Imaging Tumor Angiogenesis

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Abstract

Angiogenesis, the formation of new blood vessels, is critical for the growth and metastasis of most solid tumors. Several cytokines, cytokine receptors, and cell adhesion molecules have been identified as potential targets for cancer treatment and in general most have been inhibited through the use of monoclonal antibodies or small molecule tyrosine kinase inhibitors.

Drug development relies on the earliest possible discrimination of active and inactive new agents, and imaging has been used to assess the anti-vascular effects of new agents for over 15 years. This has been critical for the development of new agents to the extent that any vascular endothelial growth factor (VEGF) inhibitor that does not impact dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) in early drug development can be discarded as not hitting its target.

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Much further work has looked at the value of imaging in predicting benefit from anti-angiogenic agents. However, in general, conclusions have not been uniform and it is not clear at present whether imaging can be used to select patients who are most likely to benefit from VEGF pathway inhibitors. However, new imaging biomarkers are in development and are already proving novel data on the architecture and function of tumor neovasculature.

Keywords
Angiogenesis · Imaging · DCE-MRI · CT · PET · VEGF inhibitors

Introduction

Nearly all solid tumors require formation of a neovasculature that allows them to grow and spread through vascular metastasis. Following decades of observational data, which demonstrated that the density of new blood vessels in several tumors was related to the predilection to metastasis and survival (Hasan et al. 2002), the landmark identification of vascular endothelial growth factor (VEGF) (Leung et al. 1989) as the principal angiogenic growth factor led to the development of multiple new agents targeted against this cytokine or its key signaling receptor, VEGFR2. In general these drugs have improved progression free survival (PFS) in many tumors but have not uniformly impacted on overall survival. The most sensitive tumors, such as renal and neuroendocrine cancers, can be treated with single-agent VEGF pathway inhibitors (Jayson et al. 2016), clearly impacting on survival in those situations, and some trials in moderately angio-sensitive diseases have described improvements in overall survival (Hurwitz et al. 2004) while others have not (Schmoll et al. 2012).

Drug development of anti-angiogenic agents initially focused on the optimization of monoclonal antibodies that bound VEGF, culminating in the licensing of the first effective agent, the monoclonal, anti-VEGF antibody, bevacizumab. Subsequent drug development largely focused on the receptor, yielding two main classes of drug that inhibited cell signaling through antibody-mediated inhibition of the receptor (e.g., ramucirumab) or through the elucidation of small molecule VEGFR tyrosine-kinase inhibitors, of which many examples exist (Jayson et al. 2016).

Early phase drug development relies heavily on the identification of the most effective agents as early as possible and the equally important elimination of drugs that are ineffective. Three concepts have arisen, which include proof of mechanism, where investigations are performed to determine whether a defined molecular mechanism has been inhibited, and proof of principle where, as in anti-angiogenic agents, attempts are made to test whether a new drug successfully inhibits a particular phenotype such as the tumor vasculature and proof of concept, where a drug is demonstrated to improve outcome (Workman et al. 2006).

Imaging has been used extensively in the early drug development of anti-angiogenic agents to define whether a new agent achieves proof of principle, that is, to determine whether a putative agent has impacted the tumor vasculature. By far the majority of studies have deployed dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) (O'Connor et al. 2012), and this will be the principal focus of this chapter. This technology has been so uniformly useful, although susceptible to important institute-by-institute variables (O'Connor et al. 2016), that new candidate agents, which are purported to be VEGF pathway inhibitors, would be discarded if no DCE-MRI effects are described within the maximum tolerated dose of the agent. However, as we will discuss, imaging changes are necessary but not sufficient to guarantee a successful pathway to licensing.

Early phase clinical trial data inspired great confidence in imaging and its relationship to the tumor vasculature, and this led to attempts to use imaging characteristics to predict which patients would benefit from anti-angiogenic agents (O'Connor and Jayson 2012). As discussed below, this application has not been successful, and to date, no imaging biomarkers have been identified, validated, and qualified to discern which patients would benefit from VEGF

inhibitors. However, new imaging techniques are being developed and are providing novel insights into the structure and function of neovascular function.

Imaging Angiogenesis: The Technologies

The majority of imaging strategies deployed in the assessment of tumor vasculature have involved the administration and quantification of contrast during imaging. These dynamic protocols have evolved because tumor-associated vasculature is characterized by poorly functional, tortuous, dilated, and discontinuous vessels (Mancuso et al. 2006). The latter feature is a key determinant of vascular permeability; a phenotype that is critically regulated by VEGF, which itself was originally known as vascular permeability factor (Leung et al. 1989).

