The Use of Dynamic Contrast Enhanced Magnetic Resonance Imaging (DCE-MRI) To Study Tumour Angiogenesis in the Management of Metastatic Cancer

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By

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Abstract

Introduction: Dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) may act as a biomarker for successful cancer therapy. This thesis demonstrates an imaging technique developed to be simple and reproducible. PTK787/ZK222584 (PTK/ZK) is an inhibitor of vascular endothelial growth factor mediated angiogenesis. The pharmacodynamic effects of PTK/ZK were evaluated by assessing changes in contrast enhancement parameters of metastatic disease using DCE-MRI in advanced cancer patients treated in two dose escalating phase I studies using once and twice-daily dosing schedules. These studies were performed to test for 'proof of concept' of efficacy and to establish optimal biological dose and dosing regime.

Patients and Methods: A theoretical analysis was performed to optimise sequence parameters. MRI 'phantom' and clinical studies were performed to establish the validity and reproducibility of the technique. The clinical studies of PTK/ZK were performed with centralised DCE-MRI analysis. DCE-MRI was performed at baseline, day 2, and at the end of the 28-day cycle (EC1). Doses of oral PTK/ZK ranged from 50 to 2000 mg per day. Tumour permeability and vascularity were assessed by calculating the bi-directional transfer constant ($K_{\text{trans}}$). The percentage of pre-treatment $K_{\text{trans}}$ at each time point was compared with pharmacokinetic and clinical endpoints.

Results: This technique can accurately measure $R_1$ within the expected range of tumour enhancement. The reproducibility study showed a coefficient of variation (CoV) of 16% for $K_{\text{trans}}$ if tumours of diameter less than 3cm were excluded. A significant correlation was shown between changes in $K_{\text{trans}}$ and PTK/ZK oral dose, plasma levels and efficacy at both day 2 and EC1. Visual assessment of the DCE-MRI scans demonstrated similar results, but with weaker correlations to dose and efficacy.

Conclusion: The clinical findings suggest that DCE-MRI is sufficiently sensitive and reproducible to act as a useful biomarker for defining the pharmacological response and dose of angiogenesis inhibitors, such as PTK/ZK.
Preface

This thesis represents original work. I am responsible for the majority of this work. The text of this thesis excluding appendices and references is ~29,500 words. I first developed the plan for this research program in 1998. At this time there was developing interest in using dynamic contrast enhanced MRI (DCE-MRI) to investigate tumours, but very few studies had attempted to use this tool to study tumours on treatment. Further at this time, there were no published studies using DCE-MRI to study treatment effect in liver and lung tumours and no studies involving treatment by angiogenesis inhibitors. This was therefore a novel idea with several original aspects. Firstly as phase 1 trials are predominately for metastatic disease, primarily in lung and liver which undergo continual bulk movement from respiration, I decided to develop sequences already used locally for cardiac perfusion imaging, rather than existing protocols used for cancer in relatively static sites, such as brain, prostate and breast. The second original aspect is that we were able to use these techniques in a ‘first in man’ study of a novel angiogenesis inhibitor as early as 1998.

This thesis documents the sequence and protocol design, validation and clinical use of DCE-MRI in Leicester, a project I have leaded since 1998. In this time, particularly with the help of 3 key people, Professor Graham Cherryman, Dr Mark Horsfield and Professor Will Steward, I have become internationally recognised in this field, as shown by the following list of associated publications and invited presentations. This thesis involves multicentre trials involving both radiological and clinical expertise in America, Germany, Switzerland and UK. Although I am responsible for the bulk of work presented here there are many people without whom I could not have come this far. Below the list of publications, I list people who have helped in the development of this thesis.

Invited reviews:

Associated Peer Reviewed Publications:


Abstracts:


Thomas A, Morgan B, Rowark G, et al. Phase I study of the oral vascular endothelial growth factor (VEGF) receptor inhibitor PTK787/ZK222584 on a twice daily schedule in
Higginson A, Morgan B, Campbell S, et al. How does subjective visual interpretation of
dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) compare to objective
measurements of tumour enhancement during treatment with an angiogenesis inhibitor.
Rapid Assessment of Contrast Enhancement to Monitor Tumor Therapy. Proc Intl Soc
Perfusion in Normals and Advanced Liver Metastases. European Radiology 2000. 10,
D19(s9.2).

Indicators of status in field: Invited presentations
To: 13th Annual meeting of the International Society of Magnetic Resonance in Medicine
2005
2: Experience with Dose Finding with DCE- MRI with PTK787/ZK To: Spring 2005
NCI CTEP Early Drug Development Meeting Bethesda, MD
3: Imaging Hypoxia and Angiogenesis - Focus on MR Imaging. To: Dublin Molecular
Medicine Centre; research symposium 2004
4: Imaging pharmacodynamics of an angiogenesis inhibitor To: 6th international
workshop on pharmacodynamics of anticancer agents Venice – Italy 2004
5: Encouraging clinical experience in monitoring anti-angiogenic therapies by MRI.
To: International Magnetic Resonance Society Meeting Workshop “In Vivo Functional and
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I would like to acknowledge the support I have received from the following:
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DCE-MRI team
Leicester
Mark A Horsfield: Help in developing MRI sequences, MR scanning for
phantom study and writing the software required to calculate
K\text{trans} and K\text{\text{r}}. Help in further understanding of MR physics.
Jane F Utting Help with MR physics and development of manuscript under
consideration for publication from chapters 2 & 3.
Asvina Jivan Advice on sequence development in early stages
Freiberg
Juergen Hennig Performing and transmitting DCE-MRI scans from Freiberg
for & Martin Buchert clinical studies in chapters 4-6 under protocol.
Clinical MRI team (Leicester)
Antony Higginson, Shona Campbell and James Entwisle.
‘Blinded’ visual review of scans for chapter 6

Clinical team
Leicester
William P Steward Saw the promise in and supported the original idea. Advice on clinical matters. Supervision of clinical side of cancer studies.
Anne L Thomas Advice on clinical matters. Supervision of clinical side of cancer studies.

Freiberg
Joachim Drevs, Klauss Mross, Clemens Unger, Dieter Marmé Supervision of clinical side of cancer studies in Germany.

Novartis Pharma and Schering AG
Margaret Dugan Original trial sponsor for study in chapter 4. Believed in and supported the original idea.
Howard A Ball, Lucy Lee, Dirk Laurent, Andrew Henry Help with data handling for studies in chapters 4 & 5.
William Mietlowski Statistician providing the $E_{max}$ model fits in chapters 4 & 5.

I dedicate this work to my father, Dr. David Charles Morgan;
I know he would have been proud.
Chapter 1:

Introduction: Imaging Tumour Angiogenesis

1.1 Introduction

Increasing knowledge of molecular medicine and its application to the study of cancer has dramatically changed treatment approaches and the ‘imaging’ questions that these approaches bring. There is an increasing need to study biological processes \textit{in vivo} at both the pre-clinical and clinical stages of pharmacological development and application. This is particularly true for the introduction of targeted agents to inhibit specific tumour pathways such as treatments based on tumour angiogenesis.

In order to grow larger than 1 - 2 mm in diameter, solid tumours need to create a blood supply through the process of angiogenesis, the formation of new blood vessels from pre-existing vasculature \textsuperscript{1-5}. Specific inhibition of tumour-induced angiogenesis should prevent the continued growth of many solid tumours, as well as prevent their metastatic potential, thereby providing a novel approach for the treatment of cancer \textsuperscript{6}. Vascular Endothelial Growth Factor (VEGF), a potent angiogenic factor, is released by a variety of normal and neoplastic cells following a hypoxic stimulus \textsuperscript{5,7}. VEGF also induces hyper permeability of tumour vessels, a critical event in angiogenesis \textsuperscript{8}. The VEGF receptors are central to tumour angiogenesis and lymphangiogenesis \textsuperscript{9,10}, binding extracellular VEGF growth factors and triggering intracellular signalling through their tyrosine kinase domains \textsuperscript{11-14}.

Inhibition of VEGF-induced angiogenic signals selectively targets tumour-associated vessels, since cell division of endothelial cells in the normal vasculature is a rarer event. Anti-angiogenic therapy targeted at the VEGF kinase receptor is therefore expected to be a safe and well tolerated approach to cancer therapy.

Currently, there are no clinical paradigms that require information on angiogenesis status to decide diagnosis and management. However angiogenesis has been shown to be important for the prognosis and treatment of cancer and this is reflected in the fact that several anti-angiogenesis strategies are currently in clinical development \textsuperscript{15}. It is likely, in future, that anti-angiogenesis treatment protocols will be specific to the histology and stage of the disease \textsuperscript{16}. It has always been recognised that angiogenesis inhibitors may cause
tumour stasis, preventing further growth, rather than reducing tumour size. This is important since, even with standard chemotherapy, it has been demonstrated that it is probably the duration of the stable disease phase, and not tumour shrinkage that has the major effect in prolonging survival of patients.

Cancer imaging departments bear little relation to those of 25 years ago, the old equipment being replaced by advanced scanning equipment using computed x-ray tomography, ultrasound, magnetic resonance and positron emitting agents. Despite these advances in medical imaging technology, the organisation and function of most clinical cancer imaging departments remains largely unchanged. The emphasis is still on a surgical approach to disease, concentrating on the localisation, size, shape and appearance of tumour lesions. Pressure for change has been limited by the lack of innovative pharmacological approaches to cancer therapy, with surgical resection still giving the best chance of cure in most solid tumours. Tumour size measurements taken from CT scanning have shown some correlation with clinical outcome in chemotherapy and are useful for monitoring treatment where rapid size changes are expected. However, the possible absence of tumour shrinkage, and the fact that cross-sectional imaging of tumours requires a 30% reduction in size to gauge reliably the response to treatment, makes evaluation of the potential efficacy of angiogenesis inhibitors in phase I / II trials problematic. The traditional endpoint of oncology trials will therefore need to change, away from the short-term goal of improvement in tumour size and patient well being, to goals that may be related to a lack of progression of disease and a failure to metastasise. New trial designs are therefore needed.

This has lead to the search for new surrogate endpoints as markers of efficacy. Angiogenesis is a local tissue phenomenon, so attempts to measure surrogate markers in the blood, while promising, may be non-specific. Biopsies with histopathological staining are invasive, may not be representative of the whole tumour, and only give information at a single time point. Several imaging modalities potentially offer a non-invasive test, with the ability both to measure tumours spatially and to map their changes over time. Functional and molecular information is increasingly being emphasised, and the characterisation and measurement of biologic processes at the cellular and molecular level is often described as ‘molecular imaging’. Measuring vascular or other physiological changes can be described as ‘functional’ imaging. Such molecular or functional imaging could very quickly show whether the drug is working at a mechanistic / molecular level. Imaging is already changing the study of drug development...
in the pre-clinical phase, but is just starting to have an impact in the clinic. Clinical studies are particularly important, as animal studies do not directly translate to humans, and most pre-clinical studies are carried out in non wild type tumours with different locations, maturity and stages of development.

Traditionally, the purpose of a phase 1 clinical study is to establish pharmacokinetics and potential toxicity in humans, and imaging is simply used as a measure of tumour size to determine response. The extra emphasis on a molecular / mechanistic approach to drug development has increased the importance of imaging in early clinical trials, with up to four different modalities being used. The questions in early trials are not just whether the agent works in the broadest sense, but also does it have an effect via the intended mechanism (e.g., changes in vascularity) *in vivo*; does it affect the intended receptor *in vivo*; and is it getting to the right place in sufficient quantities and times in the studied dose regime? This is important for early clinical studies in advanced cancer, since it may be difficult to detect clinical efficacy by traditional methods such as survival.

