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Angiogenesis and Its Role in the Tumour Microenvironment: A Target for Cancer Therapy

Sophia Frentzas, Caroline Lum and Ting-Yu Chen

Abstract

The process of angiogenesis refers to the growth of new blood vessels from existing ones. Tumours can produce factors in the micro-environment which act on blood vessels to promote angiogenesis. It is therefore considered to be fundamental in tumour progression and metastatic dissemination. This neovascularization can be regulated by numerous endogenous factors in the tumour micro-environment. As a result, anti-angiogenic therapies have been developed in the hope of targeting this process to reduce tumour growth and progression. However, only a proportion of patients respond to therapy, indicating the presence of treatment resistance in some. In this chapter, we aim to highlight the process of angiogenesis and to review pivotal evidence for the use of anti-angiogenic therapies thus far (alone and in combination with other agents). Finally, we will illustrate recent evidence for the discovery of biomarkers for anti-angiogenic therapies and potential mechanisms of resistance to such agents.

Keywords: angiogenesis, tumour microenvironment, blood vessels, growth factor, stroma, anticancer therapies, biomarkers, resistance mechanisms

1. Introduction

Angiogenesis is a process that is important to the growth of cancers. It refers to when new blood vessels sprout from existing ones. This multi-step process is imperative to the physiological maintenance of the body such as tissue repair [1]. It is also thought to be a critical process that tumours depend on for the delivery of oxygen and nutrients, in order to facilitate growth and progression [2]. Both pro-angiogenic factors and anti-angiogenic factors play a role in modulating tumour neovascularisation. Notably, vascular endothelial growth factors (VEGF) and catecholaminergic signalling pathways have been shown to be key factors in angiogenesis, invasion and metastases [3]. Investigations into catecholaminergic signalling from the sympathetic nervous system have shown to increase VEGF and matrix metalloprotease (MMP) levels, promoting tumour growth, invasion and metastasis [4]. Since tumour angiogenesis requires the up-regulation of these factors, anti-angiogenic agents have now been developed. A multitude of trials have investigated the effect of anti-angiogenic agents on the progression of cancer as well as combination therapies to improve the current standard of care. However, not all patients respond to these, leading to studies that aim at elucidating the mechanisms of resistance.

2. The role of VEGF in tumour angiogenesis

Angiogenesis is considered to be a fundamental event in tumour progression and metastatic dissemination and is [2] regulated by numerous endogenous factors that stimulate or inhibit neovascularisation [3]. One of the most studied pathways is the vascular endothelial growth factor (VEGF) family of ligands and their receptors [5]. In humans and mice, the VEGF family consists of 5 members: VEGF-A, -B, -C, -D and placental growth factor (PIGF). These ligands demonstrate variable specificity for the three VEGF receptors (VEGFR1, VEGFR2, VEGFR3) [3, 5]. The predominant member of the VEGF family involved in tumourigenesis is VEGF-A and will be referred to as simply 'VEGF' from herein.

One of the most important stimuli for tumour angiogenesis is hypoxia, which can occur when a rapidly growing tumour exceeds the ability of the local vasculature to supply its needs. Hypoxia-inducible factor 1 (HIF-1) is a heterodimeric transcription factor, made up of two DNA binding proteins (HIF-1α and HIF-1β), which induces the transcription of many genes, including VEGF [6]. In the presence of adequate oxygen concentrations, HIF-1α is ubiquitinated and subsequently degraded by the proteasome. However, under hypoxic conditions, HIF-1α is stabilised and persistently dimerises with the other subunit, HIF-1β, to form the HIF-1 heterodimer. The stabilised HIF-1 is then able to bind the VEGF promoter, leading to persistent transcription of the VEGF gene [7]. The expression of VEGF is also stimulated by oncogenes, including Ras, c-Src, Bcr-Abl and p53 [8]. A multitude of studies have shown that VEGF is overexpressed in the majority of solid tumours and that it is a key driver of sprouting angiogenesis [9]. Furthermore, it has been demonstrated in multiple xenograft models that VEGF inhibition supresses tumour growth [10].

3. Signalling in the VEGF pathway

Binding of VEGF to the extracellular domain of VEGFR2 causes receptor dimerisation and phosphorylation of the receptor on tyrosine residues within the
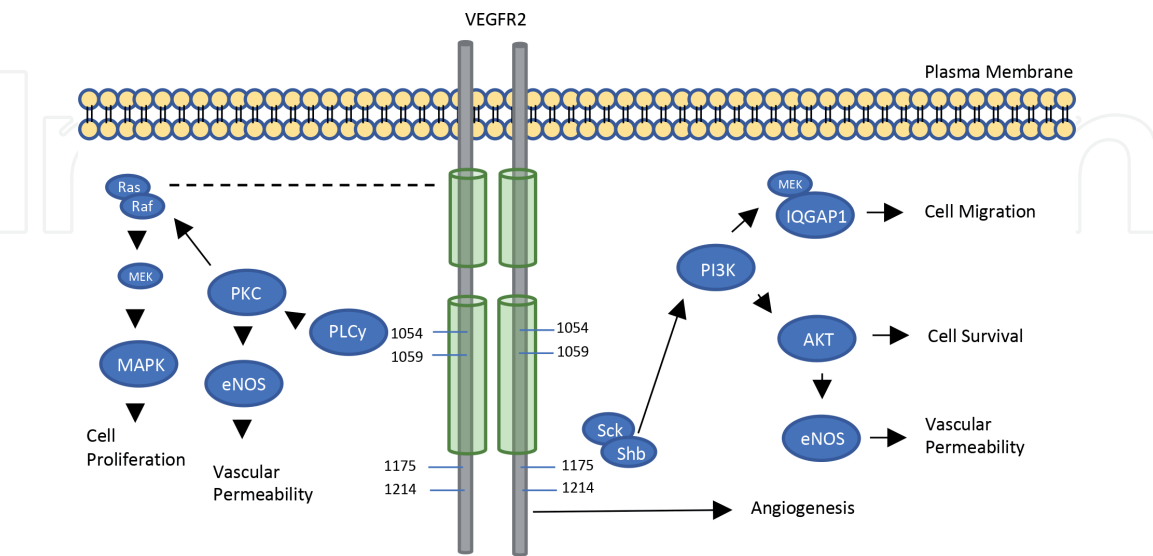
Figure 1.
Signalling from VEGFR2. The signalling cascades downstream of VEGFR2 have been the best studied and are illustrated here. VEGF binding to VEGFR2 induces dimerisation of VEGFR2 and phosphorylation of tyrosine residues (indicated by the four-digit numbers in the illustration). Pathways activated include the Ras-Raf-MEK-ERK pathway, PLCγ/PKC pathway and the PI3K/Akt pathway. Activation of downstream signalling from VEGF receptors exerts control over multiple processes required for angiogenesis including endothelial cell proliferation, migration, tube formation and vascular permeability.

intracellular domain (**Figure 1**) [11]. The Y1054 and Y1059 residues, which lie within the kinase domain, become phosphorylated in response to VEGF stimulation. These positively regulate the intrinsic kinase activity of the intracellular domain and signal to phospholipase-Cγ (PLCγ), which in turn leads to VEGFR2 internalisation [12]. The Y1175 and Y1214 residues lie in the carboxyl terminal tail. These residues become highly phosphorylated in response to VEGF. Y1214 signalling leads to endothelial cell migration and Y1175 signalling leads to PLCγ and extracellular related kinase 1/2 (ERK1/2) activation that is required for DNA synthesis and cell proliferation [13]. Activation of ERK1/2 requires the Ras-Raf-MEK-ERK1/2 signalling cascade but may also require the PLCγ/PKC/PKD pathway [14]. The roles of Y951 and Y996 residues, which lie in the kinase insert region, have not been definitively determined, but Y951 phosphorylation has been shown to increase endothelial cell migration and proliferation via both the PLC-γ and PI3K pathways [15].

4. Sprouting angiogenesis

According to the established dogma, VEGF released by tumours stimulates the growth of new vessels in the following way. The VEGF diffuses through the tissue and activates endothelial cells located in local blood vessels. Firstly, VEGF receptor activation induces the selection of sprouting endothelial cells. Proteinases such as urokinase-type plasminogen activator, uPA, and members of the matrix metalloproteinase (MMP) family mediates the dissolution of the vascular basement membrane and extracellular matrix to facilitate the infiltration of sprouting endothelial cells into the surrounding tissue [16]. Next, endothelial proliferation, migration and branching allows for the formation of new vessels. This is followed by sprout fusion and lumen formation where vessels fuse together to form a network. Finally, there is perfusion and maturation. This is where the stabilisation of new blood vessels forms a functionally perfused system, which is mediated by the recruitment of pericytes to surround the newly formed endothelial tubes; recruitment of pericytes prevents further endothelial cell proliferation and migration and also suppresses vessel leakage [17].

5. VEGF immunomodulation

Multiple possible mechanisms exist regarding immunosuppressive effects of VEGF on the tumour microenvironment. Firstly, due to the effect of VEGF on tumour vasculature, T cell migration from lymph nodes to the microenvironment may be impaired. Furthermore, the ability of T cells to migrate through vessels is negatively affected by VEGF through the down regulation of vascular endothelial selectins, adhesion molecules and promotion of Fas ligand expression. Secondly, VEGF binding to its receptor on myeloid derived suppressor cells within the tumour microenvironment results in STAT 3 signalling, with subsequent promotion of Treg cells and the down regulation of tumour specific T cells [18]. Additionally, the binding of VEGF to VEGFR2 has effects including reduced activation of cytotoxic CD8+ and CD4+ T cells, as well as the upregulation of inhibitory receptors including PD1 and CTLA4 [19]. The interaction of VEGF with VEGFR may also upregulate the programmed death ligand 1 (PDL1) on dendritic cells (DCs) [20]. Furthermore, the binding of VEGF to VEGFR1 on dendritic cells has the effect of inhibiting dendritic cell maturation [20].

6. The development of anti-angiogenic therapies

Given the key role VEGF is proposed to play in tumour angiogenesis, it is unsurprising that it has become a major drug target. Various drugs designed to inhibit VEGF signalling have been developed, including VEGF neutralising antibodies (e.g. bevacizumab), novel fusion proteins which bind pro-angiogenic growth factors (e.g. aflibercept) and VEGF receptor tyrosine kinase inhibitors (e.g. sunitinib) [5, 21]. Such agents have shown promise in the treatment of several malignancies, including mCRC, metastatic renal cell carcinoma (mRCC), metastatic lung cancer, hepatocellular carcinoma (HCC) and pancreatic neuroendocrine tumours (PNET) [22].

6.1 Bevacizumab

Bevacizumab (Avastin®) is a recombinant humanised monoclonal antibody that binds to the VEGF-A isoform of human VEGF specifically and prevents the VEGF from activating the VEGF receptor [23].

6.1.1 Bevacizumab in metastatic CRC

Trials with bevacizumab as a single agent in metastatic colorectal cancer (mCRC) failed to demonstrate activity, but early Phase I trials demonstrated that it has the potential to be combined with many chemotherapy agents [24]. In the advanced setting, several randomised Phase II and III clinical trials clearly demonstrated that bevacizumab improves response rates (ORR), progression free survival (PFS) and overall survival (OS) in mCRC, when added to standard chemotherapy in the first line setting [25, 26], and the second line setting [27] (**Table 1**). In February 2004, the US Food and Drug Administration (FDA) approved bevacizumab for the treatment of mCRC in combination with 5-fluorouracil-based chemotherapy regimens based on a pivotal Phase III study which demonstrated significant PFS and OS survival benefit [25]. Of clinical importance, bevacizumab in combination with a fluoropyrimidine has also demonstrated efficacy in elderly patients with mCRC [26].

Despite these data, only a small proportion of patients benefit from the addition of bevacizumab, and furthermore, some studies have demonstrated only an increase in PFS, with no increase in ORR or OS (**Table 1**) [28]. Additionally, even those who respond initially to bevacizumab combined with chemotherapy will inevitably develop resistance and relapse [29].

In the setting of colorectal liver-only metastasis (CRLM), it has been well demonstrated that preoperative chemotherapy improves outcome and metastatectomy rates [30]. With this in mind, and on the basis that bevacizumab can improve ORR, several groups set out to evaluate its role in the preoperative CRLM setting. Findings from a small non-randomised controlled trial of neoadjuvant conventional chemotherapy with bevacizumab in high-risk CRLM patients alluded to an improvement of CRC liver metastasis rate to 40% [31]. Data from retrospective, inter-trial studies have also suggested that the addition of bevacizumab to chemotherapy significantly improves pathological response in CRLM compared to when chemotherapy is administered alone [32]. Subgroup post hoc analyses extracted from large randomised controlled trials of unselected patients have failed to show significant improvements in resection rates with the addition of bevacizumab [33]. Without prospective randomised trials however, it is difficult to make conclusions regarding the efficacy of chemotherapy versus chemotherapy combined with bevacizumab in the CRLM setting.

The role of continuing bevacizumab beyond first progression in advanced colorectal cancer has also been examined. The results of two non-randomised

Study	Tumour	Treatment groups	ORR (%)	mPFS (months)	HR and significance for PFS	mOS (months)	HR and significance for survival
Hurwitz et al., 2004[1] Phase 3 N=813	mCRC	Irino + bolus 5FU + LV + Bev	44.8	10.6	0.54 P<0.001	20.3	0.66 P<0.001
		Irino + bolus 5FU + LV + placebo	34.8	6.2		15.6	
Kabbinavar et al., 2005[2] Phase 2 N=209	mCRC	Bolus 5FU + LV + Bev	26	9.2	0.5 P=0.0002	16.6	0.79 P=0.16
		Bolus 5FU + LV	15.2	5.5		12.9	
Saltz et al., 2008[3] Phase 3 N= 1401	mCRC	XELOX + Bev Or FOLFOX + Bev	49	9.4	0.83 P=0.0023	21.3	0.89 P=0.077
		XELOX Or FOLFOX	47	8		19.9	
Cunningham et al., 2013[4] Phase 3 N=280	mCRC	Cape + Bev	19	9.1	0.53 P<0.0001	20.7	0.79 P=0.18
		Cape	10	5.1		16.8	

Bev: bevacizumab; Cape: capecitabine; FOLFOX: Bolus 5-fluorouracil plus infusional 5-fluorouracil plus LV plus oxaliplatin; FU: fluorouracil; HR: hazard ratio; Irino: irinotecan; LV: leucovorin; mCRC: metastatic colorectal cancer; met: metastatic; mOS: medial overall survival; mPFS: median progression free survival; N: number of participants; ORR: objective response rate; PFS: progression free survival; XELOX: Capecitabine plus oxaliplatin; 5FU: 5-fluorouracil.

