Review

Tumor vascular responses to antivascular and antiangiogenic strategies: looking for suitable models

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Antiangiogenic and vascular disrupting agents are in the current cancer therapeutic armamentarium. A better understanding of the intricate mechanisms ruling neovessel survival within tumors during or after treatment is needed. Refinement of imaging and a growing knowledge of molecular biology of tumor vascularization provide new insights. It is necessary to define suitable methods for monitoring tumor response and appropriate tools to analyze data. This review compares most commonly used preclinical models, considering their recent improvements, and describes promising new approaches such as microfluidics, real-time electrical impedance based technique and noninvasive imaging techniques. The advantages and limitations of the *in vitro*, *ex vivo* and *in vivo* models are discussed. This review also provides a critical summary of emerging approaches using mathematical modeling.

Vascular remodeling: suitable models and methods for monitoring tumor response

Vasculogenesis and angiogenesis are the fundamental processes by which new blood vessels are formed. During vasculogenesis angioblasts differentiate into endothelial cells and form a primitive vascular network, whereas during angiogenesis new capillaries grow from pre-existing blood vessels. Deregulated angiogenesis has been described as a key pathological event in cancer [1].

In cancer angiogenesis abnormal amounts of stimulating factors are released. Insufficient levels of local oxygen and nutrients in a tumor >1–2 mm in diameter, coupled with genetic mutations in tumor cells, are responsible for triggering the angiogenic switch. New blood vessel formation can occur through various processes shown in Figure 1 [1].

As a means to combat cancer angiogenesis (see Table 1 for examples of antiangiogenic agents), strategies to inhibit proangiogenic or overexpress antiangiogenic molecules

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Glossary

Angioblast: primordial mesenchymal cell from which vascular endothelium cells are differentiated.
Angiogenesis: process of developing new blood vessels. Normal angiogenesis occurs during fetal development and in the uterus during the menstrual cycle, as well as around a wound or cut to help with healing. Tumor angiogenesis is the formation of new blood vessels that grow into the tumor, giving it nutrients and oxygen to assist its growth.
Cancer stem cells: multipotent cells able to differentiate into various cell types and initiate a tumor.
Conditioned medium: medium enriched with soluble factors released by cells that are cultured in it.
Endothelial cells: thin, flattened cells lining the inside surfaces of blood and lymph vessels.
Endothelial progenitor cells: multipotent cells able to differentiate into endothelial cells.
Extracellular matrix (ECM): defines the environment of cells. The ECM consists of several different macromolecules such as proteins and polysaccharides. It provides mechanical strength and protection and functions as a medium for cell communication such as growth factor signaling. ECM also provides vital cell-matrix adhesion, which regulates cell functions that are essential for wound healing or inflammatory process.
Filopodia: slender cytoplasmic projections containing actin filaments, involved in cells migration and direction.
Glioblastoma multiforme (GBM): the most malignant type of astrocytoma, composed of spongioblasts, astroblasts, and astrocytes; GBM usually occurs in the brain but may occur in the brain stem or spinal cord.
Intravital microscopy: technique used to observe biological systems *in vivo* at high resolution, characterized by a long working distance.
Intussusception: also called splitting angiogenesis, is the development and growth of transluminal pillars through the lumen of capillaries, to delineate new vascular entities through partition.
Mitotic rate: proportion of cells undergoing mitosis within a tissue.
Multiple correspondence analysis: a statistical method used to extract the major information contained in a categorical database. A categorical characteristic is not numeric information, which can take a fixed number of values.
Multi-photon laser scanning: technique using a beam of laser light, focused into a small point at the focal plane of the specimen. The beam is scanned in the X–Y direction by the mirror move. Multi-photon refers to the requirement of more than one photon to excite the fluorescent probe.
Orthotopic: natural site, opposed to heterotopic.
Perivascular cells: cell types covering the endothelium.
Response element: sequence of DNA within a gene promoter region able to bind specific transcription factor.
Tip-phenotype: VEGFR-mediated Dll4 expression dictates tip/stalk phenotype in a cell-cell dependent manner involved in sprouting angiogenesis. Tip cells, defined by the expression of Dll4, are leading the vascular sprout using filopodias as sensors for chemoattractant molecule.
Severe combined immunodeficiency (SCID): a genetic disorder in which both ''arms'' (B and T cells) of the adaptive immune system are impaired due to a defect in one of several possible genes.

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Stalk-phenotype: are endothelial cells supporting the sprouting by VEGF-induced proliferation. The stalk phenotype is maintained by lateral inhibition through Notch receptor binding to Dll4 expressed by tip cells.
Vasculogenesis: process of blood vessel formation occurring by *de novo* production of endothelial cells.
Vasculogenic mimicry: mechanism by which the tumor cells organize in vessel-like structures.
Xenotransplantation/xenograft: cells or pieces of organs or tumor grafted to a host animal whose species is different from the donor.

have been used to target tumor endothelial cells [20]. There is also a clear rationale for combining antiangiogenic molecules with other targeted agents to control tumor growth and development. Contrary to antiangiogenic strategies that inhibit the formation of new blood vessels, antineovascular therapy (ANET) disrupts existing tumor vessels. Targeting endothelial cells rather than tumor cells, provides many advantages. First, a single vessel only has to be damaged at one point to block blood flow to hundreds or thousands of tumor cells upstream and downstream [21]. Moreover, vascular endothelial cells are adjacent to the bloodstream, ensuring adequate drug delivery, whereas access to the tumor tissue is sometimes impeded by an aberrant vasculature and high interstitial pressure [21–23]. Additionally, endothelial cells lining angiogenic blood vessel of different tumors are likely to have a similar pattern of markers expression. Therefore, ANET can be used with many vascularized tumors and can also be achieved with classical cytotoxic agents through metronomic chemotherapy. Metronomic chemotherapy is a selective dosing schedule. In addition to proliferating cancer cells and various types of normal cells, such as those of the bone marrow, conventional cytotoxic chemotherapeutics affect the endothelium of the growing tumor vasculature. The antiangiogenic efficacy of chemotherapy seems to be optimized by administering comparatively low doses of drug on a frequent or continuous schedule, with no extended interruptions referred to as metronomic chemotherapy. By frequent administration at low doses, cytotoxic agents accumulate in angiogenic endothelial cells [24].

Photodynamic therapy (PDT) is another approach to tumor neovasculature targeting in addition to targeting proliferating cancer cells. Indeed, PDT effects are mediated not only through direct killing of tumor cells but also through indirect effects, which involve the destruction of tumor neovasculature [25]. Tumor neovasculature targeting appears as an approach of significant research interest for the development of active photosensitizer delivery systems able to enhance selectivity and efficiency of vascular PDT for cancer. The main molecular targets explored in the vascular-targeting PDT for cancer includes the vascular endothelial growth factor receptors (VEGFRs) such as neuropilin (NRP)-1, receptor tissue factor (TF), $\alpha_v\beta_3$ integrins, and matrix metalloproteinase (MMP) receptors [26].

