Targeting angiogenesis in melanoma: prospects for the future

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Abstract: Angiogenesis has been identified as a relevant target for melanoma experimental therapeutics, based on preclinical and clinical studies. A variety of angiogenesis inhibitors are currently being tested in both metastatic and adjuvant melanoma clinical trials. To date, the most promising evidence of benefit is based on a statistically nonsignificant trend in survival gain reported in a randomized phase II trial combining bevacizumab, a monoclonal antibody targeting vascular endothelial growth factor, with cytotoxic chemotherapy. Larger phase III studies are required to determine the true extent of clinical benefit with this class of agents. Key to these clinical trials is the need to include translational endpoints, since correlation of biological and clinical data will provide the opportunity to identify biomarkers predictive of treatment response. These biological studies will also aid our, as yet, poor understanding of the mechanism of action of angiogenesis inhibitors, as well as drug-related side effects. Finally, if these trials show meaningful clinical benefit, then careful consideration will need to be given when designing second-generation trials, in the light of novel gene-directed therapies currently showing promise in melanoma.

Keywords: angiogenesis, angiogenesis inhibitors, melanoma, treatment, vascular endothelial growth factor

Introduction

Evidence suggests that most tumours cannot grow beyond 1–2 mm without the need to establish an independent blood supply, which is generated by the process known as angiogenesis. In 1971, Judah Folkman described angiogenesis as being essential for tumour growth and proposed that its inhibition might be an effective therapeutic approach against cancer [Folkman, 1971]. Subsequently, a series of putative angiogenic factors were described. Vascular endothelial growth factor (VEGF) was cloned in 1989 and appears to be the most relevant promoter of angiogenesis in both normal conditions and malignant disease (Figure 1 and Table 1) [Ellis and Hicklin, 2008; Ferrara *et al.* 2003; Hanahan and Weinberg, 2000; Hanahan and Folkman, 1996]. It is now known that VEGF comprises a family of five glycoproteins (VEGF-A, -B, -C, -D and placental growth factor). The primary regulator of VEGF secretion is the hypoxic microenvironment, which is mediated by the transcription factor hypoxia-inducible factor 1-α (HIF-1α). VEGF-A (commonly referred to as VEGF) is overexpressed in a variety of human tumours. The VEGF ligands bind with differing affinities to the extracellular domains of three structurally similar receptor tyrosine kinases: VEGFR-1, -2 and -3. VEGFR-1 and -2 are expressed on the surface of most endothelial cells and bind VEGF-A. VEGFR-3 is expressed on lymphatic endothelial cells and is primarily involved in lymphangiogenesis. It does not bind VEGF-A, but does bind other VEGF isoforms. VEGFR-2 binding with VEGFs appears to be the major mediator of proliferation, chemotaxis, prosurvival and permeability enhancing effects in cells.

The first report of angiogenesis inhibition provided meaningful clinical benefit was published in 2004 when the combination of the monoclonal antibody, bevacizumab (Avastin), which targets VEGF, with conventional chemotherapy was shown to significantly improve survival in patients with metastatic colorectal cancer compared with chemotherapy alone [Hurwitz *et al.* 2004]. Bevacizumab has now been licensed for use in a variety of tumour types, supporting

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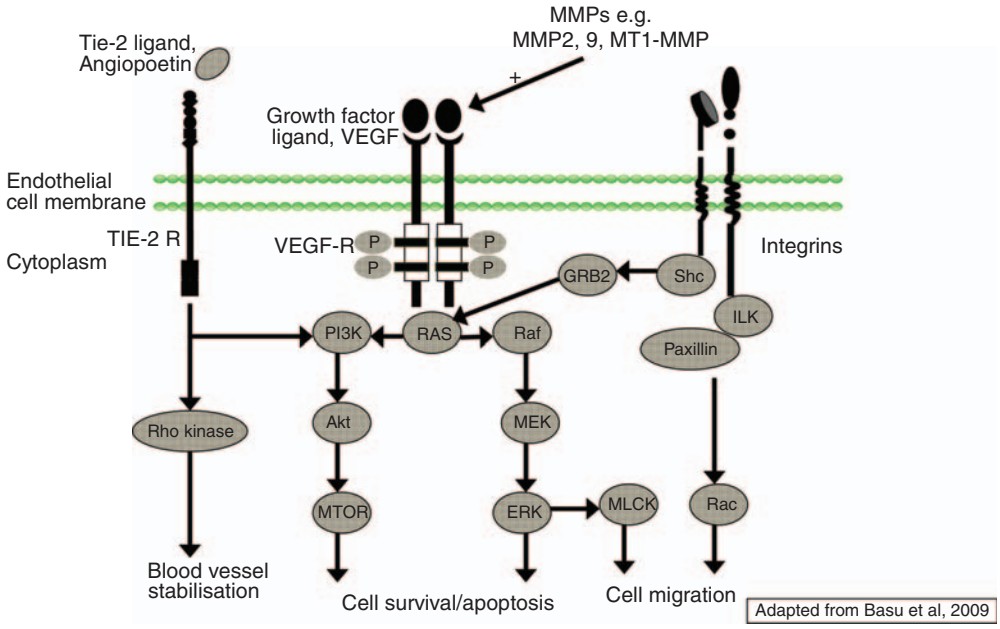

Figure 1. Pathways implicated in angiogenesis.

