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Dynamic Contrast-Enhanced MRI in Oncology Drug Development

Hai-Ling Margaret Cheng¹,²,*

¹Department of Diagnostic Imaging & The Research Institute, The Hospital for Sick Children, ²Department of Medical Imaging, University of Toronto, Toronto, Canada

Abstract: Angiogenesis, long recognized as a key factor in tumor growth and metastasis, has been the target of new anti-cancer treatment paradigms. Development of antiangiogenesis drugs is challenging, mainly due to the difficulty of determining the correct dosage and the time required to observe a clinical effect. In the past decade, imaging has shown potential to answer these questions and accelerate the drug development process by providing functional, morphological, and even molecular characterization. In this review, we describe existing challenges to modern drug development and the potential of imaging biomarkers to monitor drug bioactivity and establish early response of drug efficacy. Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI), particularly attractive for its non-invasiveness and high spatial resolution, has been useful for measuring properties of tumor microvasculature. The general methodology of DCE-MRI is described, in addition to measurable hemodynamic parameters compared to other imaging modalities. Experience with DCE-MRI in antiangiogenesis cancer therapy and results from correlative studies are examined. Current challenges for DCE-MRI, especially in relation to the required sensitivity and reproducibility, are highlighted. We conclude with an outlook on the future of DCE-MRI, including its role in the emerging field of imaging molecular markers of angiogenesis for target-specific therapy.

Key Words: Oncology, drug development, dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI), antiangiogenic cancer therapy, angiogenesis, imaging molecular biomarkers.

INTRODUCTION

Angiogenesis is the process of new blood vessel formation seen in early embryonic development and in various pathophysiologic states. In tumor formation, it plays an essential role in tumor growth beyond 2 mm³ and in initiating metastasis [1,2]. Targeting angiogenesis has thus become the new paradigm for anticancer therapies, and modern antiangiogenesis research involves both the discovery of anticancer targets and the concomitant development of drugs directed at these targets [3]. A range of targets have been identified to date, including both endogenous activators (e.g. vascular endothelial growth factor (VEGF)) and inhibitors (e.g. angiostatin, endostatin) of angiogenesis [4-6]. Many novel candidate drugs have also been developed and are in various phases of clinical testing [7], with success recently demonstrated in the first randomized trial for anti-VEGF treatment of metastatic colorectal cancer [8].

A major roadblock to the rapid development and testing of antiangiogenic drugs is that unlike traditional chemotherapeutic agents, they are cytostatic. That is, tumor stabilization rather than response is a more probable endpoint. One consequence is that neither standard dose-response relationships nor the criterion of maximum tolerated dose may be relevant, posing a challenge for selecting the most effective dose. Second, conventional measures of clinical response, such as tumor shrinkage or time to disease progression, may be much slower and thus inappropriate for efficacy assessment. This prolonged period to clinical progression or stability is detrimental to patients who are exposed to ineffective drugs. Ideally, for reasons of safety, cost, and time, development of ineffective drugs should be halted early, so that resources can be allocated to accelerate or modify more promising approaches. A key to this dilemma may be found in biomarkers of early response that reliably foreshadow subsequent positive clinical response. These would greatly aid in rapidly establishing drug efficacy and selecting the correct dosage. Biomarkers that reflect functional and metabolic responses, such as changes in blood hemodynamics, may be especially appropriate for evaluating antiangiogenic agents and determining early response.

Imaging biomarkers are particularly attractive, because changes can be assessed non-invasively and longitudinally. Various hemodynamic parameters, such as blood volume and vessel permeability, can be measured. Although several imaging modalities exist and no consensus on the most appropriate method has been reached, dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is appealing due to exceptional soft tissue contrast, high spatial anatomical detail, and functional readouts. Evidence suggests DCE-MRI is a promising biomarker of angiogenic activity in response to new anticancer treatment. However, significant challenges remain in developing robust DCE-MRI biomarkers: imaging measurements must not only be sensitive to relevant biological changes but also be accurately and reliably acquired in a standardized fashion [9].

In this review, we examine current developments and the biological rationale behind antiangiogenic cancer drugs. We then compare briefly different imaging methods and describe in detail the DCE-MRI approach for quantifying hemodynamic function. Preliminary DCE-MRI data from clinical trials of cancer therapy is presented, and their correlation to accepted prognostic factors is discussed. Current limitations

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to robust DCE-MRI assessment of angiogenesis are described, with the aim of identifying challenge areas where solutions are needed. Lastly, we discuss novel molecular MRI approaches recently demonstrated for imaging angiogenic vessels. The overall aim of this review is to provide a clear understanding of the full potential of DCE-MRI. This step is crucial to maximizing its role in pharmacological research, so that we may reliably determine biological activity and effective drug dosage, and perhaps gain insight into the mechanisms of drug action.

TUMOR ANGIOGENESIS

Tumor vasculature is distinct from that in healthy tissue in several respects, primarily due to the over-production of angiogenic factors that promotes a rapid and abundant but ill controlled formation of new vessels. The resulting vasculature has an overall high microvessel density and is spatially heterogeneous [10]. Vessel structure is mainly chaotic, characterized by: vasodilatation, tortuosity, blind ends, arteriovenous shunting, and lack of an arteriole-capillary-venule hierarchy (Fig. (1)). Blood flow also tends to be poor and does not deliver nutrients efficiently due to its spatial heterogeneity and temporal fluctuations [11]. Furthermore, vascular permeability is generally higher compared to normal tissue by an order of magnitude [12], owing to the action of angiogenic factors (e.g. VEGF) and to immature vessel formation (that results in endothelial fenestrae, discontinuous basement membranes, and lack of pericytes) [13].