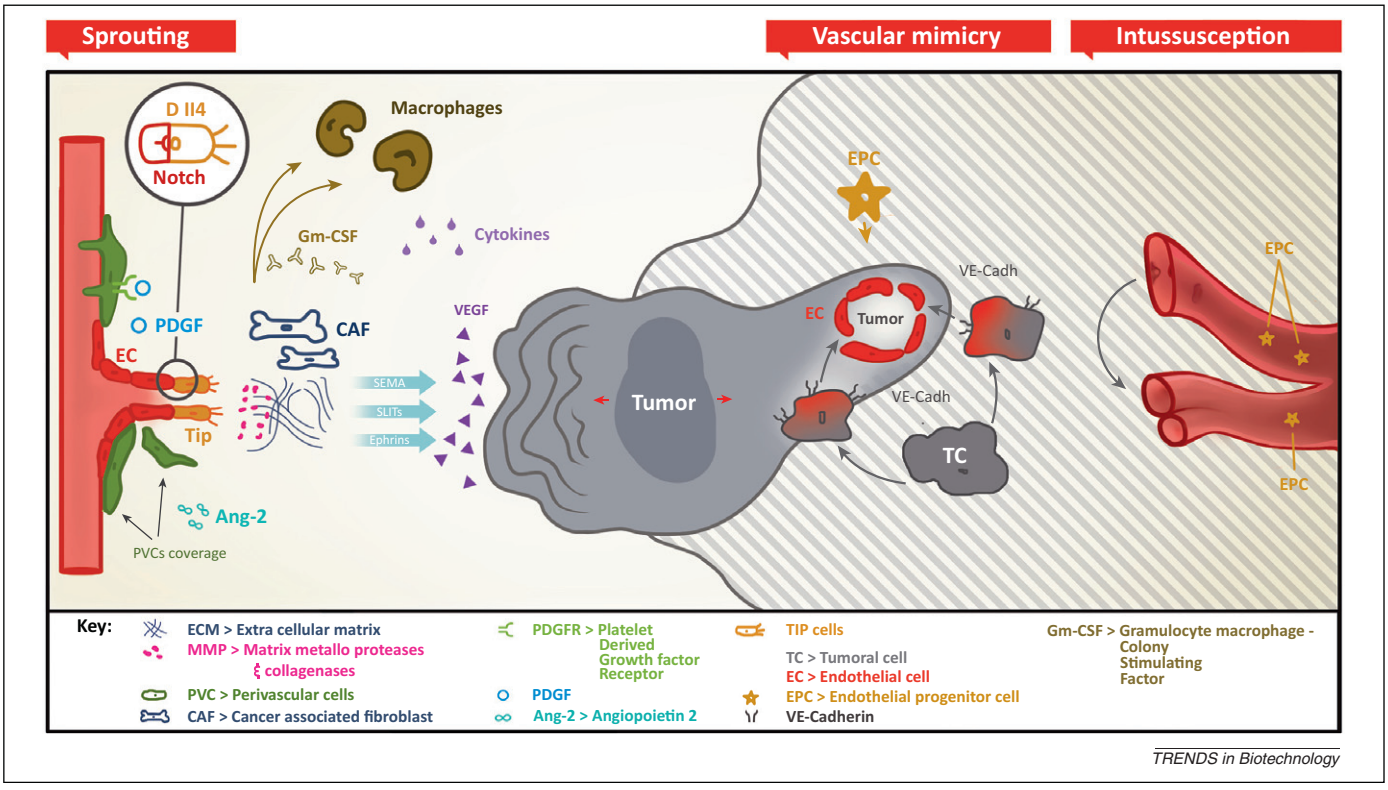

Figure 1. Schematic of new blood vessels formation through three tumor-driven processes (sprouting; vascular mimicry; intussusception). Left: illustration of the main cell types involved in the mechanism of sprouting angiogenesis, and their molecular mediators. Perivascular cell (PVC) coverage of the existing blood vessels is destabilized by angiopoietin (Ang)-2, secreted by activated endothelial cells (ECs). This allows the sprouting to grow toward the tumor as a result of the leading endothelial Tip-cells (TIP), guided by molecular cues of semaphorins, ephrins or SLITs (here represented by blue arrows) and the VEGF gradient, using their filopodia. As highlighted in the circle zoom, the Tip phenotype is maintained in ECs expression of Dll4, a ligand for Notch. Once bound, it keeps the lateral ECs in a stalk-phenotype (in red), which are cells dividing under the stimulation of VEGF secreted by tumor cells (TCs). The progression of the vascular sprout requires destruction of the extracellular matrix (ECM), mainly constituted by fibrous proteins (blue filaments). Matrix metalloproteinases (MMPs) and collagenases (in pink) are produced by ECs to digest the ECM. Cancer-associated fibroblasts (CAFs), releasing granulocyte–macrophage colony-stimulating factor (GM-CSF), recruit and activate the macrophages to produce cytokines, which participate in the proangiogenic microenvironment. Middle: vasculogenic mimicry is represented by the differentiation of TCs into ECs, likely mediated by expression of vascular endothelial cadherin (VE-Cadh) and the possibility of endothelial progenitor cell (EPC, represented as a green star-shaped cell) incorporation into the tubular structure. Right: intussusception is illustrated as a splitting, occurring in an existing blood vessel by pillar growth in the lumen.

A better understanding of the intricate mechanisms of neovessel survival within tumors during or after antiangiogenic therapies and/or ANET is necessary. Various assays and models are available to unravel the mechanism of tumor angiogenesis and vascular-targeting therapies. *In vitro* and *ex vivo* approaches have provided solid and critical data on the molecular and cellular regulations involved in tumor vascularization and have allowed target screening and drug discovery of recent decades (Table 1). As illustrated in Table 1, to acquire complementary results, it is necessary to use different experimental models. For instance, Laschke *et al.* have used the aortic ring assay to assess the inhibition of sprouting by a phenolic plant molecule [18,19]. This selection was based on the presence of different cell types (see section discussing the aortic ring model). Also, the endothelial cells of the aorta are not preselected by passaging and thus are not in a proliferative state at the time of explantation. *In vivo*, the effects of the phenolic compound have been observed using an endometriosis model in mice through the dorsal skinfold chamber [18,19]. Table 1 also highlights the diversity of molecules and biological processes that can be targeted in antiangiogenic/vascular therapies, and their mechanism of action. This variety of approaches makes the evaluation of therapies even more challenging. Indeed, it is critical to use the appropriate model when it exists.

In this review, we discuss the advantages and drawbacks, as well as the most recent technical improvements of these models and assays.

In vitro assays

Endothelial cell culture

Endothelial cells that are seeded on extracellular matrix (ECM) proteins represent the later stages of angiogenesis. These cells are extensively used to assess novel compounds for pro- or antiangiogenic effects on proliferation, migration, or morphogenesis, as well as the mechanism of action of the compound [14] (Figure 2). Although the cells can easily be probed for information on signaling pathways or gene expression, evaluation of morphological data is labor intensive and requires computer-assisted analysis.

The origin of endothelial cells has been shown to impact the results provided by *in vitro* assays, notably the origin of cells from either capillaries (human microvascular endothelial cells) or large vessels (human umbilical vein endothelial cells). Consequently, the assays have been improved to use endothelial cells activated by the tumor; they have a specific phenotype and respond differently to antiangiogenic therapies as compared to normal endothelial cells. Unfortunately, the endothelial cells phenotype tends to change rapidly after isolation. Coculture of endothelial and tumor cells could overcome these issues, because changes in gene expression and properties have been observed in such systems [27] (Figure 2).