Table 1. Role of vascular endothelial growth factor (VEGF) in promoting angiogenesis.

- Regulates new blood vessel growth by controlling endothelial cell activation, survival, migration, invasion, proliferation, chemotaxis of bone-marrow-derived progenitor cells.
- Promotes survival of immature vasculature.
- Promotes vascular function: flow (vasodilatation) and permeability.
- Stimulates lymphangiogenesis
  - growth of new lymphatic vessels often accompanies angiogenesis
  - VEGF overexpression leads to functionally abnormal lymphatic vessels in experimental models.
- Affects the immune response
  - ↓ dendritic cell maturation
  - ↑ survival and migration of immune cells.

accumulating preclinical evidence that angiogenesis is a process which is common to all cancers. There are currently over 20 VEGF-targeted agents in clinical trials and many more novel agents for which angiogenesis inhibition is thought to contribute to their mechanism of action. Melanoma is a highly vascular tumour and theoretically should be amenable to treatment with angiogenesis inhibitors. There is a good scientific rationale to support this argument; however, the clinical evidence of benefit from employing this strategy has yet to be convincingly established. This may reflect a lack of full understanding of the mechanisms responsible for the antitumour activity of angiogenesis inhibitors, as well as a need to better understand how the role of angiogenesis and the microenvironment in the different stages of melanoma progression. Thus, alongside ongoing clinical trials, biological studies are crucial to informing whether angiogenesis inhibition is likely to offer clinical benefit to melanoma patients in the coming years. While angiogenesis inhibitors are being established in clinical practice for a number of tumour types, identification of biomarkers predicting for response has proved elusive. Affordability and toxicity pose problems to both healthcare providers and patients alike. Finally, if angiogenesis inhibition is shown to be effective, their place needs to be viewed within the growing portfolio of genetically driven therapies currently in development for the treatment of melanoma.

Angiogenesis is a valid target in melanoma therapeutics

The evidence base for angiogenesis being relevant to melanoma progression and metastasis has recently been summarised in two reviews [Basu *et al.* 2009; Streit and Detmar, 2003]. The purpose of this review is therefore not to

reiterate this evidence, but the most compelling data will be summarized.

The overproduction of VEGF and its association with VEGFR expression promotes melanoma cell growth and survival through MAP kinase and phosphatidyl inositol-3-kinase (PI3K) signalling pathways [Graells *et al.* 2004]. This suggests, at least *in vitro*, that melanoma proliferation may involve a VEGF-dependent autocrine loop. Immunohistochemical studies suggest that VEGF is expressed by 20–77% of human primary melanomas [Potti *et al.* 2003; Simonetti *et al.* 2002]. Elevated VEGF expression and other soluble pro-angiogenic factors have been noted at both the mRNA and protein level, and have been demonstrated to correlate strongly with poor clinical outcome in melanoma patients [Goydos and Gorski, 2003; Poon *et al.* 2001; Ugurel *et al.* 2001]. Using immunoenzymatic techniques, raised serum VEGF levels have been observed in melanoma patients with advanced disease and a higher rate of relapse was noted in patients with resected primary melanoma whose serum VEGF increased during follow up [Osella-Abate *et al.* 2002]. In the largest tissue microarray study performed to interrogate the VEGF pathway in melanoma, over 1000 pathological specimens of melanoma and benign naevi were assessed quantitatively for expression of VEGF, VEGFR-1 and VEGFR-2. Expression of all three proteins was found to be higher in malignant melanocytes when compared with their benign counterparts samples and VEGF and VEGFR-2 were expressed in higher amounts in metastatic compared with primary melanoma [Mehnert *et al.* 2007]. Serum VEGF, VEGF-C and VEGFR-3 have also been noted to be significantly higher in metastatic melanoma patients compared with healthy controls, with higher serum VEGFR-3 levels in patients with high tumour burden and in nonresponding patients compared with responding patients, suggesting that VEGF isoforms and receptors important in lymphangiogenesis may play an important role in outcome of melanoma patients [Mouawad *et al.* 2009]. Inhibition of tumour growth has been achieved in different melanoma xenograft models by various anti-VEGF strategies [Li *et al.* 2002; Wedge *et al.* 2002; Oku *et al.* 1998].

Taken as a whole, these data justify exploratory studies targeting the VEGF pathway as a means of more effectively treating melanoma.

