Therapeutic opportunities in noncutaneous melanoma

Ther Adv Med Oncol
(2009) 1(1) 29–36
DOI: 10.1177/1758834009337664

D.K. Wilkins and P.D. Nathan

***Abstract*:** Recent evidence suggests that the biology of noncutaneous melanoma differs significantly from cutaneous melanoma and may provide therapeutic opportunity. The most frequent sites of origin of noncutaneous melanoma are the eye and mucosal surfaces. Although noncutaneous melanomas are an uncommon group of cancers (representing less than 10% of all melanomas) a greater understanding of their genetic and molecular abnormalites is being translated into novel treatment strategies. These developments are important because there is currently no effective systemic therapy for noncutaneous melanoma. Significant attention has been focused on the role of c-kit (KIT, CD117), a transmembrane receptor with tyrosine kinase activity. *In vitro* and *ex vivo* evidence suggests that c-kit is frequently expressed/over expressed/mutated in noncutaneous melanoma. Anti-tumour effects with c-kit inhibitors are seen in pre-clinical models. A variety of multitargeted kinase inhibitors which have activity against c-kit are currently in early phase clinical trials in metastatic ocular, mucosal and acral melanoma. The few case reports of significant clinical activity with targeted therapies provides hope that greater understanding of the biology of noncutaneous melanoma can be translated into effective treatment.

***Keywords*:** melanoma, noncutaneous, uveal, mucosal, c-kit, imatinib

Correspondence to:
P.D. Nathan
Consultant Medical Oncologist, Mount Vernon Cancer Centre - Medical Oncology, Rickmansworth Road, Northwood, Middlesex, HA6 2RN, UK
nathan.pd@gmail.com

D.K. Wilkins
Specialist Registrar, Medical Oncology, Mount Vernon Cancer Centre - Medical Oncology, Rickmansworth Road, Northwood, Middlesex, HA6 2RN, UK

Introduction

Melanoma is a malignant neoplasm of melanocytes. Most cases (91.2%) arise in the skin (cutaneous melanoma) however melanoma can also arise at a variety of noncutaneous sites including the eye (5.2%) and mucosal surfaces (1.3%) [Chang *et al*. 1998]. There is no effective systemic therapy for noncutaneous melanoma and novel therapies are urgently required.

Recent developments have thrown new light on the genetic and molecular mechanisms underlying noncutaneous melanoma. Current evidence suggests that the biology of noncutaneous melanoma differs significantly from cutaneous melanoma and may provide therapeutic opportunity. We describe current understanding of those molecular pathways thought to be important in the pathogenesis of noncutaneous melanoma and how this evidence is being translated into clinical trials with targeted agents.

Uveal melanoma

The most frequent site of primary ocular melanoma is the uveal tract (choroid, iris, ciliary body). Conjunctival melanoma occurs significantly less frequently than melanoma arising in the uveal tract. Uveal melanomas are the most common primary intra-ocular malignancy in adults. The largest published series on the incidence of uveal melanoma provided data from 22 European countries [Virgili *et al*. 2007]. Standardized incidence rates varied from <2 per million in Spain and Southern Italy to >8 per million in Denmark and Norway. Meta-analyses have identified light eye colour (blue or grey), fair skin colour, propensity to sunburn and personal history of welding as risk factors for uveal melanoma [Weis *et al*. 2006; Shah *et al*. 2005]. Patients with oculodermal melanocytosis have a 1 in 400 lifetime risk of developing a uveal melanoma [Singh *et al*. 1998].

Treatment of localized uveal melanoma depends on the size and location of the lesion and includes enucleation, plaque radiotherapy, transpupillary thermotherapy and local resection. Damato and colleagues reported outcomes on 356 patients treated for uveal melanoma and found that the most important risk factors for development of metastatic disease were increased basal tumour diameter, epitheloid cell type and monosomy of

chromosome 3 [Damato *et al*. 2007]. Metastatic spread is haematogenous (there are no lymphatics in the eye) and there is a striking tendency to metastasize to the liver.