In reality, radiologists are no strangers to imaging biologic processes. Imaging of the uptake of isotope labelled substances specific to thyroid (Iodine) and bone (Technetium $^{99m}$ diphosphonates) has been available for over 30 years. Furthermore, when performing CT or MRI scans with injected contrast media, radiologists have been using different contrast enhancement patterns in the liver to differentiate benign from malignant lesions for several years. However, further development of functional or molecular imaging is more problematic. Imaging studies investigating the molecular basis of cancer have been used extensively in the pre-clinical setting, but translation of this work from bench to bedside is more difficult. First, radiology departments are often focused on high turnover, clinical service work with little budget or poor organisational systems for research. Radiologists are only broadly aware of the revolution that is taking place, and may have insufficient knowledge and training. Furthermore, using complex imaging in clinical trials to study biological processes asks more time from the patient, testing their commitment. One main opportunity arises, however, because imaging has become an integral part of a patient's cancer journey with increasing sophistication of standard imaging tests. This means that the technology for many advanced functional imaging tests is often available in imaging departments.
1.2 General Angiogenesis Imaging Issues

The 'switch' to angiogenesis involves many processes and factors, which may be potential treatment targets. These range from genetic alterations, to the consequences of angiogenic factors, such as vascular endothelial growth factor (VEGF) causing changes in vascular permeability, proliferation and maturity. Vascular changes may result in increased circulation or, more commonly, the presence of inadequate vascular networks and lymphatics leading to hypoxia and high interstitial pressure. Hypoxia can be chronic 'diffusion-limited' tumour hypoxia or 'perfusion-limited' hypoxia due to a dynamic process in which the vessels periodically open and close. This hypoxia has been recognised for many years as hypodense ('necrotic') centres on contrast-enhanced CT scans (Figure 1.1). In neck lymph nodes, these areas have been correlated to hypoxia using oxygen-sensitive electrodes, and have been shown to be a marker of poor prognosis.

There are therefore several aspects of the angiogenic process that are amenable to imaging. First are the direct processes that may be manifested by over-expression of cell surface markers, falling into the realms of 'molecular imaging'. These processes may also lead to changes in circulating angiogenic factors. Less directly, there will be changes in the vasculature itself, including the vascular permeability to macromolecules (and therefore contrast agents), the perfusion of the tissue, and the maturity of the vessels in the tumour which may change in response to pharmacologic manipulation. Ultimately, failure of tissue perfusion will lead to cell death by necrosis and apoptosis, which suggests further potential imaging targets including hypoxia and apoptosis markers as well as tumour volume. Anti-angiogenic treatment may therefore have a range of effects on the vascular characteristics of a tumour but, ultimately, all should be amenable to imaging of some kind.

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**Figure 1.1**

Axial CT images before (A) and after (B) the administration of a contrast agent, which shows a supra-glottic laryngeal carcinoma (*). The administration of contrast shows a rim enhancing effect of an adjacent lymph node (arrow) around a hypo dense 'necrotic' center, known to be associated with tumor hypoxia.
There are three major mechanisms by which pharmacological targeting of tumour blood vessels could be achieved:

1. true angiogenesis inhibition
2. vascular targeting (directly destroying blood vessels)
3. non-selective anti-angiogenic effects such as has been proposed for some chemotherapeutic agents at low dose.

It is therefore clear that any imaging test that has been shown to correlate with a successful clinical outcome may be used in clinical studies of anti-angiogenesis agents, and further, that any successful cancer treatment may affect vasculature and therefore cause alteration in imaging derived vascular parameters.

It has already been stated that angiogenesis inhibitors may not result in substantial reductions of tumour volume. Other problems with imaging the response to anti-angiogenic treatments are that they may inhibit only a single positive factor, and individual tumours can express several angiogenic factors, which may lead to partial or complete resistance of the tumour vessels to therapy. This diversity encourages the use of combinations of these agents. Furthermore, advanced cancer may not be the ultimate indication, but is likely to be the target in early treatment trials. The efficacy of treatment could vary between patients and different tumour types, and the heterogeneity of delivery of drugs to solid tumours may lead to further variability in response. Without knowledge of what to target, phase 1 trials might therefore miss potential efficacy by including too diverse a group of patients and tumours. For these reasons, the aims of a single-agent phase 1 clinical trial are to demonstrate a potentially useful biological effect and to direct future choice of combination therapies, method of delivery, and target tumour types.

Phase 1 trial design is made more difficult by the lack of toxicity of these drugs, such that toxicity-based selection of dose for further development may not be optimal. Although conventional imaging techniques are still needed, since tumour size monitoring will remain an important response variable, methods to demonstrate biologic activity before reaching maximum tolerated dose, or even to show an optimal dose well below the maximum-tolerated dose, would greatly enhance the utility of such studies. There is currently no proven method of imaging the angiogenic process. The reasons for this are easy to understand: angiogenesis is a complex process involving many steps, defying a simple single method approach. Furthermore, if the individual molecular processes are to be
studied, the method has to be sensitive to microscopic changes or nanomolar concentrations of naturally occurring substances or deliverable imaging contrast agents.

For imaging to be successful, it needs to be established as a 'biomarker' or a 'surrogate endpoint' for the activity of the drug. A biological marker (biomarker) is defined as an objective measurement indicating a pharmacological response to a therapeutic intervention. A surrogate endpoint is a biomarker that is intended to substitute for a clinical endpoint, a characteristic or variable that reflects the patient's well-being. It should be noted that in a dose-escalating trial, even if a potential biomarker shows a correlation between dose and efficacy, this may be purely as a side effect or even toxicity of the drug, and does not imply cause and effect. Although many imaging tests are in the early stages of validation as biomarkers, there are, as yet, no studies validating 'functional imaging' as surrogate endpoints of clinical efficacy for anti-angiogenic treatments.

An ideal biomarker indicates the presence of a target disease in an accurate and reproducible manner, and is closely linked to success or failure of the therapeutic effect of the product being evaluated. This provides a quicker trial result, since "true" end points include parameters such as 5 year survival, and helps to avoid confounding factors, incidental to the treated disease, developing. Another advantage is that fewer patients are required as each patient can act as their own control. Figure 1.2 charts the progression of tests demonstrating specific activity of a drug through to true end points demonstrating the broader clinical efficacy of treatment. An ideal test shows both mechanistic activity and clinical efficacy.
Figure 1.2
The progression of direct to indirect tests used to monitor drug therapy.

<table>
<thead>
<tr>
<th>Molecular Imaging: Direct</th>
<th>Physiological Imaging</th>
<th>Imaging of Tumor Volume</th>
<th>Clinical Outcome: Indirect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E.g. vascularity</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Total study cost
- Relevance to clinical outcome (Efficacy)
- Specific evidence of activity
- Speed of result

An ideal surrogate clinical endpoint links evidence of Activity with evidence of Efficacy

There is therefore a balance between the importance of a specific biomarker and true clinical endpoints. On the one hand, it is useful to know whether treatments are promising in early therapeutic trials, even if advanced tumours are not the ultimate target, since this avoids rejecting a potentially useful therapy. On the other hand, in combination treatment, anti-angiogenic treatment may be expected to reduce vasculature, which may compromise the effectiveness of radiotherapy or chemotherapy. In this case, biomarkers showing specific drug activity are less helpful, and 'true' indirect clinical endpoints, such as tumour size response or delayed time to progression are required.

1.3 Types of imaging

Imaging can be performed by a variety of modalities, including X-ray computed tomography (CT), magnetic resonance imaging (MRI), radioisotope imaging (single photon emission computerised tomography - SPECT - and positron emission tomography - PET), ultrasound and optical imaging. One fundamental aspect of all imaging modalities is their resolution, which impacts on the ability to separate tissues that are different either by structure or function in a spatial and temporal manner. Resolution is related to the ratio of signal to noise, or the ratio of the information returned from the tissue to the random variation in that information due to measurement imperfections. All in vivo imaging techniques have their own strengths and weaknesses due to the different types of information returned, and therefore have varying limits of spatial and temporal resolution.
In some cases, the imaging modality alone provides information that is relevant for studying angiogenesis, e.g. Doppler ultrasound, which provides blood flow measures, or MRI using diffusion or spectroscopy techniques. However, with other techniques a contrast agent (or probe, possibly radioactively labelled) is required. In these cases, it is the attributes of the agent that largely dictate what information can be gained. The imaging test determines at what concentration, speed and spatial resolution the agent can be studied.

When a contrast agent is used, it may provide direct information related to a specific aspect of angiogenesis, or indirect (downstream) information related to a consequence of successful therapy. Most tests performed without the use of contrast media or probes are generally indirect. The multiple potential targets and treatment strategies suggest that the most useful approach may be an indirect measure of angiogenesis. The most common current indirect indicators of angiogenesis used are changes in metabolism and vascularity. These represent the expected downstream consequences of depriving the tumour of blood supply, and are particularly useful if there is uncertainty about the exact nature of the mechanisms of action of the drug, and the need to test drugs with different mechanisms in combination.

The vascularity of a tumour can be measured in terms of the blood volume (the volume of the intravascular space compared with the volume of the tumour) and perfusion (rate of blood flow into the tumour). A further aspect is the permeability of the vasculature, which is related to the ease with which substances can pass from the intravascular to the extravascular extra cellular (interstitial) space. The permeability depends on the molecular weight (size) of the substance, and angiogenesis promoters such as VEGF have been shown to increase this permeability to macromolecules. There is debate about the exact mechanism of changes in macromolecular permeability that may impact on interpretation of imaging results. One possibility is that macromolecules extravasate predominantly by an opening of the junctions between adjacent endothelial cells. However, Dvorak and colleagues argue that, although it is likely that very small hydrophilic molecules up to 3 nm in diameter pass through intact interendothelial cell junctions, in response to VEGF-A, macromolecules up to 50 to 70 nm in diameter cross the endothelium predominantly by means of a transendothelial cell pathway that involves vesiculovacuolar organelles. There are therefore potentially two distinct mechanisms for the leakage of macromolecules up to 3nm in diameter.
1.4 Magnetic resonance imaging with contrast agents

This thesis concentrates on MRI using contrast agents to study tumour vasculature. Other potential imaging modalities are discussed in chapter 7. Studies of the vasculature with MRI normally include rapid injection of a contrast agent, often referred to as Dynamic Contrast-Enhanced Magnetic Resonance Imaging, or DCE-MRI. Consequent changes in the image brightness are then used to detect and characterise lesions. DCE-MRI is already finding routine clinical application in MR mammography.

The MR image is created from the nuclei of hydrogen atoms (protons) that are mainly in water. Applying both a large static magnetic field and a series of radio-frequency pulses causes ‘excitation’ of the protons, and generates the signal. The image (a spatial location map of the proton signal) is created by applying magnetic field gradients along different directions. Although the signal intensity is largely dependent on the water concentration (or proton density), the image can be made sensitive to two different ways the signal changes or ‘relaxes’: the time constants that govern these two relaxation processes are called the $T_1$ and $T_2$ relaxation times. When the image is sensitised to one of the relaxation processes, this is called either $T_1$-weighted, or $T_2$-weighted imaging.

Contrast media are available that can cause both $T_1$ and $T_2$ to change, with a consequent change in signal intensity. The presence of contrast agent is indicated by either signal hyper intensity (with $T_1$-weighted imaging), or hypo intensity (with $T_2$-weighted imaging). For $T_1$ imaging, in normal clinical use in the brain, just a single image is acquired some time (typically 5 minutes) after contrast injection to show the distribution of the agent and to confirm opening of the blood-brain barrier. Outside the brain, however, where even in healthy tissue the contrast agent leaks from the vasculature, measuring the time course of the signal change in the tissue can be much more revealing.

Using contrast media of varying molecular weights and magnetic properties, MRI can be used to measure blood volume, perfusion and blood vessel permeability. Large molecular weight contrast agents will stay within the intravascular space, and by collecting MR images continuously as the contrast is injected, both blood volume and perfusion can be estimated. Very small molecules, such as water, will leak rapidly into the interstitial space, again providing a guide to perfusion in the dynamic phase, and the size of the interstitial space. Small to intermediate molecular weight agents, however, are neither freely diffusible nor do they remain purely in the blood pool, and the degree of signal change will be related to both flow and permeability parameters (Figure 1.3).
Starting before the injection of contrast, DCE-MR images are acquired as the contrast first ‘washes’ into the tissue, and may continue as it begins to wash out, with the plasma concentration diminishing as contrast disperses and is cleared via the kidneys. Temporal analysis of the enhancement pattern for intermediate size agents can help elucidate the separate components of blood volume, perfusion and permeability, but for low molecular weight compounds such as the standard gadolinium (Gd) chelates, available for use in humans, the enhancement pattern seen often results from an inseparable combination of flow, blood volume and permeability. As well as perfusion and permeability, the enhancement profile depends on the volume of tissue to which the contrast agent has access – the extra-vascular, extra-cellular space: the higher its volume fraction, the slower the contrast agent will equilibrate between the blood and the tissue.