The relationship between VEGF and vascular permeability led to the development of imaging protocols that could quantify such changes in vasculature and hence in situ changes in VEGF biological activity. The most widely implemented technology has been dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) (O'Connor et al. 2012). While DCE-CT has also been used to address the same issue, the advantage of DCE-MRI is that the technology avoids ionizing radiation and therefore can be repeated (O'Connor et al. 2009) without concern for radiation-associated consequences. In addition the relationship between contrast concentration and signal in MRI is not linear, with greater sensitivity at lower contrast levels, thereby affording DCE-MRI greater sensitivity at the segments of the contrast-signal curve that are relevant to drug development. On the other hand, CT scanning is globally available, and, as a result, a series of modifications to CT scan reporting have been developed, which is a factor in the changes in tumor density and ischemic effects of anti-angiogenic agents into reporting criteria (Choi et al. 2007). These are important because an anti-angiogenic agent may not change the diameter of a tumor deposit, yet it may have a profound impact on tumor biology.

Positron emission tomography (PET) has also been deployed in the evaluation of angiogenesis and anti-angiogenic agents. The technology relies on the incorporation of a positron-emitting isotope into a defined chemical structure. Positrons then encounter electrons, leading to the emission of photons that can be quantified, thereby offering the potential for absolute quantification of concentrations of PET tracer in tumors. However, other than [^{18}F]-FDG, which capitalizes on the Warburg effect in which glucose uptake by tumor is up to 20 times the background level, most tracers are not taken up by tumors to the same extent and are still of exploratory value. Thus, [^{15}O]-H_2O and [^{18}F]-FLT have been evaluated in several trials, but for reasons of very short half-life, in the case of the former and insufficiently convincing utility with respect to FLT, these technologies are not widely used.

Dynamic ultrasound, which relies on the detection of microbubbles in the circulation, has also been evaluated but is compromised largely by difficulties in quantitation of effect across multiple sites. Thus, the most widely used technology remains advanced MRI, and results obtained with this technology will be the main focus of the chapter.

Dynamic Contrast-Enhanced Magnetic Resonance Imaging (DCE-MRI)

Methodological Considerations

By far the majority of tumor vascular imaging studies have employed DCE-MRI, which identifies a number of critical vasculature-related parameters (Fig. 1). Before discussing the data from these studies, it is important to consider the technical and analytical issues that are only now being standardized to allow inter-site comparisons.

Attempts to standardize terminology (Tofts et al. 1999) and choice of DCE-MRI biomarkers (Leach et al. 2005) have been widely promoted over the past 15–20 years. However, many different approaches to deriving a given biomarker have been developed at different research institutions. This resulted in various different protocols

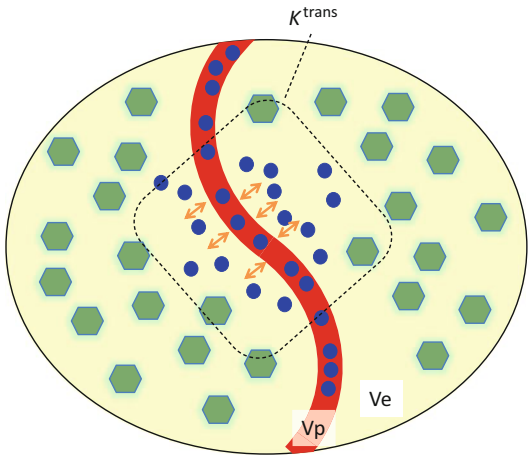

Fig. 1 DCE-MRI and derived parameters. Low molecular weight gadolinium contrast agent (*blue circles*) is injected, and images are acquired over time in the patient's tumor. Contrast in the tumor vasculature allows calculation of V_p, the tumor vascular volume. The contrast leaks out of the malformed tumor vasculature (*black dotted lines*) with kinetics dictated by the vascular permeability and endothelial surface area, parameters that are combined together to generate the analytically derived, K^{trans}. Once in the interstitial space, further analysis can also calculate a further parameter, V_e, which represents the extravascular, extracellular space. The movement of gadolinium contrast molecules is dictated by Brownian motion, and this too can be modeled through diffusion-weighted imaging (*DWI*) to identify the apparent diffusion coefficient (*ADC*). This parameter is believed to reflect cell packing and thereby potentially also serves as a biomarker of apoptosis. This is not shown here as the methodology has not been found useful in imaging angiogenesis