These underlying pathophysiological hallmarks provide a platform for tumor diagnosis: expression of angiogenic factors and their modulating effects of increased vascular density and permeability, which are recognized as more intense [14] and rapid [15] signal enhancement, respectively, are all amenable to DCE-MRI detection. These distinct vascular signatures also help define strategies for antiangiogenic cancer treatment: growth factor expression: factor expression can be targeted by anticancer drugs to restrict tumor growth and vascular access for metastasis, and resulting changes in vessel density and permeability can be monitored to assess and predict efficacy.

MODERN CANCER DRUG DEVELOPMENT: ANTI-ANGIOGENIC AGENTS

Numerous antiangiogenic cancer drugs are currently in various stages of clinical testing. Strictly speaking, these agents block new vessel formation and are classified according to the pathways and factors on which they act. Some inhibit endothelial cells directly, while others block the angiogenesis signaling cascade or the ability of endothelial cells to break down the extracellular matrix. However, they may also eliminate existing vessels and, to the extent that they do, can be considered antivascular. This rapid shutdown of existing blood supply to the tumor is mediated through a direct action on endothelial cells [16], and the primary biological marker of action is a reduction in blood flow (incidentally, imaging-derived permeability measures have little physical relevance when flow is absent). It is important to note that while the antivascular mechanism can momentarily starve the tumor, it alone may not achieve desired long-term changes in mature vessels for which antiangiogenic agents are designed. Currently, the most common antiangiogenic agent is a reduction in blood flow (incidentally, imaging-derived permeability measures have little physical relevance when flow is absent). It is important to note that while the antivascular mechanism can momentarily starve the tumor, it alone may not achieve desired long-term changes in mature vessels for which antiangiogenic agents are designed. Currently, the most common antiangiogenic

Fig. (1). Scanning electron microscope images of polymer casts of (A) normal and (B) tumor blood vessels. Notice that the organized hierarchy of arterioles, capillaries, and venules found in normal vasculature is absent in tumors. [Reprinted by permission from Macmillan Publishers Ltd: (McDonald DM, Choyke PL. Nat Med 2003; 9:713-25, Fig. 1)].

target is VEGF or its receptor. Altered hemodynamics can be mediated through a number of mechanisms, including a direct antipermeability effect and a direct antiangiogenic effect. The former can be detected on DCE-MRI as early and rapid changes in permeability [17], while the latter is seen at longer times as fewer functioning vessels [18]. Table 1 lists a few examples of antiangiogenic agents currently undergoing clinical trials.

METHODS FOR DETECTING ANGIOGENESIS

The most straight-forward assessment of angiogenesis is a direct measurement of microvessel density (MVD). However, this approach is not only invasive but suffers from biopsy sampling errors and does not quantify the degree of functional vasculature. Alternatively, indirect methods may be used, such as imaging or obtaining blood samples of circulating angiogenic factors. Imaging is particularly attractive, because it is non-invasive and provides functional information in vivo throughout an intact 3D topology. Imaging strategies that have been developed for in vivo imaging of angiogenesis include: DCE-MRI, dynamic computed tomography (CT), positron emission tomography (PET), ultrasound, and near-infrared optical imaging [19].

No single modality suits all applications, and each has its advantages and capabilities with respect to measurable hemodynamic parameters (Table 2). For example, dynamic CT is widely available, boasts the highest spatial resolution, and allows simple quantification of absolute blood volume, flow, and permeability [20]. Its main disadvantage is the use of ionizing radiation and contrast agent toxicity, both of which limit repeated intra-patient measurements. It also offers the lowest sensitivity of all imaging techniques. In contrast, PET is a very sensitive and quantitative method for measuring blood flow [21] and blood volume using 15O labeled water or 11C labeled carbon monoxide, respectively, as contrast agents. Countering this advantage, however, are poor spatial resolution (3-4 mm), short radionuclide half-life (~2 min), and limited scanner availability. Ultrasound imaging with microbubble contrast agents is useful for measuring relative blood volume and flow [22], and is popular due to its wide availability and low cost. It has lagged behind other modalities for vascular imaging, nonetheless, because of
reproducibility issues related to operator dependency. Optical techniques are emerging but are not yet widely available. Amongst imaging approaches, DCE-MRI offers a practical balance of desirable traits: unlimited anatomical access in any orientation; good spatial resolution surpassed only by CT; high sensitivity; quantification of blood volume, flow, and permeability on an absolute scale; low contrast toxicity; and no ionizing radiation. The following section describes in detail DCE-MRI methodologies and derivable hemodynamic parameters.

### IMAGING ANGIOGENESIS BY DCE-MRI

#### Basic Principles

The ability of DCE-MRI to distinguish the vasculatures of malignancies, benign tumors, and normal tissue, has been exploited in both diagnosis and treatment monitoring, including chemotherapy, radiotherapy, or newer paradigms such as antiangiogenic agents [23-25]. Imaging is performed by acquiring a series of MR images before, during, and following contrast administration. In clinical settings, a low molecular