Table 1.
Studies investigating bevacizumab in metastatic colorectal cancer in the first line.

observational cohort studies (BRiTE and ARIES) demonstrated a significant correlation between the use of bevacizumab beyond progression and substantial improvement in OS [34, 35]. Benefit of treatment beyond progression following first line treatment was later confirmed in a prospective randomised trial [36].

The efficacy of bevacizumab has also been evaluated in the adjuvant setting in CRC patients. Two large randomised studies compared survival between the following arms: adjuvant chemotherapy alone for 6 months versus adjuvant chemotherapy in combination with bevacizumab for 6 months (followed by bevacizumab alone for 6 months). Both studies demonstrated that at 1 year there was an improvement in PFS in the bevacizumab arm. However, no significant difference in OS was observed between treatment arms when assessed at 3 or 5 years [37, 38]. In fact, an analysis at 5 years in the AVANT study demonstrated a possible detrimental effect on survival with the addition of bevacizumab, documenting a higher number of relapses and deaths due to disease progression [37].

6.1.2 Bevacizumab in other tumour types

Bevacizumab in combination with cytotoxic chemotherapy has also shown significant clinical efficacy in other tumour types.

In advanced non-squamous non-small cell lung cancer (NSCLC), two randomised controlled phase III trials demonstrated significant benefit in PFS when bevacizumab was added to platinum-based doublet chemotherapy [39, 40], but only one study reported an increase in OS [40]. To further understand this discrepancy, a recent meta-analysis pooling data from several studies including the aforementioned two, deduced a modest but significant improvement in OS [41]. More recently in metastatic non-squamous NSCLC, the Impower150 phase 3 clinical trial investigated treatment with

bevacizumab plus platinum doublet chemotherapy with or without the PDL1 inhibitor atezolizumab. Treatment with atezolizumab, bevacizumab and chemotherapy compared with bevacizumab and chemotherapy resulted in a significant improvement in PFS at 6 months (66.9% vs. 36.5%) and at 12 months (56.1% vs. 18%) [42]. In an interim analysis of OS, an improvement was again seen (**Table 2**) [42].

In advanced ovarian cancer, in the first- and second-line settings, the efficacy of bevacizumab has been assessed when added to platinum-based chemotherapy doublets. Two pivotal first line phase III studies utilising the same chemotherapy doublet (ICON7/AGO-OVAR and GOG-0218 trials) demonstrated a significant improvement in PFS [43]. An updated survival analysis failed to show a significant survival benefit [43].

Bevacizumab has been investigated in glioblastoma multiforme (GBM), in the recurrent setting following first line treatment with temozolamide and radiation therapy. In this setting bevacizumab monotherapy is ineffective, however in combination with lomustine it has resulted in improvement in PFS but not OS [44]. Bevacizumab has also been investigated in the first line setting with chemo-radiation in a large randomised placebo controlled trial, but failed to improve outcomes [45].

Earlier phase III trials in RCC have demonstrated efficacy of bevacizumab in combination with sorafenib, sunitinib and interferon alpha (**Table 2**). More recently, bevacizumab has been combined with atezolizumab in metastatic RCC. A phase III randomised trial confirmed significant improvement in PFS for bevacizumab combined with atezolizumab compared with sunitinib monotherapy but mature OS data are still awaited [46].

Despite such encouraging results, bevacizumab has thus far failed to make a significant impact in several other indications, including metastatic breast cancer (mBC), melanoma, pancreatic cancer and prostate cancer. Interestingly, in breast cancer, pooled data from four large clinical trials demonstrated that it neither prolonged OS, nor delayed disease progression significantly, leading the FDA to revoke its initial approval of bevacizumab for mBC [47]. The variation in impact that bevacizumab has, not only across tumour types, but also within a single tumour type, is curious and needs to be better understood.

6.2 Ramucirumab

Ramucirumab is a fully human IgG1 monoclonal antibody that binds to the extracellular domain of VEGFR-2, blocking VEGF from activating the receptor [48]. Clinical efficacy and tolerability have been demonstrated in a number of preclinical studies and more recently in phase III trials. In the refractory metastatic gastric and gastro-oesophageal junction (GOJ) adenocarcinoma setting, ramucirumab significantly improved median OS compared with placebo but this only represented an absolute improvement of 1.4 months [49]. In the second line setting of advanced gastric and GOJ adenocarcinoma, the combination of ramucirumab and paclitaxel has become standard treatment based on the results of the pivotal RAINBOW trial showing significant improvement in OS compared with paclitaxel and placebo [50]. Ramucirumab has not shown benefit in the first line setting including combination with chemotherapy [51].

Ramucirumab has also been investigated in metastatic NSCLC but does not yet have an established role for this indication. After progression on first line platinum based chemotherapy, there was a small but statistically significant benefit in median OS of ramucirumab added to docetaxel [52]. Early results of the RELAY phase 3 clinical trial investigating ramucirumab in combination with erlotinib in the first

Study	Tumour	Treatment groups	ORR (%)	mPFS (months)	HR and significance for PFS	mOS (months)	HR and significance for survival
Sandler et al 2006[5] Phase 1L N= 878	Recurrent/ advanced NSCLC 1L	Carbo + pacli + Bev	35	6.2	0.66 P<0.001	12.3	0.79 P=0.003
		Carbo + Bev	15	4.5		10.3	
Reck et al., 2009[6] Reck et al., 2010[7] (updated analysis) Phase 3 N= 1043	Advanced NSCLC 1L	Cisplatin + gem + Bev 7.5mg/kg	37.8	6.7	Bev 7.5mg/kg vs placebo 0.75 P=0.0003	13.6	Bev 7.5mg/kg vs placebo 0.93 P=0.42
		Cisplatin + gem + Bev 15mg/kg	34.6	6.5	Bev 15mg/kg vs placebo 0.85 P=0.0456	13.4	Bev 15mg/kg vs placebo 1.03 P=0.761
		Cisplatin + gem + placebo	21.6	6.1		13.1	
Socinski et al., 2018 Phase 3 N= 1202	Met NSCLC 1L	Atezolizumab + Carbo + pacli (ACP)	NA	NA	0.62* P<0.001 (ABCP vs BCP)	NA	ABCP vs BCP 0.78 P=0.02
		Carbo + pacli + bev (BCP)	48*	6.8* 6.8** 6.1***	0.51** P<0.001	14.7*	
		Atezo + Carbo + pacli + Bev (ABCP)	63.5*	8.3* 11.3** 9.7***	0.59***	19.2*	
Rini et al., 2008[8] Phase 3 N=732	mRCC 1L	Bev + IFN	25.5	8.5	0.71 P<0.0001	NA	NA
		IFN	13.1	5.2			
Escudier et al 2007[9] Phase 3 N=903	mRCC 2L	Sorafenib	57	5.5	0.44 P<0.001	19.3	0.77 P=0.02
		Placebo	34	2.8		15.9	
Motzer et al., 2009[10] (updated analysis) Phase 3 N=750	mRCC 1L	Sunitinib	47	11	0.42 P<0.001	26.4	0.821 P=0.051
		IFN-α	12	5		21.8	

Bev: bevacizumab; C: cycle; Carbo: carboplatin; chemo: chemotherapy; gem: gemcitabine; HR: hazard ratio; IFN: interferon; mOS: medial overall survival; mPFS: median progression free survival; mRCC: metastatic renal cell carcinoma; N: number of participants; NA: not available; NR: not reached; NSCLC: non-small cell lung cancer; ORR: objective response rate; pacli: paclitaxel; PFS: progression free survival; PD-L1: programmed deat- ligand 1.

* Population with wild type (WT) genotype

** WT population with high expression of an effector T-cell

*** Patients with EGFR and ALK mutations

Table 2.
Studies investigating anti-VEGF agents in NSCLC and RCC.

line setting of metastatic EGFR mutated NSCLC have indicated an improvement in PFS however formal publication of the study findings are awaited.

Ramucirumab has also been investigated in urothelial cancers. In a phase III trial of ramucirumab plus docetaxel compared with docetaxel plus placebo in patients with advanced urothelial carcinoma who had received platinum-based chemotherapy, there was a statistically significant improvement in median PFS (4.07 months vs. 2.76 months) [53].

6.3 Aflibercept

Aflibercept is a recombinant fusion protein that binds to VEGF-A, VEGF-B and placental growth factor (PLGF), all of which have been implicated in angiogenesis and/or the survival of newly formed blood vessels [54]. As it binds to additional pro-angiogenic targets (compared to bevacizumab which binds only VEGF-A), aflibercept may provide further anti-angiogenic effects compared to targeting VEGF-A alone. In preclinical studies, it demonstrated a broad range of anti-tumour and anti-angiogenic activity both alone and in combination with chemotherapy, which was also observed in phase I clinical trials [55]. Recently, a large randomised phase III clinical trial (VELOUR) in advanced CRC patients, receiving second line therapy, demonstrated that the addition of aflibercept to systemic chemotherapy significantly improved outcomes compared to chemotherapy alone [56]. Based on this data, aflibercept was recently approved for use in the second line setting in mCRC when given in combination with chemotherapy. Importantly, results from a subanalysis of VELOUR showed that there was no significant impact of prior exposure to bevacizumab, illustrating the benefit that it provides as a multiple angiogenic factor trap, in a setting where resistance to bevacizumab may have developed [57].

6.4 Receptor tyrosine kinase inhibitors (TKIs)

Several small molecule inhibitors of VEGF receptor tyrosine kinase activity now have an established role in the treatment of certain tumour types, including mRCC, HCC and advanced CRC. These small molecule inhibitors readily diffuse through the cell membrane to compete for ATP binding to the intracellular tyrosine kinase domain of VEGF receptor 2.

6.4.1 Sunitinib

Sunitinib is an orally active multi-kinase inhibitor, which targets VEGFR1–3, PDGFRα/β, c-Kit and FLT3 [58]. Xenograft models have clearly demonstrated that as well as inhibiting new blood vessel formation, sunitinib also induces regression of newly formed immature vessels and significantly stunts tumour growth [59]. Furthermore, immunohistochemical studies performed on human tissue derived from mRCC patients treated with sunitinib have demonstrated that this agent can induce a reduction in tumour vessel density [60].

In terms of outcome in the clinical setting, sunitinib initially showed efficacy, as a single agent, for second-line therapy in single-arm, Phase II studies in mRCC [61]. Patients treated with sunitinib showed promising outcomes in terms of ORR, response duration, PFS and OS. A pivotal Phase III study was subsequently conducted comparing sunitinib with interferon-α as a first-line treatment in mRCC, which demonstrated improved OS, PFS and ORR in the sunitinib arm [62]. Based on such data, sunitinib was approved by the FDA in 2006 for the first line treatment of mRCC. Other TKI's, with similar target specificity (sorafenib, pazopanib, cabozantinib and axitinib) also have activity in mRCC. Combination with immunotherapeutic agents has also shown promising results and we are seeing the treatment algorithm for mRCC change rapidly. In a recent landmark phase 3 trial of advanced RCC in the first line setting, axitinib was combined with the PD1 inihibitor pembrolizumab and compared with sunitinib monotherapy (KEYNOTE-426). The results are promising with a significant improvement in PFS and ORR with axitinib and pembrolizumab, however more mature OS data are awaited [63].

The role of such TKIs has also being evaluated in mCRC. The anti-tumour and anti-angiogenic effects of sunitinib have been well documented in a series of CRC xenograft tumour models [64]. In the clinical setting, however, sunitinib employed either as a single agent or with combination chemotherapy, has failed to demonstrate favourable outcome, both for ORR and PFS [65].

6.4.2 Regorafenib

Recently, another TKI called regorafenib has created a lot of interest in advanced CRC. This agent inhibits VEGFR1-3, PDGFRα/β, KIT, RET, FGFR1 and Tie2. It is also a potent inhibitor of Raf-1 and suppresses both wild-type and V599E mutant BRAF activity *in vitro* and in mouse models [66]. Significant anti-tumour and anti-angiogenic effects in CRC xenograft models, both as a single agent and in combination with irinotecan chemotherapy have been reported [67]. In the clinical setting, the Phase III CORRECT trial demonstrated significant benefit for OS and PFS in advanced CRC patients, when it was used as a single agent compared to best supportive care, in a population who had failed previous standard therapy [68]. Based on this data, regorafenib was approved by the FDA as a multikinase inhibitor for metastatic colorectal cancer in the third line setting in 2012.

Regorafenib also has clinical utility in gastrointestinal stromal tumours (GIST) where it is currently employed in the third line setting after imatinib and sunitinib. This indication followed from a phase 3 randomised trial, demonstrating significantly improved PFS for regorafenib compared with placebo (4.8 months vs. 0.9 months) [69]. There was no significant difference in OS, however this trial did allow for crossover which likely impacted on this finding [69].

Regorafenib has FDA approval for second line treatment of HCC following the positive results of the phase 3 RESORCE clinical trial. Compared with placebo, regorafenib demonstrated survival benefit [70].

7. Potential mechanisms of synergy between bevacizumab and chemotherapy

Early phase clinical trials have demonstrated that bevacizumab, in combination with systemic cytotoxic chemotherapy, can potentiate treatment efficacy when given concomitantly [71]. In fact, in most clinical settings, with the exception of ovarian cancer where bevacizumab has been observed to have single agent activity [72], bevacizumab has only shown significant activity when it is combined with cytotoxic chemotherapy and the same is true for aflibercept [21].

It has been well-established that the tumour vasculature is dysfunctional and leaky, resulting in enhanced interstitial fluid pressure and thus preventing effective delivery of chemotherapy [73]. Evidence from preclinical studies showed that bevacizumab can 'normalise' the chaotic tumour vasculature, achieving reduced vessel tortuosity, reduced leakiness and reduced interstitial fluid pressure. Based on these studies, it was proposed that bevacizumab works in combination with chemotherapy to improve chemotherapy delivery [71, 73], which is now a widely accepted notion amongst many clinicians.