For patients who are not suitable for resection of liver metastases there is no systemic treatment of proven clinical value. A phase II multi-centre EORTC trial of bleomycin, vincristine, lomustine and dacarbazine (BOLD) plus interferon alpha-2b, produced no objective responses [Kivela *et al*. 2003].

Corrie and coworkers conducted a phase I trial to investigate gemcitabine and treosulfan (an alkylating agent) in patients with metastatic melanoma [Corrie *et al*. 2005]. In 5 patients with uveal melanoma no complete responses (CR) or partial responses (PR) occurred. A randomized phase II trial compared gemcitabine and treosulfan with treosulfan alone [Schmittel *et al*. 2006]. Eligible patients were permitted to have received previous cytokine therapy but not chemotherapy. The primary end-point was response rate plus stabilization, that is, CR + PR + stable disease (SD). Forty eight patients were randomized (24 into each arm). There was a trend towards improved response rate plus stabilization with combination therapy (33% *versus* 13%, $p = 0.07$). Median progression free survival was significantly higher with combination therapy (3 months *versus* 2 months, $p = 0.008$). However the absolute benefit of combination therapy was disappointing with no patients achieving a CR and only 1 patient (4%) achieving a PR.

Mucosal melanoma

Mucosal melanomas are rare and account for 1.3% of all melanomas. The most frequent sites for mucosal melanoma are the head and neck (55.4%), anal/rectal (23.8%), female genital tract (18%) and urinary tract (2.8%) [Chang *et al*. 1998]. Within the head and neck, the nasal cavity, oral cavity and sinuses are most commonly affected. The mean age at diagnosis is 67 years, somewhat older than in cutaneous melanoma in which it is 55 [Chang *et al*. 1998]. Treatment for localized/nodal disease is surgical. There is no systemic therapy of proven efficacy in the adjuvant setting or for metastatic disease.

Molecular differences between melanoma subtypes

There is an urgent need to develop effective systemic therapies for noncutaneous melanoma. A greater understanding of the molecular and genetic characteristics of these tumours is likely to form the basis of selective drug treatment.

Curtin and colleagues explored the hypothesis that different types of melanoma have distinct abnormalities in their genome and protein expression [Curtin *et al*. 2005]. They examined 126 primary melanoma specimens and divided them into four categories: acral (palms, soles, subungual), mucosal, cutaneous with chronic sun-induced damage and cutaneous without chronic sun-induced damage. They examined genome-wide alterations in the number of copies of DNA. In addition they examined mutations involving components of the mitogen-activated protein (MAP) kinase pathway and the phosphatidylinositol 3 kinase (PI3K) pathway.

Compared to either category of cutaneous melanoma, acral and mucosal melanomas were significantly more likely to have chromosomal aberrations. Furthermore because the genetic location of aberrations varied between melanoma categories acral could be distinguished from mucosal in 89% of cases and the two cutaneous categories could be distinguished in 84% of cases. In the MAP kinase pathway BRAF mutations were significantly more common in cutaneous melanoma without sun damage than in the other three categories. Over-expression of CCND1 (a protein in the MAP kinase pathway downstream of BRAF) only occurred in tumours that had amplification of the CCND1 gene or mutation of BRAF or NRAS. This highlights how over-expression of a protein can be caused by direct and upstream mechanisms. Amplifications of the CDK4 gene (CDK4 protein is a binding partner to CCND1) were significantly more common in acral and mucosal melanomas than either of the cutaneous melanomas. In the 11 tumours that had CDK4 amplification, none had aberrations in BRAF, NRAS or CCND1 which suggests that CDK4 is an independent oncogene. Gene deletions to CDKN2a (p16), a negative regulator of CDK4-CCND1 complex, were significantly more frequent in acral and mucosal melanomas than either category of cutaneous melanoma.