### Table 1. Antiangiogenic Cancer Agents in Clinical Trials

<table>
<thead>
<tr>
<th>Drug</th>
<th>Inhibition mechanism</th>
<th>Clinical trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>SU11248</td>
<td>Block activators of angiogenesis</td>
<td>Phase II advanced tumors; Phase III renal cell cancer</td>
</tr>
<tr>
<td>Anti-VEGF antibody (Bevacizumab; Avastin™)</td>
<td>Block activators of angiogenesis</td>
<td>Phase III/IV metastatic colorectal cancer; Phase III NSCLC and metastatic breast cancer; Phase I/II renal cell, pancreatic, melanoma, advanced cancers</td>
</tr>
<tr>
<td>ZD6474</td>
<td>Block activators of angiogenesis</td>
<td>Phase III NSCLC</td>
</tr>
<tr>
<td>PTK787/ZK222584 (Vatalanib)</td>
<td>Block activators of angiogenesis</td>
<td>Phase I/II advanced tumors</td>
</tr>
<tr>
<td>EMD 121974 (Cilenitide)</td>
<td>Inhibit endothelial-specific integrin/survival signaling</td>
<td>Phase II progressive or recurrent glioblastoma multiforme; Phase II prostate cancer</td>
</tr>
<tr>
<td>Combretastatin A4</td>
<td>Inhibit endothelial cells</td>
<td>Phase II advanced solid tumors</td>
</tr>
<tr>
<td>Tamoxifen Citrate</td>
<td>Inhibit endothelial cells</td>
<td>Phase III breast cancer</td>
</tr>
<tr>
<td>Dalteparin</td>
<td>Block matrix breakdown</td>
<td>Phase II ovarian cancer; Phase III pancreatic cancer</td>
</tr>
<tr>
<td>Celecoxib (Celebrex®)</td>
<td>Non-specific</td>
<td>Phase III metastatic breast or colorectal, NSCLC and advanced prostate cancer</td>
</tr>
</tbody>
</table>

* from the database of the National Cancer Institute.

b  NSCLC: non-small cell lung cancer.

### Table 2. Modalities for Imaging Angiogenesis

<table>
<thead>
<tr>
<th>Modality</th>
<th>Contrast agent</th>
<th>Sensitivity</th>
<th>Resolution</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI</td>
<td>Gd-DTPA USPIO</td>
<td>+</td>
<td>0.4-1.5 mm</td>
<td>Low contrast agent toxicity; no ionizing radiation; variety of contrast agents; measures various hemodynamic parameters.</td>
<td>Imaging indirect effect of contrast agent; instrument variation; absolute quantification can be challenging.</td>
</tr>
<tr>
<td>CT</td>
<td>Iodinated</td>
<td>+</td>
<td>&lt; 0.5 mm</td>
<td>Absolute quantification since signal attenuation is linear with contrast agent concentration.</td>
<td>Contrast agent toxicity; low sensitivity to contrast; ionizing radiation.</td>
</tr>
<tr>
<td>PET</td>
<td>H215O C15O2</td>
<td>+++</td>
<td>3-4 mm</td>
<td>Absolute quantification since emission varies directly with contrast agent concentration; whole-body imaging; nanomolar detection.</td>
<td>Expensive; limited availability; very short radionuclide half-life.</td>
</tr>
<tr>
<td>US</td>
<td>Microbubbles</td>
<td>+++</td>
<td>0.5 mm</td>
<td>Inexpensive; widely available; accurate quantification since microbubbles are confined to vessels.</td>
<td>Limited depth penetration; operator-dependent variability.</td>
</tr>
<tr>
<td>NIR</td>
<td>Indocyanine green, HbO2</td>
<td>+</td>
<td>1-2 cm</td>
<td>Inexpensive; portable; excellent contrast.</td>
<td>Limited depth penetration; non-standardized instrumentation.</td>
</tr>
</tbody>
</table>

* MRI: magnetic resonance imaging; CT: x-ray computed tomography; PET: positron emission tomography; US: ultrasound; NIR: near-infrared optical.

b  Gd-DTPA: gadopentetate dimeglumine; USPIO: ultra-small particles of iron oxide; HbO2: oxyhemoglobin.

c  Sensitivity is a relative scale of image enhancement due to contrast uptake: lowest (+) to highest (+++).
weight contrast agent is typically used, such as gadopentetate dimeglumine (Gd-DTPA). These agents are transiently confined within blood vessels during first passage but then rapidly diffuse into the extravascular extracellular space (EES) in most tissues, except the brain, testes, and retina. The rate of extravasation depends upon the endothelial permeability and surface area, and blood flow. Depending on the imaging method adopted, different hemodynamic parameters may be quantified. For example, susceptibility-weighted (T2*-weighted) techniques are used to monitor the rapid first-pass drop and recovery of signal intensity after bolus injection, from which perfusion and blood volume can be estimated. This methodology is most suitable when the contrast agent is confined within the vasculature, such as in brain applications where an intact blood-brain barrier exists [26]. In most body applications, however, considerable extravasation of contrast agent into the EES will occur, resulting in a signal increase on T1-weighted MRI. Contrast agent accumulation in the EES is best monitored using permeability (T1-weighted) imaging over longer time intervals of several minutes. Re-flux of contrast back into the vasculature may be observed if the permeability is high. This methodology yields parameters related to vessel permeability and surface area, perfusion, and the EES leakage space.

Absolute quantification of vessel properties is possible with both T1 and T2* methods and is preferable over simpler empirical analyses. However, absolute quantification is much more demanding and is feasible only if the contrast agent concentration in tissue is known accurately. In the case of T1 methods, measurement of the pre-injection intrinsic T1 is required to relate signal changes to contrast concentration. Measurement of plasma concentrations is also needed to characterize the arterial input function (AIF) under different conditions (cardiac output, injection mode, kidney function), so that their influence on the kinetics of tissue contrast changes can be accounted. Tracer kinetic models are then applied to estimate hemodynamic parameters. The following sections describe acquisition and analysis approaches of T1 and T2* DCE-MRI, hemodynamic parameters derivable from each method, and challenges to absolute quantification. A comparison of the two approaches is given in Table 3.

**Data Acquisition and Analysis**

T2*-weighted DCE-MRI – a signal decrease is observed due to magnetic field inhomogeneities created by a high concentration of contrast agent. This effect is most pronounced during the first passage, after which signal quickly recovers to its equilibrium value. These signal changes must be captured using a rapid imaging sequence, typically every 1 to 2 seconds over the first 60 seconds post-injection. Generally, a gradient-echo or spin-echo sequence [27] is used, with the latter providing greater sensitivity to microvessels. Rapid echo planar imaging (EPI) is often utilized to increase anatomical coverage while maintaining a high temporal resolution, but spatial resolution tends to be poorer. A minimum injection dose of 0.2 mmol/kg body weight is used to offset lower signal-to-noise ratios (SNR) of T2* images.

