). Survival at 2 and 3 years was better on immunotherapy (28.5% *versus* 17.9% and 20.8% *versus* 12.2%, respectively) [Robert *et al*. 2011]. Although an important advance in the treatment of melanoma, the optimal dose of ipilimumab and a basis upon which to select patients for treatment remain unresolved. The latter has implications for the cost effectiveness of treatment, as it is not possible to assess antitumour effects part way through treatment and it appears that only a minority of patients derive significant benefit from treatment. There is some evidence to suggest that monotherapy with the 10 mg/kg dose performs better than 3 mg/kg, and this is being evaluated in a randomized study now closed to recruitment. Meanwhile the standard of care is the 3 mg/kg dose.

Ipilimumab toxicity differs from that of other antibody therapies. Autoimmune events including rash, colitis, hypophysitis and hepatitis occur, and toxic deaths were recorded in the second-line study. Algorithms for managing toxicity have been developed so that the 3 mg/kg dose is now well-tolerated, but at the higher dose over half of patients experienced grade 3 or 4 toxicities.

The other CTLA-4 antibody in clinical development, tremelimumab, did not show a statistically significant survival advantage in its pivotal trials, although this result may have been affected by the

availability of ipilimumab in the United States at the time of that trial [Ascierto *et al*. 2011].

More recently promising results have been seen with antibodies that block the inhibitory T-cell receptor PD-1 or its ligand. In a large phase I trial of BMS-936558, an antibody to PD-1, 26 of 94 melanoma patients (28%) had an objective response to treatment, with two-thirds of these lasting over 1 year [Topalian *et al*. 2012]. Interestingly, tumour PD-L1 expression might provide a basis for selecting patients for treatment, as none of 17 patients with tumours negative for PD-L1 had a response. An antibody to PD-L1, BMS-936559, produced similar results with a 17% response rate amongst 55 patients with melanoma [Brahmer *et al*. 2012]. Responses have also been reported to the PD-1 antibody MK-3475 [Patnaik, 2012]. The toxicity of PD-1 targeted agents is yet to be defined, but appears to be no greater than that of ipilimumab. That being said, clinically significant episodes of interstitial pneumonitis have been reported with anti-PD-1 agents, and this will need to be monitored closely in future studies. Randomized trials to define the role of these agents in melanoma are under way or will start shortly.

Kinase inhibitors

The targeting of mutated oncogenes has been a step change in cancer therapeutics. Two-thirds of patients with melanoma have activating mutations in the oncogenic protein kinases RAF, RAS and KIT, and inhibition of kinase activity has been associated with tumour shrinkage.

The mitogen-activated protein kinase (MAPK) pathway is a key regulator in cell growth, and regulates proliferation and survival in many cancers [Garnett and Marais, 2004]. Activating mutations in the serine–threonine BRAF kinase, a constituent of the MAP kinase signal transduction pathway, were first described in 2002 and have been identified in the tumours of just under 50% of advanced melanoma patients. The two most commonly observed BRAF mutations, V600E and V600K, account for 95% of these mutations. Activated BRAF phosphorylates and activates MEK proteins, which then activate downstream MAP kinases.

BRAF. One of the first attempts to target the MAPK pathway was with the multikinase inhibitor sorafenib (BAY 43-9006) [Flaherty *et al*. 2005]. As a monotherapy, sorafenib had limited clinical activity [Wilhelm *et al*. 2004]. When combined with carboplatin and paclitaxel (CP) responses were seen in 30% of patients but there was no correlation with BRAF mutational status [Flaherty *et al*. 2008]. In the subsequent phase III randomized placebo-controlled trial of CP with or without sorafenib there was no effect on overall or progression free survival for the kinase inhibitor [Hauschild *et al*. 2009]. This lack of activity is likely explained by sorafenib's lack of specificity for BRAF. Since these initial studies MAPK inhibitors have evolved and become more selective.