Activation of the PI3K pathway was seen in 100% of tumours with NRAS mutation and 82% of samples with loss of PTEN (a negative regulator of the PI3K pathway). PTEN deletion was significantly more frequent in tumours with

BRAF mutation than NRAS mutation; this is probably because mutation to BRAF only upregulates the MAP kinase pathway whereas mutation to NRAS upregulates the MAP kinase and PI3K pathways.

C-kit and uveal melanoma

C-kit (KIT, CD117) is a transmembrane receptor with tyrosine kinase activity. Binding of stem cell factor (SCF, kit ligand) to c-kit induces dimerization of the receptor and autophosphorlyaytion of tyrosine residues. Phosphorlyation initiates down stream signaling events including activation of the MAP kinase pathway and the PI3K pathway. C-kit is an established molecular target in oncology and is pathogenically important in gastrointestinal stromal tumours (GIST). Inhibition of c-kit by imatinib, an inhibitor of several receptor tyrosine kinases including c-kit, bcr-abl and platelet derived growth factor receptor (PDGFR), has transformed the management of GIST.

C-kit expression is demonstrated in 75% of choroidal melanoma specimens and c-kit expression is positively associated with mitotic activity [Mouriaux *et al*. 2003]. Lefevre and colleagues examined the role of c-kit, its ligand SCF and the downstream kinases ERK1/2 in normal uveal melanocytes and human uveal melanoma cell lines [Lefevre *et al*. 2004]. C-kit was expressed in normal uveal melanocytes and four of six human uveal melanoma cell lines. Furthermore inhibition of c-kit expression (by c-kit specific siRNA) reduced cell proliferation in c-kit positive human uveal melanoma cell lines but not in c-kit negative cell lines or normal uveal melanocytes.

No evidence of c-kit mutations were detected in normal uveal melanocytes or human uveal melanoma cell lines. Unlike the normal uveal melanocytes all 6 human uveal melanoma cell lines secreted SCF. In normal uveal melanocytes exogenous SCF was required to induce phosphorylation of c-kit Tyr^{703} and activation of ERK1/2. However in the Mel270 cell line (a c-kit positive cell line) Tyr^{703} phosphorylation and activation of ERK1/2 was found with or without the application of exogenous SCF. These results suggest that the secretion of SCF by the Mel270 cell line causes autocrine / paracrine stimulation of the Ras/Raf/MEK/ERK1/2 pathway. Inhibition of c-kit expression or ERK1/2 activation significantly reduced cell proliferation in Mel270 cell lines.

Treatment of human uveal melanoma cell lines with imatinib greatly reduced proliferation in c-kit positive cell lines but had much less effect on c-kit negative cell lines (and no effect on normal melanocytes). Furthermore imatinib induced apoptosis in c-kit positive human uveal melanoma cell lines but not in c-kit negative human uveal melanoma cell lines. The effects on c-kit positive cells appeared to be secondary to decreased phosphorylation of c-kit Tyr^{703} and decreased activation of ERK1/2, that is, inhibition of the MAPK pathway.

The demonstration that c-kit is frequently expressed on uveal melanoma, c-kit expression is positively associated with mitotic activity, c-kit positive cells are dependent on c-kit for proliferation and that proliferation in c-kit positive cells can be inhibited by imatinib, led to c-kit being proposed as a therapeutic target.

Several investigators have examined the efficacy of imatinib in patients with choroidal melanoma. Penel and coworkers conducted a multicentre, phase II trial of imatinib in patients with metastatic uveal melanoma [Penel *et al*. 2008]. Tumours were not examined for c-kit expression or c-kit genetic aberrations. Patients received imatinib 400 mg twice daily. Patients were considered assessable for response if they had received imatinib for at least 21 days. Thirteen patients were treated but three stopped imatinib before 21 days because of early disease progression. In ten assessable patients there were no complete responses or partial responses. Only one patient achieved stable disease which lasted for 20 weeks.

Imatinib had no efficacy in these unselected patients with metastatic uveal melanoma. Unfortunately the trial did not measure relevant tumour characteristics – e.g. c-kit expression or c-kit activating mutations. Furthermore the absence of pharmacodynamic biomarker data makes it impossible to be certain what affects (if any) imatinib was having on intracellular signaling within tumour.