Although non-specific, all these factors are related to angiogenesis and micro vascular density, malignancy, and prognosis have all been correlated with enhancement parameters. Correlations are not reliable, however, probably due to the variable effects of malignancy on vascularity and vascular permeability. In tumours, where permeability is often very high, contrast enhancement will mainly depend on perfusion regardless of the contrast agent used. However, for a particular tumour, as the molecular weight of the contrast agent increases, kinetic parameters derived from DCE-MRI change in magnitude and spatial heterogeneity, suggesting that the utility of the measurement depends on optimising the size of the agent. Several studies have shown that successful therapies may result in changes in parameters derived from DCE-MRI data in animals and humans, which may prove a more accurate and earlier indication of response than standard clinical and imaging parameters.
The signal intensity from T₂-weighted MRI depends on inherent tissue properties, and requires MRI sequences that are insensitive to local magnetic field inhomogeneity (spin echo sequences). Other types of scan (T₂*-weighted imaging) are sensitised to any local magnetic field inhomogeneity and show a reduction in signal intensity in regions of poor field uniformity. Standard Gadolinium (Gd) chelates in high concentration cause shortening of the T₂* relaxation time. Such concentrations are found in the vascular tree after bolus injection, causing a decrease in observed signal intensity. This T₂* effect reduces dramatically as leakage into the extravascular space occurs. Therefore, gadolinium chelates are sometimes considered as extravascular agents with T₁-weighted imaging and as intravascular agents for T₂* sequences.

Gd chelates are used routinely in clinical practice with T₂*-weighted imaging for cerebral perfusion studies, where the blood-brain barrier prevents leakage into the extravascular space. In tumours, breakdown of the blood-brain barrier, which is essential for standard contrast enhancement, makes the T₂* effect less consistent and more difficult to interpret quantitatively. Despite this, promising results have been obtained in brain tumours with hybrid scanning that combines T₂* (perfusion-sensitive) and T₁ (leakage-sensitive) acquisitions.

Other contrast agents are now becoming available for use in humans, and these are based around iron oxide particles in a dextran coating, giving a strong T₂* change even at low concentrations. These agents, called super-paramagnetic iron oxide particle (SPIOs) or ultra small super-paramagnetic iron oxide particles (USPIOs), may have potential as blood pool markers when used in conjunction with a dynamic MRI scan. They are already proving useful in the clinic as specific lymph node markers. In a trial of eighty patients with pre-surgical prostate cancer, high-resolution MRI with highly lymphotropic super-paramagnetic nano-particles allowed the detection of small and otherwise undetectable lymph-node metastases.

These developments, and the fact that contrast-enhanced MRI is often performed routinely in cancer patients, has led to increased interest in their use to study the effects of treatment. This has met with varying degrees of success, with some clinical studies showing that DCE-MRI with standard gadolinium chelates (using a variety of imaging and analysis methods) can successfully be used to assess different types of therapy.

The choice of pulse sequence used in DCE-MRI is usually a compromise between spatial resolution, time resolution, and spatial coverage. Achieving higher spatial resolution
requires a longer scan time for any individual image and for DCE-MRI, where multiple images are sequentially acquired, the time resolution is compromised. Good time resolution is needed to fully capture the dynamic of contrast uptake, but also with increased scan times there is the risk of patient movement during the acquisition which can result in artefacts and unusable images. The choice of pulse sequence thus depends on the expected rate of uptake, which depends on the type of tumour and also the tumour locations, since anatomical areas that are easily immobilised (such the head, limbs or breast) are more suitable for high-resolution imaging.

There are many ways of quantifying the 'enhancement' of tumours after injection of a contrast agent which include semi-quantitative analysis such as measuring the slope or peak of the enhancement curve or the area under the enhancement curve. Quantitative methods include using this data to calculate the constant that determines 'passive' transfer of the contrast agent from the blood vessel into the tissue extra-cellular extra-vascular space. This can be called the bidirectional transfer constant. This is discussed in more detail in chapter 3. The most common quantitative method in current use is called $K^{\text{trans}}$ (min$^{-1}$). In order to make measurements quantitative, it is necessary to assess the tracer concentration in both the tissue of interest, and in the artery that feeds the tissue (arterial input function). There are several methods of estimating this arterial input function for MRI, none of which are completely satisfactory.

There are also different approaches to image acquisition and with different spatial and temporal resolutions and different sensitivities to tumour heterogeneity. Figure 1.4 shows how a map of enhancement can be made by calculating $K^{\text{trans}}$ for each pixel for an immobile area in the lung apex, and also how problems occur in a moving area such as the lung-liver interface.
For such mobile areas, averaging techniques may be more appropriate. Also, for follow-up studies, tumour growth or shrinkage make comparison of these ‘parameter maps’ difficult. There is no doubt however that such parameter maps increase the available information, and the heterogeneity and variability of microcirculation within the tumour rather than the average value over the tumour may be the main factor influencing therapeutic outcome in ‘staging’ scans. 66 The vascularity, vascular permeability and interstitial pressures may be very different in areas such as the tumour rim and tumour core. This means that the mechanism of contrast enhancement (and, in particular, the rate limiting step of enhancement) will be different: for example, contrast enhancement in the tumour core may depend mainly on interstitial pressure due to poor venous and lymphatic drainage whereas in the hyper vascular rim enhancement may be related to flow. Different treatments may therefore have different effects in different parts of the tumour. 59

The lack of agreement about data acquisition and analysis methods makes comparison of results between groups difficult. It is possible that different methods will lend themselves to different agents with different mechanisms of action, and too much standardisation may stifle development. An example of this is shown in figure 1.5, where a steep enhancement curve is not fitted by the standard 2-compartment model used in the estimation of $K^{\text{trans}}$, although curve b, more typical of a liver metastasis, does. The steep part of the enhancement curve is likely to represent the contribution of the tracer in the blood pool to enhancement, not taken into account in the standard analysis of $K^{\text{trans}}$, making the analysis sensitive to changes in the blood volume. It is possible to make more complex models to account for this 67, but higher quality data is required (with better signal to noise ratio).
Furthermore, in all models, assumptions are still made about the distribution of the contrast agent or isotope, which may not prove true in tumours, this issue is discussed further in chapter 3.

**Figure 1.5 A**

*MR Images showing enhancement of a liver metastasis (arrow) over time after contrast media injection; before (baseline), and after treatment with an angiogenesis inhibitor.*
1.5 Thesis aims and structure

This thesis outlines the development, validation and clinical utilisation of a DCE-MRI method to study tumour vascularity during treatment for advanced cancer in all parts of the body. The main emphasis is on advanced cancer affecting the liver. Chapter 2 outlines the MRI imaging sequence development using imaging 'phantoms' to optimise sequence parameters. Chapter 3 uses the optimised sequence in a 'normal volunteer' study and in patients with advanced cancer but not undergoing treatment to firstly confirm the applicability of the imaging protocol in a clinical study and secondly to establish the reliability and reproducibility of the imaging protocol and several possible methods for quantifying the data. Chapters 4 and 5 involve two clinical studies where the DCE-MRI protocol is used to study the affects of PTK787/ZK222584 (PTK/ZK) on advanced cancer, particularly liver metastases.

PTK/ZK is an orally active and selective inhibitor of VEGF-receptor tyrosine kinases, VEGFR-1 (Flt-1) and VEGFR-2 (KDR), under co-development by Novartis Pharma and Schering AG, which has an anti angiogenic effect on tumours. Currently available antiangiogenic therapy (anti-VEGF-A antibody) has been shown to hinder tumour-induced angiogenesis \(^{68}\). It is therefore possible that antiangiogenesis agents that target multiple
VEGF receptors, the principal downstream mediators of angiogenesis and lymphangiogenesis, would more completely inhibit tumour growth and metastases. Chapter 5 further uses DCE-MRI contrast enhancement parameters to assess optimal dosing strategy for PTK/ZK for later trials. Chapter 6 uses the MRI image sets obtained from the clinical studies (chapters 4 & 5) to assess qualitative visual analysis of DCE-MRI enhancement. Raw data is given in appendices at the end of the chapters. Data manipulation is performed using Microsoft® Excel 2002 and SPSS® 13 for Windows. The subsequent results, other imaging strategies, and future aims, are discussed in chapter 7.
Chapter 2:

Development of the DCE-MR imaging sequence

2.1 Introduction

Ideally, DCE-MRI data acquisition should have high spatial resolution allowing mapping of enhancement parameters for the tumour in a pixel by pixel manner. This is important for gaining prognostic information about a tumour where small areas of increased tumour activity could otherwise be missed \(^{69,70}\). This approach typically involves image acquisition times of between 6 and 30 seconds for each image in the dynamic series and therefore sacrifices potentially important information relating to rapid changes in enhancement.

Most DCE-MRI studies concentrate on tumours that can be easily immobilized \(^{53-55;60;71-75}\). This is not practical for many phase 1 studies as a large percentage of metastatic disease treated by chemotherapy is in parts of the body that cannot be easily immobilised, such as liver and lung. In these areas, pixel by pixel data analysis is complicated by the need for sophisticated registration of the tumour in consecutive images. Long imaging times also involve multiple breath holds, which may be difficult for a patient with advanced cancer.

This suggests an approach with short image acquisition times that can freeze motion and an analysis based on the whole tumour as the region of interest. Although this does not provide information on tumour heterogeneity, successful treatment may be expected to have an average effect on the whole tumour. Although this technique is subject to errors due to heterogeneity of enhancement, the data is easy to obtain with good ‘signal to noise ratio’. Furthermore during treatment the enhancement changes are a reflection of the biological action in the whole tumour. Our technique therefore involves a rapid, single slice technique in a coronal oblique plane designed to ‘freeze’ patient movement and keep the measured tumour ‘in-slice’.

Although enhancement patterns may be measured in terms of changes in signal intensity over a region of interest this is not ideal as the signal intensity is not a physical parameter, but depends on the type of imaging sequence used and is on an arbitrary scale. Therefore if two images are taken at different times but from the same patient without any real change in the tumour characteristics, the intensity range seen could be markedly different.
even when using the same scanner and pulse sequence. We must therefore find ways to evaluate these enhancement curves without reference to the signal intensities directly. In order to make the assessment independent of the arbitrary intensity scale, this must be 'normalised' in some way if comparisons between different patients or patient visits are required. Two simple methods of doing this are subtracting or dividing by the baseline (pre contrast) signal \(60^{72}\). The disadvantage of the first method is that it does not take into account the 'scale' of the signal intensity changes whilst for the second method, baseline signal intensity may change in follow up studies due to physiological reasons, thereby changing the apparent enhancement. For example in a trial of cancer therapy the treatment may increase oedema in a tumour. This will increase the \(T_1\) parameter of a tissue thereby lowering the pre contrast signal intensity. If signal intensities of the enhancement curve are divided by this lower value there will be an apparent increase in enhancement. Other methods of 'normalisation' could be against an intensity standard included in the image field of view (such as a water-containing vial), or against the intensities seen in the artery that feeds the tissue. This latter approach has the advantage of also countering any variation in the dose and timing of the contrast agent injection (the arterial input function – AIF). Another major hurdle to overcome when attempting quantitative assessment is that the changes in signal intensity we observe in \(T_1\)-weighted MR images are not proportional to the concentration of contrast agent \(76^{76}\). A typical response curve is shown in figure 2.1, and it should be noted that the exact form of the response depends very much on the exact pulse sequence used, and can vary from one MRI scanner to the next even with the same nominal pulse sequence implementation.

Figure 2.1

Non-linear relationship between signal intensity and contrast agent concentration [Gd] for a \(T_1\)-weighted MRI pulse sequence.