However, this concept is also highly controversial, with some work even refuting the normalisation hypothesis. For example, one group demonstrated that bevacizumab persistently reduced both tumour perfusion and chemotherapy delivery when NSCLC patients were treated with bevacizumab-containing chemotherapy [74]. Therefore, other potential explanations for synergy between bevacizumab and

chemotherapy must be considered. Current alternative theories based mostly on preclinical data include: (1) direct synergy between the anti-angiogenic effects of bevacizumab and potential anti-angiogenic effects of chemotherapy [75], (2) targeting of VEGF signalling directly in cancer cells by bevacizumab [21], (3) chemotherapy may inhibit resistance to bevacizumab, because chemotherapy suppresses the tumour recruitment of myeloid cells that have been implicated in resistance to bevacizumab [76], (4) bevacizumab may prevent tumour rebound that may occur during breaks in chemotherapy [76].

It should be noted that vessel normalisation facilitated by anti-angiogenic agents may provide therapeutic benefit through other mechanisms, which are independent of chemotherapy delivery. For example, in glioblastoma patients, vessel normalisation induced by single agent VEGF-targeted therapy may prolong survival due to other effects, such as oedema control or improved tumour oxygenation [77].

There are two other curious observations that have yet to be properly explained. Firstly, the synergistic effect of adding bevacizumab to chemotherapy does not occur in all tumour types. For example, the addition of bevacizumab does not lead to improvements in outcome in advanced breast cancer [78]. Secondly, VEGFR TKIs show single agent activity without the need for co-administration of chemotherapy [21].

Recent insight into these two curious observations has been reported. A study examining both clinical and mouse tumour tissue specimens demonstrated that tumour types utilising a vasculature surrounded by a well-developed stroma (e.g. mCRC, NSCLC) respond better to bevacizumab when it is added to chemotherapy as opposed to tumour types that utilise a vasculature without a well-developed intervening stromal component (e.g. mRCC, PNET) which respond better to VEGF TKIs alone [79]. This suggests that tumour cell interactions with different stromal components may influence response to different anti-angiogenic agents and how they synergise with concomitant drugs. However, there is still much work to be done in order to understand the mechanisms involved.

8. Synergy of anti-angiogenic agents with immunomodulatory therapy

A series of pre-clinical studies have shown that the use of anti-angiogenic agents along with immune checkpoint inhibitors (ICI) as a combination therapy has a synergistic and enhanced effect on the tumour when compared to either ICI therapy or anti-angiogenic therapy alone. Immunotherapy has emerged as a promising treatment option for many cancer types, offering hope for patients with the demonstration of improved outcomes including durable responses in some. Unfortunately, there are still many patients that either have short lived responses to such therapies or none at all. To overcome resistance mechanisms, combinations of immunotherapy with other treatments including VEGF inhibitors are being explored.

Since 2013, pre-clinical investigations in mice with various tumours have indicated that the combination of ICI and anti-angiogenic agents results in prolonged overall survival [80]. It has been observed that the VEGF can cause the upregulation of immune checkpoint molecules such as PD-1 and as a result, the use of anti-VEGF agents has been seen to reduce the expression of PD-1 on cytotoxic T lymphocytes [81]. Thus, the combination of using both anti-VEGF agents as well as anti-PD-1 agents could have a synergistic effect on inhibiting further tumour development [81]. Through the encouraging findings of pre-clinical investigations, many clinical studies have recently or are still in the process of investigating this.

There are a multitude of clinical studies supporting the role of bevacizumab in the positive immune modulation of the tumour microenvironment and its beneficial effects when combined with the immune checkpoint PD1/PDL1 and

CTLA4 inhibitors. In a study investigating melanoma patients treated with ipilimumab plus bevacizumab versus ipilimumab alone, the results showed that the combination therapy increased circulating CD4+ and CD8+ T cells compared with ipilimumab monotherapy [82]. The investigation showed that there was a greater median overall survival in patients undergoing combination therapy (25.1 months) compared to those who underwent the ipilimumab alone treatment (10.1 months) [82]. Furthermore, a separate study of patients with RCC investigating the effect that bevacizumab plus atezolizumab had versus bevacizumab alone found that the combination therapy demonstrated a reduction in neovasulature-related gene expression and decreased microvascular density. The treatment was also associated with an increased tumour infiltration of CD8+ T cells as demonstrated by immuno-histochemical staining of cells [83]. This study also demonstrated that MHC Class I is upregulated as a result of the treatment and that both intratumoural CD8+ T cells and macrophages increased as well.

In a phase II study involving patients with RCC, as compared with sunitinib monotherapy, atezolizumab and bevacizumab demonstrated improvements in PFS in patients with an immunosuppressive tumour microenvironment [84]. Whilst it was also discovered that the use of atezolizumab failed to generate an anti-tumour immune response (possibly due to myeloid-induced immune suppression), the addition of bevacizumab to atezolizumab was found to be able to overcome this suppression [84].

Both pre-clinical and clinical studies have shown that anti-angiogenic agents and immunomodulatory therapies have a synergistic affect in reducing tumour growth and a multitude of clinical trials are currently investigating this synergy further. Thus, there is promise in the use of a combination therapy with anti-angiogenic agents and immunomodulatory agents to improve on patient prognosis.

9. Potential predictive biomarkers for anti-angiogenic agents

In view of the variable outcomes seen in the clinic, there is a need for the development of validated predictive biomarkers of response for anti-angiogenic therapy. In this way, patients who will derive benefit from such agents could be appropriately selected, whilst those that will not derive benefit (either at the outset or during therapy) could be selected for alternative, more effective therapy. Such a strategy would not only improve clinical outcomes but would also reduce the unnecessary burden of (a) toxicity to the patient, and (b) cost to the economy. Despite extensive international research in this field, there is currently no biomarker which predicts benefit or resistance to anti-angiogenic agents that is approved for routine clinical practice. The following are amongst several which have been investigated in the clinical setting.

9.1 Circulating biomarkers

Circulating biomarkers are an attractive tool for patients and clinicians as 'liquid biopsies' are relatively non-invasive and easy to perform, as compared with tissue biopsies of tumour with associated risks and potential technical difficulties depending on tumour site. VEGF levels have been studied as a potential biomarker with high levels associated with poorer outcomes [85]. Findings regarding its utility as a predictive biomarker have been more inconsistent [85]. An analysis of four randomised phase 3 trials investigated circulating VEGF level as a prognostic and predictive biomarker in mCRC, lung cancer and RCC which included bevacizumab in the treatment regimen. Tumour specimens were also tested for VEGF level. This

found that higher baseline circulating VEGF levels were associated with poorer clinical outcomes but levels did not predict response to bevacizumab [86]. There is early evidence from small and exploratory studies to suggest soluble VEGFR-1, with higher levels being associated with poorer outcomes with anti-angiogenic treatments, however larger studies are required to confirm these findings [87].

Other potential circulating biomarkers have also been investigated. In mCRC, elevated IL-8 levels at baseline were associated with a shorter PFS in patients treated with chemotherapy (FOLFIRI) and bevacizumab [88]. Elevated LDH and neutrophil levels have been found to independently predict poorer survival in patients treated with chemotherapy plus bevacizumab [89]. A promising predictive biomarker for response to bevacizumab based therapy in CRC appears to be circulating endothelial cells, with studies showing that patients with lower circulating endothelial cells at baseline undergoing treatment with bevacizumab based therapy had improved PFS [90].

9.2 Levels of tumour VEGF isoforms

Levels of VEGF expression in a tumour could be a determinant of responsiveness to anti-VEGF therapy. Some small studies have demonstrated a relationship between baseline VEGF expression and response, however these findings have not been consistently replicated in large clinical trials and are often more informative as prognostic rather than predictive biomarkers [91]. Data from more recent prospective studies, however, have shown more consistency in the use of VEGF as a biomarker. A large randomised trial in patients with advanced breast cancer treated with bevacizumab demonstrated a significant association between high circulating levels of VEGF and survival benefit [78]. VEGF expression in tumours was investigated in the large phase III clinical trial of bevacizumab plus chemotherapy in mCRC, but this failed to predict outcomes [92].

There are multiple reasons why using VEGF expression as a biomarker could be problematic: (1) advanced tumours express numerous pro-angiogenic factors in addition to VEGF which could confer resistance to bevacizumab irrespective of the amount of VEGF produced [93], (2) differences in the intensity of VEGF expression might be too small to be clinically relevant, (3) hypoxia, which is promoted by anti-angiogenic therapy, is an important inducer of VEGF expression and might, therefore, lead to increased VEGF production in the presence of bevacizumab treatment; indeed, anti-angiogenic agents have been shown to induce expression of VEGF even in tumour naïve hosts [94], (4) variations in methodology across centres (including sample handling, the use of different scoring systems and non-validated antibodies) have a significant effect on biomarker trial results [95], (5) it is very challenging to standardise cut-offs for low and high VEGF levels, due to: (a) different methods used to measure VEGF at different centres and (b) differences in biology that occur between racial groups, tumour types and different stages of disease [95].

9.3 Levels of alternative pro-angiogenic growth factors

Studies which have investigated other single circulating factors (such as FGF2, and r soluble VEGFR2) have also yielded contradictory and unsatisfactory conclusions [96]. Interestingly, however, recent clinical work in mRCC patients treated with anti-angiogenic TKIs suggests that profiling multiple circulating factors in the blood could have a more powerful prognostic and predictive role than assessing levels of single factors alone [97]. In this study, when patients with mRCC were treated with the TKI pazopanib, a biomarker signature of six factors (HGF, interleukin 6 and

interleukin 8, osteopontin, VEGF and TIMP1) was able to distinguish a sub-group of patients that derived a significantly greater overall survival benefit from this agent.

9.4 VEGF polymorphisms

Polymorphisms in VEGF or VEGF receptors have been proposed to predict outcome from anti-angiogenic therapy. As these are generally binary in nature, they are attractive biomarkers since they may be easier to measure and apply prospectively. In metastatic breast cancer, polymorphisms in VEGF and VEGFR2 were analysed in several retrospective subset analyses in patients treated with chemotherapy, with or without bevacizumab. Two polymorphisms within the VEGF promoter/5′ untranslated region, VEGF alleles −2578AA and −1154AA, were significantly associated with improved OS in the bevacizumab plus paclitaxel group when compared to the −2578CA/−2578CC and −1154GA/−1154GG alleles. In contrast, they did not have prognostic power for OS in the chemotherapy-only arm [98]. The predictive power of the −2578AA and −1154AA VEGF alleles was also reported in a retrospective subset analysis of patients with metastatic colorectal cancer that received either FOLFIRI (leucovorin, fluorouracil, and irinotecan) plus bevacizumab or XELIRI (capecitabine and irinotecan) plus bevacizumab [99].

More recently, the role of VEGFR1 polymorphisms was studied in a large meta-analysis pooling DNA data from two phase III trials in patients with advanced pancreatic cancer treated with bevacizumab. VEGFR1 −1213AC/−1213CC alleles were significantly associated with poor outcome in patients receiving bevacizumab when compared to VEGFR1 −1213AA alleles [100]. To understand how this VEGFR1 polymorphism functionally affects VEGFR1 expression and how it might explain its correlation with poor outcome in patients receiving bevacizumab, Lambrechts and colleagues performed an *in vitro* study where the mutant codon of Tyr1213 was transiently overexpressed in HEK293T cells. Lysates from these cells demonstrated a significant increase in expression and signalling of VEGFR1 compared to HEK293T cells harbouring the wild type codon, thus providing a biological rationale for the role of this polymorphism as a negative predictive marker of response [100]. A significant correlation of the VEGFR −1213 with poor outcome was also corroborated by a subsequent study in patients with mRCC treated with sunitinib [101].

9.5 Radiological parameters

Functional clinical imaging, taking into account tumour vasculature or metabolic activity by utilising CT, MRI or PET scanning, either prior to commencing treatment or following brief exposure of patients to therapy, may be a useful tool for predicting response or resistance to anti-angiogenic therapy [102]. For conventional cytotoxic chemotherapy, imaging has been employed to assess therapy response based on change in tumour size, as defined by RECIST (Response Evaluation Criteria In Solid Tumours). However, biological agents, such as bevacizumab and TKIs, may be cytostatic in terms of their mechanism of action, thus size may not be the only parameter that needs to be considered when assessing response and outcome. Examination of various parameters such as blood flow and tumour morphology may provide additional important predictive information.

9.5.1 Baseline vascular perfusion on imaging

Several studies have examined pre-treatment levels of tumour perfusion and whether they can predict outcome. For example, enhanced levels of vessel perfusion

at baseline (measured by contrast-assisted tumour enhancement) in mRCC patients treated with VEGF TKIs has been shown to predict for response and survival [103].

9.5.2 Changes in vascular characteristics on imaging

Early alterations in features of the tumour vasculature on imaging after a short period of therapy have also been shown to be associated with response and outcome. For example, in studies of mRCC patients treated with anti-angiogenic TKIs, response criteria that measured both a significant reduction in tumour vascular perfusion and a significant reduction in tumour size were more predictive of outcome compared to change in lesion size alone [104].

Although the use of the above radiological criteria may seem promising as predictors of response and outcome, there are associated challenges that need to be considered before incorporating them into clinical practice. These include, (a) diversity in the methodologies used to assess potential surrogate radiological biomarkers of response between studies and across centres, and (b) insufficient comprehension of how certain radiological features correlate with the underlying tumour biology.

10. Measuring the clinical response to anti-angiogenic agents

Currently, the efficacy of any anti-neoplastic therapy is assessed by several outcome measures, which include (a) effective downsizing of tumours on clinical imaging (to facilitate curative surgery or consolidative radiotherapy for localised disease and to reduce the symptomatic burden of disease in the metastatic setting), (b) prolongation of the interval where a patient is either disease-free or progression-free, and (c) prolongation of survival.