Vemurafenib (PLX4072, RG7204, RO5185426) selectively inhibits the V600E BRAF kinase, and in its phase I study showed a 69% response rate in patients whose tumour harboured the mutation [Flaherty *et al*. 2010b]. None of the melanoma patients with wild-type BRAF responded to treatment. A phase II trial shortly afterwards demonstrated a response rate of 53% with a median duration of 6.7 months [Sosman *et al*. 2012]. In a phase III trial involving 672 patients, vemurafenib was compared with dacarbazine as first-line treatment for patients with V600E BRAF melanoma [Chapman *et al*. 2011]. At interim analysis, the data and safety monitoring board determined that vemurafenib performed statistically significantly better than dacarbazine and recommended that patients assigned to dacarbazine be allowed to cross over to vemurafenib. When first reported, median progression-free survival was 5.3 months on vemurafenib and 1.6 months on dacarbazine, with a hazard ratio of 0.26. In the subset of patients evaluable for response this too favoured vemurafenib (48% *versus* 5%), as did overall survival with a hazard ratio of 0.37. Vemurafenib caused arthralgia (21%), rash (18%) and fatigue (13%). A noteworthy finding was that 61 patients (18%) developed a cutaneous squamous cell carcinoma or keratoacanthoma, which required surgical excision. Updated results were presented at the 2012 meeting of the American Society for Clinical Oncology (ASCO) [Chapman, 2012]. Progression-free survival on vemurafenib was 6.9 months, with a hazard ratio of 0.38. Median overall survival was 13.6 months on vemurafenib, as opposed to 9.7 months on dacarbazine (hazard ratio 0.70, censoring at crossover or 0.76 without censoring), noting that a quarter of patients assigned chemotherapy crossed over to vemurafenib. The objective response rate for vemurafenib was reported as 57%, and 56% of patients remained alive at 12 months.

Although a major step forward, it has become clear that melanoma acquires resistance to vemurafenib within a few months. Tumour progression is driven by re-activation of the MAPK pathway or through the upregulation of parallel signalling pathways. Acquisition of mutations in NRAS and MEK have been described as well as overexpression of COT [Nazarian *et al*. 2010]. The MAPK pathway may also be activated when V600E BRAF splice variants lacking the RAS-binding domain develop. These dimerize in the absence of RAS activation to reactivate the pathway [Poulikakos *et al*. 2011]. Insights into mechanisms of resistance point to potential drug combinations to overcome this important clinical problem. The observation that re-activation of the MAPK pathway by multiple mechanisms is a significant component of acquired resistance to vemurafenib has promoted the concept of dual inhibition of the pathway (see below).

Dabrafenib (GSK2118436) is the second mutant BRAF inhibitor to report phase III trial results [Hauschild *et al*. 2012]. Results were similar to those for vemurafenib in that, in comparison with dacarbazine, the hazard ratio for progression-free survival was 0.30 (median 5.1 months for dabrafenib and 2.7 months for dacarbazine). Response rates were 53% for dabrafenib and 19% for chemotherapy. The side-effect profile of the two drugs looks similar, although there is a higher incidence of fever on dabrafenib, but it is not a photosensitizer like vemurafenib. Overall, it seems that there will be little to choose between the drugs as single agents.

Interestingly, the two BRAF inhibitors were evaluated in slightly different populations. Vemurafenib was tested in patients with a V600E mutation, as identified by a sensitive but specific companion diagnostic. The dabrafenib studies admitted patients with both E and K mutations. The rate of detection of different V600 mutations, and indeed other *BRAF* mutations, is highly dependent upon the technique used. Recent publications suggest that the prevalence of V600K mutations may vary with age and/or sun exposure, and, in a small number of individuals, mutation may change during the evolution of a melanoma. In ascertaining BRAF mutation status careful consideration needs to be given to both the tumour specimen tested (the most recent being preferable) and the technique used.

MEK. Downstream of RAF in the MAPK pathway is the mitogen-activated (MEK) or extracellular signal-related protein kinases (ERK). During cellular signalling as RAF travels from the cytoplasm to the cell membrane the new activated complex enables signal cascade by consecutive phosphorylation through MEK1 and MEK2. This in turn activates ERK 1 and 2 which are able to enter the nucleus and interact with several transcription factors to promote cellular growth and differentiation [Russo *et al*. 2009]. Inhibition of MEK is therefore another option for targeting the MAPK pathway [Goel *et al*. 2006; Flaherty *et al*. 2010a].

Preclinical studies of the MEK inhibitor, PD0325901, and its precursor, CI-1040, showed direct inhibition of ERK in cell lines and reduced tumour growth in animal models [Solit *et al*. 2006]. However, PD0325901 and CI-1040 were not pursued due to their toxicity in early phase trials [Rinehart *et al*. 2004; Lorusso *et al*. 2005].

