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The current state of targeted therapy in melanoma: this time it's personal

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Abstract

Treatment of metastatic melanoma has long been a challenge. Over the past 8 years significant advances have been made in understanding the genetic changes that drive melanoma development and progression. These studies have shown melanoma to be a heterogeneous group of tumors, driven by a diverse array of oncogenic mutations. There is now good evidence that activating mutations in the serine/threonine kinase *BRAF* and the receptor tyrosine kinase *KIT* constitute good therapeutic targets for restricted sub-groups of melanoma. In this article, we discuss the genetics and etiology of cutaneous and non-cutaneous melanoma and review some of the latest pre-clinical and clinical data on the new targeted therapy agents that are beginning to make an impact upon the lives of melanoma patients.

1. Introduction

Despite many years of research, disseminated melanoma remains a major clinical problem. This frustrating lack of progress led some commentators to describe melanoma as being "intrinsically therapy resistant" and there are suggestions that the resistance phenotype may be a characteristic of underlying melanocyte biology [1]. The recent years have seen an explosion in high throughput genomic profiling that have provided important new information about the molecular events that drive melanoma initiation and progression [2-4]. Of particular note is the frequent occurrence of mutations or amplifications in oncogenes that have opened opportunities for highly selective therapeutic targeting. Based upon these studies it is now clear that melanomas are a heterogeneous group of tumors with different etiologies requiring different therapeutic strategies. The current paradigm being explored in melanoma is one of targeted therapy, an approach that matches selective small molecule inhibitors to tumors expressing specific oncogenic mutations. This strategy is exemplified

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by the use of imatinib in chronic myeloid leukemia (CML) and gastrointestinal stromal tumors (GIST) and erlotinib in subsets of non-small cell lung cancer (NSCLC) that harbor activating *EGFR* mutations [5-7]. In the current review we discuss the latest research on molecular sub-grouping of melanoma and outline promising targeted therapy strategies being developed for the treatment of the molecular subtypes of melanoma.

2. The genetic sub-groupings of cutaneous and non-cutaneous melanoma

BRAF V600 mutations

The melanoma genomic revolution was kick-started by the 2002 discovery that ~50% of all melanomas harbor an activating mutation in *BRAF* [4] (Figure 1). Raf proteins constitute a 3 member family of Serine/Threonine kinases (ARAF, BRAF and CRAF) with closely overlapping functions [8]. So far over 50 distinct mutations in *BRAF* have been identified [9]. Of these, the *BRAF* V600E mutation, resulting from a valine to glutamic acid substitution, is by far the most common and accounts for over 80% of all reported *BRAF* mutations [4, 10]. Other less common, but clinically relevant variants identified from melanoma specimens are the V600K mutation (16% of all *BRAF* mutations) and V600D/V600R (3% of all *BRAF* mutations)[11]. Most of the oncogenic activity of mutant *BRAF* is mediated through activation of the mitogen activated protein kinase (MAPK) cascade, which regulates the cell cycle entry through control of cyclin D1 expression, and the suppression of $p27^{KIP1}$ [12, 13] as well as through effects upon invasion and survival [14, 15]. In experimental systems, the role of mutated *BRAF* in melanoma seems convincing with *in vitro* studies showing that the *BRAF* V600E mutation is an oncogene in immortalized mouse melanocytes [16] and that selective downregulation of the *BRAF* V600E mutation using RNAi leads to reversal of the melanoma phenotype [17, 18]. Although mutated *BRAF* is undoubtedly important for melanomagenesis, introduction of *BRAF* V600E alone is not sufficient for the transformation of primary human or mouse melanocytes[19]. Instead, melanoma development seems to require both BRAF/MAPK and activity in the PI3K/AKT pathway. This was most convincingly demonstrated in a recent transgenic mouse study showing that full transformation to melanoma occurred only when the *BRAF* V600E mutation was activated in concert with suppression of PTEN expression[20].

Although *BRAF* mutations are not ultraviolet (UV) radiation signature mutations, they have a tendency to occur on UV-exposed skin sites and are more prevalent in individuals with a poor tanning response [21]. There is also evidence that intermittent, rather than chronic sun-exposure is predictive for *BRAF* mutational status with *BRAF* mutant melanoma patients tending to be younger in age (<55 years old) with a lower cumulative UV exposure [22]. *BRAF* mutational status is also of prognostic value and is associated with inferior survival in the metastatic setting (8.5 months in BRAF wild-type vs 5.7 months for *BRAF* mutant melanoma)[23].