Conventional assessment of residual tumour volume after cytotoxic chemotherapy has traditionally been performed with the use of size-based criteria (overall response rate, ORR, by RECIST). This was based on evidence that there is good correlation between radiological information and residual viable tumour (pathological response) and good correlation with progression-free (PFS) and overall survival (OS) in patients treated with cytotoxic chemotherapy [105]. However, with the advent of biological therapies, such as bevacizumab, the value of utilising RECIST on its own as a surrogate for outcome has been questioned and new imaging criteria have been proposed [102].

10.1 RECIST criteria

For anti-angiogenic therapy employed in advanced malignant disease, retrospective clinical meta-analyses have (a) highlighted the pitfalls and limitations of using RECIST alone in the assessment of response and progression, and (b) highlighted a disassociation of RECIST from time-related endpoints of PFS and OS [105].

This curiosity was provoked by several large randomised clinical trials investigating the effect of adding bevacizumab to conventional chemotherapy in different tumour types. These have consistently demonstrated that significant improvements in PFS and OS were incongruent with modest increases in ORRs [25, 28, 40]. In their CRC meta-analysis, Grothey and colleagues specifically examined the impact of tumour response to bevacizumab (ORR) on treatment benefit (PFS, OS) and concluded that patients who did not attain a positive response according to RECIST (i.e. stable disease) in fact

showed significant benefit from bevacizumab, which was of the same magnitude as responding patients (i.e. complete or partial response) [105].

Moreover, similar concepts have consistently featured in several Phase I and II clinical trials employing antiangiogenic agents, and other molecular targeted therapies. These studies corroborate that there is little value in utilising ORR alone, particularly in predicting whether an agent will ultimately have truly meaningful effects on pathological response or in prolonging survival [106]. The underlying reason for these incongruent observations with bevacizumab and other molecular targeted therapies may be because such agents are cytostatic rather than cytotoxic [107].
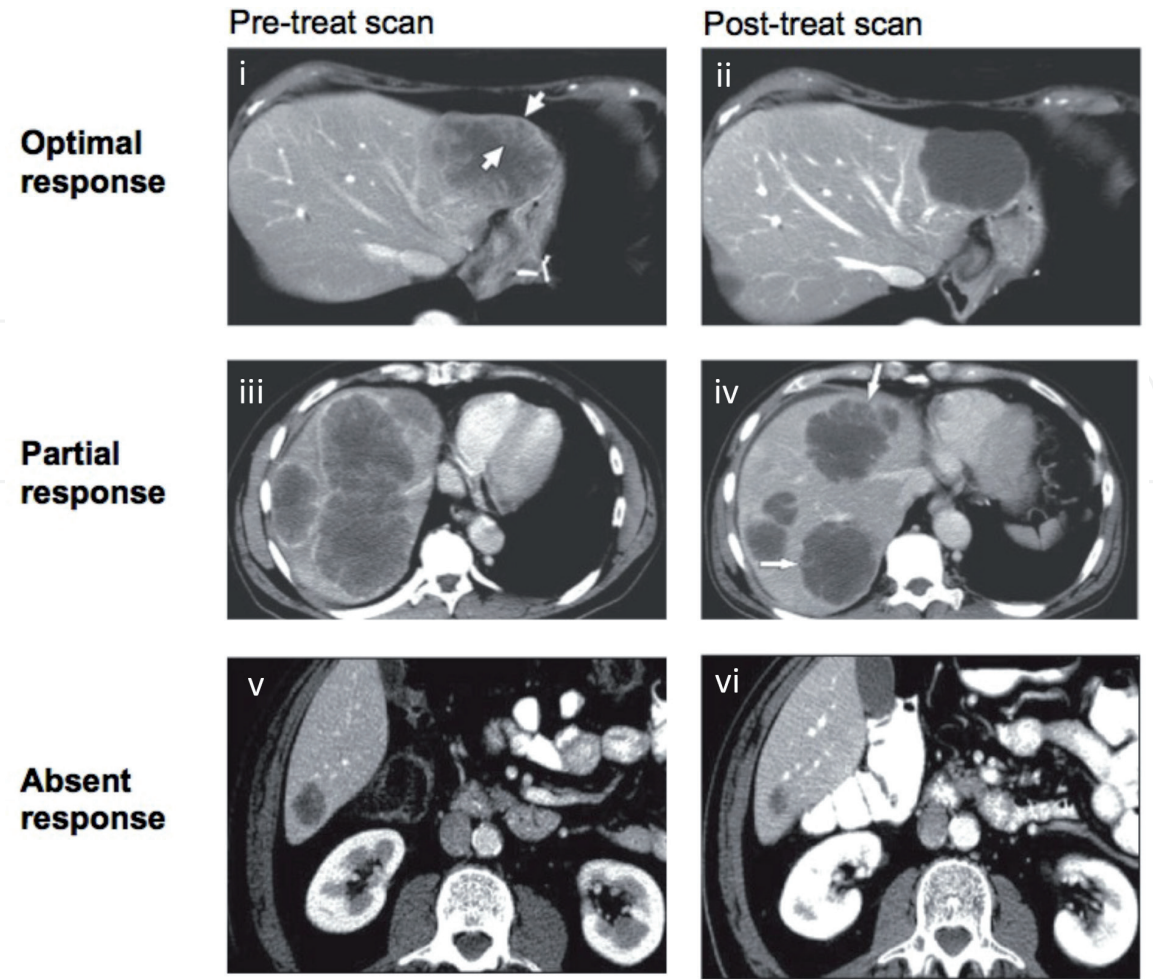
10.2 Morphological response criteria

There has been growing interest in how the appearance of lesions on clinical imaging can be utilised to accurately assess the effect of bevacizumab on tumour volume and how this appearance may correlate with other clinical end-points. In a small retrospective colorectal liver only metastasis (CRLM) patient cohort treated with bevacizumab and chemotherapy, Chun and colleagues demonstrated that novel morphological response criteria predicted more accurately for OS and pathological response than RECIST (**Figure 2**) [108]. This was subsequently validated in a larger patient population which included patients who were treated with and without bevacizumab [109]. Not only were the morphological response criteria superior to RECIST in predicting major pathological response and OS, further analyses confirmed that the morphological response criteria did not correlate with responses measured according to RECIST. Moreover, there was a significantly higher incidence of optimal responses (measured by morphological response criteria) in the patient cohort receiving bevacizumab with chemotherapy compared to the chemotherapy alone cohort [109]. These data suggest that (a) morphological response criteria and RECIST measure different biological parameters, and (b) the use of morphological response criteria represents a more sensitive tool for measuring tumour response and time-related endpoints of survival for bevacizumab. Similar findings were reported in a retrospective study of non-small cell lung cancer patients treated with bevacizumab and concomitant chemotherapy [110].

10.3 Pathological response criteria

Radiological assessment alone may not accurately reflect response to therapy because simple, unidimensional imaging parameters may overestimate or underestimate downstaging of tumour burden [111]. Furthermore, in the case of adding anti-angiogenic therapy to chemotherapy, although it has been suggested that proposed morphological imaging characteristics can accurately predict tumour response and clinical outcome, such scoring methods have not yet been validated for conventional use in clinical practice and may also be too subjective. Scoring of pathological response may therefore be a better alternative or perhaps an adjunct in assessing residual viable tumour. Moreover, in the case of preoperative chemotherapy or radiotherapy in settings such as rectal cancer and oesophageal cancer, pathological response has also been shown to correlate significantly to disease-free survival (DFS) and OS [112].

Several methodologies incorporating various parameters for scoring pathological response in resected CRLMs, treated with and without bevacizumab, have been proposed. It is still not clear from the current literature which of these classification methods may be superior.

Figure 2.
*Morphological response criteria on contrast-enhanced CT (CECT) scans as a predictor of outcome (i)
and (ii) CECT performed in a 43-year old patient before and after 10 cycles of bevacizumab containing
chemotherapy demonstrating an optimal response (OR). (i) Before therapy, the liver metastasis presented
with profound heterogeneous attenuation, a hyperattenuated peripheral rim and a thick, poorly defined
tumour-liver interface ('group 3' metastasis). (ii) After therapy, the same liver metastasis shows complete
resolution of these features (i.e. it is homogeneous, of low attenuation, with a thin, sharply defined tumor-liver
interface). Change in size of lesion is minimal. (iii) and (iv) CECT of the liver performed in a 67-year old
patient before and after 2 cycles of bevacizumab-containing chemotherapy demonstrating a partial response
(PR). (iii) Before therapy, the liver metastas is presented with features of a 'group 3' metastasis. (iv) After
therapy, the same liver metastasis shows moderate resolution of these features (i.e. it has a moderate degree
of heterogeneous attenuation, a moderately defined tumor-liver interface with a slight hyperattenuating
peripheralrim ('group 2' metastasis)). (v) and (vi) CECT of the liver performed in a 56-year old patient before
and after 2 cycles of bevacizumab-containing chemotherapy demonstrating an absent response (AR). (v) Before
therapy, the liver metastasis presented with features of a 'group 3' metastasis. (vi) After therapy, the same liver
metastas is shows a decrease in tumour size without change in attenuation or tumour-liver interface ('group 3'
metastasis). Changes in tumour morphology on CECT have been shown to correlate more significantly
with survival than the use of RECIST citeria in CRLM patients treated with bevacizumab-containing
chemotherapy.*

10.3.1 Percentage viable tumour

Microscopic assessment of the percentage residual viable tumour on haematoxy-
lin & eosin-stained sections of resected tissue has been employed as a predominant
parameter in assessing the efficacy of different pre-operative chemotherapy
regimens in tumour types such as oesophageal, gastric and rectal adenocarcinomas
[113]. Based on this methodology, Ribero and colleagues modified this scoring sys-
tem for application in CRLMs treated preoperatively, with or without bevacizumab
[114]. A semi-quantitative estimation of the percentage area of residual viable
tumour cells relative to total tumour surface area within each CRLM metastasis
was made with the analysis of four tumour cell viability subsets (<25%, 25–49%,

50–75%, >75%). This retrospective study confirmed that the addition of bevacizumab to chemotherapy yielded an incrementally greater decrease in residual viable cells within these CRLMs in comparison to those treated with chemotherapy alone but no correlation with imaging, or other clinical end-points, was made [114].

10.3.2 Tumour regression grade (TRG)

Mandard and colleagues were one of the first to establish a five-point histological scoring system for pathological response. This was based on cytological and stromal changes on haematoxylin & eosin-stained sections of primary oesophageal squamous cell carcinomas treated with chemoradiotherapy prior to resection [115].
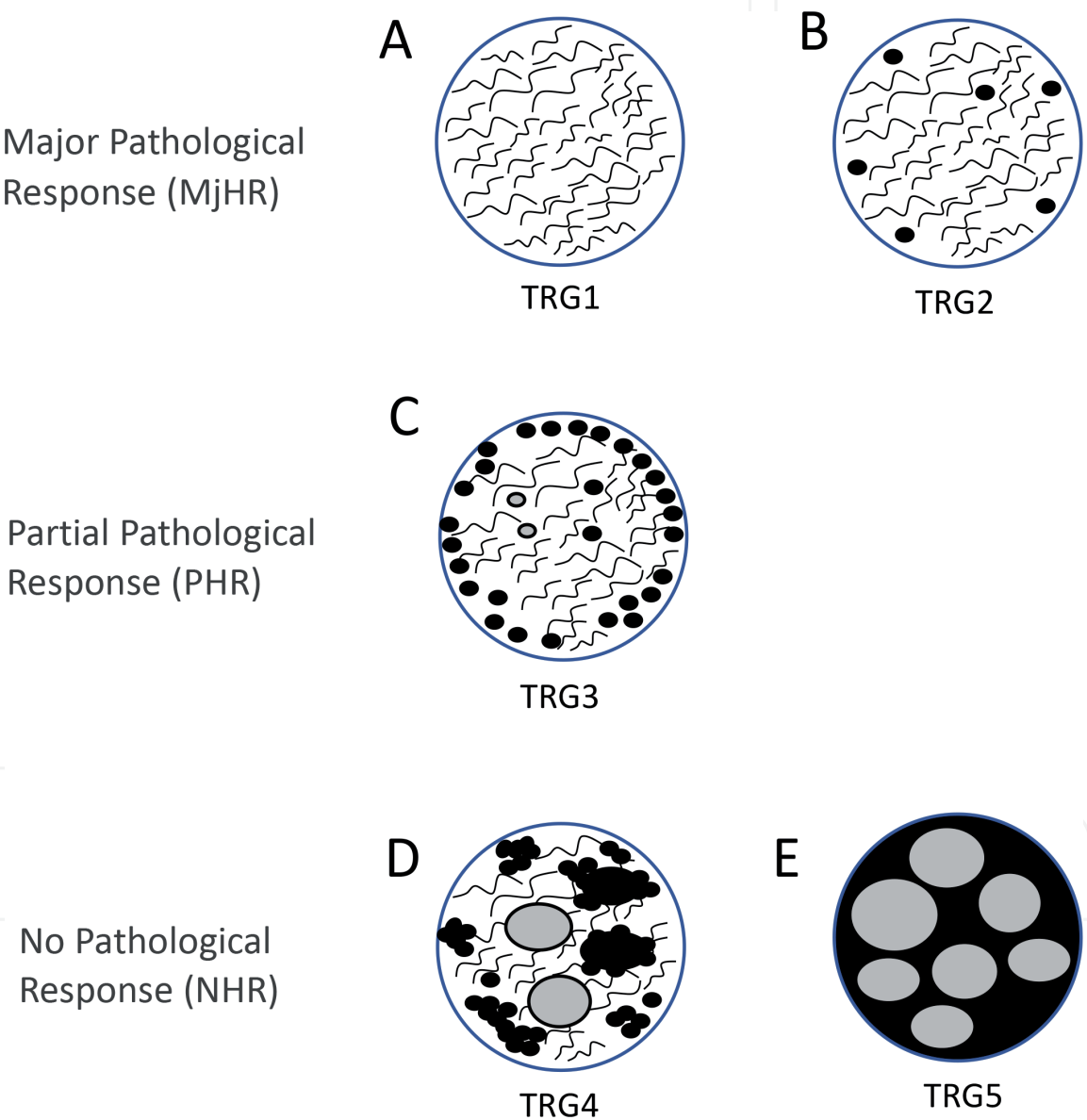
Figure 3.
Tumour regression grade (TRG) scoring system as a component of measuring pathological response in treated CRLMs. (A–E) TRG as scored on haematoxylin and eosin sections of CRLMs based on the proportion of fibrosis to viable tumour cells. The five TRGs shown in this cartoon roughly illustrate the different proportions of fibrosis (fibrils) to tumour cells (black areas). (A) TRG1. There is an absence of viable tumour cells and large amounts of fibrosis. (B) TRG2. The presence of viable tumour cells is rare and they are scattered throughout the fibrosis. (C) TRG3. There is the presence of more residual tumour cells but fibrosis predominates. (D) TRG4. Residual cancer cells predominate over fibrosis. (E) TRG5. There are no signs of tumour regression. The percentage of the CRLM surface area occupied by necrosis is also incorporated as a parameter for pathological response (grey areas). 3 main pathological response groups: TRG1-2: major response (MjHR), TRG3: partial response (PHR), TRG4-5: no histological response (NHR).