T2*-weighted images are typically restricted to brain applications where contrast leakage is minimal. As such, quantifiable parameters are denoted “cerebral”, such as cerebral blood flow (CBF) and blood volume (CBV), but can in truth be applied to non-brain areas where contrast remains quantifiable.

### Table 3. Comparison of T2*- and T1-weighted DCE-MRI Methods *

<table>
<thead>
<tr>
<th></th>
<th>T2*-weighted</th>
<th>T1-weighted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best suited for studies of</td>
<td>Capillary blood flow/volume by first-passage of contrast agent</td>
<td>Uptake or clearance of contrast agent in tissue</td>
</tr>
<tr>
<td>Type of contrast agent</td>
<td>Blood-pool agents (MW&gt;70 kD) b preferred</td>
<td>Low- to moderate-MW agents that diffuse into EES c</td>
</tr>
<tr>
<td>Contrast agent dosage</td>
<td>≥ 0.2 mmol/kg</td>
<td>0.1 - 0.2 mmol/kg</td>
</tr>
<tr>
<td>Signal intensity change</td>
<td>Decrease</td>
<td>Increase</td>
</tr>
<tr>
<td>Time resolution</td>
<td>1 – 2 s</td>
<td>2 – 25 s</td>
</tr>
<tr>
<td>Duration of imaging</td>
<td>60 – 90 s</td>
<td>5 – 10 min</td>
</tr>
<tr>
<td>Kinetic parameters measured</td>
<td>Blood flow Blood volume Transit time</td>
<td>Transfer rate constant (related to permeability, surface area, flow Blood volume Leakage space</td>
</tr>
<tr>
<td>Clinical applications</td>
<td>Primarily brain tumor applications (grading, prognosis, treatment monitoring) Other lesion characterization, including breast and liver</td>
<td>Various body tumor applications (grading, prognosis, treatment monitoring) Novel therapies, including antiangiogenic drugs</td>
</tr>
</tbody>
</table>

* DCE-MRI: dynamic contrast-enhanced magnetic resonance imaging
b MW: molecular weight
c EES: extravascular-extracellular space
largely intravascular. Analysis may be performed semi-quantitatively on signal changes to obtain parameters indirectly related to physiology. If contrast concentration is known, true quantification, either relative or absolute, is possible. Contrast concentration is calculated from observed signal changes under the assumption that concentration, $C(t)$, is linearly proportional to the change in $1/T2^*$, which can be calculated according to:

$$C(t) \propto \Delta 1 / T2^* = -\ln \left( \frac{S(t)}{S_0}\right) / TE$$

where $S(t), S_0, TE$ are the signal at time $t$, pre-injection signal, and echo time, respectively.

The relative blood volume (rCBV) and mean transit time (MTT) are then derived by calculating, respectively, the area and the “width” of the first-pass concentration profile; the relative blood flow (rCBF) is obtained through the Central Volume Theorem (rCBF= rCBV/MTT). Absolute quantification requires knowledge of the AIF: the CBF is determined through deconvolution (Eq. [2]), and CBV and MTT are computed according to Eqs. [3] and [4]:

$$C(t) = \text{CBF} \int C_s(t)R(t - \tau) d\tau$$

$$\text{CBV} = \int \frac{C(t) d\tau}{\int C_s(t) d\tau}$$

$$\text{MTT} = \frac{\text{CBV}}{\text{CBF}}$$

where $C(t), C_s(t), R(t)$ are the tissue curves, AIF, and residue function, respectively [28].

**T1-weighted DCE-MRI** – a signal increase is observed due to a shortening of the T1 relaxation time caused by the presence of contrast agent in the vasculature and the EES. A T1-weighted gradient-echo saturation or inversion recovery sequence is commonly used to monitor contrast uptake in tissue over the course of several minutes, preferably including the wash-out phase. The temporal resolution is lower compared to T2* techniques, ranging from 2 to 25 seconds, depending on the anatomy and coverage desired [29], but the spatial resolution is generally higher. In certain applications where both time and spatial resolutions are important, a 2D or EPI approach may be adopted to acquire data rapidly during contrast passage, and less rapid 3D high resolution imaging would follow to delineate anatomy. T1 approaches typically require a lower injection dose: 0.1 mmol/kg body weight shortens T1 relaxation times sufficiently in most tissues to visualize enhancement. In applications where quantitative parameters are desired, intrinsic T1 is also measured pre-injection [30].

Analysis of T1-weighted images is sensitive to endothelial permeability and the EES volume fraction, properties that are not obtainable from T2* techniques. Information on blood flow and volume may also be derived, perhaps less sensitively compared to T2* methods. Again, either signal (semiquantitative) or concentration (quantitative) changes may be assessed. Semiquantitative descriptors such as the upslope gradient, peak enhancement, and time-to-peak enhancement are straightforward to calculate. The initial area under the signal intensity or concentration curve (IAUGC) is an especially robust descriptor reflective of permeability and perfusion [31]. However, the relation of these parameters to underlying physiology is complex and not clearly understood. To obtain accurate descriptors of physiology, quantitative methods based on pharmacokinetic modeling of contrast uptake are used. Signal changes first need to be related to contrast agent concentration through measurement of the pre-injection tissue T1. Measurement of the AIF is also needed to account for individual differences in cardiac output and contrast injection rate. The tissue concentration curve, $C_i(t)$, and the AIF, $C_a(t)$, are then mathematically fitted to one of several recognized pharmacokinetic models [32-34] to derive quantitative pharmacokinetic parameters. The most commonly used model is Tofts [32], from which the volume transfer constant from blood plasma to the EES ($K_{trans}$), EES leakage volume ($v_p$), and rate constant for back-flux ($k_{ep}$) may be estimated through the following generalized equation:

$$\frac{dC_i}{dt} = K_{trans} \left( C_p - \frac{C_i}{v_p}\right) = K_{trans} C_p - k_{ep} C_i$$

where $k_{ep}=K_{trans}/v_p$ (Fig. (2)). More sophisticated models, such as extended Tofts [32] or St. Lawrence and Lee’s [34], can yield additionally plasma volume ($v_p$) and flow ($F_p$).