Angiogenesis is implicated in the mechanism of action of historical melanoma therapies

Prior to the development of specific inhibitors of angiogenesis, several drugs historically associated with melanoma treatment are now thought to exert at least some of their antitumour effect by inhibiting angiogenesis. These include thalidomide, interferon-γ and interferon-α. While thalidomide and interferon-γ have been rejected as being ineffective treatment for melanoma, interferon-α is licensed for use as adjuvant therapy and remains a standard of care at least in North America. Clinical trials consistently show that interferon-α delays time to relapse, while evidence supporting a survival benefit has only been hinted at by meta-analysis [Wheatley *et al.* 2007]. Interferon-α is a cytokine with pleiotropic cellular functions, including immunomodulatory, antiviral, antiproliferative, and anti-angiogenic effects. Clinical studies demonstrate that interferon-α treatment can induce impressive responses in angioproliferative diseases such as Kaposi's sarcoma and hemangiomas. Preclinical studies suggest that the anti-angiogenic properties may be associated with regulation of endothelial cell motility and survival, as well as inhibition of other molecules such as basic fibroblast growth factor, interleukin 8 and matrix metalloproteinases (MMPs), all of which appear to be involved in the angiogenic response [Indraccolo, 2010]. The contribution angiogenesis inhibition plays in determining how interferon-α therapy delays time to relapse in melanoma patients is not known.

VEGF-targeted agents tested in melanoma

VEGF-targeted therapies were initially developed with the notion that they would inhibit new blood vessel growth and thus starve tumours of necessary oxygen and nutrients. It has become increasingly apparent that the therapeutic benefit associated with VEGF-targeted therapy is more complex than this, involving multiple mechanisms [Ellis and Hicklin, 2008]. VEGF-targeted therapy has an impact on numerous cell types within the tumour microenvironment, including endothelial cells, haematopoietic progenitor cells, dendritic cells and tumour cells. It is not yet clear whether the various mechanisms of action are dependent on tumour type. Currently, VEGF-targeted monotherapy has only been shown to be effective in renal cell and hepatocellular carcinoma, whereas it only appears to confer a benefit in epithelial cancers when combined with cytotoxic chemotherapy.

Angiogenesis inhibitors currently in clinical practice primarily target the VEGF ligand/receptor pathway (Figure 2). They largely fall into two camps: tyrosine kinase inhibitors (recognized by their generic name ending in '-ib') and monoclonal antibodies (generic names ending in '-ab'). The two sets of agents contrast one another in that tyrosine kinase inhibitors are oral small molecules which act intracellularly and are selective rather than specific in their ability to inhibit certain tyrosine kinase receptors. Their ability to block enzyme phosphorylation of more than one target polypeptide may be advantageous in that multiple signalling pathways might be affected; however, this might also be relevant to generating drug-related side effects, which can be problematic.

Monoclonal antibodies, on the other hand, are delivered by intermittent intravenous infusion and have a specific defined inhibitory role outside of the cell or at the cell surface. The most promising agents to date intercept VEGF ligand from binding to its receptor at the cell surface. The resulting effects on tumour interstitial pressure and blood vessel permeability, a process described as 'vessel normalization', suggests that these antibodies might enhance the delivery of chemotherapy to tumour cells [Jain, 2001] in addition to possessing *de novo* antitumour activity. These antibodies are also not without side effects, the most common being hypertension, proteinuria, as well as increased incidence of thrombo-embolic events and bleeding episodes, reflecting the significant role played by VEGF signalling in regulating normal vasculature.

Both classes of drugs are being tested in melanoma.

VEGFR tyrosine kinase inhibitors

Most reports of VEGF receptor tyrosine kinase inhibitors tested in metastatic melanoma patients are based on phase II clinical trials (Table 2). The first published study of 20 patients receiving the selective VEGFR-2 inhibitor, semaxinib (SU5416, Sugen), reported no objective responses [Kuenen *et al.* 2003]. Following the groundbreaking news that a substantial proportion of melanomas carried mutations in the BRAF gene [Davies *et al.* 2002], clinical trials of sorafenib were promptly initiated. Although originally developed as a BRAF inhibitor, sorafenib also selectively inhibits VEGFR-2 and -3, as well as having some effect on platelet-derived growth factor receptor (PDGFR). Either as single agent [Eisen *et al.* 2006], or combined with conventional dacarbazine [McDermott *et al.* 2008], temozolamide [Amaravadi *et al.* 2009], or carboplatin/paclitaxel chemotherapy as first- or second-line treatment [Hauschild *et al.* 2009], sorafenib has not been shown to improve the standard of care. Even so, these studies were important in expanding our knowledge of drug-related toxicities, which are now well recognized with this class of agent: namely