Tumour response was scored according to five tumour regression grades (TRG1-5) based on the proportion of fibrosis to viable tumour cells. Later, this TRG scoring system was modified for its application in CRLMs receiving different chemotherapy backbones prior to liver resection (**Figure 3A–E**) [116]. Correlation analyses have demonstrated a significant association of major histological responders with increased survival.

Similar retrospective studies using the TRG in CRLMs were undertaken to see whether adding bevacizumab to chemotherapy would further increase pathological response rate, without necessarily increasing radiographic response rate, after liver resection. Indeed, several retrospective analyses demonstrated that a significantly increased percentage of patients treated with bevacizumab achieved a major pathological response and a significantly higher percentage area of tumour necrosis compared to chemotherapy-only treated patients [117]. Furthermore, the extent of pathological response correlated significantly with long-term-outcomes such as 3- and 5-year overall survival.

11. Mechanisms of resistance to anti-VEGF therapy

As is the case with most cancer therapeutics, drug resistance is considered to be a major factor that limits the efficacy of anti-angiogenic agents. Two 'modes' of resistance to anti-angiogenic therapy are currently recognised: intrinsic resistance, whereby the tumour fails to respond to the therapy from the outset, and acquired resistance, whereby the tumour develops means to evade the therapy after a period of response [21, 29, 118]. It is important to realise that resistance to anti-angiogenic therapy may be attributable to either the tumour cells themselves or due to interactions with their microenvironment. In terms of specific mechanisms mediating resistance to anti-angiogenic therapy, several have been proposed.
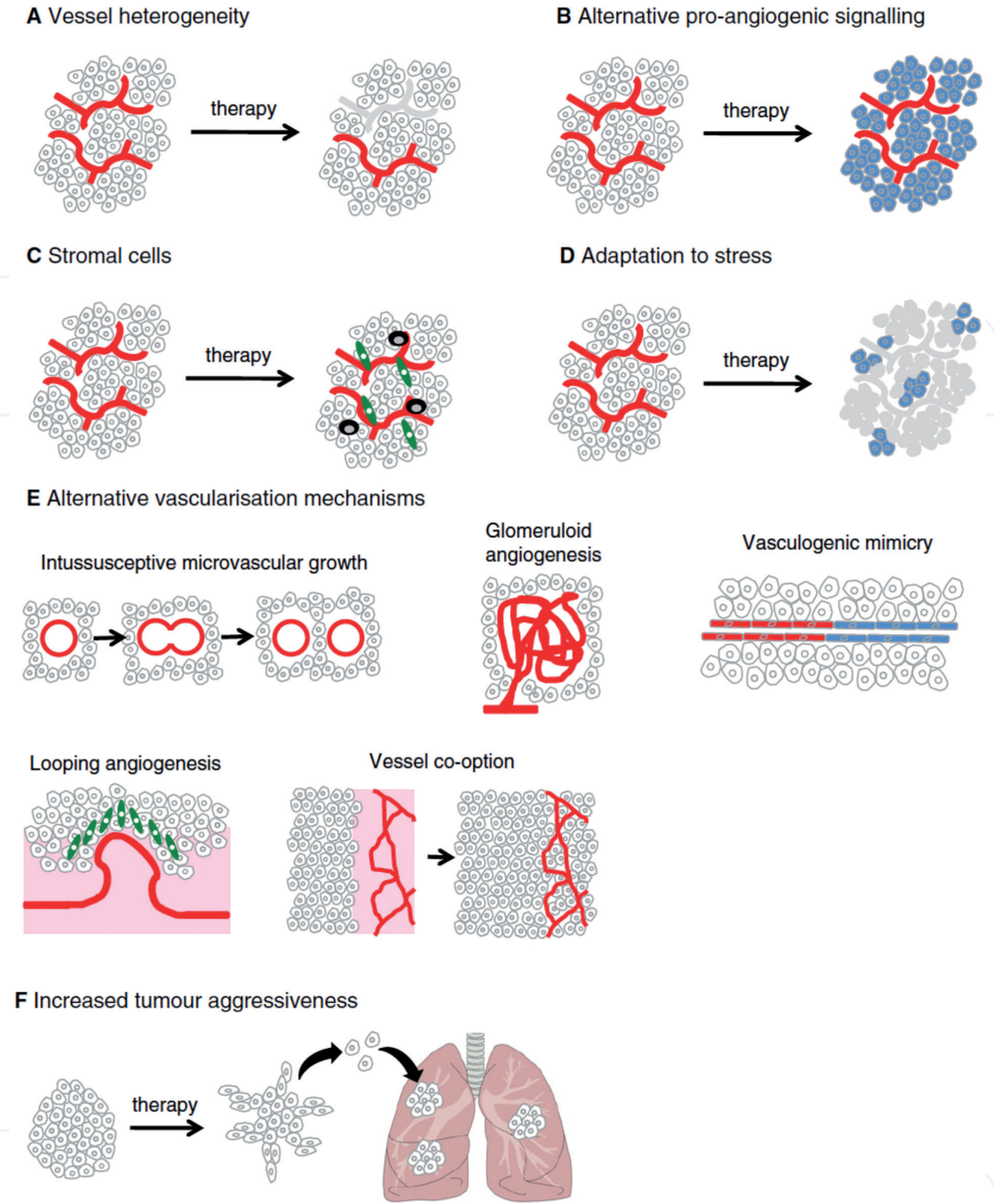
11.1 Vessel heterogeneity

Pre-clinical work has demonstrated that although anti-angiogenic agents thwart the growth of newly established tumour vessels, they are less effective against more mature blood vessels, indicating that they may be less dependent on VEGF (**Figure 4A**) [29]. This may be due to PDGF secretion mediating pericyte recruitment, allowing young vessels to mature and survive [119]. Co-inhibition of VEGF and PDGF has been shown to generate significant anti-angiogenic and anti-tumour effects than with VEGF inhibition alone [120].

11.2 Alternative pro-angiogenic signalling pathways

Alternative pro-angiogenic signalling pathways may allow tumour vascularisation to proceed when VEGF signalling is blocked (**Figure 4B**) [29]. A large body of preclinical work has identified candidate pathways that may provide such an alternative pro-angiogenic stimulus. These include fibroblast growth factors 1 and 2 (FGF1 and FGF2) [121], hepatocyte growth factor (HGF) [122] and epidermal growth factor (EGF) [123]. Most of the above preclinical work suggests that, by inhibiting both VEGF signalling and the candidate pathway, improvements in the anti-tumour efficacy can be seen. Therefore, targeting multiple pro-angiogenic pathways may prove more beneficial than employing agents that inhibit VEGF signalling alone.

Figure 4.
Proposed mechanisms of resistance to anti-angiogenic therapy. (A–F) The potential mechanisms that tumours can utilise to evade anti-angiogenic therapy. (A) Vessel heterogeneity. Tumours can contain vessels that are at different stages of maturation making some more sensitive to therapy than others. For example, here the top vessel is immature and is abolished by therapy (grey), whilst the bottom one is mature and remains viable (red). (B) Alternative proangiogenic signalling pathway scan affect the susceptibility of vessels to therapy. Here, tumour cells (blue) have up-regulated an alternative pro-angiogenic growth factor to facilitate persistent blood vessel growth and survival despite VEGF blockade. (C) Stromal cells infiltrating into of the tumour, such as myeloid progenitors (black) or fibroblasts (green), can also mediate resistance by releasing pro-angiogenic growth factors or by physically incorporating into vessels. (D) Tumour cell adaptation to stress. Subpopulations of cancer cells in the tumour (blue) can survive the hypoxic conditions and nutrient shortage resulting from vascular destruction by employing different adaptation mechanisms. (E) Alternative tumour vascularisation mechanisms. Apart from sprouting angiogenesis, tumours may utilise alternative mechanisms to recruit a vascular supply. In intussusceptive microvascular growth, new vessels are generated by the fission of pre-existing vessels. Glomeruloid angiogenesis is where tight nests of vessels, resembling the renal glomerulus, are formed. Vasculogenic mimicry is a process whereby tumour cells can create vascular-like structures themselves (blue) which are perfused as they become continuous with the host vasculature (red). In looping angiogenesis, contractile myofibroblasts (green) pull host vessels (red) out of the surrounding parenchymal tissue (pink region). Vessel co-option is a process whereby invading tumour cells engulf pre-existing vessels (red) in the normal parenchyma (pink region). (F) Selection of aggressive cells. Therapy alters the biology of the tumour cells in that they become more invasive and/or facilitate accelerated growth of metastases.

11.3 Role of stromal cells

Preclinical data suggest that cells in the tumour stroma, including fibroblasts, neutrophils, macrophages and myeloid progenitors, mediate resistance to VEGF-targeted agents (**Figure 4C**) [124]. For example, tumour-derived granulocyte-colony stimulating factor (G-CSF) mobilises myeloid cells from bone marrow, and is believed to promote pro-angiogenic Bv8 signalling by myeloid cells, which in tumours may confer resistance to anti-VEGF treatment [125]. Immunohistochemistry studies in human tumours showed expression of Bv8 in tumour-infiltrating neutrophils, which were seen in around 15% of breast carcinomas [126].

11.4 Tumour cell adaptation to stress

It is presumed that the inhibition of tumour vascularisation by anti-angiogenic agents will lead to a reduction in oxygen and nutrients available to the tumour thus causing retardation of tumour growth. However, tumours may develop a number of survival mechanisms enabling them to adapt to such hostile conditions (**Figure 4D**).

11.4.1 Metabolism

Some studies have suggested that anti-angiogenic therapy leads to metabolic reprogramming of tumour cells, allowing them to adapt to reduced vascular supply. Preclinical studies have demonstrated that treatment with anti-VEGF antibodies can lead to tumour cells relying on anaerobic metabolism and the glycolytic pathway for ATP [127]. Furthermore, the withdrawal of anti-angiogenic therapy has been shown to cause an increase in lipid metabolism, leading to a rebound in tumour growth [127].

11.4.2 Autophagy

Tumours treated with anti-angiogenic agents may also adapt to survive by activation of autophagy. Autophagy can occur in response to treatment related stressors such as hypoxia and occurs when organelles and proteins in the cell are degraded and recycled by lysosomes [128]. Autophagy-mediating molecules such as BNIP3 have been identified in GBM tumour cells after exposure (a) to hypoxic conditions in vitro, (b) to bevacizumab therapy in vivo or (c) to bevacizumab therapy in human tumours [129]. Furthermore, a recent study has reported that when MDA-MB-231 breast cancer cells were treated with an agent that induced autophagy, they exhibited increased invasiveness [130].

11.4.3 Cancer stem cells (CSCs)

It is becoming clear that many solid tumours contain relatively rare subpopulations of cancer stem cells. These are clones of tumour cells that are able to sustain self-renewal and can tolerate hostile environments [131]. Furthermore, it has been proposed that hypoxia induced by anti-angiogenic therapy can (a) select for CSCs, and (b) maintain the niche that supports the survival of CSCs [132]. Conceivably, these persistent clones of CSCs may render the tumour more invasive and metastatic and may also lead to antiangiogenic therapy resistance [133].

11.4.4 Enhanced tumour aggressiveness

Anti-angiogenic therapy has been proposed to induce hypoxic tumour microenvironments, enhancing the aggressiveness of tumour cells (**Figure 4F**) [134]. This

may help explain why the response to anti-angiogenic therapy is often transient as anti-angiogenic agents can cause initial reductions in tumour burden and a prolonged PFS, but with minimal or no improvement in OS [118]. Anti-angiogenic agents have demonstrated an ability to select for more aggressive cancer cells and enhance tumour cell invasion, growth and metastasis [135]. Moreover, it is now well accepted that some GBM patients with tumours treated with bevacizumab show an increase in tumour invasiveness [136].

11.5 Alternative vascularisation mechanisms

Despite the dogma that tumours primarily employ VEGF-dependent sprouting angiogenesis, emerging evidence now exists for alternative tumour vascularisation mechanisms, including: intussusceptive microvascular growth (IMG) (sometimes known merely as 'intussusception'), glomeruloid angiogenesis, vascular mimicry (also sometimes called 'vasculogenic mimicry'), looping angiogenesis, and vessel co-option (also sometimes called 'vascular co-option') (**Figure 4E**) [21]. These mechanisms may occur by alternative signalling pathways that may not be inhibited by VEGF-targeted therapies.

11.5.1 Intussusception

Intussusception is a mechanism whereby pre-existing vessels split into two daughter vessels without the need for endothelial cell proliferation and sprouting (**Figure 4E**). It has been observed in embryonic development and within experimental tumours recovering from anti-angiogenic therapy and radiotherapy [137]. The molecular mechanisms that control this process are still not well understood.

11.5.2 Vascular mimicry

Vascular mimicry (VM) is a process observed in clinical and preclinical studies whereby tumour cells differentiate into vascular-like structures themselves [138] (**Figure 4E**). It has been shown that basic fibroblast growth factor (bFGF) and VEGF, are incapable of inducing VM channels and networks in poorly aggressive melanoma cell lines, suggesting that VM channel formation maybe be independent of these classical pro-angiogenic growth factors [139]. However, further mechanistic detail is lacking.

11.5.3 Vessel co-option

Vessel co-option is the process whereby, when a tumour invades, existing local vessels become directly incorporated into the tumour (**Figure 4E**). Histopathological studies have indicated that colorectal and breast cancer liver metastases may utilise vessel co-option [140, 141].