Small molecule BRAF inhibitors: Sorafenib, PLX4032/4720, GSK2118436

Since the discovery of activating BRAF mutations in melanoma, a number of BRAF inhibitors have been developed and subjected to extensive *in vitro* testing [24-28]. The most extensively studied of these is the kinase inhibitor sorafenib (BAY43-9006, Nexxavar ®) [28]. Although originally developed as a CRAF inhibitor, sorafenib was also found to inhibit BRAF with moderate potency and was initially embraced as being the proof-of-concept for BRAF inhibition in melanoma[29]. In animal studies, sorafenib treatment led to limited regression of *BRAF* V600E mutated melanoma xenografts and was associated with only minor levels of apoptosis induction [29, 30]. Further pre-clinical investigations have shown sorafenib to be a relatively weak inhibitor of BRAF, with many off-target effects most notably inhibition of VEGFRs and PDGFRs [28, 31]. It now seems likely that any anti-

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melanoma activity of sorafenib is largely independent of its putative effects upon BRAF inhibition[32].

Since the evaluation of sorafenib a new generation of BRAF inhibitors has been developed. These drugs show higher potency against mutated *BRAF* and have fewer off-target effects; the list of those currently under pre-clinical investigation includes: SB590885, dabrafenib (GSK2118436), AZ628, XL281, GDC-0879 and vemurafenib (RG704, PLX4032/4720) [24, 25, 33-39]. PLX4032 (and its analogue PLX4720) are ATP-competitive RAF inhibitors (wt *BRAF* IC50 100nM, mutated *BRAF* 31nM) that selectively inhibit growth in melanoma cell lines harboring the *BRAF* V600E mutation both *in vitro* and *in vivo* mouse in xenograft models [25, 38, 40]. Henceforth we will refer to PLX4032 in the discussion of the preclinical studies and vemurafenib in the context of clinical studies. Responses to PLX4032 in melanoma xenograft models were *BRAF* V600E specific and impressive; with either partial or complete responses observed in all cases with a close relationship observed between drug exposure and response within individual xenograft models [38, 40]. Interestingly, not all *BRAF* mutated melanoma cell lines were similarly sensitive to PLX4032 and PLX4720, with a significant proportion showing varying degrees of intrinsic resistance [33, 36, 37]. Current data suggests that PLX4032/4720 induces both cell cycle arrest and apoptosis in the most sensitive cell lines and cell cycle arrest only in resistant less sensitive cell lines [33, 37]. A recent genetic study, looking for patterns of mutation and genomic amplification between PLX4032 sensitive and resistant cell lines, was unable to identify any unifying differences between the two groups [37]. It therefore seems that intrinsic resistance to BRAF inhibitors may be complex and multi-factorial [41]. There is already evidence that sub-groups of *BRAF* V600E mutated melanomas exist with alterations in *PTEN, cyclin D1, CDK2, CDK4, MITF* and *AKT3* [42, 43]. How the expression and mutational status of these genes impacts upon biological behavior and future therapy selection remains to be determined.

Another BRAF inhibitor currently exciting much interest in both the pre-clinical and clinical arenas is dabrafenib (GSK2118436), an ATP-competitive inhibitor of BRAF V600E/D/K, wild-type BRAF and CRAF [44]. The compound has been shown to have promising activity in pre-clinical models of melanoma and is now undergoing clinical evaluation [45, 46].

Low-activity (non-V600) *BRAF* mutants and CRAF-dependent melanomas—A minor sub-group of melanomas have been identified with *BRAF* mutations in positions other than 600. Many of the non-V600 *BRAF* mutants tend to show impaired BRAF kinase activation in isolated kinase assays (hence the name “low-activity” *BRAF* mutants) and required the presence of CRAF to transactivate their MAPK signaling [10]. Analysis of a large panel of melanoma cell lines and tissues revealed that ~1% of melanoma cell lines had either D594G or G469E mutation in *BRAF*, respectively and that 1% of melanoma specimens harbored a G469A mutation in *BRAF* [30]. These non-V600 *BRAF* mutated cell lines differed in their signaling from the *BRAF* V600E mutants and showed high levels of phospho-ERK, low levels of phospho-MEK and resistance to MEK inhibition [30]. Interestingly, these non-V600 *BRAF* mutants seem to form part of a broader sub-group of melanoma cell lines, including some that are *BRAF* wild-type and *BRAF* V600K mutated, that are reliant upon CRAF for their survival [47]. Studies from two independent groups have now demonstrated that shRNA knockdown of CRAF in the CRAF-dependent melanoma groups leads to MEK-independent effects upon BAD phosphorylation and Bcl-2 expression, leading in turn to apoptosis and impaired tumor growth in a mouse xenograft model [30]. Although sorafenib is a relatively weak BRAF inhibitor, it does show reasonable potency against CRAF. There is pre-clinical evidence demonstrating that sorafenib has good pro-apoptotic activity against melanoma cell lines with low-activity *BRAF* mutations, and leads to regression of these cell lines grown as mouse xenografts [30]. Furthermore the development of more selective and