conducted on a range of equipment, with a number of analytical techniques being applied, rendering more complex technologies difficult to develop internationally. We and others have published a "roadmap" describing the development of imaging biomarkers (O'Connor et al. 2016). This important protocol reviews the discovery of biomarkers via a range of scientific disciplines through to validation studies, which require implementation of the technique in a few centers with a limited number of preclinical and defined clinical studies, which allow definition of precision, bias, biological relevance, and cost-effectiveness. Successful progress through these initial steps would be followed by studies in larger numbers of research groups to define further the precision of the imaging technique across many centers while determining the key application of the imaging biomarker (e.g. in screening, diagnosis, prediction or as a pharmacodynamic test). The third step would be the qualification of the imaging biomarker with technical validation and then implementation within prospective studies, through which the health benefit and cost-effectiveness of the imaging test could be defined. Global uptake of this proposal (O'Connor et al. 2016) would be transformative for imaging bioscience and drug development.

Biological Relevance of Imaging Techniques

The dynamic process of angiogenesis leads to the formation of new blood vessels, which when assessed at the tissue level can be quantified through microvessel density (MVD). When imaging techniques to examine the vasculature were developed, many investigators attempted to correlate imaging parameters with MVD, and to some extent these studies validated the hypothesis in endometrial (Haldorsen et al. 2014) and prostate cancer (Schlemmer et al. 2004) but not initially in breast cancer (Su et al. 2003). However, subsequent multi-biomarker studies in breast (Wedam et al. 2006) and colorectal cancer (Willett et al. 2004) showed that anti-angiogenic treatment reduced MVD and radiologically quantified vasculature-related parameters together.

In retrospect some of these studies were naively optimistic, at least in humans. The resolution of DCE-MRI studies in patients is at the several millimeter level, while tissue immunohistochemical studies resolve tissues at the micrometer level, reflecting several orders of magnitude in difference. Taken in conjunction with the difficulties in maintaining the orientation of tissue specimens with respect to imaging data and the impact of tissue fixation on the ability to co-localize biological and imaging data, it is perhaps surprising that any correlations were discovered between these different modalities of tumor vascular evaluation.

Given the difficulties in comparing tissue- and imaging-derived vasculature-related parameters, there was a need to understand the clinical

significance of tumor vascular imaging. We and others described simple and more complex techniques that addressed this question. Straightforward evaluation of the enhancing fraction of a tumor, the proportion of a tumor that takes up contrast, demonstrated prognostic value in ovarian and cervix cancers (O'Connor et al. 2007; Donaldson et al. 2009), while more complex mathematical models of vascular heterogeneity provided further information on outcome in colorectal cancer (O'Connor et al. 2011; Jackson et al. 2007). Thus, despite the difficulties of comparing imaging and tissue microscopic data, imaging of tumor vasculature provides clinically relevant data.

DCE-MRI Assessment of VEGF Inhibitors

The most frequently deployed imaging strategy has been T_1-weighted DCE-MRI, which has been applied to the majority of VEGF pathway inhibitors during early phase drug development. In general, the data show that all clinically effective inhibitors, whether they are antibodies or small molecule VEGF receptor tyrosine kinase inhibitors, impact on K^{trans} (Table 1), and this derived parameter has been most frequently used as the arbiter in proof of principle studies (O'Connor et al. 2012).

Simpler models of analysis have also been developed, and these require less complex modeling for their derivation. Two of the simplest are the enhancing fraction, the volume of tumor that takes up any contrast and the $IAUC_{60}$, the area under the concentration time curve in a region of tumor in the first 60 s after injection of a gadolinium-based contrast agent. These simpler analyses have also been widely deployed, and in keeping with the original vascular density studies, which were of prognostic significance, we and others have demonstrated the prognostic significance of enhancing fraction before treatment (O'Connor et al. 2007; Jackson et al. 2007; Rose et al. 2007). More complex mathematical models have been developed to allow calculation of K^{trans}, the product of endothelial surface area, and vascular permeability as well as the other parameters shown in Fig. 1. Typically these biomarkers have been derived from the pharmacokinetic models described in other review articles (Tofts et al. 1999; Tofts 1997).