Hofmann and colleagues also examined the efficacy of imatinib in patients with metastatic uveal melanoma [Hofmann *et al*. 2009]. Patients were not selected according to c-kit expression or c-kit genetic aberration but these characteristics were assessed after trial entry. Patients received imatinib 300 mg twice daily. Twelve patients were treated and nine patients received imatinib

≥8 weeks (three patients stopped before 8 weeks because of obvious disease progression). There were no complete or partial responses, the best tumour response being stable disease in one patient that lasted 52 weeks. C-kit expression was detected on 91% of primary tumours and 100% of metastatic tumours. Furthermore SCF was produced in >70% of primary and metastatic tumours.

Given the high percentage of tumours that expressed c-kit and the possibility of autocrine stimulation by SCF, why was there no efficacy with imatinib? One possibility is that c-kit was present but functionally normal, that is, it was not constitutively activated by mutation. This hypothesis is supported by the inability to find any mutations in exons 11, 13, 17 and 18 of the c-kit gene in tumour biopsies taken prior to imatinib treatment. Furthermore Hofmann and coworkers failed to find significant quantities of downstream phosphorylated ERK, which would have been expected if there were significant activation of the c-kit/MAP kinase pathway. Other studies have also failed to find c-kit gene aberrations in uveal melanoma, both in tumour specimens [Beadling *et al*. 2008] and in human uveal melanoma cell lines [Lefevre *et al*. 2004].

A further phase II clinical trial of imatinib in c-kit positive uveal melanoma (ITEM trial) is currently recruiting patients (EudraCT Number 2007-006216-39). Early evidence of clinical activity has been seen (P.D. Nathan, personal communication) and molecular analysis of tumours from responding patients is awaited.

An alternative method to target c-kit in uveal melanoma has been the use of sunitinib. Sunitinib is a multi-targeted tyrosine kinase inhibitor which inhibits c-kit, vascular endothelial growth factor receptors, PDGFRs and fms-related tyrosine kinase 3 (FLT-3). Chan and coworkers examined the efficacy of sunitinib in 10 patients with metastatic uveal melanoma [Chan *et al*. 2008]. Tumour samples were required to have ≥10% c-kit expression. One patient (10%) had a PR, seven patients (70%) had SD and two patients (20%) had disease progression.

A phase II pilot trial of sunitinib, tamoxifen, and cisplatin as adjuvant therapy in patients with high-risk ocular melanoma is currently recruiting patients (ClinicalTrials.gov Identifier NCT00489944).

C-kit in mucosal and acral melanoma

C-kit mutations have been reported in mucosal melanoma. Curtin and colleagues investigated genetic aberrations in 102 primary melanomas [Curtin *et al*. 2006]. The tumours were mucosal, acral, and cutaneous with or without chronic sun-induced damage. Seven tumours had amplification on chromosome 4q12, the region that contains the c-kit gene. Three of these tumours (all mucosal) demonstrated mutation in exons 11, 13, 17 and 18. All had a K642E mutation, known to be oncogenic in GIST tumours [Isozaki *et al*. 2000], and one had a N566D mutation.

In addition to seven tumours with amplification on chromosome 4q12, 11 additional patients had an increased copy number of the same region. In the 95 tumours without amplification ten tumours contained mutations in exons 11, 13, 17 and 18 and one tumour contained an intronic deletion. C-kit aberrations (mutation or increased copy number) were most frequent in mucosal melanoma (39%) but did not occur at all in cutaneous melanoma without chronic sun damage. In contrast BRAF mutation was rare in mucosal melanoma (3%) but common in cutaneous melanoma without chronic sun damage (56%).