Vessel co-option has been shown to mediate resistance to VEGF inhibitors in mouse models of melanoma metastasis to the brain and in mouse models of glioblastoma multiforme, and has been observed in glioblastoma patients who have progressed on anti-VEGF therapy [142–144]. Recently, it has been demonstrated that vessel co-option plays a role in mediating resistance to anti-angiogenic therapy in colorectal cancer liver metastases [145].

In tumour samples obtained from primary lung cancer patients, gene expression arrays have been utilised to identify pathways differentially expressed between angiogenic tumours and vessel co-opting tumours [146]. Stromal expression of thrombospondin-1 appeared to be up regulated in angiogenic tumours, whilst in

vessel co-option tumours, there was increased expression of genes involved in oxidative phosphorylation in primary [146]. Surprisingly, no differences in classic hypoxia or angiogenesis related genes were found between angiogenic and non-angiogenic tumours.

In a glioma rat model of breast cancer brain and lung metastasis, co-opted blood vessels were seen in early-stage tumours and these vessels were found to overexpress angiopoietin-2, a natural antagonist of angiopoietin-1 [147]. As these tumours grew to become more hypoxic, VEGF was upregulated at the hypoxic tumour periphery and stimulated angiogenesis [147]. These observations suggest that a transition from vessel co-option to angiogenesis, or vice versa, may be dependent on the relative expression of pro-angiogenic growth factors (angiopoeitin-1, VEGF) and anti-angiogenic factors (angiopoeitin-2).

Cell adhesion molecules have been implicated in facilitating the process of vessel co-option. In a preclinical brain metastasis model, Carbonell et al. demonstrated that the β1 integrin subunit in breast cancer and lymphoma cells facilitates (a) tumour cell adhesion to the vascular basement membrane of existing brain vessels, (b) tumour cell invasion and (c) the process of vessel co-option [148]. When the function of the β1 integrin subunit was blocked, adhesion to vessels was attenuated and brain metastasis colonies failed to become established and grow [148].

Furthermore, the L1 cell adhesion molecule (L1CAM) has been shown to be involved in vessel co-option in the brain [149]. The ability of cancer cells to co-opt blood vessels was suppressed when L1CAM expression was depleted using shRNA. Conversely, when L1CAM was overexpressed, tumour cells demonstrated enhanced adherence to the outer surface of vessels and tumour growth alongside them. Although such mechanisms are likely to be more specific for vessel co-option in the brain, similar mechanisms may be at work during vessel co-option at other anatomical sites.

12. Conclusion

Tumour vascularisation is modulated by the complex interplay of several endogenous factors and processes that can be up-regulated or downregulated, depending on the tumour microenvironment and the treatment pressures that are imposed on it. A multitude of studies have shown that the majority of solid tumours exhibit an overexpression of VEGF, one of the key drivers of sprouting angiogenesis. As a result, various anti-angiogenic therapies targeting VEGF or VEGFR have now been developed and are used conventionally in the clinic. Compellingly, recent pre-clinical and clinical studies using anti-angiogenic agents in combination with immunotherapies (e.g. ICI's), have demonstrated a synergistic effect in reducing tumour growth. This highlights that there is promise, not only in incorporating anti-angiogenic therapy in the management of most cancers, but also in combining such agents with immunomodulatory agents.

However, as is the case with many cancer treatments, drug resistance can limit the efficacy of these agents. Trials of VEGF-targeted therapies in advanced malignancies have not consistently demonstrated beneficial outcomes in terms of tumour response and survival. Importantly, only a proportion of patients benefit from anti-angiogenic therapy, control of tumour growth is generally transient, there remains significant risk for therapeutic toxicity and we are still challenged by the burden of health costs.

Limited clinical outcomes with anti-angiogenic therapies are felt to be driven by either intrinsic or acquired resistance mechanisms, and several of these have now been proposed. In this chapter, we have reviewed the most commonly used anti-angiogenic agents in the clinic and have highlighted the spectrum of mechanisms

that may be involved in therapeutic resistance. However, despite the plethora of pre-clinical and clinical studies that have been undertaken, these mechanisms are yet to be entirely elucidated. Importantly, the clinically relevant mechanisms that mediate such resistance to anti-angiogenic therapy are poorly understood and we still do not have means to select patients who will benefit from these agents. Furthermore, there has been a rapid expansion in the development of multiple next generation anti-vascular agents, but there is still little clarity regarding important biological pathways that may affect their efficacy.

The data supporting the role of candidate biomarkers for response and resistance to anti-angiogenic therapies thus far have been generated from basic research, retrospective studies and limited prospective correlative studies. As such there remains a crucial need for substantial research of clinically relevant predictive biomarkers with the use of large, prospective randomised trials. This could also provide a platform for longitudinal and frequent biospecimen collection in order to further interrogate the mechanisms involved in tumour vascularisation and therapeutic resistance over time.

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References

[1] Pang RWC, Poon RTP. Clinical implications of angiogenesis in cancers. Vascular Health and Risk Management. 2006;**2**(2):97-108

[2] Folkman J. Tumor angiogenesis: Therapeutic implications. The New England Journal of Medicine. 1971;**285**(21):1182-1186

[3] Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell. 1996;**86**(3):353-364

[4] Yang EV, Sood AK, Chen M, Li Y, Eubank TD, Marsh CB, et al. Norepinephrine up-regulates the expression of vascular endothelial growth factor, matrix metalloproteinase (MMP)-2, and MMP-9 in nasopharyngeal carcinoma tumor cells. Cancer Research. 2006;**66**(21):10357-10364

[5] Tugues S, Koch S, Gualandi L, Li X, Claesson-Welsh L. Vascular endothelial growth factors and receptors: Anti-angiogenic therapy in the treatment of cancer. Molecular Aspects of Medicine. 2011;**32**(2):88-111

[6] Carmeliet P. VEGF as a key mediator of angiogenesis in cancer. Oncology. 2005;**69**(Suppl 3):4-10

[7] Brahimi-Horn C, Pouyssegur J. The role of the hypoxia-inducible factor in tumor metabolism growth and invasion. Bulletin du Cancer. 2006;**93**(8):E73-E80

[8] Takahashi H, Shibuya M. The vascular endothelial growth factor (VEGF)/VEGF receptor system and its role under physiological and pathological conditions. Clinical Science (London, England). 2005;**109**(3):227-241

[9] Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature. 2011;**473**(7347):298-307

[10] Gerber HP, Ferrara N. Pharmacology and pharmacodynamics of bevacizumab as monotherapy or in combination with cytotoxic therapy in preclinical studies. Cancer Research. 2005;**65**(3):671-680

[11] Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling—In control of vascular function. Nature Reviews. Molecular Cell Biology. 2006;**7**(5):359-371

[12] Dougher M, Terman BI. Autophosphorylation of KDR in the kinase domain is required for maximal VEGF-stimulated kinase activity and receptor internalization. Oncogene. 1999;**18**(8):1619-1627

[13] Lamalice L, Houle F, Huot J. Phosphorylation of Tyr1214 within VEGFR-2 triggers the recruitment of Nck and activation of Fyn leading to SAPK2/p38 activation and endothelial cell migration in response to VEGF. The Journal of Biological Chemistry. 2006;**281**(45):34009-34020

[14] Wong C, Jin ZG. Protein kinase C-dependent protein kinase D activation modulates ERK signal pathway and endothelial cell proliferation by vascular endothelial growth factor. The Journal of Biological Chemistry. 2005;**280**(39):33262-33269

[15] Lee SJ, Tsang PS, Diaz TM, Wei BY, Stetler-Stevenson WG. TIMP-2 modulates VEGFR-2 phosphorylation and enhances phosphodiesterase activity in endothelial cells. Laboratory Investigation. 2010;**90**(3):374-382

[16] Nelson AR, Fingleton B, Rothenberg ML, Matrisian LM. Matrix metalloproteinases: Biologic activity and clinical implications. Journal of Clinical Oncology: Official Journal of the

American Society of Clinical Oncology. 2000;**18**(5):1135-1149

[17] Abramsson A, Lindblom P, Betsholtz C. Endothelial and nonendothelial sources of PDGF-B regulate pericyte recruitment and influence vascular pattern formation in tumors. The Journal of Clinical Investigation. 2003;**112**(8):1142-1151

[18] Yang D-H, Park J-S, Jin C-J, Kang H-K, Nam J-H, Rhee J-H, et al. The dysfunction and abnormal signaling pathway of dendritic cells loaded by tumor antigen can be overcome by neutralizing VEGF in multiple myeloma. Leukemia Research. 2009;**33**(5):665-670

[19] Yang J, Yan J, Liu B. Targeting VEGF/VEGFR to modulate antitumor immunity. Frontiers in Immunology. 2018;**9**(978):1-9

[20] Martino EC, Misso G, Pastina P, Costantini S, Vanni F, Gandolfo C, et al. Immune-modulating effects of bevacizumab in metastatic non-small-cell lung cancer patients. Cell Death Discovery. 2016;**2**(16026):1-8

[21] Vasudev NS, Reynolds AR. Anti-angiogenic therapy for cancer: Current progress, unresolved questions and future directions. Angiogenesis. 2014;**17**(3):471-494

[22] Eichholz A, Merchant S, Gaya AM. Anti-angiogenesis therapies: Their potential in cancer management. OncoTargets and Therapy. 2010;**3**:69-82

[23] Ferrara N, Hillan KJ, Gerber HP, Novotny W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. Nature Reviews. Drug Discovery. 2004;**3**(5):391-400

[24] Margolin K, Gordon MS, Holmgren E, Gaudreault J, Novotny W, Fyfe G, et al. Phase Ib trial of intravenous recombinant humanized monoclonal antibody to vascular endothelial growth factor in combination with chemotherapy in patients with advanced cancer: Pharmacologic and long-term safety data. Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology. 2001;**19**(3):851-856

[25] Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. The New England Journal of Medicine. 2004;**350**(23):2335-2342

[26] Cunningham D, Lang I, Marcuello E, Lorusso V, Ocvirk J, Shin DB, et al. Bevacizumab plus capecitabine versus capecitabine alone in elderly patients with previously untreated metastatic colorectal cancer (AVEX): An open-label, randomised phase 3 trial. The Lancet Oncology. 2013;**14**(11):1077-1085

[27] Giantonio BJ, Catalano PJ, Meropol NJ, O'Dwyer PJ, Mitchell EP, Alberts SR, et al. Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: Results from the Eastern Cooperative Oncology Group Study E3200. Journal of Clinical Oncology. 2007;**25**(12):1539-1544

[28] Saltz LB, Clarke S, Diaz-Rubio E, Scheithauer W, Figer A, Wong R, et al. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: A randomized phase III study. Journal of Clinical Oncology. 2008;**26**(12):2013-2019

[29] Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. Nature Reviews. Cancer. 2008;**8**(8):592-603

[30] Nordlinger B, Van Cutsem E, Gruenberger T, Glimelius B, Poston G, Rougier P, et al. Combination of surgery and chemotherapy and the role of targeted agents in the treatment of patients with colorectal liver metastases: Recommendations from an expert panel. Annals of Oncology. 2009;**20**(6):985-992

[31] Wong R, Cunningham D, Barbachano Y, Saffery C, Valle J, Hickish T, et al. A multicentre study of capecitabine, oxaliplatin plus bevacizumab as perioperative treatment of patients with poor-risk colorectal liver-only metastases not selected for upfront resection. Annals of Oncology. 2011;**22**(9):2042-2048

[32] Gruenberger T, Arnold D, Rubbia-Brandt L. Pathologic response to bevacizumab-containing chemotherapy in patients with colorectal liver metastases and its correlation with survival. Surgical Oncology. 2012;**21**(4):309-315

[33] Okines A, Puerto OD, Cunningham D, Chau I, Van Cutsem E, Saltz L, et al. Surgery with curative-intent in patients treated with first-line chemotherapy plus bevacizumab for metastatic colorectal cancer first BEAT and the randomised phase-III NO16966 trial. British Journal of Cancer. 2009;**101**(7):1033-1038

[34] Grothey A, Sugrue MM, Purdie DM, Dong W, Sargent D, Hedrick E, et al. Bevacizumab beyond first progression is associated with prolonged overall survival in metastatic colorectal cancer: Results from a large observational cohort study (BRiTE). Journal of Clinical Oncology. 2008;**26**(33):5326-5334

[35] Grothey A, Flick ED, Cohn AL, Bekaii-Saab TS, Bendell JC, Kozloff M, et al. Bevacizumab exposure beyond first disease progression in patients with metastatic colorectal cancer: Analyses of the ARIES observational cohort study. Pharmacoepidemiology and Drug Safety. 2014;**23**(7):726-734

[36] Bennouna J, Sastre J, Arnold D, Osterlund P, Greil R, Van Cutsem E, et al. Continuation of bevacizumab after first progression in metastatic colorectal cancer (ML18147): A randomised phase 3 trial. The Lancet Oncology. 2013;**14**(1):29-37

[37] de Gramont A, Van Cutsem E, Schmoll HJ, Tabernero J, Clarke S, Moore MJ, et al. Bevacizumab plus oxaliplatin-based chemotherapy as adjuvant treatment for colon cancer (AVANT): A phase 3 randomised controlled trial. The Lancet Oncology. 2012;**13**(12):1225-1233

[38] Allegra CJ, Yothers G, O'Connell MJ, Sharif S, Petrelli NJ, Lopa SH, et al. Bevacizumab in stage II-III colon cancer: 5-year update of the National Surgical Adjuvant Breast and Bowel Project C-08 trial. Journal of Clinical Oncology. 2013;**31**(3):359-364

[39] Reck M, von Pawel J, Zatloukal P, Ramlau R, Gorbounova V, Hirsh V, et al. Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer: AVAil. Journal of Clinical Oncology. 2009;**27**(8):1227-1234

[40] Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. The New England Journal of Medicine. 2006;**355**(24):2542-2550

[41] Soria JC, Mauguen A, Reck M, Sandler AB, Saijo N, Johnson DH, et al. Systematic review and meta-analysis of randomised, phase II/III trials adding bevacizumab to platinum-based chemotherapy as first-line treatment in patients with advanced non-small-cell

lung cancer. Annals of Oncology. 2013;**24**(1):20-30

[42] Socinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, et al. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. The New England Journal of Medicine. 2018;**378**(24):2288-2301