Table 1 VEGF inhibitors impact on DCE-MRI parameters. The table shows a selection of VEGF pathway inhibitors that were evaluated with DCE-MRI through phase I/II clinical trials. The trials show near uniform reductions in a number of DCE-MRI parameters in keeping with the proposed mechanism of action of the drugs

	Phase of clinical trial	Tumor type	Change in DCE-MRI parameter	References
Antibody-related structures				
Bevacizumab (Anti-VEGF-A)	II	Colorectal	Ktrans, EF, WTV	O'Connor et al. (2009)
CDP791 (di-Fab anti-VEGFR2)	I	Multiple	No changes seen	Ton et al. (2007)
Ramucirumab (anti-VEGFR2 antibody	I	Multiple	Perfusion, Ktrans	Spratlin et al. (2010)
Protein therapeutics				
Aflibercept (VEGFR construct)	I	Multiple	Ktrans	Lockhart et al. (2010)
VEGFR tyrosine-kinase inhibitors				
Sunitinib	II	Hepatocellular	Ktrans	Zhu et al. (2009)
Sorafenib	II	Renal	Ktrans	Flaherty et al. (2008)
Cediranib	I	Multiple	IAUC60	Drevs et al. (2007)
Pazopanib	I	Hepatocellular	IAUC, Ktrans	Yau et al. (2011)

MRI Evaluation of Tumor Vasculature and Drug Development

Mechanisms of Action and Imaging

The application of MRI technologies to the assessment of VEGF inhibitors focused in large part on the reduction in K^{trans} as the key endpoint because VEGF was the principal mediator of vascular permeability, one of the terms incorporated into the calculation of K^{trans}. Because of the impact of these agents on vascular permeability and because sustained application of VEGF inhibitors reduced tumor interstitial pressure, we studied the time course of effects of the monoclonal anti-VEGF antibody, bevacizumab, on colorectal cancer liver metastases (O'Connor et al. 2009). These studies showed that the bevacizumab had an impact on vascular permeability within hours of drug administration and that to a large extent this was sustained for up to 12 days, thereby justifying the administration of the drug every 2 weeks. Interestingly the same study also showed acute changes in tumor volume within 12 days of single-agent bevacizumab administration, demonstrating the potential impact of such agents on tumor behavior.

The time course study of vascular changes in liver metastases revealed a reduction in K^{trans}, enhancing fraction and plasma volume fraction (Vp). Together these data were suggestive of reduced perfusion to patients' tumors. Preclinical work using thyroid models at the same time suggested that the vessels rapidly regressed when treated with VEGF inhibitors and that those that were left had a more normal structure, the concept of vascular normalization (Mancuso et al. 2006). This was then studied by Jain and colleagues in human brain studies, which suggested that one of the principal modes of action of anti-angiogenic VEGF inhibitors was vascular normalization (Batchelor et al. 2007, 2010). Further evidence suggested that vascular normalization might be of key importance in determining the optimum time to administer radiotherapy (Winkler et al. 2004).

The Search for Predictive Biomarkers

Given the strong dynamic relationship between pharmaceutical VEGF inhibition and changes in DCE-MRI, much work focused on the evaluation of pretreatment imaging characteristics as potential predictive biomarkers, tests that could be used to select the patients most likely to benefit from such drugs. However, except for some hints of predictive value in tumors that were highly sensitive to VEGF inhibitors, such as glioma, no predictive value has been detected (O'Connor and Jayson 2012).

Alternative attempts to develop predictive imaging biomarkers have focused on the quantitative evaluation of tissue VEGF concentrations through the development and evaluation of the PET tracer, [^{89}Zr]-bevacizumab. Zr was used in this imaging agent because its decay is much longer than other widely available PET isotopes. Yet this issue is still an important confounding factor in the development of the tracer. The half-life of bevacizumab in the circulation is approximately 20 days (Lu et al. 2008), whereas that of 89Zr is 3.3 days (Zhang et al. 2011).Thus, inevitably the intravascular content of [89Zr]-bevacizumab will confound interpretation of tissue levels of the tracer. Nevertheless, in the acute imaging setting specific uptake of bevacizumab has been demonstrated in vivo, where control immunoglobulin was not taken up (Nagengast et al. 2007). Further imaging studies have demonstrated uptake of the tracer in breast (Gaykema et al. 2013) and renal cancer (Oosting et al. 2015) and anti-angiogenic agents that impacted tracer uptake, and in renal cancer, patients with the largest SUV had the longest progression free survival with VEGF inhibitors (Oosting et al. 2015). Thus, despite concerns over the confounding issues of intravascular imaging agent and the need to determine bound versus free drug in the vasculature, this imaging agent appears to hold some promise but requires further validation.