The signal intensity saturates at high concentrations, making quantification of [Gd] difficult in the artery that feeds the tumor.
True quantification requires the signal intensities to be converted to $R_1$ values ($R_1 = 1/T_1$) since, in the case of standard Gadolinium Chelates the change in $R_1$ is proportional to the contrast agent concentration. ($R_1 = R_{10} + r_1[Gd]$ where $r_1$ is the spin-lattice ($T_1$ weighting) constant and $R_{10}$ is the relaxivity pre-contrast). However assumptions are made that the tissue micro-environment does not change this relation, that water exchange is fast \(^{77}\) and that changes in $R_2$ due to Gadolinium chelate do not significantly effect the signal intensity of the image ($R_2 = R_{20} + r_2[Gd]$ where $r_2$ is the spin-spin ($T_2$ weighting) relaxivity and $R_{20}$ is the relaxivity pre-contrast) \(^{78,80}\).

The calculation of $R_1$ from signal intensities is possible, although it is challenging to devise methods that can quantify concentration of the gadolinium-based contrast agent over the wide range seen in slowly-enhancing tumours and in the feeding arteries.

When planning a multi-centre clinical trial for patients with advanced cancer, a DCE-MRI technique that is quick, does not involve multiple breath holds and is applicable in all areas of the body has obvious advantages. A validated, straightforward way of analysing the data from DCE-MRI would allow wider application of the technique. This chapter shows the rationale for developing a single slice DCE-MRI technique and investigates different approaches to standardising the measured signal intensities, and analyzing the time course of signal intensities from a region of interest (ROI) that covers the whole tumour cross section.

### 2.2 Methods

#### 2.2.1 Pulse sequence

To achieve high temporal resolution and avoid the need for breath holding, we used scan parameters with individual image acquisition times of less than 500 ms, effectively freezing motion due to respiration. To achieve this, a repetition time (TR) of 5 ms or less is necessary for 100 phase encoding steps. $T_1$ weighting can then be created by altering the flip angle (gradient recalled echo, GRE sequence or fast low angle shot, FLASH) or applying a magnetisation preparation pulse (Snapshot FLASH, magnetisation prepared spoiled gradient echo) \(^{81}\) either with a 180° pulse (inversion recovery, IR) or a 90° pulse (saturation recovery, SR) \(^{82}\).

Signal changes using these techniques after injection of low molecular weight Gadolinium-chelates depend mainly on $T_1$ shortening and to a lesser extent on $T_2^*$.
shortening. These $T_2^*$ effects are of minor importance using a $T_1$-weighted sequence with a short time to echo (TE). Wilke et al. found a decrease in the signal intensity for an IR Snapshot FLASH with a time to echo (TE) of 3.0 ms only with concentrations above 5 mmol due to the $T_2^*$ effect, whereas peak concentrations of about 3 mmol would be expected after standard doses of Gadolinium-chelates.

The signal characteristics for these sequences can be predicted using the following equations assuming minimal $T_2^*$ effect. See appendix 2.5.1 for the derivation of these equations at the end of this chapter.

**2.2.1.1 Spoiled Gradient Recalled Echo / FLASH**

\[
\text{Signal} = \psi(C)^{m-1} + \frac{(1 - ER)((C)^{m-1} - 1)}{(C - 1)} \quad \text{[Eqn. 2.1]}
\]

Where \( ER = e^{\frac{TR}{T_1}} \), \( C = ER \cdot \cos \alpha \) and \( m = \) number of phase encoding steps to the centre of \( k \) space, \( \psi \) is the calibration constant (for a given flip angle \( \alpha = \Omega M_0 \cdot \sin \alpha \) and \( \Omega \) is a constant depending on receiver gain.

**2.2.1.2 Inversion recovery Snapshot Spoiled Gradient Recalled Echo / FLASH**

\[
\text{Signal} = \psi \left[ C^{(n-1)} \left( 1 - EI \right) \left( 1 + \frac{1 - ED(1 - C^{N_k}(1 - EI)) + \frac{ED(ER - 1)(C^{N_k} - 1)}{C - 1}}{1 + ED \times C^{N_k} \times EI} \right) \right] + \left( 1 - ER \right) \left( \frac{C^{(n-1)} - 1}{C - 1} \right) \quad \text{[Eqn. 2.2]}
\]

Where \( EI = e^{\frac{TI}{T_1}} \) and \( ED = e^{\frac{TD}{T_1}} \), \( N_k \) is the number of lines of \( k \) space and \( TD \) is the delay between the end of image acquisition and the next inversion pulse and \( TI \) is the interval from the inversion pulse to the first \( \alpha \) pulse. The sum of \( TI \), \( TD \) and \( (TR \times N_k) \) is \( TR_0 \), the time between successive inversion pulses.
2.2.1.3 Saturation recovery Snapshot Spoiled Gradient Recalled Echo / FLASH

\[ Signal = \psi \left[ (1 - e^{-\frac{T_{\text{rec}}}{T_1}})(C)^{m-1} + \frac{(1 - E R)[(C)^{m-1} - 1]}{(C - 1)} \right] \]  

[Eqn. 2.3]

where \( T_{\text{rec}} \) is the time between the saturation pulse and the first \( \alpha \) pulse.

Assuming similar noise and receiver gain settings for these sequences they are then explored for differing flip angles and values of \( \alpha, T_1, T_{\text{rec}} \) and \( T_{R0} \) for the best contrast characteristics between \( R_1 \) values of 1 and 3 s\(^{-1} \) (\( T_1 = 1 \) to 0.3 s).

2.2.2 Calculation of \( R_1 \)

In order to provide data that is comparable between patient visits, it is necessary to convert signal intensities to quantitative data. Ideally calculating \( R_1 \) does this, as changes in \( R_1 \) are proportional to changes in \([\text{Gd}]\). This can be done in a variety of ways, principally by determining the calibration constant (\( \psi = \Omega M_0 \sin \alpha, \Omega \) is a constant depending on receiver gain). If a ‘standard’ of known \( R_1 \) is placed in the imaging field this can establish these values but only in the region of the ‘standard’, which may be different to the site of tissue of interest. Other methods use variable inversion or saturation techniques or multiple flip angles but both these methods can be relatively time consuming. Our method is by acquiring an image without the inversion pulse applied, after the contrast enhanced run, removing a major component of \( T_2 \) weighting. This is the \( S_0 \) signal and we approximate it to \( \psi \), the calibration constant.

The ratio of signal intensities with (\( S \)) and without (\( S_0 \)) the inversion pulse is then related to the \( R_1 \) of the tissue by Eq. 2.2. It can be seen that for equation 2.2 if the time between successive inversion pulses increases (\( T_{R0} \to \infty \)) and the flip angle (\( \alpha \to 0 \)), then Eq. 2.2 simplifies to Eq. 2.4, from which \( R_1 \) may be determined, where \( T_{\text{eff}} \) is the time from the inversion pulse to the centre of \( k \) space.

\[ Signal = S_0 \left( 1 - 2e^{-\frac{T_{\text{eff}}}{T_1}} \right) \]  

[Eqn. 2.4]

We used an inversion pulse for magnetisation preparation; this pulse was non slice selective to minimise the effects of through-plane motion and blood inflow. The parameters of the sequence used were: \( TR = 3.3 \) ms, echo time (TE) = 1.4 ms, flip angle
(α) = 8°, k space matrix 100 phase encoding steps × 128 points in the readout direction, inversion time (TI) = 655 ms, and time between successive inversion pulses (TR₀) = 3000 ms.

2.2.3 Phantom Study

This approach to $R_1$ estimation was tested in a phantom study. The phantom consisted of vials of water doped with different concentrations of Gd-DTPA giving a range of $R_1$ values from 0.4 to 10 s⁻¹. The $R_1$ values were accurately measured using an IR-prepared turbo Spin Echo (TSE) sequence with TE=10ms, TR=5000ms, echo train length = 5, 128x128 matrix, 1.56 mm in plane resolution, 5 mm slice thickness and inversion times of 23, 123, 323, 723 and 1520 ms. Phantom work was done using a Siemens Symphony 1.5 T system (Siemens, Erlangen, Germany). Based on Eq. 2.2 this sequence effectively nulls signal at an $R_1$ of 0.53 s⁻¹. Where $R_1$ was less than 0.53 s⁻¹, the positive signal of the magnitude images was inverted. In a DCE-MRI study, rectified signal can be recognized if the signal reduces in the first few images of contrast enhancement. The estimated $R_1$ values, using the chosen DCE-MRI sequence, and Eqn. 2.2 & 2.4, were compared with the accurately measured $R_1$ values.

2.3 Results

2.3.1 Choosing the sequence

The optimal contrast characteristics for the FLASH, Snapshot FLASH with saturation recovery and inversion recovery sequences (SR- and IR- FLASH) with image acquisition of ~ 500 ms are given by figure 2.2 and table 2.1.
Figure 2.2
The optimal contrast characteristics for the FLASH (GRE), Snapshot FLASH with saturation recovery and inversion recovery sequences (SR- and IR- FLASH) with an image acquisition time of ~ 500 ms for (a) R₁ of 0-10 s⁻¹ and (b) R₁ of 1 to 3 s⁻¹.
Table 2.1

Parameters to achieve optimal contrast-noise between $R_1$ values of 1 to 3 s$^{-1}$.

<table>
<thead>
<tr>
<th></th>
<th>Flip angle $\alpha$</th>
<th>$T_{rec}$</th>
<th>$T_{rec}$</th>
<th>TI</th>
<th>TI eff</th>
<th>TR$_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLASH</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR-FLASH</td>
<td>13</td>
<td>90 ms</td>
<td>340 ms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR-FLASH</td>
<td>10</td>
<td></td>
<td></td>
<td>650 ms</td>
<td>900 ms</td>
<td>3000 ms</td>
</tr>
</tbody>
</table>

Another advantage of 'non slice-selective' magnetization-prepared sequences such as snapshot FLASH is that they have less inflow effects $^{78}$. This is shown by comparing enhancement curves using snapshot FLASH and FLASH (standard gradient recalled echo sequence (GRE) as shown in Figure 2.3.

Figure 2.3

*Signal intensity readings of the abdominal aorta after injection of contrast using a Snapshot FLASH (IR) sequence and a FLASH (GRE) sequence showing greater variability due to inflow effects from systole and diastole for the FLASH (GRE) sequence.*

For this project the inversion recovery snapshot FLASH sequence was chosen. The next sections concern the optimisation of this sequence.
2.3.2 Choosing the Flip Angle

In order to quickly calculate $R_1$ it is helpful to know proton density. This could be done by a long acquisition with long TR, short TE and Flip angle of 90°. Alternatively a similar approach calculates $R_1$ at baseline by using multiple flip angles. For inversion recovery (or saturation recovery) if the flip angle is low enough the effect of flip angle could potentially be ignored and $T_1$ could be calculated based on equation 2.4. However as the flip angle is reduced signal (based on $\Omega M_0 \sin \alpha$) reduces.

Figure 2.4 shows how the IR sequence using a flip angle of 4, 8 and 12 performs. The $S_0$ line (based on the signal achieved without the inversion pulse) should be as flat as possible (i.e. have no $T_1$ weighting). A flip angle of 8 gives a compromise of signal / noise and a $S_0$ trace proportional to proton density (i.e. flat).

Figure 2.4
A comparison of the relative signal intensities achieved for increasing $R_1$ for 3 different flip angles.
2.3.3 Choosing the Inversion Time (TI)

Liver tumours have a baseline $T_1$ value of approximately 1.4 seconds ($R_1 = 0.71$)\(^8\). If the TI is too short then contrast enhancement will result in an initial reduction in signal requiring allowance for phase, which is problematic for rapidly enhancing tumours. Figure 2.5 shows the performance of an IR sequence with $\alpha = 8$, $TR = 3.5$ ms, and varying effective TI. An effective TI of 850 ms is the shortest time that gives positive enhancement for $R_1$ values greater than $0.7s^{-1}$ ($T_1 <1.4$ s).