Similarly Beadling and coworkers have demonstrated that the frequency of c-kit aberrations varies significantly between different types of melanoma and is most common in acral and mucosal melanoma [Beadling *et al*. 2008]. They examined 189 melanoma specimens (primary lesions and metastases) for c-kit mutations, c-kit copy number, c-kit expression, BRAF mutations and NRAS mutations. Mutations in c-kit exons 11, 13 and 17 were most frequent in acral melanomas (23%) and mucosal melanomas (16%). Similarly increased c-kit copy number was most frequent in acral melanomas (27%) and mucosal melanomas (26%). No choroidal melanomas had a c-kit mutation or an increase in c-kit copy number. In mucosal melanomas 25% had NRAS mutations but none had BRAF mutations.

Ashida and colleagues examined characteristics of the c-kit gene (mutation in exons 11, 13, 17 and 18 and copy number) and c-kit protein (expression, activation i.e. phosphorylation at Tyr^{719}) in mucosal and acral melanoma samples [Ashida *et al*. 2009]. C-kit expression was moderate (2+) or strong (3+) in 13 of 27 examined

samples (48%). Nineteen tumours (68% with moderate/strong c-kit expression) were examined for c-kit mutation. One tumour had a K642E mutation; a second had a D820Y mutation. Both tumours with c-kit mutation were acral. In addition fresh frozen tumour specimens were available from 13 patients. C-kit activation (phosphorylation of c-kit Tyr^{719}) was detected in 8 of 13 samples (62%). Of the eight samples with c-kit activation five had no c-kit gene aberration (mutation or amplification), two had c-kit gene mutation and one had kit gene amplification. In these eight samples with c-kit activation five of six examined (83%) had strong (3+) expression of SCF and six of seven examined (86%) had moderate (2+) or strong (3+) expression of c-kit. It is possible that in cells without c-kit gene aberration c-kit was activated by an autocrine or paracrine SCF loop. The *in vitro* effects of imatinib and sunitinib on acral melanoma cell lines were examined. In a c-kit positive cell line containing the D820Y mutation, sunitinib (but not imatinib) significantly reduced cell proliferation. In a c-kit positive cell line that does not have a c-kit gene mutation but does proliferate significantly in response to SCF (via c-kit activation) both sunitinib and imatinib caused a significant reduction in proliferation. In a control c-kit negative cell line imatinib had no effect but high-concentration sunitinib (10 μM) caused a significant reduction in proliferation, possibly due to inhibition of other tyrosine kinases.

The *in-vitro* effects of imatinib on mucosal melanoma cell cultures have also been investigated [Jiang *et al.* 2008]. Mucosal melanoma samples from three patients were used to produce three cell cultures. One contained an activating c-kit mutation (exon 11, V559A), one overexpressed c-kit and one had an increased copy number in the region of the c-kit gene. None of the cell lines had mutations in BRAF or NRAS. In the c-kit mutant cell line imatinib significantly reduced proliferation, induced G1 arrest, induced apoptosis, inhibited the MAPK pathway and inhibited the PI3K pathway. None of these effects occurred when imatinib was applied to c-kit cell lines that did not have a c-kit mutation.

There is emerging anecdotal evidence that imatinib can produce significant clinical responses in some patients with mucosal and acral melanoma. Hodi and coworkers reported on a 79-year old woman with metastatic c-kit positive rectal melanoma who had a significant reduction in tumour activity on FDG-PET/CT after 4 weeks of treatment with imatinib [Hodi *et al.* 2008]. Examination of c-kit gene exons 11, 13 and 17 revealed a seven codon duplication mutation within exon 11. A significant clinical response was also reported in a 69-year old woman with extensive loco-regional metastases from a primary anal melanoma [Lutzky *et al.* 2008]. Biopsies of both the primary tumour and a metastasis revealed strong c-kit expression. Treatment with imatinib 400 mg daily induced a complete clinical response. The disease relapsed after 5 months but a second complete clinical response was induced by increasing the imatinib dose to 600 mg daily. Examination of c-kit exons 11, 13, 17 and 18 revealed a mutation in exon 13 (K642E). In addition there was amplification of the mutated allele. Kim and colleagues reported on a 66-year old man with c-kit positive metastatic acral melanoma who had a partial response after receiving imatinib [Kim *et al.* 2008]. No mutations were detected in c-kit exons 9, 11, 13, 15 or 17. Amplification of the c-kit gene was not examined.