Proof of Principle

A selection of illustrative early phase clinical trials that incorporated imaging into the evaluation of VEGF pathway inhibitors are listed in Table 1.

To a variable extent, these trials demonstrated that there was a dose-response effect. In other words, the higher the dose or dose level of VEGF pathway inhibitor, the greater the impact on the MRI imaging parameter. A further observation from these data was that there appeared to be a threshold effect in which clinical responses or disease stabilization were only seen in patients whose tumors manifested greater than 50% reduction in K^{trans} or $IAUC_{60}$ (O'Connor et al. 2012) following treatment with a VEGF inhibitor.

The observation of a dose-response effect increased confidence in DCE-MRI technology. Further support for the technology was derived from the observation that a di-Fab anti-VEGFR2 fragment (Ton et al. 2007) did not impact on DCE-MRI and at least in early phase evaluation did not demonstrate the same clinical efficacy or toxicity signals that were observed with other VEGF inhibitors. Critically the results seen with the di-Fab construct contrast with those seen with an intact anti-VEGFR2 antibody, ramucirumab (Spratlin et al. 2010), suggesting that the Fc domain of the antibody is of critical importance as the effector part of the drug.

Much of the 2000–2010 decade of imaging research in angiogenesis focused solely on the tumor vasculature as the critical target for VEGF inhibitors. Toward the end of this period, the relationship between VEGF and the immune system became clearer (Motz and Coukos 2011). These studies showed that VEGF inhibitors could increase immune reactivity, and thus a potential mode of action of this class of drug is through increasing the potency of the immune system. Given the rapidly developing interest in the potential for immunotherapy to augment the efficacy of radiotherapy (Sharabi et al. 2015), there remains a critical need for further mechanistic studies to understand the synergy between radiotherapy and VEGF inhibitors in vivo and in humans, albeit with increased toxicity.

To resolve the question of whether imaging analysis of the vasculature reports epiphenomenonological data whereas the principal mode of action of VEGF inhibitors is through the immune system, one approach would be to determine if other drugs with a proposed anti-vascular mechanism of action also have imaging effects. Several drugs that inhibit other vascular targets have been evaluated in the clinic, and selected studies are listed in Table 2.

The exemplary studies cited in Table 2 show that inhibition of a number of vascular targets results in changes in imaging that are related to the dose of the agent. Further, the fact that several antibody-based structures, which specifically target particular cytokines or pathways, cause imaging effects argues against the thesis presented above and assert that VEGF inhibitors induce imaging effects through an anti-vascular mode of action that is the core mechanism of action of the drugs. Taken in conjunction with other mechanistic studies (Wedam et al. 2006; Willett et al. 2004), these data demonstrate that DCE-MRI is a reproducible and sensitive method for determining whether a candidate drug has anti-vascular activity. This conclusion has been introduced into early phase drug development to the extent that some would consider that a drug with putative VEGF inhibitory characteristics that did not reduce K^{trans} within its maximum tolerated dose was not hitting its target.

Imaging Effects Are Necessary But Not Sufficient

The early success seen with bevacizumab, in particular, and the parallel interest in the emerging positive imaging data led to an exaggerated reliance on imaging for later phase clinical trial decision making. Thus, the results of early phase clinical trials of some of the agents listed in Tables 1 and 2 led to phase III clinical trials that were subsequently negative. Examples of such agents include PTK/ZK where positive imaging studies (Morgan et al. 2003) led to phase III studies that were ultimately negative (Hecht et al. 2011; Van Cutsem et al. 2011). Additional negative phase III trials of drugs that had shown positive imaging effects in phase I/II evaluation included cediranib (Schmoll et al. 2012; Batchelor et al. 2007, 2010; Hoff et al. 2012), cilengitide

Table 2 Imaging effects of drugs targeted at systems other than VEGF/VEGFR. The table shows the clinical and imaging effects of non-VEGF inhibitor, anti-angiogenic agents in early phase evaluation. In general the data show that inhibition of a number of vascular targets results in vascular changes in imaging