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potent inhibitors of CRAF may also offer benefit for melanomas and other malignancies expressing these low-activity/non-V600 mutations in *BRAF*.

NRAS, KRAS and HRAS—RAS proteins constitute a large family of low molecular weight GTP-binding proteins that localize to the plasma membrane. Three of the RAS family members, *NRAS, HRAS* and *KRAS* are often mutated in human cancers, and >20% of all tumors harbor activating mutations in one of their RAS genes [48]. Mutations in *NRAS, KRAS and HRAS* have been identified in 20%, 2% and 1% of all melanomas, respectively [49]. Mutations in *NRAS* are most commonly the result of a point-change leading to the substitution of leucine to glutamine at position 61 [4, 50], with mutations at positions 12 and 13 being reported less frequently [2]. Large-scale analysis of melanoma samples and cell lines have shown that although *BRAF* V600E and *NRAS* mutations are mutually exclusive, there is overlap between low activity *BRAF* mutations and *NRAS* mutations [2, 51]. Mechanistically, mutations in *NRAS* lead to impairment of GTPase activity, so that the GTPase is locked into its "On" position. In its GTP-bound state RAS binds to and activates a number of effector enzymes involved in proliferation, the best characterized of these being CRAF [52]. Thus, melanomas harboring activating *NRAS* mutations differ from melanomas with *BRAF* mutations in that they rely upon CRAF to induce their MAPK pathway activity [52]. RAS is also known to activate the phospho inositide-3-kinase (PI3K)/AKT pathway, which contributes to tumor progression via the modulation of growth and survival of transformed cells [13]. In addition to MAPK and PI3K/AKT, mutated *NRAS* can also activate other intracellular signaling pathways important for malignant transformation. In particular, recent studies have demonstrated the importance of Ral guanine nucleotide exchange factors (Ral-GEFs) in the anchorage-independent growth observed following the *NRAS*-mediated melanocytes transformation [53]. A role for *RAS* mutations in melanoma initiation has been confirmed in animal models, where the introduction of mutated *HRAS* or *NRAS* (Q61K) leads to melanoma in transgenic mice lacking expression of the CDK inhibitor $p16^{INK4A}$ [54, 55].

Farnesyltransferase inhibitors, dual MEK/PI3K inhibition

NRAS is a small GTPase, and thus a difficult target for conventional drug discovery [48]. Farnesyltransferase inhibitors (FTIs), a class of drugs that prevent the membrane localization (and thereby activation) of small G-proteins, were originally developed as agents to target oncogenic Ras signaling [56]. Despite an extensive research effort, these compounds have shown little single-agent activity, even in colorectal carcinoma where ~40% of the tumors have activating mutations in *KRAS* [56]. FTIs have never been evaluated in a clinical trial of melanoma patients selected for their *NRAS* status. Although there is limited evidence that FTIs may have some activity against melanoma cell lines *in vitro*, these studies preceded the era of molecular sub-grouping melanoma cell lines [57]. Attention has now turned to pathways that are downstream of Ras activation that may be more tractable to pharmacological intervention. Pre-clinical evidence suggests that simultaneous blockade of the MEK and PI3K pathways leads to the regression of Ras-driven tumors in animal models [58, 59]. Other experimental studies have shown that dual inhibition of BRAF and CRAF or BRAF and PI3K (using shRNA knockdown) was effective at reducing the growth and survival of *NRAS*-mutated human melanoma xenografts [60]. Although *NRAS* mutated melanomas are known to rely upon CRAF for their MAPK signaling, there is little evidence that sorafenib is any more effective on the *NRAS* mutants than melanoma cell lines with *BRAF* mutations [30].