**Fig. (2).** Distribution of a low molecular weight contrast agent within an individual voxel of tissue. Contrast passes from the plasma space, $v_p$, to the extravascular-extracellular space (EES), $v_e$. The rate of forward transfer and back-flux are described by the volume transfer constant ($K_{trans}$) and the rate constant ($k_{ep}=K_{trans}/v_p$), respectively.

Despite the physiological relevance of quantitative parameters, their derivation imposes significant demands on data acquisition and analysis. Reliability also becomes an issue when contrast concentration measurements or modeling assumptions are inaccurate. The impact of these errors has been difficult to establish, mainly due to a lack of reliable clinical gold standards for comparing kinetic and physiological parameters. These issues were discussed during a consensus meeting on DCE-MRI in anticancer therapy assessment: the T1-weighted approach was recommended, with $K_{trans}$ as the primary quantitative endpoint and IAUGC as a semiquantitative alternative [35].
Hemodynamic Parameters from DCE-MRI Data

**Blood Volume** – can be estimated accurately when the contrast agent is confined within the vasculature. This is true of Gd-DTPA in normal brain tissue. Outside of the brain and in tumors, macro-molecular contrast agents may be necessary [36]. T2* measurement of the area under the first-pass curve is the most reliable MRI approach for estimating blood volume, provided contrast leakage is absent or minimal. Otherwise, substantial error can arise, in which case blood volume should be derived alternatively using a pharmacokinetic model [32]. However, implicit in this latter approach are lower temporal resolutions, simultaneous estimation of other parameters (e.g. Ktrans), and competing mechanisms on MR signal contrast, all of which decrease accuracy and reproducibility of blood volume estimates.

**Blood Flow** – can be derived accurately from DCE-MRI data if contrast leakage is minimal. As with estimating blood volume, the T2* approach is the most common, which involves deconvolving the tissue concentration curve on the AIF [28]. A T1-based approach can also be adopted, and blood flow is obtained from Tofts’ Ktrans in flow-limited regimes under high permeability conditions. The drawback to this method is the difficulty of assessing the degree of permeability and, hence, validity of assuming equivalence between Ktrans and blood flow. A much less common but more accurate T1 approach is St. Lawrence and Lee’s [34]. Blood flow is estimated directly along with Ktrans in any flow regime; however, the accuracy and reproducibility of this method has not been established. A fourth approach, different from DCE-MRI in that an endogenous agent (labeled water) is employed, is arterial spin labeling for absolute flow measurements [37].

**Capillary Permeability** – is generally obtained through T1-based DCE-MRI estimates of Ktrans. This parameter reports on the size of the contrast agent relative to the endothelial wall, but its interpretation depends on the balance between permeability and blood flow. If contrast delivery is limited by permeability (i.e. low permeability relative to blood flow), Ktrans represents the product of the permeability and surface area of the capillary endothelium. At the other extreme, if delivery is limited by flow (i.e. high permeability relative to flow), Ktrans represents blood flow per unit volume of tissue. Accurate assessment of permeability may be difficult, because the underlying physiology is often unknown. For example, although angiogenic tumor vessels tend to be more permeable than normal vasculature, changes in flow rate may account for Ktrans differences that are incorrectly ascribed to permeability differences. For this reason, high molecular weight contrast agents have been useful for monitoring permeability changes in tumor vasculature [38].

**Leakage Space** – is obtained only through pharmacokinetic modeling of T1-based images and describes the space to which the contrast agent has access. For the agent Gd-DTPA, this space is the EES (vE). Although not a descriptor of vessel function, the leakage space may reflect cellular density and provides valuable information on cell integrity in necrotic and inflammatory conditions. Its reproducibility has been validated in studies of various cancers [39,40].

Challenges of DCE-MRI Quantification

Although quantitative DCE-MRI can provide physiological insight not possible with semiquantitative methods, its application presents a number of challenges. To address or circumvent these challenges, a variety of approaches have been proposed, with the result that great variability currently exists in both acquisition and analysis amongst different imaging centers. This lack of standardization makes comparisons between studies extremely difficult. Herein we review the major limitations and suggest practical solutions where appropriate.

In DCE-MRI, both high temporal and spatial resolutions are desired to characterize contrast kinetics and delineate heterogeneous vascular patterns. Trade-offs must be made on these competing requirements and should consider the anatomy and methodology. For T2*-weighted methods, high temporal resolution is essential for capturing the first-pass bolus. In T1-weighted imaging, observation of contrast leakage allows slower acquisitions, but even here, theory predicts improved accuracy through faster sampling [41]. However, the simultaneous reduction in spatial resolution and/or coverage may adversely affect diagnostic specificity. There is no consensus on what trade-offs are appropriate; the impact of these factors on the accuracy and specificity in different applications remains to be investigated.