Target	Drug	Phase of Trial	Tumor type	Clinical effect	Imaging effect	References
Angiopoietin	AMG 386, trebananib, peptidobody	I	All	Response in ovarian cancer	Reduction in Ktrans	Herbst et al. (2009)
VEGFR2, Tie2, PDGFRβ and FGFR2	Regorafenib, low molecular weight TKi	I	All	Response in renal, colorectal and sarcoma	Dose response reduction in IAUC60	Mross et al. (2012)
PDGFRβ	CDP860, di-Fab anti-PDGFRb	I	Ovarian and colorectal	Increased ascites and peripheral edema	Increased enhancing fraction	Jayson et al. (2005)
Vascular integrins	Cilengitide, cyclic anti-vascular integrin penta-peptide	I	glioma	CR and PR seen in glioma	Association between perfusion, PK and response	Nabors et al. (2007)
	Anti-vascular integrin antibody	I	All	PR in angiosarcoma	None seen	Mullamitha et al. (2007)
Tubulins (anti-vascular agents)	Combretastatin	I	All	CR in anaplastic thyroid	Reduction in perfusion	Dowlati et al. (2002)

(Nabors et al. 2007; Stupp et al. 2014), and to a lesser extent (because of a modestly positive phase III trial) trebananib (Herbst et al. 2009; Monk et al. 2014).

The explanations for the failure of positive proof of principle imaging early phase studies to translate into positive phase III clinical trials are diverse. Suggestions to account for this failure include pharmacokinetic differences, the critical nature of the target ligand and/or lack of biomarkers to identify the patients who most benefit. On the other hand, those agents that have yielded positive results in phase III trials have all demonstrated positive imaging studies. Thus, taken together the data suggest that positive imaging data are necessary but not sufficient to identify drugs that will yield positive phase III trial outcomes for anti-vascular agents.

Why This Technology Remains Important

The above discussion has shown that imaging technology has been useful for selecting agents that are or are not biologically active but that the technology does not predict a positive outcome from phase III evaluation. Nevertheless, studies conducted over time demonstrate that VEGF inhibitors cause vascular changes that can be detected through imaging. Given the lack of predictive biomarkers for VEGF inhibitors, on-treatment changes like these are potentially the best way of detecting biological effects of these drugs. This is important because we are now entering the era of combination regimens of biologically targeted agents. Recent trials have demonstrated the efficacy of the combination of cediranib, a VEGF receptor tyrosine kinase inhibitor and olaparib, a PARP inhibitor (Liu et al. 2014). Such combinations appear active even in the absence of underlying germline BRCA gene mutations. However, this combination, which will be continued until progression, is expensive, and there is a critical need for efficacy biomarkers that can monitor and optimize use of such combinations if they are to be used in multiple health-care systems.

Pharmacodynamic PET Scanning

PET scanning relies on the incorporation of positron-emitting isotopes into chemical structures that can be administered to humans. Positrons collide with electrons to release two photons that can be quantitatively detected. Thus, the major advantage of PET scanning over most of the other imaging technologies is that it is perhaps the most quantifiable of all the techniques. That said, there are some critical logistic problems associated with the technology. These include the very short half-life of many positron-emitting isotopes, the need to often quantify the arterial input of the tracer to an imaged organ to generate entirely quantitative data, the requirement for real-time pharmacokinetic analysis to demonstrate that a novel tracer is chemically intact at the time of imaging, as well as the need for a GMP radiochemistry facility, a cyclotron and imaging equipment particularly for studies that deploy the less frequently used tracers. Thus, other than the most well-established of imaging tracers such as [18F]-FDG-PET studies, few other imaging tracers have been comprehensively studied to quantify tumor vasculature.

Studies of [^{18}F]-FDG-PET

FDG-PET has been evaluated in several studies of anti-angiogenic agents. The premise on which its use is predicated is that tumors in general take up glucose at a far greater level than surrounding tissues other than those with high background uptake, such as the brain. This differential uptake, based on the Warburg effect, allows PET imaging to be conducted.