KIT—Melanomas developing on body sites with low-levels of environmental ultraviolet radiation exposure, such as on the soles of the feet or subungual sites (acral melanomas) or the mucous membranes (mucosal melanomas) are known to have a low incidence of *BRAF*

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mutations [61]. Instead, these more rare histological sub-types of melanoma are often associated with genetic amplification of and/or activating mutations in the receptor tyrosine kinase *KIT*. A recent landmark study showed that 21% of mucosal melanomas, 11% of acral melanomas and 17% of melanomas arising on sun-damaged skin harbor activating mutations in *KIT*, with many of these occurring at the imatinib-sensitive juxatmembrane position [61]. Sequencing of *c-KIT* exons 11, 13, 17 and 18 revealed the most prevalent mutations to be K642E, L576P, D816H and V559A, that interestingly are enriched at different locations from KIT mutations in GIST or hematological malignancies [62]. In most cases, the acquisition of a *KIT* mutation was accompanied by an increase in copy number and some degree of genomic amplification. There were also instances where *KIT* was amplified in the absence of a mutation [61]. Since the initial report of *KIT* aberrations in melanoma, a number of further studies have validated this finding [63-65]. Pooling of the currently available data suggests the *KIT* mutational frequency to be 14% in acral melanoma and 18% in mucosal melanoma [66]. Given that acral and mucosal melanomas each represent only 2% of all melanomas, the total percentage of melanomas with *KIT* mutations is likely to be quite low. Moreover in an Australian population where over 40% of melanomas are associated with chronic sun damaged skin KIT mutations were observed at a similar low frequency of 2% [67].

In experimental studies, introduction of the D814Y mutant of *KIT* into non-tumorigenic immortalized melanocytes did not lead to either oncogenic transformation or enhanced proliferation [68]. The lack of proliferation seen in the *KIT* mutated melanocytes was unexpected, but is likely to be a consequence of *KIT* constituting only one oncogenic “hit”. In agreement with this idea, it was recently shown that the two most common *c-KIT* mutations found in melanoma (K642E and L576P) were only able to transform melanocytes when grown under hypoxia or following the introduction of exogenous hypoxia-inducible factor 1α(HIF-1α) [69]. Mechanistically, it seemed that introduction of mutated *c-KIT* activated PI3K/AKT signaling but not MEK/ERK and that the combination of hypoxia and mutated *c-KIT* was required to fully activate both pathways. Interestingly, these data again support the transformation model seen in *BRAF* mutated melanoma where dual MAPK/AKT signaling is required for tumor initiation and progression [69].

Imatinib, sunitinib and dasatanib

The relative scarcity of melanoma cell lines harboring activating *KIT* mutations has made pre-clinical studies a challenge. It is becoming clear that the presence of a *KIT* mutation, rather than genomic amplification is predictive of response to small molecule KIT inhibitors [66]. It further seems likely that the nature of the *KIT* mutation dictates which KIT inhibitor should be used [66]. To date, only two pre-clinical studies have been published on melanoma cell lines derived from either acral or mucosal melanomas [65, 70]. The first of these characterized 3 primary mucosal melanoma cell lines, of which one was noted to have an exon-11 V559D mutation in *c-KIT* [65]. Treatment of this cell line with imatinib led to cell cycle arrest and apoptosis induction and was associated with inhibition of JAK/STAT, PI3K/AKT and MAPK signaling and the inhibition of Bcl-2, survivin and Mcl-1 expression [65]. A second study reported the identification of a mucosal melanoma cell line with a D820Y exon-17 mutation in *c-KIT* (the mutation often associated with imatinib resistance in GIST) with sensitivity to sunitinib (only at high concentrations) [70]. One other recent publication reported the identification of a non-acral/non-mucosal melanoma cell lines harboring an L576P *KIT* mutation [71]. In this instance, the cell line was found to be resistant to imatinib, nilotinib and sorafenib but sensitive to dasatanib [71]. There is also limited evidence suggesting that the presence of constitutive KIT activity (as shown by phospho-KIT) may be predictive of KIT inhibitor responsiveness [72].