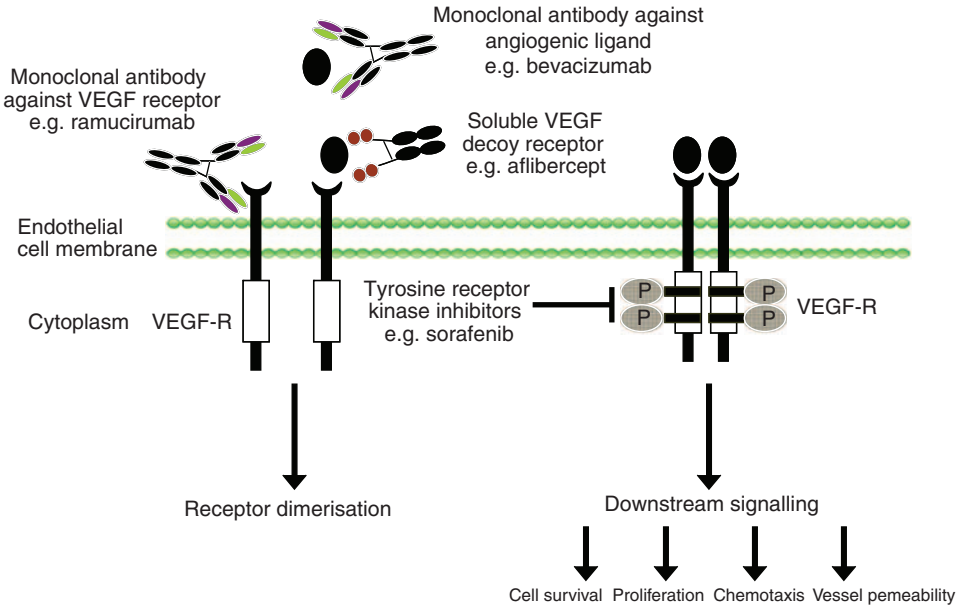

Figure 2. Vascular endothelial growth factor (VEGF) signalling inhibition.

hand–foot skin reactions, hypertension, fatigue and gastrointestinal toxicities. Studies combining sorafenib with other cytotoxic drugs, hormonal therapies, immunotherapy and targeted agents such as bevacizumab are still ongoing.

Axitinib (AGO13736, Pfizer), a multikinase pan-VEGFR inhibitor (inhibiting VEGFR- 1, -2 and -3), as well as PDGFR and c-kit, was tested in a phase II trial in metastatic melanoma, but the overall response rate of 15.6% and median overall survival (OS) of 6.8 months were unimpressive [Fruehauf *et al.* 2008]. Again, common treatment-related adverse effects included fatigue, hypertension and diarrhoea. In this study, an unplanned retrospective subgroup analysis of patients treated with axitinib in this and other studies involving other tumour sites identified longer OS in patients who developed hypertension (diastolic pressure >90 mmHg). The investigators proposed that hypertension might be a marker of treatment response [Rini *et al.* 2008]. This hypothesis has not been substantiated in other trials testing similar agents. Even so, Pfizer has not progressed further trials of axitinib in melanoma.

Table 2. Results of phase II and III* trials testing selective vascular endothelial growth factor receptor tyrosine kinase inhibitors in metastatic melanoma.

Author	Description	Regimen	Number of patients	Response rate (PRs only) (%)	Stable disease (%)	Median PFS	Median OS
Kuenen *et al.* [2008]	First line	Semaxanib	20	0			
Fruehauf *et al.* [2008]	First line and previously treated	Axitinib	32	16		2.3 months	6.8 months
Cook *et al.* [2010]	First line and previously treated	Vatalanib	34	3	32	1.8 months	6.5 months
Decoster *et al.* [2009]	Previously treated	Sunitinib	18	11	28		
Eisen *et al.* [2006]	Pre-treated	Sorafenib	37		19		11 weeks
Kim *et al.* [2009]	Pre-treated	Sorafenib and temsirolimus	21		42.8		
Hauschild *et al.* [2009]	Previously treated	Sorafenib and paclitaxel and carboplatin	135	12		17.9 weeks	
		Placebo and paclitaxel and carboplatin	135	11		17.4 weeks	
Amaravadi *et al.* [2007]	First line (no brain metastases)	Sorafenib and temozolomide (standard dose)	38	24	39		
	(known brain metastases)	Sorafenib and temozolomide (extended dose)	40	15	55		
	Previously treated(no brain metastases)	Sorafenib and temozolomide (standard dose)	35	17	49		
		Sorafenib and temozolomide (extended dose)	34	0	27		
Eisen *et al.* [2007]	First line	Sorafenib and dacarbazine	83	10	41	14 weeks	41 weeks
McDermott *et al.* [2008]	First line	Sorafenib and dacarbazine	51	24		21.1 weeks	
		Placebo and dacarbazine	50	12		11.7 weeks	

IFN, interferon; OS, overall survival; PFS, progression-free survival; PRs, partial responses.

Other negative trials testing various VEGFR selective tyrosine kinase inhibitors, including sunitinib [Decoster *et al.* 2009], dovitinib [Kim *et al.* 2008] and vatalanib [Cook *et al.* 2010], have followed. Clearly, these results are disappointing. However, they were probably destined to fail from the outset for the following reasons. First, the negative outcomes may simply reflect the chemoresistant nature of metastatic melanoma, which has hitherto plagued our ability to identify any effective systemic therapy for these patients. Second, it is now apparent that tyrosine kinase inhibitors are not likely to be cytotoxic, but cytostatic in nature, thus applying conventional trial design using an objective response rate as the primary endpoint is not ideal. Third, attempting to block angiogenesis in already established metastatic tumours is probably the equivalent of trying to lock the gate once the horse has bolted.