Two measurements crucial to DCE-MRI quantification are the intrinsic T1 and the AIF. Unfortunately, because these measurements pose additional time constraints and are generally difficult to obtain, they are often foregone at the expense of parameter accuracy. Intrinsic T1 measurement is necessary in T1-weighted DCE-MRI to relate signal changes to contrast agent concentration, but traditional methods are time-consuming and have inherent inaccuracies in vivo. In the absence of T1 measurements, linearity between signal and concentration is often assumed, or a baseline T1 value is adopted. Neither alternative is ideal, and every effort should be made to measure T1 pre-contrast accurately. Amongst the more rapid T1-mapping methods available [42,43], Cheng et al. [30] has recently reported on a rapid and accurate 3D method amenable to clinical imaging and independent of system imperfections. Measurement of the AIF is likewise challenging. A large vessel may not be present in the imaging volume; a simple solution of increasing the field-of-view to include a vessel is often unacceptable due to a concomitant lowering of spatial resolution. When a vessel is present, measurement of contrast concentration in blood is challenged by flow effects and partial volume errors. Several AIF measurement methods have been proposed to address these issues, but their implementation often requires specialized sequences and are not widely available [44,45]. Furthermore, because the AIF is measured in a large vessel, it may not represent the true arterial input to the voxel if delay and dispersion are significant. These complicating factors often necessitate that either an assumed function [46] or a population-averaged value [47] be adopted in place of an AIF measurement. The latter approach has shown improved reproducibility in DCE-MRI parameter estimation over using an assumed function. However, the ideal is to obtain AIF measurements individually when possible so that depend-
ence on cardiac output, injection mode, and kidney function can be eliminated. A few AIF measurement techniques are available for both T2* [45,48] and T1 methods [49], the latter being relatively simple and readily implemented in the clinic.

Other issues at the acquisition stage include motion compensation strategies, method of contrast injection, and type of contrast agent. Motion is especially problematic in liver and lung imaging, but errors may be minimized using registration [50] or navigator [51] techniques. The speed of contrast injection also affects contrast kinetics. A rapid bolus injection is preferred for better parameter accuracy; a short and consistent bolus width can be achieved using a power injector. However, if temporal resolution is low and cannot adequately capture the kinetics of first-passage in highly permeable tissue, a slow infusion may be preferred. The choice of contrast agent is also very important. It must be compatible with the tumor physiology and the properties to be measured. Non-specific agents spanning a wide range of molecular weights may be employed to assess different vascular properties. For example, macromolecular agents can assess the permeability of very leaky tumors without flow contamination in T1-weighted approaches; they can also improve estimates of blood flow and volume in T2*-weighted approaches. Agents in this category include Gd chelates and superparamagnetic compounds. A second category is tissue-specific contrast agents. These agents represent the new direction in contrast agent development and are intended to improve the specificity of contrast distribution. Example targets include the reticuloendothelial system, lymphatic tissue, or sites of active angiogenesis.

The analysis of DCE-MRI images involves various considerations to ensure accurate quantification. In T1-weighted approaches, one consideration is validity of the chosen model and its assumptions to describe pharmacokinetics. Only certain biological conditions, not all, can be described by any model. For example, Patlak’s model [33] is suited to low-permeability situations where back-flux is negligible at early times, whereas more permeable vessels are better suited to Tofts [32]. The model chosen must be appropriate for the physiology investigated, but model fit failures can still arise due to additional mechanisms that are not well understood. For instance, the discrepancy often noted between actual data and Tofts model, particularly in the first couple of passages of contrast, may involve a few factors. The AIF may be inaccurate, possibly due to low temporal sampling or an incorrectly assumed form. Multiple tissue compartments may exist, a very probable scenario in tumors, which would invalidate ‘Tofts’ two-compartment assumption. Third, the assumption of a single T1 relaxation rate may be inappropriate to relate signal changes to concentration. This is equivalent to a departure from the regime of fast proton exchange, such that water (proton) movement between compartments is now comparable to differences in relaxation rates, and multiple relaxation rates exist. Unfortunately, exchange is not simple to quantify, and although interstitial-intracellular water movement is generally in fast exchange [52], this assumption may not hold across the vascular-interstitial interface. The effects of exchange can be minimized by maintaining a low blood contrast concentration with a low injection dose or by selecting proper acquisition settings [52]. Fourth, the model chosen should provide robust estimates for the number of physiological parameters desired; an increased number of free parameters will increase sensitivity to image noise and undermine reliability in derived measurements. As a result, the full adiabatic model [34] that estimates flow and blood volume in addition to permeability is seldom used even in research applications. In T2*-weighted approaches, accuracy is limited by contrast recirculation and leakage. Recirculation is usually addressed by fitting a gamma-variate function to extract the first passage of contrast bolus [26]. Leakage may be handled by several solutions. Post-processing may be employed to extract the residual leakage profile [53]. However, most solutions are implemented at the acquisition stage. For example, since loss of contrast compartmentalization results in T1 enhancement that counteracts T2* signal drop, one solution is to use non-Gd-based agents that have a weak T1 effect, such as ultrasmall particle iron oxides. If only Gd-based agents are available, the tissue may be presaturated to saturate the EES, or T1-minimized sequences can be employed. A final consideration common to both T1- and T2*-weighted DCE-MRI is whether to analyze on a pixel-by-pixel or region-of-interest (ROI) basis. The latter generally yields robust parameter estimation due to good SNRs; it is also more immune to motion. However, tumor heterogeneity is not delineated. Thus, pixel-wise analysis is preferred despite the increased likelihood of model-fit failures. Pixel maps may be further analyzed using histogram or principal components analysis to quantify tumor heterogeneity.