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Another subtype of melanoma that seems to be associated with activation of *KIT* signaling are those arising in the pigmented cells of the eye. Uveal melanoma is the most common primary eye tumor in adults; these derive from the melanocytes of the choroid, ciliary body and iris. In common with other forms of non-cutaneous melanoma, uveal melanomas generally lack activating mutation in *BRAF* and *NRAS* [73] and instead maintain expression of c-KIT in up to 87% of cases. Although unlike acral and mucosal melanomas, uveal melanomas typically lack activating c-*KIT* mutations [74]. Cell culture experiments have demonstrated that uveal melanoma cell lines harbor phospho-KIT expression and undergo imatinib-mediated cell cycle arrest [75]. Clinical results of KIT inhibitors in uveal melanoma have not been reported.

GNAQ and GNA11—Activation of MAPK signaling seems to be a requisite for melanoma development. In uveal melanomas, most of which lack *BRAF* and *RAS* mutations, there is emerging evidence that MAPK activity is driven through activating Q209 mutations in the heterotrimeric G-protein alpha subunit GNAQ [76] or the equivalent residue in the closely related G-protein alpha subunit GNA11. Mutations at Q209 of GNAQ are analogous to those seen in *NRAS* (at Q61), and result in impaired GTPase activity leading to constitutive signaling. Large scale molecular profiling has identified GNAQ Q209 mutations in 46-49% of uveal melanoma samples and 27% of uveal melanoma cell lines [76, 77]. *In vitro* studies showed that although introduction of GNAQ-Q209L alone was unable to transform primary human melanocytes, it did lead to anchorage-independent growth when transfected into immortalized melanocytes (mutated p53/CDK4 and hTERT) [76]. Although no small molecule inhibitors of GNAQ currently exist, the potential therapeutic relevance of this G-protein was demonstrated by that fact that siRNA knockdown of GNAQ led to increased cell death in uveal melanoma cell lines that harbored the mutation [76].

4. Matching treatments to mutational profiles: clinical data

The preclinical data described above has led to a number of hypothesis-driven clinical trials to target the oncogenic mutations found in melanoma. The initial attempts to target oncogenic mutations in melanoma were to treat all melanomas regardless of genotype with targeted agents. This has now been replaced by clinical studies where patients are selected based on mutational profile that is leading to some striking results.

MEK inhibitors

The findings of frequent mutations in *BRAF* and *NRAS* in melanoma led to the evaluation of MEK inhibitors in melanoma including AZD6244, CI-1040 and PD0325901. These early studies did not select patients based on genotype and it was not clear if these agents were able to reliably inhibit MEK and phosphorylation of ERK in melanoma cells at the maximum tolerated doses[78-80]. Results were disappointing with ~10% objective response rates and retrospective analyses of genotypes in a subset of treated patients failing to predict clinical benefit. Dosing with MEK inhibitors has been limited by diarrhea and visual disturbance including retinal vein thrombosis in a small subset of patients. Recently the MEK inhibitor GSK1120212 with more reliable and sustained inhibition of MEK and pERK at clinically achievable doses has been evaluated in melanoma patients harboring *BRAF* V600E mutations with preliminary data indicating response rates in excess of 20% [81, 82]. Definitive randomized studies with this agent are planned that should determine the duration and rate of clinical response to inhibition of MEK in patients with advanced disease.

BRAF inhibitors

Sorafenib was the first RAF inhibitor to enter clinical development in patients with melanoma. In these initial studies patients were not selected on the basis of genotype. Due to

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its poor selectivity for the active conformation of BRAF induced by the V600E mutation, coupled with significant "off-target" activity, results were disappointing with very low response rates [83]. Randomized studies of sorafenib in combination with chemotherapy have given variable results with low response rates but provided no evidence that clinical effects were mediated through inhibition of BRAF [31, 84].

In contrast, the development of BRAF-inhibitors that are selective for the active conformation of the kinase have given very encouraging results. The BRAF-inhibitor vemurafenib (PLX4032) when delivered at the maximum-tolerated dose, induces strong inhibition of downstream signaling as determined by inhibition of pERK, reduction in Ki67 and inhibition of glucose uptake into melanoma metastases as measured by FDG-PET [40, 85] (Figure 2). In a recent phase III trial of individuals with previously untreated *BRAF* V600E mutant melanoma (n=675) vemurafenib treatment (960mg BID twice daily) led to responses in 48% of patients [86]. The response rate of the dacarbazine control group was 5%. At 6 months, the overall survival was 84% and 64% for the vemurafenib and dacarbazine treated groups, respectively[86]. Similar impressive effects on signaling and response have been observed with another selective BRAF inhibitor GSK2118436 [45] that has also entered phase III clinical trials.