Figure 2.5
*A comparison of the relative signal intensities achieved for increasing $R_1$ for different effective inversion times (TI) from 400 to 1000 ms.*

2.3.4 Pulse Sequence, Quantification of $R_1$ and Phantom study

Figure 2.6 shows simulated signal intensities for the IR snapshot FLASH sequence, using Eqn. 2.2 and the simplified Eqn. 2.4 for the sequence described above.
Although there is considerable difference between the signal intensities predicted by the two expressions the ratio of $S$ to $S_0$ remains similar and so Figure 2.7 shows that $R_1$ can be estimated accurately for $R_1$ values between 1 and 5 s$^{-1}$ using either Eqn. 2.2 or Eqn. 2.4. The marked error occurring at $R_1$ values above 5 s$^{-1}$ is due to almost complete relaxation during the TI interval when $S$ approaches $S_0$, as shown in Figure 2.6.
Thus, with this sequence, selection of sequence parameters to accurately measure relatively short $R_1$ compromises the ability to measure long $R_1$ values accurately. The chosen $T_{I\text{eff}}$ is therefore a compromise based on the pre-contrast and range of $R_1$ seen in tumours during DCE-MRI.
2.4 Discussion

There are many potential sources of error using this sequence to calculate $R_1$<sup>78</sup> but DCE-MRI using an inversion recovery snapshot FLASH sequence with a relatively long $T_{I eff}$ of 815 ms and an interval of 3 seconds between subsequent images, provides a rapid, reasonably accurate measure of $R_1$ for values between 1 and 5 s<sup>-1</sup>, and therefore of Gd-DTPA concentration during contrast enhancement.

Using a long $T_{I eff}$ is helpful as it provides good contrast to noise for low levels of enhancement such as may occur in necrotic tumours. However, this has the disadvantage that arterial contrast concentration cannot be accurately measured due to rapid relaxation during the long $T_{I eff}$ period. Sequences designed to measure blood concentration of contrast agent<sup>88</sup> have a very short $T_{I eff}$ (150 ms), which may show little or no enhancement in necrotic tumours since they often have a relatively short pre-contrast $R_1$. Measurement of arterial Gd-DTPA concentration (AIF) is considered important when assessing absolute values of tumour microcirculation<sup>76</sup>. However, the AIF may not be so important for measuring a treatment effect in patients, unless there is a change in cardiac function or haemodynamics on therapy.

The simplified equation (Eqn. 2.4) used to convert signal intensities into $R_1$ values does not take into account the signal saturation due to successive inversion pulses. However, with a low flip angle ($8^\circ$) and $T_{R0}$ of 3 seconds, this equation still allows an acceptable estimate of $R_1$ to be made (Figure 2.7). If the $T_{R0}$ were to be shortened further, in order to increase temporal resolution, a previous study<sup>85</sup> has shown that the estimated $R_1$ would become progressively more inaccurate and equation 2.2 would be required to estimate $R_1$. Imaging of tumours with a short pre-contrast $R_1$ can be a problem with this sequence since, in some cases enhancement may be negative in the first few images of the dynamic series. In a DCE-MRI study, rectified signal can be recognized if the signal reduces in the first few images of contrast enhancement. This problem could be overcome by using saturation recovery rather than inversion recovery, at the expense of lower contrast at shorter $R_1$ values.
2.5 Appendices

2.5.1 Derivation of the relationship of MR signal intensity to changing $R_1/T_1$ assuming minimal effect from transverse magnetization.

2.5.1.1 Spoilt Gradient recalled echo sequence or fast low angle shot (FLASH)

Initially the relationship of signal intensity to changing $T_1$ will be derived for a single train of alpha pulses. This will then be modified for magnetisation prepared sequences where the alpha pulse train starts at a modified longitudinal magnetisation. This sequence consists of a series of alpha pulses with different phase encoding to acquire the image, with a spoiling gradient applied to remove any persisting phase coherence between alpha pulses. The time between alpha pulses is the repetition time (TR). The contrast characteristics of the image depend on the signal intensity when phase encoding gradients are used relating to the centre of $k$ space. For the sequences described the centre of $k$ space is acquired half way through the alpha pulse run (phase encoding steps). So for $N_k$ lines of $k$ space the centre $(m)$ will be after $N_k/2$ steps.

The MR signal ($S$) is related to the longitudinal magnetization given by the equation A1 where $Mz_{(n)}$ = longitudinal magnetization at the nth line of $k$ space, $\alpha$ is the flip angle of the alpha pulse and $\Omega$ is the constant depending on receiver gain:

$$ S = Mz_{(n)} \cdot \sin \alpha \cdot \Omega \quad [A1] $$

After an alpha pulse ($\alpha$) the longitudinal magnetization ($Mz$) changes:

$$ Mz^+_{(n)} = Mz^-_{(n)} \cdot \cos \alpha \quad [A2] $$

$Mz$ will then return to equilibrium ($M0$) in the manner$^{89}$:

$$ \frac{\delta Mz}{\delta t} = -\frac{(Mz - M0)}{T_1} \quad [A3] $$

$Mz^+_{(n+1)}$ after the interval TR is therefore given by:

$$ \int_{Mz^-_{(n)}}^{Mz^+_{(n+1)}} \frac{1}{(Mz - M0)} \cdot \delta Mz = -\frac{1}{T_1} \int_0^{TR} 1 \cdot \delta t = -\frac{TR}{T_1} \quad [A4] $$
So \[ -\frac{TR}{T_1} = \ln(Mz'(n+1) - M0) - \ln(Mz'(0)\cos\alpha - M0) \] \[ \text{[A5]} \]

So \[ e^{\frac{TR}{T_1}} = \frac{Mz'(n+1) - M0}{Mz'(0)\cos\alpha - M0} \] \[ \text{[A6]} \]

So \[ Mz'(n+1) = [Mz'(0)\cos\alpha e^{\frac{TR}{T_1}} + M0(1 - e^{\frac{TR}{T_1}})] \] \[ \text{[A7]} \]

At steady state $Mz'(n+1) - Mz'(0)$ approaches zero, therefore the $Mz$ of a spoiled gradient echo sequence when steady state has been achieved can be calculated:

\[ Mz = M0.\frac{(1 - e^{\frac{TR}{T_1}})}{(1 - \cos\alpha e^{\frac{TR}{T_1}})} \] \[ \text{[A8]} \]

This however does not apply if steady state is not achieved by the time the centre of $k$ space is read. In this case the signal intensity will be related to the signal available immediately prior to the centre ($m^{th}$ line) of $k$ space. Solving equation [A7] for subsequent lines of $k$ space can be simplified to the recursive formula, which has been described previously:

\[ Mz(n) = [Mz(1)\cdot(C)^{n-1} + M0.(1 - ER)\sum_{0}^{n-2}(C)^{i}] \] \[ \text{[A9]} \]

or \[ Mz(n) = [Mz(1)\cdot(C)^{n-1} + \frac{M0.(1 - ER)[(C)^{n-1} - 1]}{(C - 1)}] \] \[ \text{[A10]} \]

Where $ER = e^{\frac{TR}{T_1}}$ and $C = ER\cos\alpha$

The available signal at the centre of $k$ space ($m^{th}$ line) can therefore be written as:

\[ S = M0.\sin\alpha.\Omega.\frac{[(C)^{m-1} + (1 - ER)[(C)^{m-1} - 1]]}{(C - 1)} \] \[ \text{[A11]} \]

The product of $\Omega M0.\sin\alpha$ is the calibration constant (for a given flip angle $\alpha$) and can be expressed as `$\psi$'.
2.5.1.2 Saturation recovery spoilt Gradient recalled echo

$T_1$ contrast can be further modified by applying a magnetization preparation pulse (saturation pulse of 90°) a defined time ($T_{rec}$, recovery time) prior to the alpha pulse train of phase encoding steps. The effect of the saturation pulse is always to make $M_z = 0$.

Therefore after a 90° saturation pulse, $M_z$ is given by the equation:

$$M_z^+ = M_z' \cos \alpha = 0. \quad [A12]$$

Using the same steps as above, $M_z(t)$ after an interval $T_{rec}$ is given by:

$$M_z(t) = M_z(t) - M_0 \left[ e^{-\frac{T_{rec}}{T_1}} \right] \quad [A13]$$

So

$$-\frac{T_{rec}}{T_1} = \ln(M_z(0) - M_0) - \ln(0 - M_0) \quad [A14]$$

And

$$e^{\frac{T_{rec}}{T_1}} = \frac{M_z(0) - M_0}{-M_0} \quad [A15]$$

And

$$M_z^-(t) = M_0 \left(1 - e^{-\frac{T_{rec}}{T_1}} \right) \quad [A16]$$

As for spoiled gradient echo, signal will be modified by the alpha pulse train as per equation [A10] where equation [A16] can be inserted as the 1st value $M_z(t)$. The available signal at the centre of $k$ space ($m^{th}$ line) for a saturation recovery sequence can therefore be written as:

$$S = M_0 \sin \alpha \Omega \left[ (1 - e^{-\frac{T_{rec}}{T_i}})(C)^{m-1} + \frac{(1-ER)[(C)^{m-1}-(C-1)]}{(C-1)} \right] \quad [A17]$$

2.5.1.3 Inversion Recovery Sequence spoilt Gradient recalled echo.

$T_1$ contrast can be also be modified by applying a different type of magnetization preparation pulse; an inversion pulse of 180°, a defined time ($TI$, time from inversion) prior to the alpha pulse chain of phase encoding steps. The effect of the inversion pulse is to make $M_z^+ = -M_z^{-}$ (reverse the polarity). Following the process above $M_z(t)$ after an interval TI is given by:
\[
\frac{Mz^{(0)} - M0}{(Mz - M0)} = \frac{1}{T1} \int \delta(t) dt = -\frac{TI}{T1}
\]  

[Equation A18]

So

\[
-\frac{TI}{T1} = \ln(Mz^{(0)} - M0) - \ln(-M0 - M0)
\]  

[Equation A19]

And

\[
e^{\frac{TI}{T1}} = \frac{Mz^{(0)} - M0}{-2M0}
\]  

[Equation A19]

And

\[
Mz^{(0)} = M0(1 - 2e^{-\frac{TI}{T1}})
\]  

[Equation A20]

As for gradient echo, signal will be modified by the alpha pulse train as per equation [A10]. Saturation recovery is not complicated by the time between magnetization preparation pulses and subsequent alpha pulse trains. However for inversion recovery if TI is short relative to T1, the interval between inversion pulses (TR0) must be considered. These issues have been addressed in a previous paper (Jivan et al) and the ‘steady state’ reduction in Mz can be given as:

\[
Mz^{(0)} = M0\left[1 - 2e^{-\frac{TR0}{T1}}\right]\sin \alpha
\]  

[Equation A21]

As can be seen if TR0 is large relative to T1 eqn. [A21] tends to eqn. [A20].

Mz is also affected by the effect of the previous alpha pulse train and this has also been taken into account in the Jivan study showing available signal at the centre of k space (mth line) for a saturation recovery sequence can be written as:

\[
S = M0 \sin \alpha \Omega \left[ \frac{C^{(m-1)}}{C^{(m-1)} - 1} \left( 1 - EI \left( 1 + \frac{ED(1 - C^{Nk}(1 - EI)) + ED(ER - 1)(C^{Nk} - 1)}{C^{Nk} - 1} \right) \right) \right] + (1 - ER) \frac{C^{(m-1)} - 1}{C - 1}
\]  

[Equation A22]

where ER = \(e^{-\frac{TR}{T1}}\), EI = \(e^{-\frac{TI}{T1}}\), ED = \(e^{-\frac{TD}{T1}}\), C = ER \cos \alpha, Nk is the number of lines of k space (phase encoding steps), and TD is the delay between the end of image acquisition and the next inversion pulse.
The sum of TI, TD and (TR×Nk) is TRo, the time between successive inversion pulses. As TRo → ∞ and α → 0, then equation [A22] simplifies to equation [A20] except TI_eff is the time from the inversion pulse to the centre of k space not the start of the alpha pulse train, this can then be presented for MR signal thus:

\[ S = M_0 \sin \alpha \Omega \left(1 - 2e^{-\frac{TI_{\text{eff}}}{T_1}}\right) \]  

[A23]

Appendix 2.5.2 Data from phantom study.
Twenty-four vials of distilled water doped with increasing concentration of Gadolinium Chelate. Accurate R1 was measured by IR-prepared turbo spin echo and the signal intensities where measured using the described sequence from which R1 values were estimated using equations 2.2 and 2.4.