A phase II trial of imatinib in patients with c-kit mutant metastatic melanoma (mucosal, acral or cutaneous with chronic sun-induced damage) is currently recruiting patients (ClinicalTrials.gov Identifier NCT 00424515).

In addition to imatinib several other tyrosine kinase inhibitors with activity against c-kit are being investigated in patients with advanced mucosal melanoma. Two phase II trials are assessing the activity of sunitinib in patients with advanced mucosal melanoma (ClinicalTrials.gov Identifier NCT 00631618 and ClinicalTrials.gov Identifier NCT 00577382). The primary endpoint of both trials is objective response rate. NCT 00631618 requires patients to have tumour with aberration of the c-kit gene or receptor. Patient eligibility in NCT 00577382 does not depend on c-kit status but tumour tissue will be examined for c-kit mutation.

Nilotinib (AMN107) inhibits Bcr-Abl and c-kit [Weisberg *et al.* 2005] and is licensed for the treatment of adults with chronic phase and accelerated phase Philadelphia chromosome positive chronic myeloid leukaemia (CML) who have resistance or intolerance to prior therapy including imatinib. A phase II trial (ClinicalTrials.gov Identifier NCT 00788775) is examining nilotinib

in patients with c-kit mutated or amplified metastatic melanoma (mucosal, acral or cutaneous with chronic sun-induced damage). Patients are required to have progressed on imatinib or be intolerant of it. The primary trial endpoint is the percentage of patients who are alive and without disease progression 4 months after commencing nilotinib.

Dasatinib has activity against multiple tyrosine kinases including Bcr-Abl and c-kit [Steinberg, 2007]. It is licensed for the treatment of adults with chronic, accelerated or blast phase CML with resistance or intolerance to prior therapy including imatinib. Dasatinib is also indicated for the treatment of adults with Philadelphia chromosome positive acute lymphoblastic leukaemia and lymphoid blast CML with resistance or intolerance to prior therapy. A phase II trial of dasatinib is investigating the objective response rate in patients with advanced mucosal, acral or cutaneous with chronic sun-induced damage melanoma (ClinicalTrials.gov Identifier NCT 00700882). Metastatic tumour samples from eligible patients will be examined for c-kit mutation or amplification.

GNAQ

GNAQ is a gene that encodes a G-protein α-subunit, necessary for transmitting signals from G-protein coupled transmembrane receptors to intracellular signaling pathways. GNAQ has recently been identified as an oncogene in uveal melanoma.

Van Raamsdonk and coworkers examined 48 uveal melanomas and found GNAQ mutations in 46% [Van Raamsdonk *et al*. 2009]. No mutations to GNAQ were found in normal tissue adjacent to the tumour, indicating that the mutations were somatically acquired. All mutations occurred at codon 209 and all resulted in the substitution of the original glutamine. They examined the effect of transfecting normal and genetically modified melanocytes with $GNAQ^{Q209L}$. (The genetically modified melanocytes had an extended lifespan because of transduction with telomerase and inactivation of p53 and p16/CDK4/RB pathways.) Transfection of genetically modified melanocytes with $GNAQ^{Q209L}$ led to anchorage independent growth, although this did not occur when normal melanocytes were transfected. Melanocytes (melan-a cells) transfected with $GNAQ^{Q209L}$ (but not wild-type GNAQ) produced tumours in nude mice. There was also evidence that $GNAQ^{Q209L}$ activates the MAP kinase pathway, as evidenced by increased levels of phosphorylated ERK. Activation of the MAP kinase pathway did not appear to be secondary to mutations in BRAF or NRAS, since no mutations were found in these genes. It is likely that replacement of glutamine at codon 209 causes loss of GTPase activity and results in constitutive activation. Furthermore in a uveal melanoma cell line containing the $GNAQ^{Q209L}$ mutation, inhibition of GNAQ by siRNA caused decreased levels of phosphorylated ERK and a reduction in cell number.