New agents with novel and distinct antiangiogenic profiles are being identified in drug development programmes which inhibit kinases across multiple different signalling pathways and show 'tandem' inhibition of kinases within the same signalling path. The attraction of this method of inhibition is that a single molecule may bypass the activity of downstream effectors that have undergone feedback upregulation. An example of this concept is regorafenib (Bayer), which inhibits both VEGFR-2 and the Tie-2 receptor tyrosine kinase. Tie-2 receptor activation by its ligand, angiopoietin, appears to be involved in blood vessel stabilization and VEGF is also able to activate Tie-dependent signalling pathways (Figure 3). The dual receptor inhibitory effect of regorafenib potentially offers advantages over inhibition of the VEGF axis alone, which have yet to be evaluated in clinical trials.

When testing these agents, the trial endpoints used to evaluate efficacy need to be carefully considered. If, as many believe, for ethical reasons these agents need to be tested in advanced disease in the first instance, the trials should incorporate endpoints other than clinical objective response. Disease control rate (the proportion of all complete responses, partial responses and stable disease) or progression-free survival (PFS) may be more relevant endpoints to evaluate agents predicted to generate cytostasis. Inclusion of pharmacodynamic endpoints is critical to provide some early biological, pharmacological or functional signal of proof of principle, in order to avoid discarding drugs inappropriately from further testing in melanoma (Table 3). For inhibitors of angiogenesis, novel imaging tools are available which can reliably assess tumour vascularity and permeability. Thus, dynamic contrast enhanced (DCE) MRI and DCE ultrasound have been incorporated into early phase studies of dovitinib [Kim *et al.* 2008], vatalanib [Cook *et al.* 2010] and sorafenib combined with temozolamide [Robert *et al.* 2009] give some early indication that tumour devascularization may be associated with a subset of melanoma patients who might respond to treatment. Key to the new era of personalized medicine is the ability to identify individuals most likely to respond to a specific treatment. In the context of VEGF-targeted therapy, identification of biomarkers

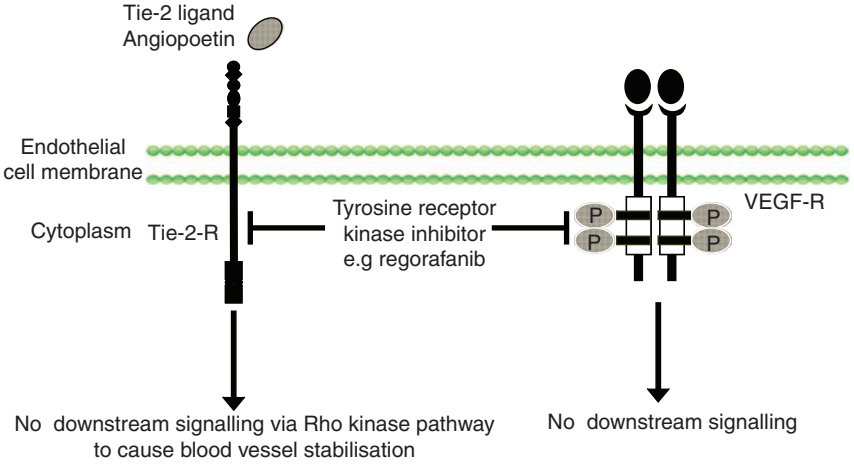

Figure 3. Tandem kinase inhibition.

predictive for response has proved elusive to date [Murukesh *et al.* 2010]. Until they are discovered and validated, drug costs may remain prohibitive to clinical use in most cases, and certainly within the UK National Health Service.

Monoclonal antibodies to VEGF

While there is now a plethora of small molecule tyrosine kinases targeting angiogenesis, the number of monoclonal antibodies remains remarkably small. Bevacizumab, a humanized monoclonal IgG antibody directed against VEGF ligand, remains the only agent in this class licensed for use in several cancer types. A growing number of small studies conducted in metastatic melanoma have been undertaken combining bevacizumab with interferon or cytotoxic chemotherapy and have shown modest activity (Table 4). Recently, the results of a larger randomized phase II trial tested carboplatin and paclitaxel chemotherapy with or without bevacizumab as first-line therapy for metastatic melanoma (the BEAM trial) were reported [O'Day *et al.* 2009]. The addition of antibody to chemotherapy improved PFS by 22% (95% confidence interval [CI]: 0.55–1.13; $p = 0.19$), and OS by 21% (hazard ratio 0.78) compared with chemotherapy alone. The primary endpoint of the study was improvement in PFS, which was not met, but the OS gain was intriguing. A subsequent definitive trial is planned.

Bevacizumab combined with a cremaphor-free nanoparticle albumin-bound paclitaxel formulation (Nab paclitaxel, Abraxane) designed to improve paclitaxel tumour cell penetration [Boasberg *et al.* 2009] has been investigated as first-line therapy in patients with metastatic melanoma. Over half of the patients enrolled had extremely poor prognosis disease, yet the 12-month survival rate of 83% was extremely encouraging.