Clinical Validation

Both T2* and T1-weighted DCE-MRI have shown value in tumor diagnosis and staging, with T2* being most reliable in brain applications. Sensitivity of DCE-MRI parameters to differences in microvessel density, size, and permeability have enabled lesion differentiation [54], staging [55], prognosis [56], and detection of relapse [57] in a variety of conditions. Validation has also been attempted in various DCE-MRI studies, despite the lack of a gold standard, with most comparing against clinical outcome such as response [58,59] or survival [60], or histopathology such as tumor grade and size [61], metastases [62], MVD [14,63-67], or VEGF expression [68-70]. Because MVD is a good prognostic indicator for many cancers, its correlation with DCE-MRI parameters is perhaps the most common, but significant associations are not always found [65,68]. This discrepancy can be explained by the fact that MVD measures the number of vessels, while DCE-MRI parameters are sensitive to the number and size of functional vessels. In fact, the sensitivity of DCE-MRI to vessel properties other than density makes it potentially more powerful than immunohistochemical assays. For example, its sensitivity to VEGF-induced permeability in angiogenic vessels [15] has been explored in several studies, with positive correlations found in cancer diagnosis [71] and tumor response to anti-VEGF therapy [72,73]. Despite the usefulness of DCE-MRI, however, there are a number of limitations, including overlap between malignant and benign disease, and inconsistent prediction of clinical outcome.

The main shortcoming with many of these correlative studies is variability in DCE-MRI implementation and patient populations. Thus, it has been difficult to compare re-
results from different studies for determining which DCE-MRI parameters are most accurate and robust. It has been equally difficult to reconcile why correlation with expected histological indices is sometimes absent. These challenges must be addressed if DCE-MRI is to be useful for assessing treatment response, whether by conventional chemotherapy or by newer paradigms such as antiangiogenic treatment, which is the topic of the following section. The current capabilities of DCE-MRI suffice if only sensitivity to overall treatment-induced changes is required, since most tumor therapies ultimately result in vessel collapse, which effectively decreases all vascular indices (i.e. flow, blood volume, permeability). On the other hand, if underlying mechanisms of, say, drug action need to be elucidated, DCE-MRI needs to distinguish accurately the various indices. Technological advances for quantitative DCE-MRI are required, and standardized DCE-MRI methods must be adopted in multi-center clinical trials.

**DCE-MRI MONITORING OF ANTIANGIOGENIC TREATMENT**

**Current Status**

Sensitivity of DCE-MRI to microvessel properties has attracted much interest in its use as a biomarker for assessing antiangiogenic tumor therapy. Several phase I trials of antiangiogenic cancer drugs have included DCE-MRI to determine its potential as a predictive marker [73-80]. A number of these correlative studies have shown early evidence of drug action through DCE-MRI, with subsequent positive clinical response. The DCE-MRI parameter in which significant changes are most commonly seen is $K_{\text{trans}}$, most likely because of the permeability-wielding effect of many antiangiogenic agents (Fig. (3)). For example, in studies of the VEGF receptor tyrosine kinase inhibitor PT787/ZK 222584 on colorectal and liver metastases [73], anti-VEGF bevacizumab on breast cancer [75], or anti-VEGF AG-013736 on various solid tumors [79], decreases in $K_{\text{trans}}$ were related to plasma levels of drug and were significantly lower after one cycle of treatment. Furthermore, responders showed greater changes in $K_{\text{trans}}$ than non-responders both at early times and after treatment [73]. Such early vascular changes are not unexpected, as both xenograft [81] and human [82] studies have shown reduced microvessel permeability occurring within hours after application of an anti-VEGF agent, despite the much longer time period over which the drug exerts an effect. In fact, drug efficacy may be predicted based on early changes on DCE-MRI, as demonstrated by Morgan et al. [73].

Measurable response by DCE-MRI is not always observed, however. In a phase I study of SU5416, a VEGFR2 inhibitor, on various solid tumors, no changes in $K_{\text{trans}}$ or $v_e$ were seen [76]. The most probable culprit is that permeability changes induced by SU5416 may have been too small to be detected by DCE-MRI. Other contributing factors include a small patient population, a ROI-analysis approach that failed to distinguish heterogeneous drug activity, and an inappropriate patient population for the drug tested. This last point is important, because the tumors examined in that study and in many phase I trials are often at an advanced stage with mature vessels that are less responsive to antiangiogenic treatment. Recently emergent tumors with new, proliferating vessels that are more responsive to this type of treatment would be a more appropriate platform to evaluate DCE-MRI.

Despite the varying results of these studies, ample evidence exists that DCE-MRI can assess treatment efficacy and may be predictive of later clinical response. The following section discusses existing challenges for DCE-MRI. These challenges account for variable results observed and, at the same time, define the standards required of DCE-MRI as a biomarker for anticancer therapy assessment.

**Challenges for DCE-MRI**

The effort to standardize DCE-MRI techniques is confounded by not only technological issues, as discussed earlier, but also biological issues. Biological unknowns such as
heterogeneity amongst and within tumors, individual variations in response to treatment, and a dose-response effect different from traditional therapy, can all determine the effectiveness of a DCE-MRI strategy. In many studies a threshold dose has been reported, rather than a consistent dose-response curve. That is, changes in DCE-MRI parameters are not seen until a minimum dose level is reached, beyond which an expected graded dose-response effect is absent [78]. This minimum dose, however, is very different from the optimum biologically active dose we seek to identify, which may be obscured by tumor heterogeneity. The influence of biological variations may be more easily managed if the quantitative capability of DCE-MRI were improved.