Selumetinib (AZD6244, ARRY-142886) had unimpressive results in a randomized phase II multicentre study comparing it with temozolomide. The MEK inhibitor had a 12% objective response rate, which was unaffected by the BRAF or NRAS mutation status of the tumour [Kirkwood *et al*. 2012]. An issue for selumetinib may be its relatively short half-life, meaning that at tolerable doses there is always likely to be some time without MEK inhibition. Several other MEK inhibitors are in clinical development that have better pharmacokinetic profiles. Amongst these, trametinib (GSK1120212) has reported results from a randomized phase III trial comparing it with chemotherapy in patients with V600 mutant BRAF melanoma [Flaherty *et al*. 2012]. In this study, 322 patients were assigned 2:1 to trametinib or chemotherapy (dacarbazine or paclitaxel). The kinase inhibitor gave improved progression free (hazard ratio 0.45) and overall survival (hazard ratio 0.54) despite crossover to trametinib of 51 out of 108 patients assigned chemotherapy). Median progression-free survival on trametinib was 4.8 months, suggesting that the problem of acquired resistance seen with BRAF inhibitors also holds for drugs targeting MEK.

A further attraction of MEK inhibition, since this targets wild-type protein, is the possibility that this provides a means of treating NRAS mutant melanoma. In a phase II trial, 3 out of 13 evaluable

patients with NRAS mutations responded to MEK162. Insufficient data exist to judge whether single-agent MEK inhibition is worth pursuing [Ascierto *et al*. 2012].

KIT. As the receptor for stem-cell factor, the C-KIT receptor tyrosine kinase is important in the development of melanocytes. In anatomical sites of low ultraviolet exposure such as the palms of the hands, soles of the feet and mucous membranes, melanoma still occurs and these acral or mucosal melanomas have a moderate incidence of activating KIT mutations [Curtin *et al*. 2006]. The specific KIT mutations identified in melanoma subtypes are those commonly reported in gastrointestinal stromal tumours (GIST) [Ashida *et al*. 2009]. Dramatic tumour responses have been reported in two melanoma patients following treatment with imatinib [Hodi *et al*. 2008; Lutzky *et al*. 2008]. Following this, trials have been conducted on the use of selective KIT inhibition with imatinib on melanoma patients harbouring KIT mutations or amplification with overall response rate of 23% [Guo *et al*. 2011]. A further phase II multicentre trial observed durable responses in 4 of the 25 patients [Carvajal *et al*. 2011]. Two studies of nilotinib are now open: in the UK the NICAM trial is a single-arm assessment of the activity of the drug in patients with KIT mutated melanoma, and an international study is randomizing such patients between nilotinib and dacarbazine.

PI3K–AKT–MTOR axis. The phosphatidylinositol-3-kinase (PI3K) pathway is frequently dysregulated in melanoma, in particular through loss of the tumour suppressor gene phosphate and tensin homolog deleted on chromosome ten (PTEN) [Davies and Gershenwald, 2011]. PTEN loss has been noted in 30–50% of melanomas [Birck *et al*. 2000]. The predominant AKT isoform, AKT3 has been shown to be overexpressed in 60% of melanomas [Stahl *et al*. 2004]. Inhibitors of the pathway have not provided grounds for their development in melanoma along the lines of MAPK pathway inhibitors. However, there is crosstalk between the MAPK and PI3K pathways, so that the latter may modulate sensitivity and/or resistance to MAPK inhibitors [Smalley *et al*. 2006]. In particular, BRAF or MEK inhibition of BRAF mutant melanoma cells *in vitro* renders them critically dependent upon AKT for their survival.

Combination therapies

Combining targeted agents is attractive for the reasons outlined above. The combination of MAPK and PI3K pathway inhibitors is the subject of many clinical trials. These are only now reporting doses for further study and/or preliminary efficacy so that there are as yet no results in melanoma to guide pivotal trial design.

Dual MAPK pathway inhibition is further advanced. Dabrafenib and trametinib can be combined at the full single-agent doses for each drug, and have demonstrated impressive efficacy in early phase trials. Amongst 77 BRAF mutant melanoma patients treated with this combination, 63% responded with a median duration of 11.3 months. This offers the possibility of a longer lasting effect than can be achieved with BRAF inhibitors alone. Of equal interest is the absence of hyperproliferative skin toxicity with the combination. This side effect, which results in squamous cell cancers and keratoacanthomas in 18–24% of patients treated with BRAF inhibitors, is driven by paradoxical activation of the MAPK pathway in the presence of RAS activation [Heidorn *et al*. 2010]. One would predict that simultaneous MEK inhibition would reduce this effect, and <2% of patients developed squamous cell cancers on combination treatment. Phase III trials to compare dabrafenib and trametinib with single-agent BRAF inhibitors are under way.