The results from clinical studies of bevacizumab in metastatic melanoma are not yet robust enough to change practice and larger phase III randomized trials are still required. Even so, with the knowledge that angiogenesis is an essential prerequisite for the establishment of systemic metastases, a natural question to investigate is whether anti-VEGF treatment should be administered prior to development of metastatic disease, in the preventative setting. Large numbers of patients undergo surgical resection of primary melanoma, which for many will be curative. However, a significant proportion of patients remain at high risk of recurrence due to invasion of melanoma early into the dermal tissues. Despite huge international efforts to identify effective treatment to improve outcomes after surgery ('adjuvant therapy') in melanoma patients at high risk of recurrence, no systemic therapy has yet been shown convincingly to improve survival. Most studies have focused on immunotherapy approaches. Alternatively, inhibition of VEGF might suppress the angiogenic switch to malignancy and prevent micrometastases from establishing themselves. This hypothesis is being tested in the currently recruiting United Kingdom Adjuvant Trial of AVASTin in high-risk Melanoma (AVAST-M), where bevacizumab is being given for a period of 1 year to melanoma patients in the adjuvant setting and

Table 3. Potential biomarkers for evaluating angiogenesis inhibitors.

Noninvasive	Minimally invasive	Invasive
Computed tomography imaging	Blood circulating endothelial cells	Tissue biopsy
Positron emission tomography imaging	Blood circulating endothelial progenitor cells	Interstitial fluid pressure measurement
Dynamic contrast enhanced MRI or ultrasound	Protein levels in plasma (ie. VEGF, bFGF	Measurement of tissue oxygenation
Urine protein (MMP, VEGF)	Protein levels in ascites/ pleural effusion	Skin wound healing
BP monitoring	HU177 levels (cryptic collagen epitope)	

bFGF, basic fibroblast growth factor; BP, blood pressure; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor.

Table 4. Results of phase II clinical trials with bevacizumab in metastatic melanoma.

Author	Description	Regimen	Number of patients	Response rate (%)	Partial response (%)	Stable disease (%)	Median PFS (months)	Median OS (months)
Varker *et al.* [2007]	Previously treated	Bevacizumab	16				3	8.5
		Bevacizumab and IFN	16				3	10
González-Cao *et al.* [2008]	Previously treated	Bevacizumab and weekly paclitaxel	12	17	17	58	3.7	7.8
Perez *et al.* [2009]	Previously treated	Bevacizumab and weekly paclitaxel and carboplatin	53	17	17	57	6	12
Peyton *et al.* [2009]	Previously treated	Bevacizumab and everolimus	28	4	4	68	3.5	
Si *et al.* [2009]	Previously treated acral	Bevacizumab and temozolomide and sorafenib	11	18	18	54.5		
Wyman *et al.* [2007]	First line and Previously treated	Bevacizumab and erlotinib	29	9	9	22	3.2	
Munzone *et al.* [2007]	First line	Bevacizumab and dacarbazine	8	20	20	40		
Boasberg *et al.* [2009]	First line	Bevacizumab and nab-paclitaxel	41				6.25	91% (6 months) 83% (12 months)
O'Day *et al.* [2009]	First line	Bevacizumab and paclitaxel and carboplatin	143	25.5	23.4	50.4	5.6	12.3
		Paclitaxel and carboplatin	71	16.4	14.9	37.4	4.2	8.6

IFN, interferon; OS, overall survival; PFS, progression-free survival.

survival will be compared with a randomized control group who undergo routine follow up after their surgery [Corrie *et al.* 2009].

This trial is important for a number of reasons. First, with 1320 patients entered, it will be the largest UK adjuvant melanoma trial conducted to date, and one of the largest worldwide. Second, it will be the largest existing dataset of patients treated with bevacizumab monotherapy, thus affording the opportunity to closely monitor and study drug-related side effects. Third, archived tumour tissue, paired normal tissue and blood are being collected on all recruited patients prior to entry, with additional samples being collected at relapse. This unique tissue bank will provide the opportunity to study the relevance of angiogenesis in melanoma progression as well as identification of novel markers of both prognosis and treatment response. The trial is recruiting on time, is expected to complete accrual in late 2012, with a first interim analysis planned by the end of 2013.

VEGF-trap (soluble VEGF receptor)

Other routes of intercepting the VEGF ligand/VEGFR axis are actively being investigated in melanoma. Aflibercept is a fusion protein that incorporates portions of human VEGFR-1 and VEGFR-2, fused to the constant region of human IgG_1. This acts as a soluble decoy VEGF receptor which, in preclinical studies, showed a favourable profile over other VEGF inhibitors, with increased binding affinity (dissociation constant 0.5 picomolar) for VEGF-A, as well as binding of placental growth factor [Holash *et al.* 2002]. Its efficacy has been tested in a phase II study of treatment-naive patients with metastatic melanoma and early results of the interim analysis of the first 21 patients (which included patients with ocular melanoma) were reported recently, in which one patient

achieved a complete response [Tarhini *et al.* 2009].