Interestingly all BRAF inhibitors including sorafenib, vemurafenib, GSK2118436 and XL281 that have been evaluated clinically have induced proliferative squamous lesions in the skin that closely resemble squamous cell carcinomas of the keratoacanthoma type [39, 45, 85-87]. These lesions are frequently rapidly growing and can be managed with surgery or other local measures. Although the mechanism by which inhibition of RAF kinases induces these lesions remains to be fully elucidated there is now clinical evidence that the paradoxical activation of MAPK signaling, arising as a result of BRAF inhibition, may play a role in SCC development (see below) [46].

KIT inhibitors

Inhibition of KIT by tyrosine kinase inhibitors has been reported to induce clinical responses in melanomas harboring KIT mutations. Case reports and small series have reported objective responses to the KIT-inhibitors imatinib and sorafenib [88-92]. Definitive randomized studies are underway to determine if KIT-inhibition improves progression-free survival in melanoma (clinicaltrials.gov number NCT01028222). As 5 KIT inhibitors are currently approved for GIST or CML (imatinib, dasatnib, nilotinib, sorafenib and sunitinib) that have varying inhibitory profiles against a range of *KIT* mutations, it may be possible to match an individual *KIT* mutation to a particular drug. Indeed clinical response has been reported to sorafenib in a case of metastatic anal melanoma with the imatinib resistant mutation D820Y[91].

5. Resistance and the development of combination therapies

Although the presence of an activating *BRAF* V600E mutation generally predicts for sensitivity to BRAF inhibition, not all patients with *BRAF* V600E mutant melanoma respond to vemurafenib and there is evidence that some patients may be intrinsically resistant [85]. Recent preclinical studies have demonstrated that amplification of cyclin D1 (in up to 17% of *BRAF* V600E mutant melanomas) or loss of the tumor suppressor RB together with loss of the tumor suppressor phosphatase and tensin homolog (PTEN) may both contribute to intrinsic BRAF inhibitor resistance [93-95]. In the case of PTEN loss, BRAF inhibition was found to paradoxically activate AKT which prevented cell death by suppressing the levels of the pro-apoptotic protein BIM [94].

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Almost all *BRAF* mutant melanoma patients who respond to vemurafenib ultimately fail therapy and become resistant. These observations mirror the pattern of response seen to targeted therapy in CML, GIST [96, 97] and most recently medulloblastoma [98, 99], where an initial period of tumor regression is later followed by relapse. Even before the development of BRAF specific inhibitors it was already known that both growth factors and cytokines could rescue melanoma cells from apoptosis following the siRNA-induced knockdown of BRAF[100, 101, 82]. A number of studies have now begun to address the mechanisms of acquired BRAF inhibitor resistance in both melanoma cell lines and specimens from *BRAF* mutant melanoma patients failing vemurafenib therapy. So far a large number of potential acquired resistance mechanisms have been reported; these include upregulated receptor tyrosine kinase signaling (both PDGFRβ and IGF1R), the emergence of apparently *de novo* mutations in *NRAS*, acquisition of novel mutations in *MEK1* and the increased expression of MAP3K8 (otherwise known as COT) [102-106]. Although the resistance mechanisms reported so far are diverse, nearly all involve the reactivation of a common set of signaling pathways already known to be important for melanoma progression, such as MEK/ERK and PI3K/AKT/mTOR[106]. There is already preclinical data showing that dual BRAF + MEK inhibition may prevent or delay the onset of resistance to PLX4720 and may overcome resistance mediated by MEK1 mutations, COT overexpression and the acquisition of *de novo NRAS* mutations [104, 107, 108]. In contrast, resistance mediated through IGFR1 signaling can be overcome by dual MEK + PI3K inhibition and resistance mediated through PDGFR signaling can be ameliorated through the targeting of the mTOR/PI3K/AKT pathway [103]. As a number of these mechanisms reactivate MEK phase I/II trials of the BRAF inhibitor GSK2118436 in combination with the MEK inhibitor GSK1120212 (NCT01072175) and vemurafenib combined with the MEK inhibitor GDC-0973 (NCT01271803) are underway. There are already indications that these combinations may be effective. In a recent presentation at ASCO, the phase I/II trial of the GSK112012 + GSK2118436 combination was associated with objective response rates (complete response + partial response) of 77% at a dose level of 150mg GSK118436/1mg GSK112012 and 74% at the dose level of 150mg GSK118436/2mg GSK1120212 [46]. Even more significantly, the combination of the BRAF + MEK inhibitor was associated with significantly reduced levels of squamous cell carcinoma (<1%, n=109) [46]. Other clinical studies combining BRAF inhibitors with inhibitors of the mTOR/PI3K/AKT pathway are due to commence in the near future.