The MAPK pathway is important in determining cellular responses to stress, including DNA damage. MEK inhibitors may therefore synergize with cytotoxic chemotherapies and radiotherapy. This effect is seen preclinically, irrespective of BRAF and NRAS, as ERK activation is ubiquitous in melanoma [Smalley and Flaherty, 2009]. Early phase trials of MEK inhibition and radiotherapy are being performed. Studies of combinations of chemotherapy and selumetinib have recently been completed in melanoma, but results are not yet available. These randomized phase II trials will compare dacarbazine against dacarbazine and selumetinib in V600E mutant melanoma and docetaxel against docetaxel and selumetinib in wild-type disease. Of interest, docetaxel and selumetinib performed better than docetaxel alone in KRAS mutant non-small cell lung cancer, although the increase in overall survival from 5.4 to 9.2 months was not statistically significant in this small randomized phase II study [Janne *et al*. 2012]. A similar trial of trametinib and paclitaxel

versus paclitaxel alone in wild-type BRAF melanoma is currently recruiting in the United Kingdom.

Combinations of immune checkpoint and BRAF inhibitors are also being evaluated. The different kinetics of response suggest that these might be complementary, and there is some suggestion that BRAF inhibition can increase tumour T-cell infiltrates, which might benefit immunotherapy [Wilmott *et al*. 2012].

Clinical prospects

The last 2 years have seen unprecedented success in identifying promising new treatments for melanoma. That being said, patients with metastatic melanoma still die of their disease within months, so that there is much still to do to improve their lot. The checkpoint and kinase inhibitors offer different problems. The latter benefit only a subpopulation but do so rapidly and for the majority, but briefly. The former have a more durable impact upon a minority of patients, take time to exert their effect and, as yet, offer no basis upon which to select patients for therapy. The rapid dissection of acquired resistance to treatment offers the promise of more effective combination regimens in the near future. The key will be identifying those mechanisms that matter and the best time to intervene.

Pending the development of combination regimens uncertainty remains as to the optimal sequencing of kinase inhibitors and ipilimumab. In many territories the practicalities of access to, and funding for, treatments will effectively decide this question. Where both are available there are no data to guide decision making, although there is consensus that patients with symptomatic metastatic disease harbouring a BRAF mutation should receive a BRAF inhibitor first.

The use of these drugs at an earlier stage of melanoma will also be explored. Results from an adjuvant study of ipilimumab in high-risk resected melanoma are expected in 2013 or 2014, and trials of BRAF inhibitors or combined BRAF and MEK inhibition in this setting are starting now. For the latter, the development of squamoproliferative lesions where RAS abnormalities pertain remains a concern and will require close monitoring. Two other trials in the adjuvant setting will report their results soon: the DERMA study, of MAGE-3 vaccination, and AVAST-M, looking at bevacizumab, have both completed accrual.

We can expect many more changes to the treatment of melanoma in coming years. The excitement generated by recent results is as much a reflection of the new avenues for exploration being opened up as of their activity against the disease.

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References

Ascierto, P., Marincola, F. and Ribas, A. (2011) Anti-Ctla4 monoclonal antibodies: the past and the future in clinical application. *J Transl Med* 9: 196.

Ascierto *et al*. (2012) Efficacy and safety of oral MEK162 in patients with locally advanced and unresectable or metastatic cutaneous melanoma harboring *BRAFV600* or *NRAS* mutations. *J Clin Oncol* 30: (suppl; abstr. 8511).

Ashida, A., Takata, M., Murata, H., Kido, K. and Saida, T. (2009) Pathological activation of KIT in metastatic tumors of acral and mucosal melanomas. *Int J Cancer* 124: 862–868.

Atkins, M., Kunkel, L., Sznol, M. and Rosenberg, S. (2000) High-dose recombinant interleukin-2 therapy in patients with metastatic melanoma: long-term survival update. *Cancer J Sci Am* 6(Suppl. 1): S11–S14

Atkins, M., Middleton, M. and Chapman, P. (2009) Chemotherapy-based treatment of metastatic melanoma. In: Atkins, M. (ed.), *Cutaneous Melanoma*, 5th edn. St. Louis, MO: Quality Medical Publishing, Inc..