Mutations in GNAQ were also detected in 83% of blue naevi. Blue naevi only rarely become malignant and so it appears that mutation in GNAQ alone is insufficient to cause a malignant phenotype (a situation analogous to mutation in BRAF or NRAS). Mutations in GNAQ were not detected in acral melanomas or mucosal melanomas.

Conclusion

Recent evidence has begun to reveal the genetic and molecular events underlying noncutaneous melanoma. Some of these insights have already been translated into early clinical trails. It is becoming apparent that significant biological differences exist between noncutaneous melanoma and cutaneous melanoma (and also between different categories of cutaneous melanoma). It is likely that ultimately, this will lead to different systemic therapies for different types of melanoma.

A thorough understanding of the biology is likely to be a requirement for clinical success. Identification of appropriate patients by prospective identification of the presence of the relevant drug target is likely to be required to enhance the chances of detecting clinical activity in trials. If this is not possible, phase II trials should be designed and appropriately powered with the knowledge that only a certain percentage of the study population is likely to have the molecular lesion that confers sensitivity to the drug under investigation.

Incorporation of relevant pharmacodynamic biomarkers into early phase clinical trials will aid interpretation of both responders and non-responders.

The few case reports of significant clinical activity with targeted therapies provide hope that a

greater understanding of the biology of these rare diseases, together with the increasing availability of appropriately targeted agents, will lead to significant improvements in treatment for these hitherto untreatable conditions.

Authorship

Both authors (D.K. Wilkins and P.D. Nathan) contributed to the design and content of this manuscript. The authors did not have assistance from any other people in the preparation of the manuscript.

Conflict of interest statement

The authors declare that there is no conflict of interest.

References

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

Beadling, C., Jacobson-Dunlop, E., Hodi, F.S., Le, C., Warrick, A., Patterson, J. *et al.* (2008) KIT Gene Mutations and Copy Number in Melanoma Subtypes. *Clin Cancer Res* 14: 6821–6828.

Chan, K.R., Gundala, S., Laudadio, M., Mastrangelo, M., Yamamoto, A. and Sato, T. (2008) A pilot study using sunitinib malate as therapy in patients with stage IV uveal melanoma. *J Clin Oncol* (suppl 20) 26: abstract 9047.

Chang, A.E., Karnell, L.H. and Menck, H.R. (1998) The National Cancer Data Base report on cutaneous and noncutaneous melanoma. A summary of 84,836 cases from the past decade. *Cancer* 83: 1664–1678.

Corrie, P.G., Shaw, J., Spanswick, V.J., Sehmbi, R., Jonson, A., Mayer, A. *et al.* (2005) Phase I trial combining gemcitabine and treosulfan in advanced cutaneous and uveal melanoma patients. *British Journal of Cancer* 92: 1997–2003.

Curtin, J.A., Fridlyand, J., Kageshita, T., Patel, H.N., Busam, K.J., Kutzner, H. *et al.* (2005) Distinct Sets of Genetic Alterations in Melanoma. *N Engl J Med* 353: 2135–2147.

Curtin, J.A., Busam, K., Pinkel, D. and Bastian, B.C. (2006) Somatic Activation of KIT in Distinct Subtypes of Melanoma. *J Clin Oncol* 24: 4340–4346.

Damato, B., Duke, C., Coupland, S.E., Hiscott, P., Smith, P.A., Campbell, I. *et al.* (2007) Cytogenetics of Uveal Melanoma: A 7-Year Clinical Experience. *Ophthalmology* 114: 1925–1931.

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

Hofmann, U.B., Kauczok-Vetter, C.S., Houben, R. and Becker, J.C. (2009) Overexpression of the KIT/SCF in uveal melanoma does not translate into clinical efficacy of imatinib mesylate. *Clin Cancer Res* 15: 324–329.