The approach being taken to manage therapeutic escape in melanoma differs from the current model of treating acquired targeted therapy drug resistance seen in other cancers, where resistance is often associated with secondary mutations in drug target proteins. The most well known examples of this phenomenon are imatinib resistance in CML and GIST that arises as the result of *de novo* “gatekeeper” mutations in BCR-ABL and c-KIT, respectively [96, 109, 110]. Although preclinical studies identified Threonine-529 to be the BRAF gatekeeper site, there is currently little evidence that chronic treatment of melanoma patients with vemurafenib leads to acquisition of secondary mutations in *BRAF* at the gatekeeper site or at any others [32, 85, 102].

6. Future perspectives

The importance of patient selection

A future can be envisaged where the molecular profiling of patient tumors will become an integral part of therapy selection for medical oncologists. The importance of matching the right targeted therapy to the correct melanoma genotype is illustrated by recent pre-clinical studies showing that inhibitors of BRAF paradoxically activate MAPK signaling in tumors that lack activating *BRAF* mutations. Reports from at least 6 independent groups have shown that BRAF inhibition activates MAPK in cell lines with *NRAS* and *KRAS* mutations as well as those cell lines where the MAPK pathway is activated through other oncogenes

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such as *HER2* [34, 111-115]. Mechanistic studies showed that although vemurafenib and other BRAF inhibitors were able to profoundly inhibit the activity of *BRAF* V600E containing complexes in melanoma cells they instead promoted the activity of CRAF-CRAF dimers in cells with RAS mutations, leading in turn to MEK activation [34, 115]. There is also evidence that PLX4032 increases the invasive potential of *NRAS*-mutated melanoma cells through the through the activation of ERK and FAK signaling [113]. Additional studies demonstrated that BRAF inhibitors may even contribute to the progression of *NRAS* mutated melanomas in part by suppressing apoptosis through the modulation of Mcl-1 expression [114]. Following these observations a new generation of BRAF inhibitors were recently unveiled that apparently prevent the paradoxical activation of MAPK signaling. Although data is currently lacking on these new drugs, it is hoped that their improved selectivity profile may prevent the onset of SCC and delay the time to resistance.

These studies are extremely important in the approaching the development of new cancer therapies as they indicate that simple empiric evaluation of novel cancer therapeutics in patients could be associated with adverse outcomes. Instead they affirm the approach of rationally developing therapies in cancer patients based on strong preclinical data and individual patient molecular profiling. It is indeed time to get personal in treatment of melanoma.

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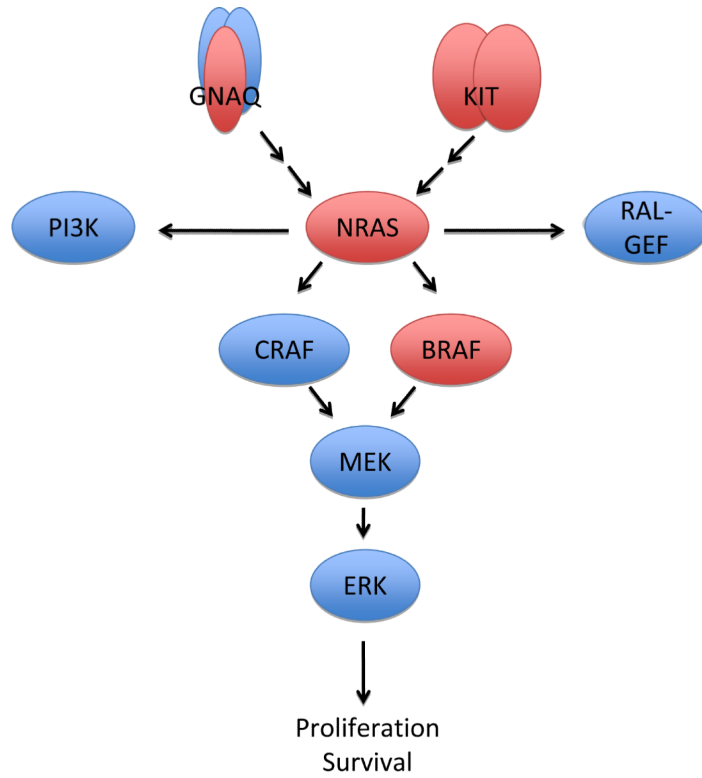

Figure 1.
Sample scheme showing some of the important molecular pathways important for melanoma progression. Genes with activating mutations in melanoma are highlighted in red.