Birck, A., Ahrenkiel, V., Zeuthen, J., Hou-Jensen, K. and Guldberg, P. (2000) Mutation and allelic loss of the PTEN/MMAC1 gene in primary and metastatic melanoma biopsies. *J Investigative Dermatol* 114: 277–280.

Brahmer, J., Tykodi, S., Chow, L., Hwu, W., Topalian, S., Hwu, P. *et al*. (2012) Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med*, PMID: 22658128.

Carvajal, R., Antonescu, C., Wolchok, J., Chapman, P., Roman, R.-, Teitcher, J. *et al*. (2011) KIT as a therapeutic target in metastatic melanoma. *JAMA* 305: 2327–2334.

Chapman, B. (2012) Updated overall survival (OS) results for BRIM-3, a phase III randomized, open-label, multicenter trial comparing BRAF inhibitor vemurafenib (Vem) with dacarbazine (DTIC) in previously untreated patients with BRAFV600E-mutated melanoma. *Proc Amer Soc Clin Oncol*, Abstract 8502.

Chapman, P., Hauschild, A., Robert, C., Haanen, J., Ascierto, P., Larkin, J. *et al.* (2011) Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med* 364: 2507–2516.

Curtin, J., Busam, K., Pinkel, D. and Bastian, B. (2006) Somatic activation of KIT in distinct subtypes of melanoma. *J Clin Oncol* 24: 4340–4346.

Davies, M. and Gershenwald, J. (2011) Targeted therapy for melanoma: a primer. *Surg Oncol Clin N Am* 20: 165–180.

Eggermont, A., Suciu, S., Santinami, M., Testori, A., Kruit, W., Marsden, J. *et al.* for EORTC Melanoma Group (2008) Adjuvant therapy with pegylated interferon alfa-2b versus observation alone in resected stage III melanoma: final results of EORTC 18991, a randomised phase III trial. *Lancet* 372: 117–126.

Flaherty, K., Hodi, F. and Bastian, B. (2010a) Mutation-driven drug development in melanoma. *Curr Opin Oncol* 22: 178–183.

Flaherty, K., Puzanov, I., Kim, K., Ribas, A., McArthur, G., Sosman, J. *et al.* (2010b) Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med* 363: 809–819.

Flaherty, K., Redlinger, M., Schuchter, L., Lathia, C., Weber, B. and O'Dwyer, P. (2005) Phase I/II, pharmacokinetic and pharmacodynamic trial of BAY 43-9006 alone in patients with metastatic melanoma. *J Clin Oncol* 23: 3037.

Flaherty, K., Robert, C., Hersey, P., Nathan, P., Garbe, C., Milhem, M. *et al.* (2012) Improved survival with MEK inhibition in BRAF-mutated melanoma. *N Engl J Med*, in press.

Flaherty, K., Schiller, J., Schuchter, L., Liu, G., Tuveson, D., Redlinger, M. *et al.* (2008) A phase I trial of the oral, multikinase inhibitor sorafenib in combination with carboplatin and paclitaxel. *Clin Cancer Res* 14: 4836–4842.

Garnett, M. and Marais, R. (2004) Guilty as charged: B-RAF is a human oncogene. *Cancer Cell* 6: 313–319.

Goel, V., Lazar, A., Warneke, C., Redston, M. and Haluska, F. (2006) Examination of mutations in BRAF, NRAS, and PTEN in primary cutaneous melanoma. *J Investigative Dermatol* 126: 154–160.

Guo, J., Si, L., Kong, Y., Flaherty, K., Xu, X., Zhu, Y. *et al.* (2011) 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.

Hauschild, A., Agarwala, S., Trefzer, U., Hogg, D., Robert, C., Hersey, P. *et al.* (2009) 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.

Hauschild, A., Grob, J., Demidov, L., Jouary, T., Gutzmer, R., Millward, M. *et al.* (2012) Phase III, randomized, open-label, multicenter trial (Break-3) comparing the BRAF kinase inhibitor dabrafenib (GSK2118436) with dacarbazine (DTIC) in patients with BRAF V600E -mutated melanoma. *Proc Amer Soc Clin Oncol*, Abstract LBA8500.

Heidorn, S., Milagre, C., Whittaker, S., Nourry, A., Niculescu-Duvas, I., Dhomen, N. *et al.* (2010) Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. *Cell* 140: 209–221.

Hodi, F., Friedlander, P., Corless, C., Heinrich, M., Mac Rae, S., Kruse, A. *et al.* (2008) Major response to imatinib mesylate in KIT-mutated melanoma. *J Clin Oncol* 26: 2046–2051.