Isozaki, K., Terris, B., Belghiti, J., Schiffmann, S., Hirota, S. and Vanderwinden, J.M. (2000) Germline-activating mutation in the kinase domain of KIT gene in familial gastrointestinal stromal tumors. *Am J Pathol* 157: 1581–1585.

Jiang, X., Zhou, J., Yuen, N.K., Corless, C.L., Heinrich, M.C., Fletcher, J.A. *et al.* (2008) Imatinib targeting of KIT-mutant oncoprotein in melanoma. *Clin Cancer Res* 14: 7726–7732.

Kim, K.B., Eton, O., Davis, D.W., Frazier, M.L., McConkey, D.J., Diwan, A.H. *et al.* (2008) Phase II trial of imatinib mesylate in patients with metastatic melanoma. *Br J Cancer* 99: 734–740.

Kivelä, T., Suciu, S., Hansson, J., Kruit, W.H., Vuoristo, M.S., Kloke, O. *et al.* (2003) Bleomycin, vincristine, lomustine and dacarbazine (BOLD) in combination with recombinant interferon alpha-2b for metastatic uveal melanoma. *Eur J Cancer* 39: 1115–1120.

Lefevre, G., Glotin, A.L., Calipel, A., Mouriaux, F., Tran, T., Kherrouche, Z. *et al.* (2004) Roles of stem cell factor/c-Kit and effects of Glivec/STI571 in human uveal melanoma cell tumorigenesis. *J Biol Chem* 279: 31769–31779.

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

Mouriaux, F., Kherrouche, Z., Maurage, C.A., Demailly, F.X., Labalette, P. and Saule, S. (2003) Expression of the c-kit receptor in choroidal melanomas. *Melanoma Res* 13: 161–166.

Penel, N., Delcambre, C., Durando, X., Clisant, S., Hebbar, M., Negrier, S. *et al.* (2008) O-Mel-Inib: a Cancéro-pôle Nord-Ouest multicenter phase II trial of high-dose imatinib mesylate in metastatic uveal melanoma. *Invest New Drugs* 26: 561–565.

Schmittel, A., Schmidt-Hieber, M., Martus, P., Bechrakis, N.E., Schuster, R., Siehl, J.M. *et al.* (2006) A randomized phase II trial of gemcitabine plus treosulfan versus treosulfan alone in patients with metastatic uveal melanoma. *Ann Oncol* 17: 1826–1829.

Shah, C.P., Weism, E., Lajous, M., Shields, J.A. and Shields, C.L. (2005) Intermittent and chronic ultraviolet light exposure and uveal melanoma: a meta-analysis. *Ophthalmology* 112: 1599–1607.

Singh, A.D., De Potter, P., Fijal, B.A., Shields, C.L., Shields, J.A. and Elston, R.C. (1998) Lifetime prevalence of uveal melanoma in white patients with oculo(dermal) melanocytosis. *Ophthalmology* 105: 195–198.

Steinberg, M. (2007) Dasatinib: a tyrosine kinase inhibitor for the treatment of chronic myelogenous

leukemia and philadelphia chromosome-positive acute lymphoblastic leukemia. *Clin Ther* 29: 2289–2308.

Van Raamsdonk, C.D., Bezrookove, V., Green, G., Bauer, J., Gaugler, L., O'Brien, J.M. *et al.* (2009) Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. *Nature* 457: 599–602.

Virgili, G., Gatta, G., Ciccolallo, L., Capocaccia, R., Biggeri, A., Crocetti, E. *et al.* (2007) Incidence of uveal melanoma in Europe. *Ophthalmology* 114: 2309–2315.

Weis, E., Shah, C.P., Lajous, M., Shields, J.A. and Shields, C.L. (2006) The association between host susceptibility factors and uveal melanoma: a meta-analysis. *Arch Ophthalmol* 124: 54–60.

Weisberg, E., Manley, P.W., Breitenstein, W., Brüggen, J., Cowan-Jacob, S.W. and Ray, A. (2005) Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. *Cancer Cell* 7: 129–141.