[43] Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, et al. Incorporation of bevacizumab in the primary treatment of ovarian cancer. The New England Journal of Medicine. 2011;**365**(26):2473-2483

[44] Taal W, Oosterkamp HM, Walenkamp AM, Dubbink HJ, Beerepoot LV, Hanse MC, et al. Single-agent bevacizumab or lomustine versus a combination of bevacizumab plus lomustine in patients with recurrent glioblastoma (BELOB trial): A randomised controlled phase 2 trial. The Lancet Oncology. 2014;**15**(9):943-953

[45] Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. New England Journal of Medicine. 2014;**370**(8):699-708

[46] Rini BI, Powles T, Atkins MB, Escudier B, McDermott DF, Suarez C, et al. Atezolizumab plus bevacizumab versus sunitinib in patients with previously untreated metastatic renal cell carcinoma (IMmotion151): A multicentre, open-label, phase 3, randomised controlled trial. The Lancet. 2019;**15**;393(10189):2362-2364

[47] Rossari JR, Metzger-Filho O, Paesmans M, Saini KS, Gennari A, de Azambuja E, et al. Bevacizumab and breast cancer: A meta-analysis of first-line phase III studies and a critical reappraisal of available evidence. Journal of Oncology. 2012;**2012**:417673

[48] Spratlin JL, Cohen RB, Eadens M, Gore L, Camidge DR, Diab S, et al. Phase I pharmacologic and biologic study of ramucirumab (IMC-1121B), a fully human immunoglobulin G1 monoclonal antibody targeting the vascular endothelial growth factor receptor-2. Journal of Clinical Oncology. 2010;**28**(5):780-787

[49] Fuchs CS, Tomasek J, Yong CJ, Dumitru F, Passalacqua R, Goswami C, et al. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): An international, randomised, multicentre, placebo-controlled, phase 3 trial. The Lancet. 2014;**383**(9911):31-39

[50] Wilke H, Muro K, Van Cutsem E, Oh S-C, Bodoky G, Shimada Y, et al. Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): A double-blind, randomised phase 3 trial. The Lancet Oncology. 2014;**15**(11):1224-1235

[51] Fuchs CS, Shitara K, Bartololmeo MD, Lonardi S, Al-Batran SE, Van Cutsem E, et al. Ramucirumab with cisplatin and fluoropyrimidine as first-line therapy in patients with metastatic gastric or junctional adenocarcinoma (RAINFALL): A double-blind, randomised, placebo-controlled, phase 3 trial. The Lancet Oncology. 2019;**20**(3):420-435

[52] Garon EB, Ciuleanu T-E, Arrieta O, Prabhash K, Syrigos KN, Goksel T, et al. Ramucirumab plus docetaxel versus placebo plus docetaxel for second-line treatment of stage IV non-small-cell lung cancer after disease

progression on platinum-based therapy (REVEL): A multicentre, double-blind, randomised phase 3 trial. The Lancet. 2014;**384**(9944):665-673

[53] Petrylak DP, de Wit R, Chi KN, Drakaki A, Sternberg CN, Nishiyama H, et al. Ramucirumab plus docetaxel versus placebo plus docetaxel in patients with locally advanced or metastatic urothelial carcinoma after platinum-based therapy (RANGE): A randomised, double-blind, phase 3 trial. The Lancet. 2017;**390**(10109):2266-2277

[54] Fischer C, Jonckx B, Mazzone M, Zacchigna S, Loges S, Pattarini L, et al. Anti-PlGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. Cell. 2007;**131**(3):463-475

[55] Byrne AT, Ross L, Holash J, Nakanishi M, Hu L, Hofmann JI, et al. Vascular endothelial growth factor-trap decreases tumor burden, inhibits ascites, and causes dramatic vascular remodeling in an ovarian cancer model. Clinical Cancer Research. 2003;**9**(15):5721-5728

[56] Van Cutsem E, Tabernero J, Lakomy R, Prenen H, Prausova J, Macarulla T, et al. Addition of aflibercept to fluorouracil, leucovorin, and irinotecan improves survival in a phase III randomized trial in patients with metastatic colorectal cancer previously treated with an oxaliplatin-based regimen. Journal of Clinical Oncology. 2012;**30**(28):3499-3506

[57] Tabernero J, Van Cutsem E, Lakomy R, Prausova J, Ruff P, van Hazel GA, et al. Aflibercept versus placebo in combination with fluorouracil, leucovorin and irinotecan in the treatment of previously treated metastatic colorectal cancer: Prespecified subgroup analyses from the VELOUR trial. European Journal of Cancer. 2014;**50**(2):320-331

[58] Faivre S, Demetri G, Sargent W, Raymond E. Molecular basis for sunitinib efficacy and future clinical development. Nature Reviews. Drug Discovery. 2007;**6**(9):734-745

[59] Mendel DB, Laird AD, Xin X, Louie SG, Christensen JG, Li G, et al. In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: Determination of a pharmacokinetic/pharmacodynamic relationship. Clinical Cancer Research. 2003;**9**(1):327-337

[60] Griffioen AW, Mans LA, de Graaf AM, Nowak-Sliwinska P, de Hoog CL, de Jong TA, et al. Rapid angiogenesis onset after discontinuation of sunitinib treatment of renal cell carcinoma patients. Clinical Cancer Research. 2012;**18**(14):3961-3971

[61] Escudier B, Roigas J, Gillessen S, Harmenberg U, Srinivas S, Mulder SF, et al. Phase II study of sunitinib administered in a continuous once-daily dosing regimen in patients with cytokine-refractory metastatic renal cell carcinoma. Journal of Clinical Oncology. 2009;**27**(25):4068-4075

[62] Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Oudard S, et al. Overall survival and updated results for sunitinib compared with interferon alfa in patients with metastatic renal cell carcinoma. Journal of Clinical Oncology. 2009;**27**(22):3584-3590

[63] Rini BI, Plimack ER, Stus V, Gafanov R, Hawkins R, Nosov D, et al. Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. New England Journal of Medicine. 2019;**380**(12):1116-1127

[64] Christensen JG. A preclinical review of sunitinib, a multitargeted receptor tyrosine kinase inhibitor

with anti-angiogenic and antitumour activities. Annals of Oncology. 2007;**18**(Suppl 10):x3-x10

[65] Carrato A, Swieboda-Sadlej A, Staszewska-Skurczynska M, Lim R, Roman L, Shparyk Y, et al. Fluorouracil, leucovorin, and irinotecan plus either sunitinib or placebo in metastatic colorectal cancer: A randomized, phase III trial. Journal of Clinical Oncology. 2013;**31**(10):1341-1347

[66] Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Research. 2004;**64**(19):7099-7109

[67] Wilhelm SM, Dumas J, Adnane L, Lynch M, Carter CA, Schutz G, et al. Regorafenib (BAY 73-4506): A new oral multikinase inhibitor of angiogenic, stromal and oncogenic receptor tyrosine kinases with potent preclinical antitumor activity. International Journal of Cancer. 2011;**129**(1):245-255

[68] Grothey A, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): An international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet. 2013;**381**(9863):303-312

[69] Demetri GD, Reichardt P, Kang Y-K, Blay J-Y, Rutkowski P, Gelderblom H, et al. Efficacy and safety of regorafenib for advanced gastrointestinal stromal tumours after failure of imatinib and sunitinib (GRID): An international, multicentre, randomised, placebo-controlled, phase 3 trial. The Lancet. 2013;**381**(9863):295-302

[70] Bruix J, Qin S, Merle P, Granito A, Huang YH, Bodoky G, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): A randomised, double-bind, placebo-controlled, phase 3 trial. The Lancet. 2017;**389**(10064):56-66

[71] Jain RK. Molecular regulation of vessel maturation. Nature Medicine. 2003;**9**(6):685-693

[72] Kaye SB. Bevacizumab for the treatment of epithelial ovarian cancer: Will this be its finest hour? Journal of Clinical Oncology. 2007;**25**(33):5150-5152

[73] Jain RK. Normalization of tumor vasculature: An emerging concept in antiangiogenic therapy. Science. 2005;**307**(5706):58-62

[74] Van der Veldt AA, Lubberink M, Bahce I, Walraven M, de Boer MP, Greuter HN, et al. Rapid decrease in delivery of chemotherapy to tumors after anti-VEGF therapy: Implications for scheduling of anti-angiogenic drugs. Cancer Cell. 2012;**21**(1):82-91

[75] Kerbel RS. Antiangiogenic therapy: A universal chemosensitization strategy for cancer? Science. 2006;**312**(5777):1171-1175

[76] Shaked Y, Henke E, Roodhart JM, Mancuso P, Langenberg MH, Colleoni M, et al. Rapid chemotherapy-induced acute endothelial progenitor cell mobilization: Implications for antiangiogenic drugs as chemosensitizing agents. Cancer Cell. 2008;**14**(3):263-273

[77] Kamoun WS, Ley CD, Farrar CT, Duyverman AM, Lahdenranta J, Lacorre DA, et al. Edema control by cediranib, a vascular endothelial growth factor receptor-targeted kinase inhibitor, prolongs survival despite persistent brain tumor growth in

mice. Journal of Clinical Oncology. 2009;**27**(15):2542-2552

[78] Gianni L, Romieu GH, Lichinitser M, Serrano SV, Mansutti M, Pivot X, et al. AVEREL: A randomized phase III trial evaluating bevacizumab in combination with docetaxel and trastuzumab as first-line therapy for HER2-positive locally recurrent/metastatic breast cancer. Journal of Clinical Oncology. 2013;**31**(14):1719-1725

[79] Smith NR, Baker D, Farren M, Pommier A, Swann R, Wang X, et al. Tumor stromal architecture can define the intrinsic tumor response to VEGF-targeted therapy. Clinical Cancer Research. 2013;**19**(24):6943-6956

[80] Yasuda S, Sho M, Yamato I, Yoshiji H, Wakatsuki K, Nishiwada S, et al. Simultaneous blockade of programmed death 1 and vascular endothelial growth factor receptor 2 (VEGFR2) induces synergistic anti-tumour effect in vivo. Clinical and Experimental Immunology. 2013;**172**(3):500-506

[81] Malo CS, Khadka RH, Ayasoufi K, Jin F, AbouChehade JE, Hansen MJ, et al. Immunomodulation mediated by anti-angiogenic therapy improves CD8 T cell immunity against experimental glioma. Frontiers in Oncology. 2018;**8**(320):1-6

[82] Hodi FS, Lawrence D, Lezcano C, Wu X, Zhou J, Sasada T, et al. Bevacizumab plus ipilimumab in patients with metastatic melanoma. Cancer Immunology Research. 2014;**2**(7):632-642

[83] Wallin JJ, Bendell JC, Funke R, Sznol M, Korski K, Jones S, et al. Atezolizumab in combination with bevacizumab enhances migration of antigen-specific T-cells in metastatic renal cell carcinoma. Nature Communications. 2016;**7**:12624

[84] McDermott DF, Huseni MA, Atkins MB, Motzer RJ, Rini BI, Escudier B, et al. Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus sunitinib in renal cell carcinoma. Nature Medicine. 2018;**24**(6):749-757

[85] Zhu AX, Duda DG, Sahani DV, Jain RK. HCC and angiogenesis: Possible targets and future directions. Nature Reviews. Clinical Oncology. 2011;**8**(5):292-301

[86] Bernaards C, Hegde P, Chen D, Holmgren E, Zheng M, Jubb AM, et al. Circulating vascular endothelial growth factor (VEGF) as a biomarker for bevacizumab-based therapy in metastatic colorectal, non-small cell lung, and renal cell cancers: Analysis of phase III studies. Journal of Clinical Oncology. 2010;**28**(15_suppl):10519

[87] Duda DG, Willett CG, Ancukiewicz M, di Tomaso E, Shah M, Czito BG, et al. Plasma soluble VEGFR-1 is a potential dual biomarker of response and toxicity for bevacizumab with chemoradiation in locally advanced rectal cancer. The Oncologist. 2010;**15**(6):577-583

[88] Kopetz S, Hoff PM, Morris JS, Wolff RA, Eng C, Glover KY, et al. Phase II trial of infusional fluorouracil, irinotecan, and bevacizumab for metastatic colorectal cancer: Efficacy and circulating angiogenic biomarkers associated with therapeutic resistance. Journal of Clinical Oncology. 2010;**28**(3):453-459

[89] Cetin B, Kaplan MA, Berk V, Ozturk SC, Benekli M, Isikdogan A, et al. Prognostic factors for overall survival in patients with metastatic colorectal carcinoma treated with vascular endothelial growth factor-targeting agents. Asian Pacific

Journal of Cancer Prevention. 2012;**13**(3):1059-1063

[90] Manzoni M, Mariucci S, Delfanti S, et al. Circulating endothelial cells and their apoptotic fraction are mutually independent predictive biomarkers in Bevacizumab-based treatment for advanced colorectal cancer. Journal of Cancer Research and Clinical Oncology. 2012;**138**(7):1187-1196

[91] Jubb AM, Miller KD, Rugo HS, Harris AL, Chen D, Reimann JD, et al. Impact of exploratory biomarkers on the treatment effect of bevacizumab in metastatic breast cancer. Clinical Cancer Research. 2011;**17**(2):372-381

[92] Jubb AM, Hurwitz HI, Bai W, Holmgren EB, Tobin P, Guerrero AS, et al. Impact of vascular endothelial growth factor-a expression, thrombospondin-2 expression, and microvessel density on the treatment effect of Bevacizumab in metastatic colorectal. Cancer. 2006;**24**(2):217-227

[93] Relf M, LeJeune S, Scott PA, Fox S, Smith K, Leek R, et al. Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor beta-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis. Cancer Research. 1997;**57**(5):963-969

[94] Ebos JM, Lee CR, Christensen JG, Mutsaers AJ, Kerbel RS. Multiple circulating proangiogenic factors induced by sunitinib malate are tumor-independent and correlate with antitumor efficacy. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(43):17069-17074

[95] Maru D, Venook AP, Ellis LM. Predictive biomarkers for bevacizumab:

Are we there yet? Clinical Cancer Research. 2013;**19**(11):2824-2827

[96] Jain RK, Duda DG, Willett CG, Sahani DV, Zhu AX, Loeffler JS, et al. Biomarkers of response and resistance to antiangiogenic therapy. Nature Reviews Clinical Oncology. 2009;**6**(6):327-338

[97] Tran HT, Liu Y, Zurita AJ, Lin Y, Baker-Neblett KL, Martin AM, et al. Prognostic or predictive plasma cytokines and angiogenic factors for patients treated with pazopanib for metastatic renal-cell cancer: A retrospective analysis of phase 2 and phase 3 trials. The Lancet Oncology. 2012;**13**(8):827-837

[98] Schneider BP, Wang M, Radovich M, Sledge GW, Badve S, Thor A, et al. Association of vascular endothelial growth factor and vascular endothelial growth factor receptor-2 genetic polymorphisms with outcome in a trial of paclitaxel compared with paclitaxel plus bevacizumab in advanced breast cancer: ECOG 2100. Journal of Clinical Oncology. 2008;**26**(28):4672-4678

[99] Koutras AK, Antonacopoulou AG, Eleftheraki AG, Dimitrakopoulos FI, Koumarianou A, Varthalitis I, et al. Vascular endothelial growth factor polymorphisms and clinical outcome in colorectal cancer patients treated with irinotecan-based chemotherapy and bevacizumab. The Pharmacogenomics Journal. 2012;**12**(6):468-475

[100] Lambrechts D, Claes B, Delmar P, Reumers J, Mazzone M, Yesilyurt BT, et al. VEGF pathway genetic variants as biomarkers of treatment outcome with bevacizumab: An analysis of data from the AViTA and AVOREN randomised trials. The Lancet Oncology. 2012;**13**(7):724-733

[101] Beuselinck B, Karadimou A, Lambrechts D, Claes B, Wolter P, Couchy G, et al. VEGFR1 single

nucleotide polymorphisms associated with outcome in patients with metastatic renal cell carcinoma treated with sunitinib—A multicentric retrospective analysis. Acta Oncologica. 2014;**53**(1):103-112

[102] O'Connor JP, Jayson GC. Do imaging biomarkers relate to outcome in patients treated with VEGF inhibitors? Clinical Cancer Research. 2012;**18**(24):6588-6598

[103] Fournier LS, Oudard S, Thiam R, Trinquart L, Banu E, Medioni J, et al. Metastatic renal carcinoma: Evaluation of antiangiogenic therapy with dynamic contrast-enhanced CT. Radiology. 2010;**256**(2):511-518

[104] Vasudev NS, Goh V, Juttla JK, Thompson VL, Larkin JM, Gore M, et al. Changes in tumour vessel density upon treatment with anti-angiogenic agents: Relationship with response and resistance to therapy. British Journal of Cancer. 2013;**109**(5):1230-1242

[105] Grothey A, Hedrick EE, Mass RD, Sarkar S, Suzuki S, Ramanathan RK, et al. Response-independent survival benefit in metastatic colorectal cancer: A comparative analysis of N9741 and AVF2107. Journal of Clinical Oncology. 2008;**26**(2):183-189

[106] Kerbel RS. Clinical trials of antiangiogenic drugs: Opportunities, problems, and assessment of initial results. Journal of Clinical Oncology. 2001;**19**(18 Suppl):45S-51S

[107] Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, et al. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. Nature. 1993;**362**(6423):841-844

[108] Chun YS, Vauthey JN, Boonsirikamchai P, Maru DM, Kopetz S, Palavecino M, et al. Association of computed tomography morphologic criteria with pathologic response and survival in patients treated with bevacizumab for colorectal liver metastases. JAMA: The Journal of the American Medical Association. 2009;**302**(21):2338-2344

[109] Shindoh J, Loyer EM, Kopetz S, Boonsirikamchai P, Maru DM, Chun YS, et al. Optimal morphologic response to preoperative chemotherapy: An alternate outcome end point before resection of hepatic colorectal metastases. Journal of Clinical Oncology. 2012;**30**(36):4566-4572

[110] Lee HY, Lee KS, Hwang HS, Lee JW, Ahn MJ, Park K, et al. Molecularly targeted therapy using bevacizumab for non-small cell lung cancer: A pilot study for the new CT response criteria. Korean Journal of Radiology. 2010;**11**(6):618-626

[111] Schott AF, Roubidoux MA, Helvie MA, Hayes DF, Kleer CG, Newman LA, et al. Clinical and radiologic assessments to predict breast cancer pathologic complete response to neoadjuvant chemotherapy. Breast Cancer Research and Treatment. 2005;**92**(3):231-238

[112] Bouzourene H, Bosman FT, Seelentag W, Matter M, Coucke P. Importance of tumor regression assessment in predicting the outcome in patients with locally advanced rectal carcinoma who are treated with preoperative radiotherapy. Cancer. 2002;**94**(4):1121-1130

[113] Ajani JA, Mansfield PF, Crane CH, Wu TT, Lunagomez S, Lynch PM, et al. Paclitaxel-based chemoradiotherapy in localized gastric carcinoma: Degree of pathologic response and not clinical parameters dictated patient outcome. Journal of Clinical Oncology. 2005;**23**(6):1237-1244

[114] Ribero D, Wang H, Donadon M, Zorzi D, Thomas MB, Eng C, et al. Bevacizumab improves pathologic

response and protects against hepatic injury in patients treated with oxaliplatin-based chemotherapy for colorectal liver metastases. Cancer. 2007;**110**(12):2761-2767

[115] Mandard AM, Dalibard F, Mandard JC, Marnay J, Henry-Amar M, Petiot JF, et al. Pathologic assessment of tumor regression after preoperative chemoradiotherapy of esophageal carcinoma. Clinicopathologic Correlations. Cancer. 1994;**73**(11):2680-2686

[116] Rubbia-Brandt L, Giostra E, Brezault C, Roth AD, Andres A, Audard V, et al. Importance of histological tumor response assessment in predicting the outcome in patients with colorectal liver metastases treated with neo-adjuvant chemotherapy followed by liver surgery. Annals of Oncology. 2007;**18**(2):299-304

[117] Klinger M, Tamandl D, Eipeldauer S, Hacker S, Herberger B, Kaczirek K, et al. Bevacizumab improves pathological response of colorectal cancer liver metastases treated with XELOX/FOLFOX. Annals of Surgical Oncology. 2010;**17**(8):2059-2065

[118] Ebos JM, Kerbel RS. Antiangiogenic therapy: Impact on invasion, disease progression, and metastasis. Nature Reviews Clinical Oncology. 2011;**8**(4):210-221

[119] Franco M, Roswall P, Cortez E, Hanahan D, Pietras K. Pericytes promote endothelial cell survival through induction of autocrine VEGF-A signaling and Bcl-w expression. Blood. 2011;**118**(10):2906-2917

[120] Erber R, Thurnher A, Katsen AD, Groth G, Kerger H, Hammes HP, et al. Combined inhibition of VEGF and PDGF signaling enforces tumor vessel regression by interfering with pericyte-mediated endothelial

cell survival mechanisms. FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology. 2004;**18**(2):338-340

[121] Welti JC, Gourlaouen M, Powles T, Kudahetti SC, Wilson P, Berney DM, et al. Fibroblast growth factor 2 regulates endothelial cell sensitivity to sunitinib. Oncogene. 2011;**30**(10):1183-1193

[122] Shojaei F, Lee JH, Simmons BH, Wong A, Esparza CO, Plumlee PA, et al. HGF/c-Met acts as an alternative angiogenic pathway in sunitinib-resistant tumors. Cancer Research. 2010;**70**(24):10090-10100

[123] Cascone T, Herynk MH, Xu L, Du Z, Kadara H, Nilsson MB, et al. Upregulated stromal EGFR and vascular remodeling in mouse xenograft models of angiogenesis inhibitor-resistant human lung adenocarcinoma. The Journal of Clinical Investigation. 2011;**121**(4):1313-1328

[124] Shojaei F, Wu X, Qu X, Kowanetz M, Yu L, Tan M, et al. G-CSF-initiated myeloid cell mobilization and angiogenesis mediate tumor refractoriness to anti-VEGF therapy in mouse models. Proceedings of the National Academy of Sciences of the United States of America. 2009;**106**(16):6742-6747

[125] Shojaei F, Wu X, Malik AK, Zhong C, Baldwin ME, Schanz S, et al. Tumor refractoriness to anti-VEGF treatment is mediated by CD11b+Gr1+ myeloid cells. Nature Biotechnology. 2007;**25**(8):911-920

[126] Jubb AM, Soilleux EJ, Turley H, Steers G, Parker A, Low I, et al. Expression of vascular notch ligand delta-like 4 and inflammatory markers in breast cancer. The American Journal of Pathology. 2010;**176**(4):2019-2028

[127] Xu J, Wang J, Xu B, Ge H, Zhou X, Fang JY. Colorectal cancer cells refractory to anti-VEGF treatment are vulnerable to glycolytic blockade due to persistent impairment of mitochondria. Molecular Cancer Therapeutics. 2013;**12**(5):717-724

[128] Viry E, Paggetti J, Baginska J, Mgrditchian T, Berchem G, Moussay E, et al. Autophagy: An adaptive metabolic response to stress shaping the antitumor immunity. Biochemical Pharmacology. 2014

[129] Hu YL, DeLay M, Jahangiri A, Molinaro AM, Rose SD, Carbonell WS, et al. Hypoxia-induced autophagy promotes tumor cell survival and adaptation to antiangiogenic treatment in glioblastoma. Cancer Research. 2012;**72**(7):1773-1783

[130] Indelicato M, Pucci B, Schito L, Reali V, Aventaggiato M, Mazzarino MC, et al. Role of hypoxia and autophagy in MDA-MB-231 invasiveness. Journal of Cellular Physiology. 2010;**223**(2):359-368

[131] Kasai T, Chen L, Mizutani A, Kudoh T, Murakami H, Fu L, et al. Cancer stem cells converted from pluripotent stem cells and the cancerous niche. Journal of Stem Cells and Regenerative Medicine. 2014;**10**(1):2-7

[132] Conley SJ, Gheordunescu E, Kakarala P, Newman B, Korkaya H, Heath AN, et al. Antiangiogenic agents increase breast cancer stem cells via the generation of tumor hypoxia. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**(8):2784-2789

[133] Ebos JM, Lee CR, Cruz-Munoz W, Bjarnason GA, Christensen JG, Kerbel RS. Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. Cancer Cell. 2009;**15**(3):232-239

[134] Sullivan R, Graham CH. Hypoxia-driven selection of the metastatic phenotype. Cancer Metastasis Reviews. 2007;**26**(2):319-331

[135] Paez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Vinals F, et al. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer Cell. 2009;**15**(3):220-231

[136] Zuniga RM, Torcuator R, Jain R, Anderson J, Doyle T, Ellika S, et al. Efficacy, safety and patterns of response and recurrence in patients with recurrent high-grade gliomas treated with bevacizumab plus irinotecan. Journal of Neuro-oncology. 2009;**91**(3):329-336

[137] Gianni-Barrera R, Trani M, Reginato S, Banfi A. To sprout or to split? VEGF, Notch and vascular morphogenesis. Biochemical Society Transactions. 2011;**39**(6):1644-1648

[138] Folberg R, Hendrix MJ, Maniotis AJ. Vasculogenic mimicry and tumor angiogenesis. The American Journal of Pathology. 2000;**156**(2):361-381

[139] Paulis YW, Soetekouw PM, Verheul HM, Tjan-Heijnen VC, Griffioen AW. Signalling pathways in vasculogenic mimicry. Biochimica et Biophysica Acta. 2010;**1806**(1):18-28

[140] Vermeulen PB, Colpaert C, Salgado R, Royers R, Hellemans H, Van Den Heuvel E, et al. Liver metastases from colorectal adenocarcinomas grow in three patterns with different angiogenesis and desmoplasia. The Journal of Pathology. 2001;**195**(3):336-342

[141] Stessels F, Van den Eynden G, Van der Auwera I, Salgado R, Van den Heuvel E, Harris AL, et al. Breast adenocarcinoma liver metastases, in contrast to colorectal cancer

liver metastases, display a non-angiogenic growth pattern that preserves the stroma and lacks hypoxia. British Journal of Cancer. 2004;**90**(7):1429-1436

[142] Kienast Y, von Baumgarten L, Fuhrmann M, Klinkert WE, Goldbrunner R, Herms J, et al. Real-time imaging reveals the single steps of brain metastasis formation. Nature Medicine. 2010;**16**(1):116-122

[143] Leenders WP, Kusters B, Verrijp K, Maass C, Wesseling P, Heerschap A, et al. Antiangiogenic therapy of cerebral melanoma metastases results in sustained tumor progression via vessel co-option. Clinical Cancer Research. 2004;**10**(18 Pt 1):6222-6230

[144] di Tomaso E, Snuderl M, Kamoun WS, Duda DG, Auluck PK, Fazlollahi L, et al. Glioblastoma recurrence after cediranib therapy in patients: Lack of "rebound" revascularization as mode of escape. Cancer Research. 2011;**71**(1):19-28

[145] Frentzas S, Simoneau E, Bridgeman VL, Vermeulen PB, Foo S, Kostaras E, et al. Vessel co-option mediates resistance to anti-angiogenic therapy in liver metastases. Nature Medicine. 2016;**22**(11):1294-1302

[146] Hu J, Bianchi F, Ferguson M, Cesario A, Margaritora S, Granone P, et al. Gene expression signature for angiogenic and nonangiogenic non-small-cell lung cancer. Oncogene. 2005;**24**(7):1212-1219

[147] Holash J, Maisonpierre PC, Compton D, Boland P, Alexander CR, Zagzag D, et al. Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science. 1999;**284**(5422): 1994-1998

[148] Carbonell WS, Ansorge O, Sibson N, Muschel R. The vascular basement membrane as "soil" in brain metastasis. PLoS One. 2009;**4**(6):e5857

[149] Valiente M, Obenauf AC, Jin X, Chen Q, Zhang XH, Lee DJ, et al. Serpins promote cancer cell survival and vascular co-option in brain metastasis. Cell. 2014;**156**(5):1002-1016