Janne *et al.* (2012) Phase II double-blind, randomized study of selumetinib (SEL) plus docetaxel (DOC) versus DOC plus placebo as second-line treatment for advanced *KRAS* mutant non-small cell lung cancer (NSCLC). *J Clin Oncol* 30: (suppl; abstr. 7503).

Kirkwood, J., Bastholt, L., Robert, C., Sosman, J., Larkin, J., Hersey, P. *et al.* (2012) Phase II, open-label, randomized trial of the MEK1/2 inhibitor selumetinib as monotherapy versus temozolomide in patients with advanced melanoma. *Clin Cancer Res* 18: 555–567.

Lorusso, P., Adjei, A., Varterasian, M., Gadgeel, S., Reid, J., Mitchell, D. *et al.* (2005) Phase I and pharmacodynamic study of the oral MEK inhibitor CI-1040 in patients with advanced malignancies. *J Clin Oncol* 23: 5281–5293.

Lutzky, J., Bauer, J. and Bastian, B. (2008) Dose-dependent, complete response to imatinib of a metastatic mucosal melanoma with a K642E KIT mutation. *Pigment Cell Melanoma Res* 21: 492–493.

Mocellin *et al.* (2010) Interferon alpha adjuvant therapy in patients with high-risk melanoma: a systematic review and meta-analysis. *J Natl Cancer Inst* 102: 493–501.

Nazarian, R., Shi, H., Wang, Q., Kong, X., Koya, R., Lee, H. *et al.* (2010) Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. *Nature* 468: 973–977.

Patnaik, A. (2012) Phase I study of Mk-3475 (anti-PD-1 monoclonal antibody) in patients with

advanced solid tumors. *Proc Amer Soc Clin Oncol*, Abstract 2512.

Poulikakos, P., Persaud, Y., Janakiraman, M., Kong, X., Ng, C., Moriceau, G. *et al.* (2011) RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). *Nature* 480: 387–390.

Rinehart, J., Adjei, A., Lorusso, P., Waterhouse, D., Hecht, J., Natale, R. *et al.* (2004) Multicenter phase II study of the oral MEK inhibitor, CI-1040, in patients with advanced non-small-cell lung, breast, colon, and pancreatic cancer. *J Clin Oncol* 22: 4456–4462.

Robert, C., Thomas, L., Bondarenko, I., O'Day, S., Weber, J., Garbe, C. *et al.* (2011) Ipilimumab plus Dacarbazine for Previously Untreated Metastatic Melanoma. *N Engl J Med* 364: 2517–2526.

Russo, A., Torrisi, E., Bevelacqua, Y., Perrotta, R., Libra, M., McCubrey, J. *et al.* (2009) Melanoma: molecular pathogenesis and emerging target therapies (review). *Int J Oncol* 34: 1481–1489.

Smalley and Flaherty (2009) Integrating BRAF/MEK inhibitors into combination therapy for melanoma. *Br J Cancer* 100: 431–435.

Smalley, K., Haass, N., Brafford, P., Lioni, M., Flaherty, K. and Herlyn, M. (2006) Multiple signaling pathways must be targeted to overcome drug resistance in cell lines derived from melanoma metastases. *Mol Cancer Therapeut* 5: 1136–1144.

Solit, D., Garraway, L., Pratilas, C., Sawai, A., Getz, G., Basso, A. *et al.* (2006) BRAF mutation predicts sensitivity to MEK inhibition. *Nature* 439: 358–362.

Sosman, J., Kim, K., Schuchter, L., Gonzalez, R., Pavlick, A., Weber, J. *et al.* (2012). Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. *N Engl J Med* 366: 707–714.

Stahl, J., Sharma, A., Cheung, M., Zimmerman, M., Cheng, J., Bosenberg, M. *et al.* (2004) Deregulated AKT3 activity promotes development of malignant melanoma. *Cancer Res* 64: 7002–7010.

Topalian, S., Hodi, F., Brahmer, J., Gettinger, S., Smith, D., McDermott, D. *et al.* (2012) Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*, PMID: 22658127.

Wilhelm, S., Carter, C., Tang, L., Wilkie, D., McNabola, A., Rong, H. *et al.* (2004) 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.

Wilmott, J., Long, G., Howle, J., Haydu, L., Sharma, R., Thompson, J. *et al.* (2012) Selective BRAF inhibitors induce marked T-cell infiltration into human metastatic melanoma. *Clin Cancer Res* 18: 1386–1394.