There is a biological problem that probably accounts for the inconsistent findings associated with the use of FDG-PET in studying human tumor vasculature. The uptake of FDG is affected by the delivery of the PET tracer to a tumor, that is, by tumor perfusion, as well as the uptake of FDG into tumor cells. One of the major effects of anti-angiogenic therapy is to decrease blood supply to tumors; thus one might expect the impact on imaging would be a reduction in uptake of the tracer into tumors that have been treated with anti-angiogenic agents. On the other hand, we know that the consequence of anti-angiogenic agents at the tissue level is the induction of hypoxia, which itself can induce the expression of the glucose transporter and thereby increase uptake of the FDG radio-tracer into tumor cells. These discordant biological effects therefore compromise interpretation of FDG effects, and in accordance with this conceptual problem, the clinical data associated with anti-angiogenic agents have largely been inconsistent.

The first studies of FDG-PET in patients treated with VEGF inhibitors did not show any clear effect although these were very small investigations (Willett et al. 2004). The situation is more complicated when evaluating small molecular weight VEGF receptor tyrosine kinase inhibitors as they target kinases in addition to those of the VEGF receptor and therefore can induce greater effects on FDG imaging through direct antitumor control. However, despite this issue, imaging with conventional or Choi et al. (2007) modifications to conventional CT scan reporting were associated with greater response detection in gastrointestinal stromal tumors than FDG-PET (Benjamin et al. 2011; Yap et al. 2013; Judson et al. 2014).

Anti-angiogenic agents have been developed to target a number of cytokines or their receptors. However, despite correlative studies that suggest, for example, that FDG uptake reflects angiopoietin expression in colorectal cancer (Strauss et al. 2008), FDG has for the most part not been deployed in the pharmacodynamics evaluation of these other drugs.

Together the conflicting biological interpretation of the impact of anti-angiogenic agents on FDG uptake has led to only a few studies incorporating this technology, and largely these have not yielded consistent or useful results. Hence, FDG has not been widely used to evaluate anti-angiogenic effects.

Studies of [^{15}O]-H_2O and [^{18}F]-FLT

From a conceptual point of view, measurement of tumor perfusion through administration and quantification of [^{15}O]-H_2O should represent one

of the best strategies for the assessment of anti-angiogenic agents. Indeed early studies of water-PET with the vascular disrupting agent, combretastatin, revealed the profound effect of the drug on the tumor vasculature (Anderson et al. 2003).

Despite the significant potential to quantify perfusion through the use of [15O]-H_2O, the major limitation of the technology is that the half-life of the isotope is only 2 min, and thus the tracer has to be generated at the point of infusion into the patient, requiring major investment in infrastructure. For this reason and because of the substantial evidence base supporting MRI studies, [15O]-H_2O has not established a position in the evaluation of anti-angiogenic agents.

One of the potential consequences of successful inhibition of angiogenesis should be reduced proliferation in tumors. Thus, it was of interest to evaluate a potential tracer, the uptake of which was related to proliferation. [^{18}F]- 3′-deoxy-3-′-fluorothymidine (FLT) was developed for this purpose and has been evaluated to a limited extent in solid tumor oncology and in particular angiogenesis. The tracer is taken up by cells where it is then phosphorylated by thymidine kinase 1, thereby preventing its egress from the cell.

Correlative studies in lung cancer suggest that FLT uptake (determined by the SUV) correlates with the proliferation marker, Ki67 and CD105-determined microvessel density (Yang et al. 2012). In renal cancer, FLT-PET uptake reduced after 1–2 weeks of treatment with the VEGF receptor tyrosine kinase inhibitor, sunitinib (Horn et al. 2015), and then increased upon withdrawal of the drug (Liu et al. 2011). Thus, these limited data suggest that FLT might be a useful tracer in the evaluation of anti-angiogenic agents. However, there are two important confounding factors: Whereas the uptake of FDG is profoundly increased through the Warburg effect, this is not the case for FLT. Thus, the impact of an effective agent on tracer uptake is likely to be less apparent and harder to detect. Secondly, much drug development focuses at least at the early stages on patients with metastatic disease. With respect to FLT, background uptake of FLT in the liver is significant, and thus only the most intensive and rigorous of imaging protocols can detect changes in FLT uptake in liver metastases, which are frequently present and evaluable in patients participating in early phase clinical trials.

Emerging Imaging Technologies

We argued above that PET scanning offers the most quantitative technology for the evaluation of tumor vasculature. However, because of the number of technical issues involved in quantitative PET and the extensive literature that has arisen through MRI, the latter technology has become more widely used in the study of tumor vasculature.