Two aspects are relevant in DCE-MRI quantification: reproducibility and validity. Reproducibility is as important as accuracy, because the change expected to occur in response to treatment must be larger than variations in the chosen DCE-MRI parameter. This issue has been investigated in several studies [39,83], and the consensus is that two baseline measurements should be incorporated before drug administration [39]. Unfortunately, reproducibility estimates in many studies performed to date cannot be compared due to different approaches in DCE-MRI acquisition and analysis. Further investigation is required to determine and define the factors that influence reproducibility, such as modeling techniques and AIF measurement. In general, however, quantitative parameters are more sensitive to physiological and machine noise compared to semi-quantification. Amongst quantitative parameters, \( v_c \) exhibits lower variability compared to \( K_{trans} \) in the presence of physiological changes [39]. In addition to being reproducible, a DCE-MRI parameter must be valid: that is, it should accurately reflect the physiology interrogated. Quantification based on pharmacokinetic modeling, such as estimating \( K_{trans} \) and \( v_c \), can provide insight into underlying physiology and is the preferred method. In fact, if the issues discussed in “Challenges of DCE-MRI Quantification” are addressed, we can remove dependence on instrument settings, cardiac output, tissue type, and other sources of error. Essentially, this means adopting the following minimum set of requirements: baseline T1 measurement, AIF measurement, and a temporal resolution of 1 minute or less, depending on the resolution, anatomic coverage, and signal-to-noise required [9]. Pixel-by-pixel is preferred over ROI analysis where motion is not a complicating factor, so that heterogeneous patterns of response can be observed. Adopting this minimum set of guidelines may benefit studies in which positive correlations between DCE-MRI and accepted clinical or biological endpoints were not observed [76]. Even studies where correlations were seen will benefit from improved accuracy and reproducibility. Perhaps the greatest challenge remaining is separating different physiological phenomena that are inexorably tied together in one DCE-MRI parameter. The best example is \( K_{trans} \). Despite a significant decrease in this parameter at 2 days and at the end of the first cycle following anti-VEGF therapy, the precise mechanisms were unknown [73]. Successful inhibition of VEGF receptors may have resulted in either reduced permeability or vascularity, or both, within hours after treatment onset. Distinction of these two separate phenomena would lend better insight into the drug’s action and help optimize treatment regimens. However, this distinction requires improved and perhaps novel modeling techniques and the use of appropriate contrast agents.

To be useful as a surrogate biomarker of drug response, DCE-MRI parameters must indicate changes shortly after treatment is initiated, show a dose-response relationship, and predict later clinical response. Proof-of-concept in each of these areas has already been discussed. We can anticipate that consistent performance will result from improvements in both parameter accuracy and reproducibility. This would require a concerted effort across imaging centers to standardize their overall DCE-MRI approaches. For any particular disease, both treatment and imaging strategy may need to be specifically tailored if meaningful evaluation is to be undertaken. Clear imaging endpoints that are biologically relevant must be defined prospectively, and then compared against clinical outcome.

**NOVEL MRI APPROACHES: MOLECULAR MARKERS OF ANGIOGENESIS**

In addition to imaging microvessel function using non-target-specific MR contrast agents, angiogenic vessels can be imaged using agents that bind to characteristic molecular markers of angiogenesis. Developments in molecular MR contrast agents are fairly recent, and most have targeted the \( \alpha_\beta \) integrin, which is a protein expressed specifically on neovascular vessels. Other proteins selectively expressed by angiogenic vessels, and therefore provide potential markers for imaging tumors, include the \( \alpha_\beta \) integrins [84], VEGF and its receptor VEGFR-2 [85], CD105 (endoglin) [86], CD36 (thrombospondin-1 receptor) [87], Thy-1 [88], and tumor endothelial markers [89]. Molecular imaging adds another layer of specificity to assessing the efficacy of drug application. It does so by revealing the mechanisms behind functional changes observed. For example, imaging the molecular expression of membrane proteins or receptors following anti-VEGF treatment informs on the changing composition of tumor vessels, which imaging of flow or permeability changes alone cannot provide.

Molecular markers offer dual-function imaging capability. Not only can contrast agents be targeted to active endothelial cells for imaging angiogenic tumor vessels, but also drugs can be targeted to the same sites for selective therapeutics [90]. In fact, treatment and imaging can be performed simultaneously by conjugating the drug to the imaging agent. This would allow the spatial distribution and pharmacokinetics of drug uptake to be visualized. Incomplete or ineffective tumor treatment may be more accurately assessed when it is known where and how much drug was delivered.

The principal limitation with molecular imaging techniques is the low concentration of many of these markers. To be useful, imaging agents must bind to these markers with high specificity and be detectable at low concentrations by the imaging modality. Compared to more established molecular imaging techniques such as PET and SPECT, MRI is much less sensitive. However, recent studies have demonstrated feasibility of molecular MRI of angiogenesis using paramagnetic MRI agents that are conjugated to an antibody for detecting angiogenic markers, such as the \( \alpha_\beta \) integrin [91,92] (Fig. 4). A second issue is that the affinity of the
cross-site standardization of imaging protocols and analysis methods must be established. Issues related to accurate quantitative imaging must also be addressed, including: T1 and AIF measurements, robust data analysis, and statistical data interpretation. Adherence to these measures would ensure reproducibility of DCE-MRI parameters. Prospective, multicenter clinical trials adopting these standards may then be conducted to evaluate the validity and predictive value of DCE-MRI. An understanding of therapy-induced changes in vessel function can be augmented by incorporating molecular MRI to monitor specific molecular and cellular actions of antiangiogenic agents.

REFERENCES


CONCLUDING REMARKS

DCE-MRI holds much promise as an imaging biomarker in novel cancer drug development such as antiangiogenic therapy. Not only can DCE-MRI fulfill an urgent need to understand in more detail the action of angiogenesis inhibitors, but also it may provide sensitive indication of changes in tumor vessels after treatment. Correlative studies have demonstrated the potential of DCE-MRI to measure non-invasively vascular changes in response to therapy, such as changes in blood volume, flow, and permeability. Prediction of subsequent clinical response has also been shown in some therapeutic trials. However, the biomarker performance of DCE-MRI needs to be confirmed definitively. To do so,


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