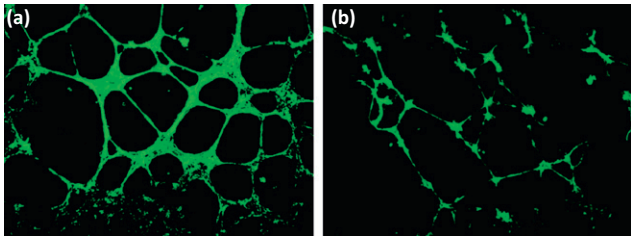

Figure 2. *In vitro* evaluation of human umbilical endothelial vein endothelial cells (HUVECs) ability to form tube-like structure on Matrigel coating. The endothelial network thickness has been assessed using fluorescence intensity (Nikon AZ100) of PECAM (Platelet endothelial cell adhesion molecule-1) antibody coupled to Alexa 488, after 6 h culture in low-serum medium [**(a)** – 2% fetal calf serum (FCS)], or coculture with FaDu cells [**(b)** 200 000 cells/cm^2]. HUVECs were seeded on Matrigel at 150 000 cells/cm^2 and fixed using 4% paraformaldehyde (reproduced, with permission, from [14]).

Microfluidic technologies

To develop multiple microtissues with perfused capillaries based on vasculogenic process, microfluidic technologies were developed. They are chip-systems reproducing capillary networks through the integration of channels (as small as 5 μm in diameter), and bifurcations. Endothelial cells can be cultured and maintained in laminar flow conditions in these devices used to mimic *in vivo* conditions. The major advantages of microfluidic devices are to control precisely the spatial and temporal composition of the liquid (e.g., growth factor gradients), and the ability to visualize cellular events such as cell–cell interactions (homo or heterotypic) or particle–cell interactions by coupling the device to imaging techniques using fluorescence. Developments of microfluidic devices include the creation of 3D networks, using patterns of microvascularization reproduced from *in vivo* imaging. Progress is promising, as the applications for these devices continue to expand, and the devices allow the observation of very subtle biological events [28].

Migration assays

Conventional methodologies used for endothelial cell migration detection are the transwell migration assay or the tube formation assay. Transwell assays allow quantification of cells moving through a membrane, from one compartment to another, classically guided by a gradient or a growth factor [18]. Tube formation is a morphogenesis assay and is used to evaluate the effect of a molecule or a medium on the organization of endothelial cells seeded on a matrix (collagen-based or more complex such as Matrigel) [14]. Quantification then requires cell counting or computer-assisted image analysis. The xCELLigence system, based on real-time cell analysis, is a new, versatile device for invasion and migration studies, and is at present the only device able to measure quantitatively the invasive and migratory behavior of cells in real time without exogenous labels. As cells migrate from the upper chamber through the membrane into the bottom chamber, they contact electronic sensors on the underside of the membrane, resulting in an increase in impedance. The impedance intensity correlates to increasing numbers of migrated cells, and cell-index values reflecting impedance changes are continuously recorded. This system can notably evaluate the influence of a cellular protein on the migration potential of a wide range of cell lines. These findings could be the starting point for further investigations to understand the cellular processes involved in migration at the molecular level [29].

Table 1. Examples of experimental models to evaluate the vascular effects of antiangiogenic and/or antivascular agents in preclinical studies

Models		Antiangiogenic agents	Therapeutic targets		Molecule class/mechanism of action	Refs
in vitro/ex vivo	*in vivo*					
HUVECs, tube formation	DSFC/IVM	Bevacizumab	VEGF	**Proangiogenic factors**	Humanized IgG1 mAb	[2]
	Mice xenografts/IHC	VEGF-Trap, Aflibercept			Composite VEGF decoy receptor	[3]
	Rabbit cornea	Thalidomide	VEGF, b-FGF		IMiD	[4]
	DSFC/IVM	Nadroparin (LMWH)			Heparin or heparan sulfate mimetic	[5]
	Angiosponge model	PG545			–	[6]
Aortic ring assay	GEM models and microbubble Contrast-enhanced ultrasound imaging	Sunitinib malate (SU11248)	VEGFR-1, -3, PDGFR a/b, RET	**RTK**	Multikinase inhibitor, including RTK	[7]
	CAM Mice xenografts	AL3810, CHIR-258	VEGFR2, PDGFR, FGFR1			[8] [9]
	Mice xenografts/IHC	AMG-706, Pazopanib GW786034	VEGFR-1, -2, -3 PDGFR, c-Kit			[10] [11]
HBMvEC culture	Mice xenografts/IHC	ABT-510	Endothelial CD-36	**Proliferation**	Peptidic analog of TSP-1	[12]
3D bovine aortic EC–Matrigel culture	Mice Matrigel plug angiogenesis assay	RGDechiHCit	$\alpha_v\beta_3$ et $\alpha_v\beta_5$	**Integrins**	Antagonist	[13]
HUVEC–Matrigel culture	–	Cetuximab	EGFR	**Other factors with vascular effects**	IgG1 mAb	[14]
	DSFC/IVM Mice Matrigel plug angiogenesis assay Retinopathy murine model	CL1-R12	Human and murine CD160		IgG1 mAb Caspase-dependent EC apoptosis	[15]
	Mice xenografts/IHC	TPC-Ahx-ATWLPPR	Nrp-1		Conjugate photosensitizer-heptapeptide	[16]
3D EC melanoma spheroidal model	–	Resveratrol	HIF-1α, p53		Polyphenol	[17]
Aortic ring assay Murine EC culture	DSFC/IVM CAM	4-Hydroxybenzyl alcohol (HBA)	Anti-MMP-9, VEGF		Phenol	[18] [19]

CAM, chick chorioallantoic membrane; CD, cluster of differentiation; DSFC, dorsal skinfold chamber; EC, endothelial cell; EGFR, epithelial growth factor receptor; FGF, fibroblast growth factor; GEM, genetically engineered mice; HBMvEC, human brain microvascular endothelial cells; HIF, hypoxia-inducible factor; HUVEC, human umbilical vein endothelial cell; IHC, immunohistochemistry; IMiD, immunomodulatory drug; IVM, intravital microscopy; LMWH, low-molecular-weight heparin; mAb, monoclonal antibody; MMP, matrix metalloproteinase; Nrp, neuropilin; PDGFR, platelet-derived growth factor receptor; R-1, receptor-1; RET, REarranged during Transfection (neurotrophic factor receptor); RTK, receptor tyrosine kinase; TSP, thrombospondin; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

Ex vivo organ culture assays: toward the complexity of tumor angiogenesis

The most widely used *ex vivo* model for angiogenesis is aortic explants from rodents. Pieces of aorta are cultured in a 3D matrix. New vessels appear spontaneously within 3 days. After exposure of aortic rings to growth factors or new antiangiogenic compounds, the angiogenic sprouting can be assessed. This easy-to-use assay has become popular because of its good correlation with *in vivo* angiogenesis: it summarizes different levels involved in sprouting angiogenesis from endothelial cell biology to paracrine regulation by the different cell types present in the aorta [30]. Some studies have demonstrated the existence of vascular-wall resident stem-like cells in the adventitia of the human internal thoracic artery. The rodent aortic ring might be a good model to study a possible role for progenitors in tumor angiogenesis, because pluripotent cells are located in the aortic adventitia of adult mice [31]. The main drawbacks for an aortic ring model are variability caused by either the rodent genetic heterogeneity, or the lack of reproducibility of handling of the aorta. Quantifying the newly formed vessels is also difficult, especially due to the 3D growth. Moreover, sprouting from large vessels such as the aorta may be very different than from microvessels occurring in tumors [30]. Finally, we could comment that the different cell types in this assay do not characterize a tumor population. Pericyte coverage is decreased in the tumor environment, and the patterns of expression in tumor-associated fibroblasts or cancer-associated macrophages are modified similarly to those in endothelial cells (Figure 1).