VEGFR antibodies

Anti-angiogenic antibodies have also been directed against the extracellular domain of VEGFRs thereby preventing the binding interaction with VEGF ligand. A fully humanized anti-VEGFR-2 IgG1 monoclonal antibody, ramucirumab (IMC-1121B, Imclone), raised interest as a potential therapeutic option in melanoma when a phase I dose-escalation study of IMC-1121B in 37 patients with advanced cancer showed promising results, with one partial response lasting 31 weeks seen in a melanoma patient [Spratlin *et al.* 2010]. On the basis of this, a multicentre, randomized, open-label phase II study of ramucirumab with or without dacarbazine has enrolled treatment-naive metastatic melanoma patients and results are awaited with interest.

Other novel strategies targeting angiogenesis

MMPs

Remodelling of the extracellular matrix during angiogenesis is accomplished largely through the activity of MMPs. Angiogenic mitogens such as basic fibroblast growth factor (bFGF) and VEGF can stimulate the production of MMPs by capillary endothelial cells. MMP-2 and MMP-9 levels have frequently been reported to be elevated in human malignancies and MMP inhibition using BB-94 (batimastat), has been shown to reduce vascularity and growth of liver metastases in a B16F1 mouse melanoma model [Chirivi *et al.* 1994]. These findings suggest that MMP activity is critical to both the initiation of angiogenesis and to the maintenance of the growing vascular bed required to support tumour growth and metastasis. However, MMP activity has been shown to generate endogenous inhibitors of angiogenesis, including angiostatin and endostatin, so MMPs may act as both positive and negative regulators of angiogenesis in cancer. This may explain why MMP inhibitors have proved so unsuccessful in clinical practice to date. Marimastat was one of the first agents to enter the clinic, was tested in a variety of cancer types including melanoma [Quirt *et al.* 2002], all of which singularly failed, largely due to a lack of specificity. Peptidomimetic MMP inhibitors mimic the structure of collagen at the MMP cleavage site and musculoskeletal damage to normal tissues has limited patient tolerance.

More recent evidence suggests that some MMPs may possess antitumour properties of their own, for instance MMP8 has been identified as a tumour suppressor in melanoma, so broad spectrum blockade of multiple MMPs would not be expected to be beneficial [Palavalli *et al.* 2009]. Trials testing newer generations of more specific inhibitors of the MMPs that are unambiguously implicated in the progression of melanoma and angiogenesis may prove more fruitful [Pavlaki and Zucker, 2003].

As argued with other inhibitors of angiogenesis, it may be that MMP inhibitors would actually be more effective in earlier stages of cancer progression, which might also allow smaller, better tolerated doses to be delivered to patients. This hypothesis was borne out in the RIP-Tag2 model of pancreatic carcinogenesis, when the broad spectrum MMPI, batimastat, was effective in the early stages of progression. While research is ongoing to better understand the functions of MMPs and to identify effective MMP-targeted therapy, a variety of drugs with MMP inhibitory action are in clinical trial. Of interest to melanoma is a current study of genestein, the soy isoflavone with MMP-2 and -9 inhibitory activity, currently being tested in a phase II prevention study involving melanoma patients among other tumour types [Roy *et al.* 2009].

Anti-integrin strategies

Integrins are members of the immunoglobulin superfamily, comprising noncovalently linked heterodimers of α- and β-subunits. They act as cell surface receptors for extracellular membrane proteins and, via these interactions, they mediate cell attachment and migration. Integrins on endothelial cells have been implicated in the control of cell growth, migration and survival during angiogenesis. The αvβ3 integrin has been shown to be upregulated in endothelial cells by angiogenic factors in response to inflammation, wound healing and tumourigenesis [Mahabeshwar and Byzova, 2007; Brooks *et al.* 1994]. Since then, numerous members of the integrin family have been implicated in angiogenesis.

Antagonism of integrins using function blocking antibodies, peptides and small molecule inhibitors has been tested in a several melanoma clinical trials. The best-characterized inhibitors are the function-blocking anti-integrin antibodies such as etaracizumab (MEDI-522, Abegrin), a humanized antibody against αvβ3. Its early

prototype, Vitaxin was well tolerated in a phase I trial [Gutheil *et al.* 2000]. Etaracizumab has since been evaluated in a randomized phase II trial as monotherapy versus a combination of etaracizumab plus dacarbazine in 112 metastatic melanoma patients. Although no objective responses were demonstrated in the etaracizumab-only arm compared with 13% in the combination arm, surprisingly, patients who received etaracizumab alone had a median OS greater than 12 months, compared with 9.4 months for the combination arm. The 1-year survival rate was 53% for patients receiving etaracizumab alone and 42% for those on combination treatment, while both groups were noted to have better survival than is usually reported or most first-line regimens in metastatic melanoma. Unfortunately, these early promising results were not borne out with longer patient follow up and this antibody is no longer being investigated in metastatic melanoma [Schadendorf *et al.* 2009].

The fully human monoclonal antibody, intetumumab (CNTO 95, Centocor), blocks both αvβ3 and αvβ5 integrins, and inhibits angiogenesis and tumour growth by 80% in human melanoma xenografts in vivo [Trikha *et al.* 2004]. A four-arm randomized phase II study compared two doses of intetumumab given alone with the higher dose of intetumumab combined with DTIC (dacarbazine) versus DTIC alone in metastatic melanoma patients. At 2 years of follow up, a trend towards improved PFS and OS with the highest dose of antibody with or without dacarbazine has been reported [Loquai *et al.* 2009]. Further evaluation of this agent is justified.