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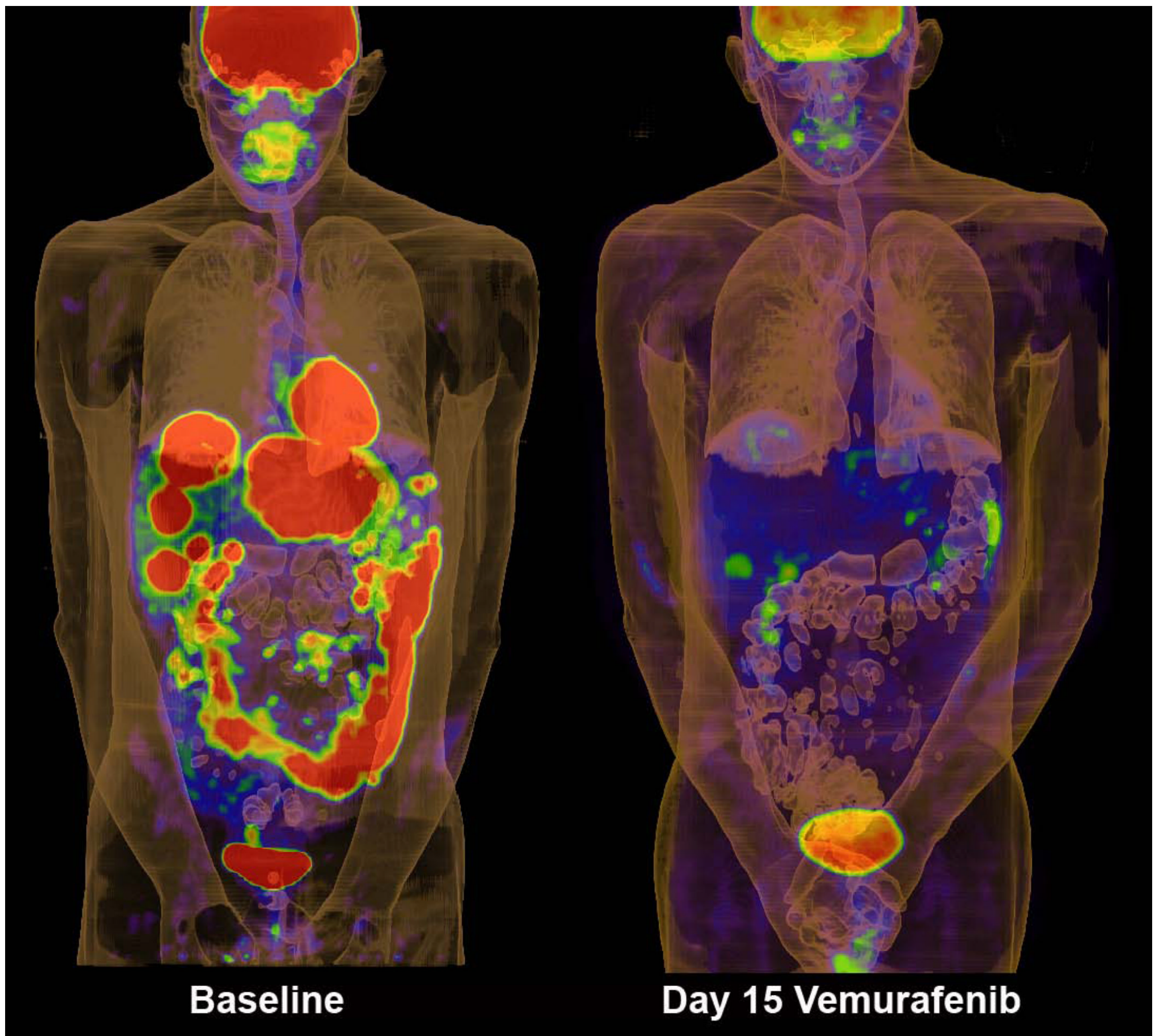

Figure 2.
FDG-PET scans of patient on the phase I trial of vemurafenib. Panels show scans of tumor burden at baseline and after 15 days of treatment at 960mg bid.