In vivo angiogenesis assays and models

Zebrafish

Zebrafish *(Danio rerio)* is an exciting vertebrate model for angiogenesis because of its significant degree of genetic homology with humans. Moreover, zebrafish are amenable to direct intravital imaging with high spatial and temporal resolution through fluorescent tools. The embryo transparency is a valuable characteristic, and transplantation of human tumor cells has been recently developed. However, the embryo can only bear a limited number of cancer cell grafts; they interfere with development and can be lethal. Angiogenesis assays on zebrafish embryos are very amenable for pharmacological *in vivo* screening, because embryos can be maintained in 96-well plates. Zebrafish has been shown to discriminate between highly and poorly angiogenic tumors [32]. Despite their similarity, some discrepancies between zebrafish and mammalian models have been observed, notably in the pattern of expression of some markers. Similarly, hypoxia induces increased vascular density and tumor cell dissemination in tumor-bearing zebrafish maintained in hypoxic conditions [33]. Contrary to tumor hypoxia occurring at a tissue level, hypoxia in the zebrafish is applied to the whole body through water containing 7.5% oxygen. In comparison, *in vitro* hypoxia uses up to 1% of oxygen. This emphasizes the need to optimize zebrafish protocols for human cancer models, and the requirement of a better knowledge of zebrafish embryos physiology.

Chorioallantoic membrane (CAM)

The CAM model takes advantage of the surface-level capillary network developing within the chick extra-embryo membrane mediating gas and nutrients exchanges before hatching. Major advantages are a very low cost, reproducibility, and its ease of use [34]. Transferred into a Petri dish, the embryo *ex ovo* can receive multiple grafts, reducing the cost even more, which is offset by limited survival. The CAM allows a medium throughput screening of the effects of pharmacological compounds on tumor vascularization (that can be administered through slow-release polymer carriers or intravenous injection). The introduction of gridded collagen on plants in shell-less CAM allows easier scoring of angiogenesis, and the incorporation of various materials such as cells or testing compounds. The vertical growth of newly formed vessels within the 3D matrix enables discrimination from the pre-existing vasculature [35].

One must naturally use caution when extrapolating from a model using chicken cells. Similarly, even in the case of tumor grafts or cell inoculation, the possibility cannot be excluded of a bias in studies conducted in these kinds of models while the embryo is still developing. Embryonic stem cells (ESCs) may have a more dramatic impact on vessel formation in embryonic tissues than in adult tissues. ESCs can spontaneously differentiate into miscellaneous cell types depending on the environment and the surrounding factors. When cultured in suspension, aggregating human ESCs form embryoid bodies, which have the potential to differentiate into endothelial cells. These embryoid bodies, when implanted in severe combined immunodeficiency (SCID) mice, are able to constitute functional microvasculature within the host tissue [36]. Caution should be used when using developing embryos for angiogenic assays.

Mouse models

In vivo preclinical angiogenesis studies in cancer mostly use subcutaneous xenografts on mice. However, the choice of the tumor model is a critical point for angiogenesis assays to be predictive of clinical situations. Notably, important differences between subcutaneous xenografts and orthotopic models have been pointed out. Orthotopic models could be more predictive of antiangiogenic/vascular therapy mechanisms of action and efficacy [37]. The discrepancies between preclinical and clinical data have recently been reviewed. Most patients receiving VEGF inhibitors have late stage or metastasis diseases, whereas *in vivo* assays are mainly realized using animals bearing early-stage or primary tumors [38]. Preclinical studies using tumor-bearing mice suffer a lack of relevant and specific markers to assess the effects of antiangiogenic/vascular therapies. The endpoint commonly used is tumor microvessel density (MVD), which is the number of vessels in a given surface. To evaluate MVD, immunohistochemical techniques are used with markers such as CD31, CD34, or lectins. However, MVD is controversial, because the tumor can regress during treatment, without any change in MVD [39].

To overcome these limitations, noninvasive *in vivo* imaging such as magnetic resonance imaging (MRI), whole

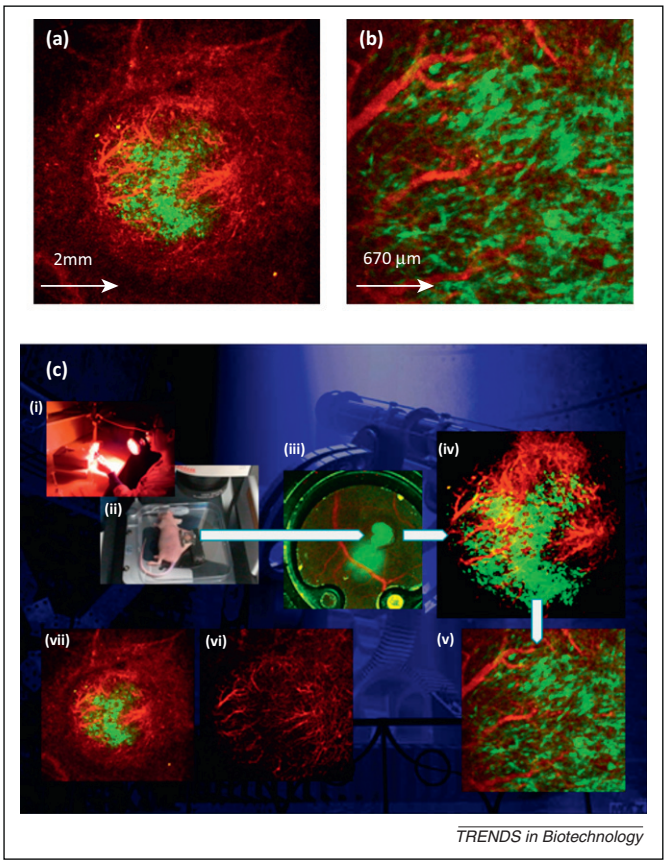

Figure 3. Application of intravital confocal microscopy on nude mice (around 12 weeks old), bearing a dorsal skinfold chamber (day 22 after surgery, small dorsal kit from APJ Trading Co. Inc., CA, USA) with a U87-GFP tumor chunk (20 days post-graft, green, 488 nm). Images performed on anesthetized mice (ketamine–xylazine, 10 µl/g body weight) 5 min after intravenous injection of 120 µl nanoparticles conjugated with chlorin as photosensitizer (red, 650 nm). Multiphoton illumination was coupled with a confocal microscope (PTIBC-IBISA NANCY PPIA 7561 CNRS), objective (2×), Macrozoom (2×) for **(a)** and (60×) for **(b)** to detect fluorescence (bead from 520 to 550 nm, excitation at 488 nm and nanoparticles with chlorine from 630 to 670 nm, excitation at 800 nm). **(c)** Intravenous injection of nanoparticles through the caudal vein (i), position of a mouse bearing the dorsal skinfold chamber on the microscope plate (ii). First, mutiphoton illumination is optimized on the whole field (iii) to detect both GFP fluorescence (green) and chlorin fluorescence (red). Then, the tumor tissue is specifically imaged using the Macrozoom [2× in (iv) and 60× in (v)], and the chlorin-fluorescence channel can be selected for vascular imaging analysis (vi). (vii) shows the tumor tissue (U87-GFP in green) and the surrounding microvasculature (chlorin fluorescence in red).

body fluorescence and bioluminescence, or intravital microscopy approaches are being improved. Imaging tools have the potential to evaluate patient response clinically. I*n vivo* multispectral fluorescent imaging, for example, is used to detect a distinct signature associated with blood vessels in fluorescent tumors in mice, enabling the imaging of tumor vasculature and quantification without vascular probes [40].