<table>
<thead>
<tr>
<th>Accurately measured R1 (see text)</th>
<th>Signal Intensities</th>
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</table>
Chapter 3:
Validation of a snapshot FLASH DCE-MRI imaging protocol to assess tumour contrast enhancement in advanced cancer

3.1. Introduction

The previous chapter describes a DCE-MRI technique that is quick, does not involve multiple breath holds and is applicable in all areas of the body. This technique may prove useful in multi centre trials of cancer therapies. This sequence is capable of showing the contrast enhancement parameters of normal tissues and tumours at high temporal resolution. As stated in chapter 1 there are numerous methods of evaluating contrast enhancement parameters.

In order to plan clinical studies it is important to know the inherent variability of the results from a DCE-MRI approach in order to assess the significance of individual changes, and to use power statistics to determine the cohort sizes required to give statistical significance for a desired treatment effect. In this chapter two clinical studies are described. Firstly the chosen imaging sequence was used to test the utility of this technique for assessing liver and spleen enhancement in normal volunteers. Spleen enhancement is a useful gauge of the dynamic range of the sequence as it has a pre-contrast $T_1$ value similar to liver metastases and has rapid enhancement. Liver enhancement is used to assess the reproducibility of different analysis methods, assuming normal liver enhancement is similar in the volunteers. Secondly the chosen imaging sequence was performed in cancer patients on 2 occasions one week apart, without treatment, in order to assess reproducibility of DCE-MRI data with different approaches of standardising the measured tumour signal intensities, and of analysing the time course of signal intensities from a region of interest (ROI) that covers the whole tumour cross section.
3.1.1 Quantification of DCE-MRI

Simple methods of analyzing the data include measuring peak enhancement or the peak slope of the enhancement curve. As the concentration in the feeding artery is constantly changing the peak enhancement depends on different physiological parameters at different times. The peak enhancement after a minute will depend mainly on the perfusion and permeability of the vasculature whilst after 10 minutes the peak enhancement may depend more on the extravascular extracellular space. Peak slope can also be a problem as, in rapidly enhancing tumours, it may be difficult to define and, in heterogeneous tumours, there may be more than one component to the initial slope. Perhaps the simplest semi-quantitative approach to assessment of contrast dynamics is to evaluate the area under the enhancement curve out to a certain fixed time after contrast injection. This measurement has no direct physiologic meaning but is a robust measurement that depends on the vascularity of the tumour in its broadest sense. This is often termed the initial area under the enhancement curve (IAUC). Unless the signal intensities have been ‘normalised’ in some way, such as by being converted to R1 values as in chapter 2, it is usual to divide the IAUC by the area under the enhancement curve for a feeding artery (arterial input function, AIF) out to the same time point. In fact any ideal analysis method must be able to take the AIF into account, although in follow up studies, where relative changes from pre treatment values are important, there may be less need to measure the AIF.

A more sophisticated approach attempts to quantify the perfusion/permeability in terms of a non-specific leakage rate constant and also the extra-vascular extra-cellular space volume fraction. These methods make a few assumptions. Firstly, that the imaged tissue is composed of compartments, the vascular space, the extracellular extravascular space and intracellular space (as in figure 1.3). As contrast does not enter red blood cells and tissue cells in normal cells these spaces can be simplified to a two compartment model consisting of the vascular plasma space and the extracellular extravascular space, these spaces can be given as their fractional volumes \( v_p \) (plasma space is the vascular space less the haematocrit), \( v_e \) (the fractional extracellular extravascular space) and \( v_i \) (the fractional volume of the intracellular space) so \( v_p + v_e + v_i = 1 \) \(^{90}\). Other assumptions include that there is no active distribution mechanism and therefore flux depends on concentration gradients \(^{91}\).

The distribution from the vascular to the extravascular compartment can then be described by the difference in the concentration in these compartments modified by a coefficient,
which depends on the permeability (leakiness) of the capillary or venule membrane and the effective surface area of this membrane available for distribution. Again this is a simplification. In chapter 1 the various mechanisms of permeability are discussed implying that overall leak is a combination of different mechanisms of distribution. Further the available surface area is modified by potential restriction in perfusion and therefore flow of tracer across the membrane.

Currently, there is no complete consensus of the best method of applying this model, although many studies use similar methods to estimate the transfer constant for Gd-chelate as it passes into the interstitial space including $K^{\text{trans}}$, $K_i$, $K_{ep}$ and $K_2$. The imaging community is working to develop uniformity of imaging and analysis protocols, but this will remain difficult until successful treatments are available by which to assess the utility of different analysis methods in different tumours and organ systems.

Currently the most commonly used method is to calculate the bi-directional transfer constant $K^{\text{trans}}$ using a two compartment model.

$$\frac{dC_t}{dt} = K^{\text{trans}} \left( C_p(t) - \frac{C_t(t)}{v_e} \right) \quad \text{Eqn 3.1}$$

where $C_p$ is the concentration of contrast agent in the blood plasma (i.e., the AIF); $C_t$ is the concentration of contrast agent in the tissue, averaged over the whole tissue; $K^{\text{trans}}$ is the transfer constant; and $v_e$ is the tissue extra-vascular extra-cellular space volume fraction.

The solution to Eqn. [3.1] is (ignoring the vascular component of tissue contrast concentration):

$$C_t(t) = K^{\text{trans}} \int C_p(\tau) e^{-\frac{K^{\text{trans}}(t-\tau)}{v_e}} d\tau \quad \text{Eqn 3.2}$$

Knowledge of $C_p(t)$ (either through measurement or estimation by other means) and measurements of $C_t(t)$ allows $K^{\text{trans}}$ and $v_e$ to be estimated. The vascular component of tissue contrast concentration is often ignored as it is considered to be low in normal tissue and may be underestimated due to $T_2^*$ effects due high tracer concentrations. In tumours this may not be a ‘safe’ assumption and the model can be expanded to include the vascular component of tissue contrast concentration, but this requires better quality data (see figure 1.5, chapter 1).
Equation 3.2 can be simplified by assuming that there is only a uni-directional flux of contrast in the early part of contrast enhancement. This approach can only be used for the early part of the contrast enhancement curve.

The process of calculating $K_{\text{trans}}$ and $v_e$ from a tissue enhancement curve and an AIF curve enables any variability in the AIF to be accounted for, with the result that $K_{\text{trans}}$ and $v_e$ are parameters that are interpretable in a physically-meaningful way, independent of the technique used.

Under certain idealized circumstances of a very short, tight bolus injection of contrast, the value of $K_{\text{trans}}$ is numerically the same as the ratio of the initial upslope of the tissue enhancement curve to the instantaneous contrast agent concentration in the plasma. However, as the signal intensities change rapidly in the artery, and certain tumours can also enhance rapidly; acquiring images fast enough to measure these slopes and peaks accurately is difficult.

For the clinical studies in chapters 4 & 5 the method described by Larsson et al was used originally using the term $K_i$ in units of mls/100g/min. This has a linear relationship to the value $K_{\text{trans}}$ which is becoming a more accepted term. As the results are presented as relative changes, and $K_i$ is linearly proportional to $K_{\text{trans}}$, results will be expressed as $K_{\text{trans}}$ for the rest of this text for consistency.

### 3.2 Methods

#### 3.2.1 Pulse sequence

To achieve high temporal resolution and avoid the need for breath holding, the scan parameters chosen in Chapter 2 have an image acquisition time of less than 500 ms, effectively freezing motion due to respiration. The inversion pulse for magnetisation preparation (Snapshot FLASH) was non slice selective to minimise the effects of through-plane motion and blood inflow. Since respiratory motion in the abdomen and lower part of lung is largely in the cranio-caudal direction, a coronal or sagittal oblique plane was used to keep the tumour within the imaged slice during the dynamic run. The DCE-MRI sequence was then run with 100 measurements, 3 seconds apart (TR$_o$=3000ms). The parameters of the sequence used were: TR = 3.3 ms, echo time (TE) = 1.4 ms, flip angle ($\alpha$) = 8°, $k$ space matrix 100 phase encoding steps $\times$ 128 points in the readout direction, inversion time (TI) = 655 ms. Low molecular weight Gadolinium-chelate 0.1
mmol/kg (Omniscan, Nycomed) was injected as a rapid bolus through an arm vein in less than 5 seconds. Injection commenced after the first 4 measurements to allow magnetization to reach a steady state. At the end of the dynamic run, the sequence was repeated with the inversion pulse switched off to acquire the $S_0$ image.

### 3.2.2 Reproducibility in Normal Volunteers

Four volunteers had a DCE-MRI scan. All were informed about the investigational nature of the study according to institutional and regional guidelines, and subsequently gave informed consent prior to start of the study. Permission of local ethics regulatory bodies was obtained.

All had an approximately 4 hours fast. All MR images were acquired using a 1.5 Tesla whole body magnet equipped with 25 mTm$^{-1}$ gradient coils and phased array surface coils (Siemens Magnetom Vision, Erlangen, Germany). A single axial slice was planned to include liver, spleen and aorta. The DCE-MRI sequence was then run with 100 measurements, 3 seconds apart ($TR_0=3000$ms) as described above. At the end of the dynamic run, the sequence was repeated with the inversion pulse switched off to acquire the $S_0$ image. Signal intensities were taken from regions of interest drawn around the liver and spleen avoiding blood vessels and aorta separately using an image analysis package, Analyze™ (Mayo clinic, Rochester, MN, USA).

### 3.2.3 Reproducibility in metastatic tumours

Eleven patients with advanced cancers, including colorectal cancers with liver metastases, were recruited as part of two ‘phase I’ trials. Patients had two DCE-MRI scans one week apart, without treatment. All patients were informed about the investigational nature of the study according to institutional and regional guidelines, and subsequently gave informed consent prior to start of the study. Permission of local ethics regulatory bodies was obtained.

All MR images were acquired using a 1.5 Tesla whole body magnet equipped with 25 mTm$^{-1}$ gradient coils and phased array surface coils (Siemens Magnetom Vision, Erlangen, Germany). All patients underwent standard transverse breath hold spoilt gradient recalled echo (GRE) $T_1$ and turbo spin echo (TSE) $T_2$-weighted imaging of the tumour region (GRE, typically $TR = 150$ ms, $TE = 4$ ms, $\alpha = 70^\circ$ and matrix 256 x 256, TSE typically $TR = 5000$ ms, $TE = 90$ ms, $\alpha = 90^\circ$, echo train length = 23). In regions where respiratory motion was a factor, the $T_1$ weighted multi-slice GRE examination was repeated during
gentle respiration to improve slice positioning for the dynamic series, which was also performed during gentle respiration. Since respiratory motion in the abdomen and lower part of lung is largely in the cranio-caudal direction, a coronal or sagittal oblique plane was used to keep the tumour within the imaged slice during the dynamic run. A representative disease site was selected (target lesion) and a single coronal oblique slice was planned to bisect the midline of the tumour and a major blood vessel (usually aorta). This became the imaged tumour for all subsequent scans. The DCE-MRI sequence was then run with 100 measurements, 3 seconds apart (TR0=3000ms) as described above. At the end of the dynamic run, the sequence was repeated with the inversion pulse switched off to acquire the $S_0$ image.