Attempts to extract more information from MRI studies included the development of larger molecular weight imaging agents, which could capitalize on the leaky vasculature that characterizes human cancer. These newer tracers have included gadolinium-albumin conjugates, iron oxide tracers, and nanoparticles (summarized in Barrett et al. 2006). However, while theoretically and preclinically exciting, the development of these novel reagents into licensed imaging agents has largely not occurred, and there remains a reluctance to combine two novel factors in one clinical trial, the anti-angiogenic agent and the imaging molecule. Thus to a large extent, this field has not progressed. It is hoped that the publication of guidelines for the development and validation of newer imaging agents will be accepted and will help to introduce new imaging tracers into the clinic more efficiently (O'Connor et al. 2016).

Two further MRI technologies that do not require contrast can be used to image the vasculature. However, they have not been widely used in the study of angiogenesis or anti-angiogenic agents. They include arterial spin labeling (Barrett et al. 2007) (ASL) where a volume of blood is magnetized and its entry into an organ and the subsequent mixing with water in imaged tissue are investigated. To a large extent, this technology has been applied to vascular studies of the brain,

where motion artifact is least likely to be problematic. However, occasional studies that are not focused on the brain have highlighted the potential for this technique to quantify the tumor vasculature, e.g., in renal cancer (Zhang et al. 2016).

The second imaging approach that has been evaluated is blood oxygen level-dependent imaging (BOLD), which relies on the paramagnetic signal of deoxyhemoglobin. However, interpretation of this signal, which again has most frequently been applied in the brain, is difficult because of the confounding influences of flow, perfusion, and deoxygenation (Padhani et al. 2007). Recent refinements of the technology have demonstrated its potential to discriminate between different grades of glioma (Wiestler et al. 2016), but further studies are needed if the technology is to become more widely used in the evaluation of anti-angiogenic agents.

Tissue studies of tumor vasculature revealed a range of vascular maturity that appeared to be a relevant determinant of response to VEGF inhibitors (Sitohy et al. 2011). Vascular maturity in part reflects the degree of pericyte coverage, and thus the vessels' capacity to respond to vasodilatory influences such as carbon dioxide. This understanding led to attempts to administer CO_2 during BOLD-MRI to assess the maturity of tumor vessels as CO_2 should cause vasodilatation of mature blood vessels that are coated in pericytes. Trials were conducted in air containing 5% CO_2 or in 95% oxygen/5% CO_2 (carbogen). However, these techniques can be distressing for patients because of claustrophobia and perceived oxygen deprivation, and, despite the reported increase in tumor perfusion, these confounding factors have impacted significantly on the potential to exploit this technology further (Padhani et al. 2007; Taylor et al. 2001).

Future Directions

Several developments over the last few years have highlighted the critical need for predictive and pharmacodynamic imaging biomarkers if we are to exploit the tumor vasculature as a target for cancer treatment. One of the most exciting developments was the recent report of the combination of VEGF inhibitors with the PARP inhibitors in ovarian cancer (Liu et al. 2014). The activity seen with these two oral agents was striking and critically did not correlate with the presence or absence of germline BRCA gene mutations, which have traditionally been used to select patients for treatment with a PARP inhibitor. A second critical development focuses on the new class of immunotherapeutic agents that target a number of immune checkpoints. Given that VEGF inhibitors have potent immunomodulatory potential (Motz and Coukos 2011), there is a clear need to understand and develop combinations of VEGF inhibitors and checkpoint inhibitors (Wallin et al. 2016).

These studies are underway and it is likely that additivity will be detected. However, in both examples presented here, the cost of treating patients with combination regimens will be significant and, for many health-care organizations, prohibitive. Thus, there is a critical need for predictive and/or pharmacodynamic biomarkers that can be used to direct and then optimize therapy for our patients. As both combinations represent new paradigms in cancer treatment, e.g., there will be many further studies that evaluate novel combinations of VEGF inhibitors with other DNA repair inhibitors, one can foresee the development of multiple new, effective but expensive combination regimens. It is therefore mandatory for investigators and the pharmaceutical industry to incorporate suitable biomarker studies into the drug development strategy if we are to afford to treat our patients with these regimens.

Cross-References

- ▶ Biomarkers for Anti-angiogenic Therapy
- ▶ Mechanisms of Anti-angiogenic Therapy
- ▶ Mechanisms of Tumor Angiogenesis
- ▶ Pathology of Tumor Angiogenesis
- ▶ The Role of the VEGF Signaling Pathway in Tumor Angiogenesis

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