Bioluminescence imaging (BLI) is another noninvasive approach, which is expanding for *in vivo* imaging of vasculature. Bioluminescence is the emission of photons in the visible range of wavelengths through the oxidation of an enzyme such as luciferase (North American firefly luciferase, 560 nm) [41]. This imaging technique provides non-quantitative data but its recent development in tomography is a seductive approach to track cells (like progenitor cells) by whole body imaging because of its high sensitivity.

Intravital microscopy can use *in situ* samples, acute (exteriorized) tissue or chronic transparent chambers. Transparent chambers can be used to elucidate molecular, cellular, anatomical, and functional aspects of angiogenesis (Figure 3) with high spatial and temporal resolution. Coupled to fluorescent tools (reporter genes, fluorescent dyes or antibodies), tumors implanted in transparent chambers (dorsal skinfold or cranial windows) constitute a powerful and highly informative model for tumor vascularization and follow-up of antiangiogenic therapy effects. Computer-assisted image analysis further enables quantification such as vessel length, number or diameter by morphological measures, as well as dynamic changes of blood flow, permeability, or shear stress by administration of specific dyes [42]. Adding location methods, the same area can be observed through hours, days or weeks and thus, intravital microscopy can be used for real-time and time-course imaging. Time-course imaging within tumors in living animals might provide critical data to improve treatment efficacy through dynamic parameters that can be monitored before versus after treatment. Additionally, intravital methods and transparent chambers, coupled to sophisticated microscopy techniques such as multi-photon laser scanning (MPLS) microscopy, reveal very fine biological processes such as tumor cell mobility, arrest/adhesion or extravasation, as well as hemodynamics, tissue oxygen level using phosphorescence or even fibrillar collagen structure with second harmonic imaging [43]. A major advantage of multi-photon microscopy is 3D imaging (Figure 3). Intravital observation of tumor vascularization, using a transparent chamber combines many advantages; mostly, the possibility to acquire information from living animals repeatedly on the same region of interest for up to several weeks. This highly dynamic and functional approach, can provide numerous quantitative data, although offset by the slight invasiveness, the cost and the requirement of image analysis methods [42,43]. Dynamic contrast-enhanced MRI (DCE-MRI) uses a contrast material and fast imaging. Regular MRI only shows pictures of the tumor, whereas DCE-MRI also gives information about the blood vessels of the tumor. Noninvasive DCE-MRI reveals progressive development of new vessels in a standardized murine angiogenesis model [44].

Computational and mathematical methods

Understanding the inhibition of angiogenesis in response to therapy is a challenge, given the complexity of the processes involved at the molecular, cellular and tissue levels. In this context, mathematical models provide a powerful analysis tool to dissect the biological mechanisms and the role of tumor growth in angiogenesis and the consequences of its inhibition. Moreover, the model can provide insight for effective and robust treatment protocols by predicting and explaining their successes (or failures), while reducing the number of time-consuming and expensive experiments.

Many mathematical models for tumor angiogenesis have been presented in the literature from the 1970s [45]. From simple partial differential equations to complex multiscale models [46–56], each has provided new insights into our understanding of the angiogenesis process and its

Box 1. Major characteristics of mathematical antiangiogenic models

These features are used in the multiple correspondence analysis whose results are presented in Figure I.

- **Model structures**. The model structure defines the type of equations used in the mathematical models. Two main categories are usually used to model antiangiogenic treatment responses: the nonlinear differential equation (NDE) and the partial differential equation (PDE). The former class is used to describe the temporal dynamics of key variables such as the tumor and endothelial volumes, whereas the latter explains the spatiotemporal behavior by predicting the 2D or 3D vasculature patterns.
- **Validity tests**. Has the mathematical model been validated by statistical tests based on *in vitro* or *in vivo* data? Without any relevant validation tests, credibility of mathematical models remains limited.
- **Model size**. The model size can be measured by two numerical indicators: the number of equations and the number of model parameters.
- **Multiscale dimension**. A multiscale model provides numerical results at different biological scales such as gene expression, protein concentrations, cell proliferation, and tumor dynamics. Such a model is characterized by its number of biological scales and the most challenging task is to model the cross-scales interactions.
- **Determinism versus stochasticity**. Some models are only based on deterministic equations coming from physical laws such as fluid mechanics and do not account for uncertainty in their predictions. Uncertainty sources are multiple: measurement noise, environmental disturbances, and inappropriate modeling assumptions etc. The awareness of such random phenomena is taken into account by the introduction of stochastic variables in the models.
- **Theoretic versus empiric**. Equations may be derived from physical/chemical fundamental principles (inference models, also called first-principle or mechanistic models) but they can also be completely deduced from experimental data (deductive models, also called black-box or data-driven models).

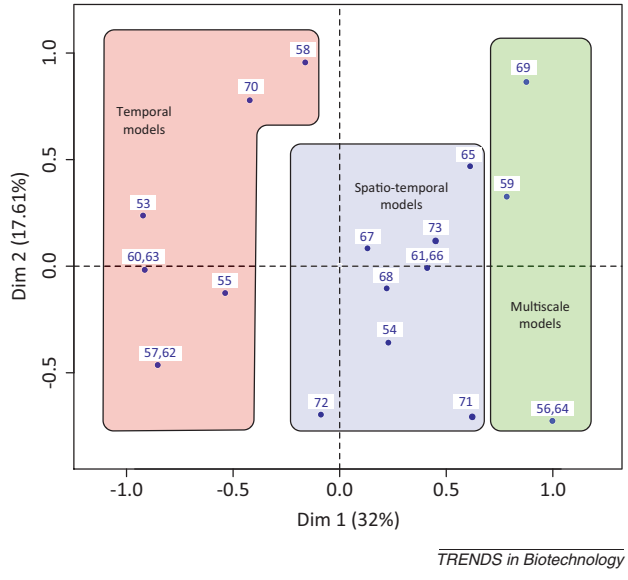

Figure I. Results of a multiple component analysis applied to six modeling characteristics of nineteen mathematical models [50–56,58–73] used to describe the effects of antiangiogenic treatments. Three main model classes appear indicated by blue, red, and green sets.

effects on natural tumor growth. Antiangiogenic models may be classified according to three main categories depending on their purposes: (i) therapy oriented; (ii) understanding of the antiangiogenic process; and (iii) theoretical analysis of tumor-vasculature systems. The first class is therapy oriented. Models are used to suggest new therapeutic strategies [50], to test the effectiveness of new antiangiogenic treatment [51–56], to design optimal model-based control schemes in drug therapies [57], to optimize drug administration in chemotherapy [58] and treatment planning systems in radiotherapy [59,60], and to measure the impact of anticancer agents on metastatic spreading [61].

The second category describes biological insight, such as the prediction of the capillary networks formation [51,62], transport of macromolecules in the tumor [58,63], analysis of competition between angiogenesis and antiangiogenesis processes [53,64,65], or understanding how migrating tumor cells respond to chemoattractants at multiple biological levels [66]. The third category is devoted to the theoretical analysis of tumor-vasculature systems with investigations on the influence of delays on the tumor eradication [50,65], or the mathematical analysis of inherent properties of angiogenesis such as stability [69].