Signal intensities were taken from regions of interest drawn around the tumour and a major blood vessel (e.g. aorta) separately using an image analysis package, Analyze™ (Mayo clinic, Rochester, MN, USA). For the tumour, the ROI was drawn based on the pre-contrast images after review of the contrast-enhanced images so that the whole tumour was included but large blood vessels and non tumour tissues were avoided. In liver metastases the ROI was not extended if there was abnormal contrast enhancement outside the area of abnormality on the pre-contrast image. This is because abnormal tumour ‘rim enhancement’ has been associated with non cancerous tissue, possibly due to compression or local angiogenic factors. For the major blood vessel, the ROI was drawn based on the peak arterial enhancement phase of imaging. The positions of the ROIs were corrected on a time point by time point basis for any movement during the dynamic run. The shapes of the ROIs were not changed throughout the dynamic run. The maximum diameter and appearance on $T_1$- and $T_2$-weighted images of the target lesion was recorded. The slice position for the dynamic run was checked on both $T_1$- and $T_2$-weighted images to ensure consistency between scans and care was taken to ensure the ROIs had the same anatomical positions and sizes for both scans.

3.2.4 Analyses of Signal Intensities

For both studies the signal intensities were pre-processed in 3 forms as summarised in Table 3.1 (below). First, the raw signal intensities were used unchanged. In this form, any variability caused by different coil sensitivity or receiver gain on the two visits, or by different contrast bolus characteristics remained uncorrected. Secondly, the signal intensities were divided by the initial area under the arterial enhancement curve (IAUC-A) for a defined period of time. The IAUC-A was calculated by first subtracting the average
pre-contrast signal intensities in the artery from the arterial time series, then using the trapezium rule to calculate the total area under the resulting change in enhancement out to times of 60 and 180 seconds (IAUC-A[60] and IAUC-A[180]) after contrast injection. Thirdly, the signal intensities were converted to $R_1$ values using Eqn. 2.4 (chapter 2) and the $S_0$ image collected after the end of the dynamic run.

These three data sets were then used to calculate peak enhancement (PE) and the initial area under the enhancement curve for the tumour, calculated as above, for 60 and 180 seconds (IAUC[60] and IAUC[180]). Since the imaging parameters are optimized for tumour rather than arterial enhancement, it was not possible to calculate $R_1$ accurately for the arterial ROI ($C_p(t)$). $K_{trans}$ was therefore measured in two ways: first using the raw signal intensities from the tumour and arterial ROIs, and secondly using the $R_1$ values from the tumour, and a standard data set for the arterial input function (AIF).

This standardised AIF was based on cases performed using the above sequence and previous work $^{84,88}$ to establish average first pass kinetics for the dose of contrast agent used. This was also compared with a simple model of contrast kinetics as shown in equation 3.3 $^{92}$:

$$C_p(t) = D(3.99e^{-0.144t} + 4.78e^{-0.111t})$$  \hspace{1cm} \text{Eqn 3.3}$$

where $D$= gadolinium chelate dose in mmol/kg and $t$= time in minutes since injection.

The standard AIF generated is shown in figure 3.1. The timing of the start of this standard arterial input function (AIF) was measured from the artery imaged in the study.
In clinical DCE-MRI, measurement of $S_0$ at every time point would result in unacceptably low temporal resolution. Therefore, we performed a single measurement of $S_0$ at the end of the dynamic run, by acquiring an image without the inversion preparation pulse. Eq. 2.2 shows that measured $S_0$ depends on $R_1$ as there is still some $T_1$ weighting, due to spin relaxation during the TR intervals, even when $T_1 \to \infty$ (the equivalent of turning the inversion pulse off). A single reading of $S_0$ therefore produces inaccurate estimates of $R_1$ when $R_1$ differs from its value at the time when $S_0$ is measured. An estimate of the maximum error introduced by the assumption of a constant $S_0$ was made using estimates of $R_1$ pre-contrast and at maximum enhancement. Eq. 2.2 was used to predict the true $S_0$ at each of these time points. $R_1$ calculations were then performed using Eq. 2.4, firstly with the single measured $S_0$ value and second using the true $S_0$ values (corrected for $R_1$). This error analysis was performed for typical enhancement curves seen in the clinical study.

### 3.2.5 Statistical analysis

DCE-MRI is applied to assess changes caused by treatment, with patients acting as their own controls. Our work has shown a wide inter-patient coefficient of variation (CoV) of 61% in pre-treatment values for $K_{\text{trans}}$, equivalent to $K_{\text{trans}}$. CoV is equivalent to the standard deviation of a group expressed as a percentage of the mean. This considerable variability in the pre-treatment enhancement of tumours makes the percentage rather than
absolute changes of enhancement parameters more amenable to analysis. Statistical analysis was therefore performed on the percentage changes of the observed parameter between the two scans. The percentage change data was tested for normality of distribution by the Shapiro-Wilk test. The mean change and CoV were calculated and expressed as percentages\textsuperscript{101}. Assuming that the post treatment variability is similar, the CoV can be used to assess the statistical power of studies, or anticipate the patient numbers required for a study to demonstrate a given degree of treatment effect. The repeatability value was also evaluated\textsuperscript{101}, which is defined as the range within which 95% of measurements will fall, assuming no treatment effect, and is therefore helpful in assessing the significance of individual patient results.

3.3 Results

3.3.1 Reproducibility in Normal Volunteers

Figure 3.2 shows a typical normal splenic enhancement curve. This shows that the temporal resolution of the sequence, and its dynamic range, are suitable for imaging of the spleen. As stated in the introduction this is a good model for metastatic disease.
Figure 3.3 shows four contrast enhancement curves for normal liver presented as signal intensities and as $R_1$. The graphs show that for this imaging system converting the signal intensity data to $R_1$ values improves the consistency of the enhancement curves both in terms of the pre-contrast value and the scaling of the enhancement curve.

![Figure 3.3](image1.png)

**Figure 3.3**
Contrast enhancement curves for four normal livers presented as signal intensity (a) and $R_1$ (b) against time.

![Figure 3.3](image2.png)

Table 3.1 shows the CoV (%) of the studied enhancement parameters for four liver ROIs. This gives a reflection of the reproducibility of the result with a lower score representing improved reproducibility.
Table 3.1

COV(%) of the studied enhancement parameters for four liver ROIs.

PE = Peak Enhancement. IAUC[t] = Initial area under the tumour contrast enhancement curve for first t seconds. IAUC-A[t] = Initial area under the arterial contrast enhancement curve for first ‘t’ seconds.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CoV (%)</th>
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<tbody>
<tr>
<td><strong>Un-Scaled Signal Intensity Data</strong></td>
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<tr>
<td>PE</td>
<td>33.2</td>
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<td>IAUC[180]</td>
<td>33.8</td>
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<tr>
<td><strong>Signal Intensity related to measured arterial input function</strong></td>
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<tr>
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<tr>
<td>κ_{trans}</td>
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</table>

The normal volunteer study therefore suggests that ‘normalising’ data to the AIF or preferably R₁ improves consistency of the results for normal liver. The improved consistency for R₁ over using AIF may be due to the fact that this sequence is not optimised for measuring the large changes in enhancement seen in the feeding artery (abdominal aorta).

### 3.3.2 Reproducibility in metastatic tumours

Of the eleven patients recruited, one patient was excluded due to incorrect positioning of the imaging slice on the second scan. Despite breathing motion, it was possible to maintain the size and shape of the tumour and arterial ROI in all remaining cases. Although all slices included a major artery (aorta or iliac artery), ghosting from motion artefact in the phase-encoded direction was not observed. The minimum observed pre-contrast $R₁$, averaged over the whole ROI, was $0.88 \, \text{s}^{-1} \, (T₁ = 1.14 \, \text{s})$. The maximum $R₁$ was $3.4 \, \text{s}^{-1} \, (T₁=0.3 \, \text{s})$.

From Fig. 3.4, it can be seen that the smoothness of the curves improves with increasing tumour size. Since $S₀$ is not continuously measured as $R₁$ changes, errors are introduced in
the dynamic measurement of $R_1$, which can be estimated as described earlier. For lesion A (pre-contrast $R_1 = 1 \text{ s}^{-1}$, maximum $R_1 = 3.4 \text{ s}^{-1}$, and $R_1$ at $S_0$ signal acquisition = 3 s$^{-1}$), the error in the maximum change of estimated $R_1$ (2.4 s$^{-1}$) is 0.13 s$^{-1}$ or 5.4%. For lesion B (gradually increasing enhancement throughout, pre-contrast $R_1 = 1 \text{ s}^{-1}$, maximum $R_1 \sim 2 \text{ s}^{-1}$) the error in the maximum change of estimated $R_1$ (1 s$^{-1}$) is approximately 0.009 s$^{-1}$, or less than 1%. For lesions C and D the maximum estimated error is also less than 1%.

Figure 3.4
The $R_1$ time courses for four DCE-MRI series, covering the range of contrast enhancement and tumor size. The highest variation in $R_1$ between data points is seen for tumor A, which is the smallest measured tumor. Although the smoothness of the enhancement curve is related to tumor size, all cases are amenable to analysis.

In this study there was correlation between the difference in the enhancement parameters of repeated measurements in a single patient and their mean value for that patient (for $k_{trans}$ $r=0.42$, Pearson correlation coefficient). This causes a skew of the ‘normal’ distribution and a transformation of the data should be performed in these circumstances. As stated in the methods section, the data were transformed to percentage difference from the first scan to the second. There was no significant correlation between the percentage difference and the mean measured parameters and therefore no further data transformation was required. For all listed parameters expressed as percentage change, there was no significant evidence against a normal distribution (Shapiro-Wilk test). The mean difference, CoV and repeatability are shown for all measured parameters in Table 3.2.
Table 3.2

Summary of parameters derived from two DCE-MRI scans, performed one week apart, without therapeutic intervention. PE = Peak Enhancement. IAUC[t] = Initial area under the tumour contrast enhancement curve for first t seconds. IAUC-A[t] = Initial area under the arterial contrast enhancement curve for first t seconds.

<table>
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<th>Parameter</th>
<th>Mean Change (%)</th>
<th>CoV (%)</th>
<th>Repeatability (%)</th>
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<tr>
<td>PE</td>
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<td><strong>Signal Intensity related to measured arterial input function</strong></td>
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<td>70.3</td>
</tr>
<tr>
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<td>37.8</td>
<td>72.1</td>
</tr>
<tr>
<td>$k^{\text{trans}}$</td>
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<td>34</td>
<td>75.0</td>
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<td><strong>R$_1$ Data</strong></td>
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<tr>
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<td>15.9</td>
<td>29.5</td>
</tr>
<tr>
<td>IAUC[60]</td>
<td>1.7</td>
<td>15.8</td>
<td>29.5</td>
</tr>
<tr>
<td>IAUC[180]</td>
<td>0.8</td>
<td>16.1</td>
<td>29.9</td>
</tr>
<tr>
<td>$k^{\text{trans}}$</td>
<td>3.1</td>
<td>19.1</td>
<td>36.1</td>
</tr>
</tbody>
</table>

In this study, use of raw signal intensities for calculating peak enhancement and IAUC resulted in high CoV, which is improved by dividing by the arterial IAUC. Even though the peak enhancement is taken from just a single data value along the enhancement curves, rather than an integration of many more data points, as is the case with IAUC, the reproducibility is very similar to IAUC. This is a reflection of the smoothness of the tumour enhancement curves. The $k^{\text{trans}}$ calculated from tumour and arterial signal intensities showed a higher CoV (34%) than the $k^{\text{trans}}$ from tumour $R_1$ and a standard AIF (19.1%). This is probably a reflection of the fact that any real variation in the AIF between the two scans is outweighed by the fact that the AIF is measured inaccurately with this sequence.

The individual patient data for two commonly used parameters, $k^{\text{trans}}$ and IAUC[60], calculated from $R_1$ values, are given in Table 3.3.
Table 3.3

Individual patient data showing tumour size, mean difference, coefficient of variation (CoV) and repeatability for $K_{\text{trans}}$ and IAUC[60] for 2 scans, 1 week apart, without therapeutic intervention. Parameters were calculated using $R_1$ values and a standardized arterial input function for $K_{\text{trans}}$.