The αv integrins have also been targeted using a cyclic peptide inhibitor of integrins αvβ3 and αvβ5, cilengitide, which in preclinical studies was observed to increase the antitumour activity of temozolomide against melanoma [Tentori *et al.* 2008]. Cilengitide was well tolerated in phase I trials and mature data are awaited from a phase II trial that has been completed in melanoma patients [Kim *et al.* 2007]. A chimeric mouse–human anti-α5β1 antibody M200 (volociximab), was developed, since β1 integrin on endothelial cells appears to be required for ligation of fibronectin during angiogenesis. It has been tested in melanoma patients in combination with dacarbazine with evidence of response in 62% of patients [Kuwada, 2007]. While it is too early to judge how useful integrin inhibitors will be in clinical practice, it is of note that the side-effect profile of these agents is generally mild. This favourable attribute would suggest their use in combination with other drugs such as anti-VEGF agents would be justified, to provide a broader coverage of angiogenesis inhibition.

Future challenges for angiogenesis inhibition

The list of angiogenesis inhibitors discussed here is by no means exhaustive. As the mechanisms governing angiogenesis are unravelled, new opportunities for therapeutic intervention are being generated. Other classes of agents currently in clinical trial include those mimicking endogenous angiogenesis inhibitors (e.g. endostatin) [Cui *et al.* 2009; Ling *et al.* 2007], inhibitors of placental growth factor [Fischer *et al.* 2007], and agents blocking HIFα, the key molecule in hypoxia signalling [Patnaik *et al.* 2009; Semenza, 2007; Hewitson and Schofield, 2004]. Many of these classes of agents are still at the early stages of their development and over the next decade, the clinical impact of targeting angiogenesis in melanoma will become apparent.

As already alluded to, key to their evaluation will be the need to design clinical trials appropriately, testing in the most appropriate patient population and ensuring pharmacodynamic endpoints are given adequate consideration when judging signals for further development, since the likelihood of generating objective responses over and above the level seen consistently with conventional therapy for metastatic melanoma is extremely remote. Pharmacodynamics are also crucial for identifying biomarkers of response.

A precedent has already been set for evaluating angiogenesis inhibitors at an early stage of melanoma progression. Furthermore, there is increasing evidence that the angiogenic process begins in the premalignant stages of most cancers. In premalignant melanoma, microvessel density has been reported to be significantly increased in dysplastic nodules compared with benign naevi. Thus, angiopreventive strategies warrant exploration, if appropriate agents could be identified [Menakuru *et al.* 2008].

Melanoma is entering a new era of genetically driven treatment, affording the potential for ‘stratified’ therapy in future years. The first trials of treatments being tested specifically in

BRAF mutant and c-kit mutant patients are now underway. Even so, the majority of melanoma patients will not carry these mutations, and the benefit of treatment in those who do may be short lasting, so the need to find more effective treatments and combination regimens for this devastating disease remains absolutely paramount.

While focusing on this first generation of angiogenesis inhibitor trials for signals of activity, melanoma investigators must maintain an awareness of findings with similar agents tested in other cancer types. Drug-related toxicities including hypertension, skin toxicity and fatigue limit patient tolerance of VEGF-targeted therapy. These need to be better understood and managed effectively. Questions about optimal duration of therapy [Wolmark et al. 2009] and the practicality of delivering what might be long-term treatment over many years have been raised from the first trials of adjuvant therapy with bevacizumab undertaken in colon cancer. Finally, emerging resistance to long-term treatment [Hanahan and Bergers, 2008] will inevitably pose further challenges to clinical application.

Summary

Treatment of metastatic melanoma remains unsatisfactory, since, to date, no systemic therapy has been shown to improve patient survival. There is therefore a clear case for testing new therapies in the first-line setting. VEGF is the principal ligand thought to regulate angiogenesis in human tumours. In melanoma, altered expression of VEGF and other proangiogenic ligands, in addition to their receptors, has been found to correlate with both disease stage and progression. However, strategies to block VEGF ligands and receptors have not yet translated into meaningful clinical benefit to patients. The most promising evidence of activity in melanoma is based on a single randomized phase II study with the VEGF-directed monoclonal antibody, bevacizumab, combined with cytotoxic chemotherapy. The VEGF ligand/receptor pathway is by no means the only mechanism for inhibiting angiogenesis and many new agents with diverse mechanisms of action are at different stages of preclinical and clinical development. Careful attention needs to be paid to the design of key clinical trials to ensure both clinical and pharmacodynamic endpoints are weighted appropriately when making go/no-go decisions to develop new treatments. Whether or not these trials generate evidence of benefit to patients, they will surely contribute significantly to the understanding of angiogenesis-directed therapy in melanoma specifically, with wider implications for its use in all cancers where there is an established role.

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