These mathematical models can take various forms, classified according to six characteristics presented in Box 1. Twenty-one models have been analyzed with respect to these characteristics, completed by a multiple correspondence analysis, which clearly reveals the presence of three main classes of mathematical models in antiangiogenic therapies: temporal [50,52,54,55,57,59,60,67], spatiotemporal [51,58,62–65,68–70], and multiscale models [53,56,61,66] (Box 1, Figure 1). All the established models are built up from physical equations, that is, they are mechanistic models, and very few have been directly validated and compared with *in vivo* data [50,56]. For instance, a validated multiscale model of tumor growth was applied to demonstrate the power of *in silico* techniques to make predictions of antitumor activity [56]. In particular, this model was suggested to test the antitumor activity of a clinically used angiogenesis inhibitor and a vascular disrupting agent currently undergoing clinical trial testing. The results highlight that the mathematical model can make predictions in agreement with preclinical and clinical data, and can also be used to gain more insight into the treatment protocols. The results suggest that vascular-targeting agents, as currently administered, cannot lead to cancer eradication, although a highly efficacious agent may lead to long-term cancer control [56]. However, few models have been confronted with real data. Testing procedures may be decomposed in qualitative and quantitative validations. In the latter class, preclinical/clinical and model responses (generally tumor growth responses with and without treatment) are compared [50,55,57], and statistical tests are sometimes applied to assess the relevance of the model predictions. In the qualitative validation procedures, model predictions are indirectly compared to experimental data (published results) through their ability to conclude in the same directions [51,56,58,59,68]. The number of studies in which modeling results are compared to experimental data is relatively low.

The integration of multi-resolution imaging and computational modeling could yield novel insights into a range of diseases involving the pathological vasculature. Image-based multiscale modeling derives morphological data in

Box 2. Summary of critical points: challenges for the most suitable model

In recent decades, knowledge on angiogenesis has progressed greatly; growing from a pretty basic morphological description to a very detailed elucidation of molecular processes involved in blood vessel formation. Knowledge in biotechnology, notably in imaging, has extensively contributed to these progresses, especially with boundaries being pushed further in microscopy techniques, allowing refinement of models and angiogenesis assays at the same time. Nevertheless, some challenges still need to be tackled to reach a better understanding of angiogenesis and mechanism of action of antiangiogenic strategies and antivascular therapies.

- **Efficacy assessment**. The best indicator of antiangiogenic and antivascular effects has yet to be characterized. Vessel number or length might not be the most relevant endpoints to assess treatment efficacy [39]. Notably, vessels that are not functional are frequent within tumor tissue and are included in the measurement of MVD [77]. Thus, MVD can decrease while blood flow is maintained supporting tumor growth. Functionality must be considered through hemodynamic (velocity, wall shear stress) and blood flow parameters to reflect fully vascular-targeted therapy effects. It could be interesting to consider a composite marker combining anatomical and functional aspects. Computational and mathematical approaches may be able to contribute to reach such markers by integrating parameters of different natures.
- **Technical aspects of angiogenesis models**. The degree of prediction from preclinical studies to clinical situation needs to be improved through an appropriate choice of experimental conditions. Improvements in *in vitro* and *ex vivo* assays will likely come from the use of tumor material such as tumor endothelial cells or cancer-associated fibroblasts [27]. The presence of activated or reprogrammed stromal cells might be required to mimic better the tumor environment. Even if genetically engineered mice provide strong evidence of some molecular factors involved in the regulation of angiogenesis, these rodent models are unlikely to represent the pathological situation in humans [7]. Similarly, addressing links and interdependence between factors is a challenging task to tackle in order to control fully angiogenesis assays, as well as to determine the presence and involvement of cell types that could have a dramatic impact on angiogenesis assay outcomes. For example, it is important to know the contribution of BMDCs and endothelial progenitor cells or the role of ESCs in assays using *in vivo* models from different embryonic stages. For this last point however, methods allowing the detection of BMDCs have reached a limit that needs to be overcome, and a lack of standardization in the studies conducted makes the involvement of bone-marrow derived cells (BMDCs) controversial [78].
- **Informative assays**. Mathematical models and time course observation might provide further knowledge on release rate of angiogenic factors, as well as their concentration and distribution. Such data should help to identify resistance phenomenon and improve delivery of therapy, particularly with a temporal scale to adapt treatment schedule. Theoretical mathematical models need to be confronted and validated with experimental data, if not entirely built from experimental observation.

the organ or tumor vasculature from one of several high-resolution and 3D imaging methods [74]. According to the authors, multiple imaging techniques can be handled such as confocal microscopy (microscopic scale), μ-CT and μ-MRI (mesoscopic scale) and *in vivo* MRI (macroscopic scale). These technologies can be exploited to evaluate antitumor and antiangiogenic treatment scenarios, as well as guide the administration of these agents.

The modeling work demonstrates how increasingly sophisticated modeling technology, driven by computational simulation and calibrated with experimental data, can be developed to provide an investigative tool complementary to traditional methods previously presented in the above subsection *Mouse models*. Intravital microscopy could be also an ideal partner for this approach, capable of providing quantitative measurements of dynamic events *in vivo* [75]. Finally, the computational advances have the potential to: (i) contribute to a systems-level view of angiogenesis [76]; (ii) increase the efficacy of administration of systemic pharmaceutical agents; and (iii) support the development of clinical blood biomarkers (e.g., cancer proteins secreted into the blood) for cancer detection.

Concluding remarks

A correct and in-depth understanding of the intricate mechanisms of neovessel survival within tumors during or after antiangiogenic or antineovascular treatments has become a real challenge. The main challenges for the most suitable model are summarized in Box 2. Appropriate *in vivo* models and noninvasive imaging approaches of tumor vascularization such as BLI provide essential tools. Hence, the cost effective and noninvasive character of optical imaging approaches could make them ideal modalities for repeated measurements and to follow treatment response of antiangiogenic agents and ANET over time. Moreover, the development of computational and mathematical modeling will improve the powerful contribution of the emerging treatments. These models will also give helpful insights in the establishment of robust treatment protocols by predicting and explaining their success or failure. Computational models have greatly refined the understanding of the microenvironmental changes that accompany tumor angiogenesis such as changes in tumor blood flow, oxygen diffusion, VEGF expression and extracellular pH. However, high-resolution 3D imaging data that simultaneously quantify tissue morphology and the molecular players involved are not all available. Simultaneously visualizing changes in the complex angiogenic microenvironment at different spatial scales remains a challenge due to the lack of integration between micro- and macroscopic imaging data [74]. Moreover, these developments are helping us gain a fundamental understanding of the cellular and molecular regulation of tumor angiogenesis that will benefit the development of new cancer therapies.

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References

1 Bussolati, B. *et al.* (2011) Tumor exploits alternative strategies to achieve vascularization. *FASEB* 25, 2874–2882
2 Borgström, P. *et al.* (1996) Complete inhibition of angiogenesis and growth of microtumors by anti-vascular endothelial growth factor neutralizing antibody: novel concepts of angiostatic therapy from intravital videomicroscopy. *Cancer Res.* 56, 4032–4039
3 Holash, J. *et al.* (2002) VEGF-Trap: a VEGF blocker with potent antitumor effects. *Proc. Natl. Acad. Sci. U.S.A.* 99, 11393–11398
4 D'Amato, R.J. *et al.* (1994) Thalidomide is an inhibitor of angiogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 91, 4082–4085

5 Debergh, I. *et al.* (2010) The low-molecular-weight heparin, nadroparin, inhibits tumour angiogenesis in a rodent dorsal skinfold chamber model. *Br. J. Cancer* 102, 837–843
6 Dredge, K. *et al.* (2011) PG545, a dual heparanase and angiogenesis inhibitor, induces potent anti-tumour and anti-metastatic efficacy in preclinical models. *Br. J. Cancer* 104, 635–664
7 Olson, P. *et al.* (2011) Imaging guided trials of the angiogenesis inhibitor sunitinib in mouse models predict efficacy in pancreatic neuroendocrine but not ductal carcinoma. *Proc. Natl. Acad. Sci. U.S.A.* 108, 1275–1284
8 Zhou, Y. *et al.* (2012) AL3810, a multi-tyrosine kinase inhibitor, exhibits potent anti-angiogenic and antitumor activity via targeting VEGFR, FGFR, and PDGFR. *J. Cell Mol. Med.* 16, 2321–2330
9 Trudel, S. *et al.* (2005) CHIR-258, a novel, multitargeted tyrosine kinase inhibitor for the potential treatment of t(4;14) multiple myeloma. *Blood* 105, 2941–2948
10 Polverino, A. *et al.* (2006) AMG 706, an oral, multikinase inhibitor that selectively targets vascular endothelial growth factor, platelet-derived growth factor, and kit receptors, potently inhibits angiogenesis and induces regression in tumor xenografts. *Cancer Res.* 66, 8715–8721
11 Sloan, B. and Scheinfeld, N.S. (2008) Pazopanib, a VEGF receptor tyrosine kinase inhibitor for cancer therapy. *Curr. Opin. Investig. Drugs* 12, 1324–1335
12 Anderson, J.C. *et al.* (2007) ABT-510, a modified type 1 repeat peptide of thrombospondin, inhibits malignant glioma growth in vivo by inhibiting angiogenesis. *Cancer Biol. Ther.* 6, 454–462
13 Santulli, G. *et al.* (2011) Evaluation of the anti-angiogenic properties of the new selective αVβ3 integrin antagonist RGDechiHCit. *J. Transl. Med.* 9, http://dx.doi.org/10.1186/1479-5876-9-7
14 Jouan-Hureaux, V. *et al.* (2012) Modulation of endothelial cell network formation *in vitro* by molecular signaling of head and neck squamous cell carcinoma (HNSCC) exposed to cetuximab. *Microvasc. Res.* 83, 131–137
15 Chabot, S. *et al.* (2011) A novel antiangiogenic and vascular normalization therapy targeted against human CD160 receptor. *J. Exp. Med.* 208, 973–986
16 Bechet, D. *et al.* (2010) Neuropilin-1 targeting photosensitization-induced early stages of thrombosis via tissue factor release. *Pharm. Res. Mar.* 27, 468–479
17 Trapp, V. *et al.* (2010) Anti-angiogenic effects of resveratrol mediated by decreased VEGF and increased TSP1 expression in melanoma-endothelial cell co-culture. *Angiogenesis* 13, 305–315
18 Laschke, M.W. *et al.* (2011) In vitro and in vivo evaluation of the anti-angiogenic actions of 4-hydroxybenzyl alcohol. *Br. J. Pharmacol.* 163, 835–844
19 Lim, E.J. *et al.* (2007) Anti-angiogenic, anti-inflammatory and anti-nociceptive activity of 4-hydroxybenzyl alcohol. *J. Pharm. Pharmacol.* 59, 1235–1240
20 Eichhorn, M.E. *et al.* (2004) Anti-vascular tumor therapy: recent advances, pitfalls and clinical perspectives. *Drug Resist. Updat.* 7, 125–138
21 Eberhard, A. *et al.* (2000) Heterogeneity of angiogenesis and blood vessel maturation in human tumors: implications for antiangiogenic tumor therapies. *Cancer Res.* 60, 1388–1393
22 Thorpe, P.E. *et al.* (2003) The first international conference on vascular targeting: meeting overview. *Cancer Res.* 63, 1144–1147
23 Augustin, H.G. (1998) Antiangiogenic tumour therapy: will it work? *Trends Pharmacol. Sci.* 19, 216–222
24 Gasparini, G. (2001) Metronomic scheduling: the future of chemotherapy. *Lancet Oncol.* 2, 733–740
25 Benachour, H. *et al.* (2011) Targeting strategies in photodynamic therapy. In *Brain Cancer, Targeting Strategies and Cervical Cancer* (Salvatti, E.K., ed.), pp. 1–38, Nova Science Publishers
26 Tirand, L. *et al.* (2006) A peptide competing with VEGF165 binding on neuropilin-1 mediates targeting of a chlorin-type photosensitizer and potentiates its photodynamic activity in human endothelial cells. *J. Control. Release* 111, 153–164
27 Cryan, L.M. and Rogers, M.S. (2011) Targeting the anthrax receptors, TEM-8 and CMG-2, for anti-angiogenic therapy. *Front. Biosci.* 16, 1574–1588
28 Wong, K. *et al.* (2012) Microfluidic models of vascular functions. *Annu. Rev. Biomed. Eng.* 14, 205–230
29 Bird, C. and Kirstein, S. (2009) Real-time, label-free monitoring of cellular invasion and migration with the xCELLigence system. *Nat. Methods* 6
30 Nicosia, R.F. (2009) The aortic ring model of angiogenesis: a quarter century of search and discovery. *J. Cell. Mol. Med.* 13, 4113–4136
31 Passman, J.A. *et al.* (2008) A sonic hedgehog signaling domain in the arterial adventitia supports Sca1+ smooth muscle progenitor cells. *Proc. Natl. Acad. Sci. U.S.A.* 105, 9349–9354
32 Nicoli, S. and Presta, M. (2007) The zebrafish/tumor xenograft angiogenesis assay. *Nat. Protoc.* 2, 2918–2923
33 Lee, S.L.C. *et al.* (2009) Hypoxia-induced pathological angiogenesis mediates tumor cell dissemination, invasion and metastasis in a zebrafish tumor model. *Proc. Natl. Acad. Sci. U.S.A.* 106, 19485–19490
34 Ribatti, D. (2008) Chick embryo chorioallantoic-membrane as a useful tool to study angiogenesis. *Int. Rev. Cell Mol. Biol.* 270, 181–224
35 Deryugina, E.I. and Quigley, J.P. (2008) Chick embryo chorioallantoic membrane models to quantify angiogenesis induced by inflammatory and tumor cells or purified effector molecules. *Methods Enzymol.* 444, 21–41
36 Hanjaya-Putra, D. and Gerecht, S. (2009) Vascular engineering using human embryonic stem cells. *Biotechnol. Prog.* 25, 2–9
37 Loi, M. *et al.* (2011) The use of the orthotopic model to validate antivascular therapies for cancer. *Int. J. Dev. Biol.* 55, 547–555
38 Ebos, J.M.L. and Kerbel, R.S. (2011) Antiangiogenic therapy: impact on invasion, disease progression, and metastasis. *Nat. Rev. Clin. Oncol.* 8, 210–221
39 Tozer, G.M. (2003) Measuring tumour vascular response to antivascular and antiangiogenic drugs. *Br. J. Radiol.* 76, 23–35
40 Mayes, P. *et al.* (2008) Non-invasive vascular imaging in fluorescent tumors using multispectral unmixing. *Biotechniques* 45, 459–464
41 Oudina, K. *et al.* (2011) Noninvasive bioluminescent quantification of viable stem cells in engineered constructs. In *Mammalian Cell Viability: Methods and Protocols, Methods in Molecular Biology* (Vol. 740) (Stoddart, M.J., ed.), pp. 165–178, Humana Press
42 Baron, T.V. *et al.* (2011) Intravital microscopy in the mouse dorsal skin chamber model for the study of solid tumors. *Am. J. Cancer Res.* 5, 674–686
43 Fukumura, D. *et al.* (2010) Tumor microvasculature and microenvironment: novel insights through intravital imaging in preclinical models. *Microcirculation* 17, 206–225
44 Wittenborn, T. *et al.* (2012) Ultrahigh-field DCE-MRI of angiogenesis in a novel angiogenesis mouse model. *J. Magn. Reson. Imaging* 35, 703–710
45 Gabhann, F.M. (2011) *Modeling Tumor Vasculature: Molecular, Cellular, and Tissue Level Aspects and Implications* (Jackson, T.L., ed.), pp. 37–53, Springer
46 Thompson, J.R. and Brown, B.W. (1987) *Cancer Modeling* (Marcel Dekker ed.), pp. 1–440, Springer Press
47 Chaplain, M.A.J. (1987) From mutation to metastasis: the mathematical modelling of the stages of tumour development. In *A Survey of Models for Tumor-Immune System Dynamics* (Adam, J.A. and Bellomo, N., ed.), pp. 187–236, Birkhäuser
48 Peirce, S.M. (2008) Computational and mathematical modeling of angiogenesis. *Microcirculation* 15, 739–751
49 Rejniak, K.A. and McCawley, L.J. (2010) Current trends in mathematical modeling of tumor-microenvironment interactions: a survey of tools and applications. *Exp. Biol. Med.* 235, 411–423
50 Hahnfeldt, P. *et al.* (1999) Tumor development under angiogenic signaling: a dynamical theory of tumor growth, treatment response, and postvascular dormancy. *Cancer Res.* 59, 4770–4775
51 Chaplain, M.A. (2000) Mathematical modelling of angiogenesis. *J. Neurooncol.* 50, 37–51
52 Arakelyan, L. *et al.* (2003) Multi-scale analysis of angiogenic dynamics and therapy. In *Cancer Modelling and Simulation* (Preziosi, L., ed.), pp. 205–239, CRC Press
53 Billy, F. *et al.* (2009) A pharmacologically based multiscale mathematical model of angiogenesis and its use in investigating the efficacy of a new cancer treatment strategy. *J. Theor. Biol.* 260, 545–562
54 D'Onofrio, A. and Gandolfi, A. (2009) A family of models of angiogenesis and anti-angiogenesis anti-cancer therapy. *Math. Med. Biol.* 26, 63–95
55 Ribba, B. *et al.* (2011) A model of vascular tumour growth in mice combining longitudinal tumour size data with histological biomarkers. *Eur. J. Cancer* 47, 479–490

56 Gevertz, J.L. (2011) Computational modeling of tumor response to vascular-targeting therapies – part I: validation. *Comput. Math. Methods Med.* http://dx.doi.org/10.1155/2011/830515
57 Gorelik, B. *et al.* (2008) Efficacy of weekly docetaxel and bevacizumab in mesenchymal chondrosarcoma: a new theranostic method combining xenografted biopsies with a mathematical model. *Cancer Res.* 68, 9033–9040
58 Finley, S.D. *et al.* (2011) Pharmacokinetics and pharmacodynamics of VEGF-neutralizing antibodies. *BMC Syst. Biol.* 21, 193–213
59 Finley, S.D. and Popel, A.S. (2012) Predicting the effects of anti-angiogenic agents targeting specific VEGF isoforms. *AAPS J.* 14, 500–509
60 Ledzewicz, U. and Schattler, H. (2008) Optimal and suboptimal protocols for a class of mathematical models of tumor anti-angiogenesis. *J. Theor. Biol.* 252, 295–312
61 Stéphanou, A. *et al.* (2005) Mathematical modelling of flow in 2D and 3D vascular networks: Applications to anti-angiogenic and chemotherapeutic drug strategies. *Math. Comput. Model.* 41, 1137–1156
62 Sachs, R.K. *et al.* (2001) Simple ODE models of tumor growth and anti-angiogenic or radiation treatment. *Math. Comput. Model.* 33, 1297–1305
63 Ergun, A. *et al.* (2003) Optimal scheduling of radiotherapy and angiogenic inhibitors. *Bull. Math. Biol.* 65, 407–424
64 Benzekry, S. *et al.* (2012) Modeling the impact of anticancer agents on metastatic spreading. *Math. Model. Nat. Phenomenon* 7, 306–336
65 Plank, M.J. and Sleeman, B.D. (2003) A reinforced random walk model of tumour angiogenesis and anti-angiogenic strategies. *Math. Med. Biol.* 20, 135–181
66 McDougall, S.R. *et al.* (2002) Mathematical modelling of flow through vascular networks: implications for tumour-induced angiogenesis and chemotherapy strategies. *Bull. Math. Biol.* 64, 673–702
67 Habbal, A. (2005) A Nash topology game for tumoral anti-angiogenesis. *Struct. Multidisciplinary Optimization* 30, 404–412
68 Jain, R.K. *et al.* (2007) Effect of vascular normalization by antiangiogenic therapy on interstitial hypertension, peritumor edema, and lymphatic metastasis: insights from a mathematical model. *Cancer Res.* 67, 2729–2735
69 Vital-Lopez, F.G. *et al.* (2011) Modeling the effect of chemotaxis on glioblastoma tumor progression. *AIChE J.* 57, 778–792
70 Karasözen, B. *et al.* (2009) A model of angiogenesis by hybrid systems with delay on the piecewise constant part. *J. Process Control* 19, 1257–1264
71 Jain, R.K. and Baxter, L.T. (1988) Mechanisms of heterogeneous distribution of monoclonal antibodies and other macromolecules in tumors: significance of elevated interstitial pressure. *Cancer Res.* 48, 7022–7032
72 Delgado, M. *et al.* (2010) An angiogenesis model with nonlinear chemotactic response and flux at the tumor boundary. *Nonlin. Anal. Theory Methods Appl.* 72, 330–347
73 Anderson, A.R. and Chaplain, M.A. (1998) Continuous and discrete mathematical models of tumor-induced angiogenesis. *Bull. Math. Biol.* 60, 857–899
74 Kim, E. *et al.* (2012) Multiscale imaging and computational modeling of blood flow in the tumor vasculature. *Ann. Biomed. Eng.* 40, 2425–2441
75 van de Ven, A.L. *et al.* (2012) Integrated intravital microscopy and mathematical modeling to optimize nanotherapeutics delivery to tumors. *AIP Adv.* 2, http://dx.doi.org/10.1063/1.3699060
76 Snoeks, T.J.A. *et al.* (2010) In vivo optical approaches to angiogeneisis imaging. *Angiogenesis* 13, 135–147
77 White, S.M. *et al.* (2011) Automated computation of functional vascular density using laser speckle imaging in a rodent window chamber model. *Microvasc. Res.* 82, 92–95
78 Patenaude, A. *et al.* (2010) Involvement of endothelial progenitor cells in tumor vascularization. *Microvasc. Res.* 79, 217–223