<table>
<thead>
<tr>
<th>SITE</th>
<th>Primary</th>
<th>Size (cm)</th>
<th>$K_{\text{trans}}$ (min$^{-1}$)</th>
<th>% change</th>
<th>IAUC[60]</th>
<th>% change</th>
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<tbody>
<tr>
<td></td>
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<td>Scan 1</td>
<td>Scan 2</td>
<td>Scan 1</td>
<td>Scan 2</td>
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<tr>
<td>Liver</td>
<td>Colorectal</td>
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<td>0.111</td>
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<td>10.1</td>
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<td>0.014</td>
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<td>1.2</td>
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<td>Colorectal</td>
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<td>9.3</td>
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<tr>
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<td>Colorectal</td>
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<td>0.192</td>
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<td>16.6</td>
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<td>Liver</td>
<td>Colorectal</td>
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<td>0.037</td>
<td>0.044</td>
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<td>3.6</td>
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<td>0.081</td>
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<td>Lung</td>
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<td>0.171</td>
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<td>12.6</td>
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<td>Melanoma</td>
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<td>0.49</td>
<td>0.40</td>
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<table>
<thead>
<tr>
<th>K_{\text{trans}}</th>
<th>IAUC[60]</th>
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<tr>
<td>All cases</td>
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<tr>
<td>Mean Change %</td>
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<tr>
<td>CoV %</td>
<td>19.1</td>
</tr>
<tr>
<td>Repeatability %</td>
<td>36.1</td>
</tr>
</tbody>
</table>

| Size>3cm          |          |
| Mean Change %     | 6.1      | 4.3     |
| CoV %             | 15.5     | 13.9    |
| Repeatability %   | 30.6     | 26.5    |

Although no correlation was seen between $T_2$ signal intensity and enhancement parameters, the second case in Table 3.3 had very high $T_2$ compared with the other cases, consistent with a cystic nature of the metastasis. There is a tendency for greater variability with reducing size, and excluding lesions less that 3 cm in diameter reduced CoV. The colorectal liver metastases group also had lower CoV and repeatability values ($K_{\text{trans}}$ 14.2% & 26.5% and IAUC[60] 11% and 21.3% respectively), although this may be related to the fact that this group had relatively larger tumours.

3.4 Discussion

DCE-MRI using an inversion recovery snapshot FLASH sequence with a relatively long $T_{\text{eff}}$ of 815 ms and an interval of 3 seconds between subsequent images, performed in a
coronal oblique plane provides a rapid, reasonably accurate measure of $R_1$ for values between 1 and 5 s$^{-1}$, and therefore of Gd-DTPA concentration during contrast enhancement. The $R_1$ values seen in the clinical arm of this study were within this range. Since $S_0$ is not continuously measured as $R_1$ changes, there are errors of up to 6% in the estimated $R_1$. Systematic errors also occur in estimating dynamic parameters based on signal intensity, since MRI signal intensity is not proportional to Gd-DTPA concentration. These errors do not necessarily affect the reproducibility of contrast enhancement parameters but may affect the assessment of change in enhancement due to treatment.

Using a long $T_{1\text{eff}}$ is helpful as it provides good contrast to noise for low levels of enhancement such as may occur in necrotic tumours. However, this has the disadvantage that arterial contrast concentration cannot be accurately measured due to rapid relaxation during the long $T_{1\text{eff}}$ period. Measurement of arterial Gd-DTPA concentration (AIF) is considered important when assessing absolute values of tumour microcirculation. However, the AIF may not be so important for measuring a treatment effect in patients, unless there is a change in cardiac function or haemodynamics on therapy. Techniques that allow accurate, reproducible measurement of the AIF as well as tissue enhancement may well improve the reproducibility of these parameters, but this study shows that sub optimal measurement of AIF makes the reproducibility of the study worse than if a standardised AIF is used.

Imaging of tumours with a short pre-contrast $R_1$ can be a problem with this sequence since, in some cases enhancement may be negative in the first few images of the dynamic series. In a DCE-MRI study, rectified signal can be recognized if the signal reduces in the first few images of contrast enhancement. In practice, this is rarely a problem in liver metastases and this correction was not required in the clinical arm of this study as pre-contrast $R_1$ was always greater than 0.53 s$^{-1}$. This problem could be overcome by using saturation recovery rather than inversion recovery, at the expense of lower contrast at shorter $R_1$ values.

Although respiratory motion causes the tumour to move throughout the series of image acquisitions, the coronal oblique plane allowed positioning of the ROI for all images without change in size or shape. One possible criticism is that the position of the tumour relative to the selected slice will constantly change throughout the series of images leading to partial volume errors. Although this is true, the smooth enhancement curves generated suggest that this does not significantly affect the measured intensities. This is despite the
fact that liver metastases often have large areas of necrosis and therefore considerable variation in enhancement within the tumour volume. The use of a single region of interest for the whole tumour does not allow discrimination of regions within the tumour, but it does give a good signal-to-noise ratio by averaging the signal from a large volume of tissue and minimises partial volume effects.

The CoV of the repeated measures depends on the pre-processing (use of raw signal intensities or conversion to \( R_1 \)), analysis method (peak enhancement, IAUC or \( K^{\text{trans}} \)) and patient cohort used. Peak enhancement is a simple measure, shown to have similar reproducibility to IAUC. However, peak enhancement is much more a reflection of the extra-vascular, extra-cellular space volume fraction (\( v_e \)) rather than \( K^{\text{trans}} \), since changes in \( K^{\text{trans}} \) shift the position of the peak of the enhancement curve more than its amplitude, while peak enhancement is, in the limit of large \( K^{\text{trans}} \), proportional to \( v_e \). The validity of using peak enhancement as a biomarker for angiogenesis would therefore seem questionable.

The study shows it is important to use some method for 'standardising' the signal intensities from the dynamic image series. The results using calculated \( R_1 \) values for IAUC[60] and IAUC[180] and \( R_1 \) values with a standard AIF for \( K^{\text{trans}} \), show a CoV ranging from 11% to 19.1%. Assuming the post-treatment CoV is similar, this value can be used to assess the statistical power of studies or anticipate the patient numbers required for a study. Interestingly, our previous published data 100 shows a 58% mean reduction of \( K_i \) (equivalent to \( K^{\text{trans}} \)) with an SEM of 5.2% for 15 patients with colorectal liver metastases treated with PTK/ZK with 1000mg or more. This is consistent with a CoV of 20% (the CoV is equivalent to the Standard Deviation (SD) of percent changes and the SEM is the SD divided by square root of the number of cases). Re-analysis of our other published work 104 for patients with liver metastases treated with PTK/ZK with 1000mg or more (n=14) showed a 56% mean reduction in \( K^{\text{trans}} \) and a CoV of 21.1%. A CoV of ~20%, for both the reproducibility study and treatment effect, implies a cohort of 10 patients would be expected to show a 25% treatment effect with statistical significance (power 0.8). If the CoV is reduced to 14%, as for IAUC[60] in tumours greater than 3cm diameter, then an 18% treatment effect would show significance. Conversely, if a 40% treatment effect is expected then only 3-4 patients would be required to expect a statistically significant result to p<0.05.

Evelhoch et al. 105 measured the median IAUC parameter (similar to our IAUC[60]) in 19 human tumours with a 7.2 second image acquisition time. They demonstrated pre-
treatment tumour inter-patient CoV of 64% and intra-patient CoV, in repeated measurements without treatment, of 18%, similar to this study. The high CoV for inter-patient tumour measurement supports the notion of assessing percentage rather than absolute changes. Galbraith et al. assessed reproducibility in 16 patients with tumours 3cm in diameter or greater. They use an 11 second image acquisition time. Their data is presented in a slightly different manner and uses both pixel by pixel and ROI analysis. For ROI analysis the data can be summarised to show that for a cohort of 16 patients, IAUC can measure greater than 12% changes and $K_{\text{trans}}$ can measure 14-17% changes. Similarly, our data extrapolated for 16 patients and tumours 3cm or greater, (IAUC[60] CoV=14% and $K_{\text{trans}}$ CoV=16%) would be sensitive to 14% and 16% changes respectively. Both studies use similar methodology and do not measure AIF, but our study has an image acquisition time of less than 500ms as opposed to 7.2 and 11 seconds, dropping the requirement for multiple breath holds and increasing temporal resolution, but at the expense of signal to noise of any given image.

The repeatability varied from 26.5% for IAUC[60] (tumours of diameter greater than 3cm) to 36.1% for $K_{\text{trans}}$ (whole group). This is a measure of the significance of an individual result. From our previously published data a 40% change in enhancement parameters is considered to be clinically significant (the change required to predict a tumour response in colorectal liver metastases). A 40% change in an individual patient can therefore be considered both a statistically and a clinically significant finding. Both $K_{\text{trans}}$ and IAUC are shown to give similar results in the clinical application of this technique and the improved reproducibility of IAUC in this study suggests it is a valuable, straightforward method of evaluating contrast dynamics from DCE-MRI.

In this study, DCE-MRI failed in one patient due to incorrect positioning of the slice. The incorrect placement was demonstrated by studying the reference slice on both $T_1$- and $T_2$-weighted images but was more apparent on $T_2$-weighted imaging as central tumour necrosis could be seen. In our previous study failures to collect data of sufficient quality to perform DCE-MRI analysis was related to tumour size with three of the four patients out of 39 who had a failed study having tumours less than 3cm in diameter. When selecting the target lesion, avoiding metastases with very high $T_2$-weighted signal intensity is suggested to avoid purely necrotic / cystic tumours and to select metastatic deposits with a diameter of greater than 3cm.
In summary, this technique provides a rapid, straightforward, robust method of measuring tumour enhancement to monitor therapy. All stages of analysis are simple to perform if Eqn. 2.4 (see chapter 2) is used to calculate $R_1$ and IAUC is used to assess tumour enhancement. The speed of image acquisition freezes motion, allowing a wide variety of tumour applications. Also, since multiple breath holds are not required, the scanning protocol is easier both for patients and scanning technicians.
### 3.5 Appendices

#### 3.5.1 Data from normal volunteer study.

Initial slope (s\(^{-1}\)), PE = Peak Enhancement. IAUC[t] = Initial area under the tumour contrast enhancement curve for first t seconds. IAUC-A[t] = Initial area under the arterial contrast enhancement curve for first 'f' seconds. Units for signal intensity data are arbitrary.

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3.5.2: Data from reproducibility study.

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Chapter 4:

A clinical study using DCE-MRI, as a biomarker for the anti-angiogenic effect of a novel inhibitor of VEGF-receptor tyrosine kinases, in patients with liver metastases from colorectal carcinoma.

4.1 Introduction

This chapter involves using the techniques described in chapters 2 and 3 in coordinated phase 1 studies of a novel cancer agent. These studies have expanded to involve over 100 patients and multiple tumour sites. This sub study of the larger trials involves using DCE-MRI to assess the changes in vascular parameters of liver metastases from colorectal primary tumours on treatment with a novel cancer therapy.

PTK787/ZK222584 (PTK/ZK) is a potent, orally active and selective inhibitor of VEGF-receptor tyrosine kinases, VEGFR-1 (Flt-1) and VEGFR-2 (KDR), under co-development by Novartis Pharma and Schering AG. PTK/ZK has been shown to inhibit growth and reduce microvasculature in sub-cutaneously implanted human tumour xenografts in nude mice 107-109. Its intended indication is for the treatment of patients with solid tumours known to over-express VEGF and VEGF-Receptor, including carcinomas of the gastrointestinal tract 110. Phase I to III studies are ongoing to evaluate the safety, pharmacokinetics, pharmacodynamic effects and biologic activity of PTK/ZK in several advanced cancers including colorectal cancer, breast cancer, glioblastoma multiforme, prostate, lung and renal cancer where VEGF is known to play a role 111,112.

As stated in chapter 1 successful chemotherapy or radiotherapy will typically result in a reduction of the cross sectional diameter of a tumour when measured on serial computed tomography (CT) scans, but these changes and their relationship to efficacy may be slow and unreliable 21,113. Measurement of the efficacy of targeted biological agents, including inhibitors of angiogenesis, is even more difficult because they may not cause rapid involution of tumours 1 and may simply slow or stop tumour growth. This provides a problem for a phase 1 study because the biologically active dose may be difficult to predict
in humans, as it may be considerably lower than the maximum-tolerated dose of drugs having a safe toxicology profile.

Dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) has been used to study the pathophysiology of tumours \(^\text{114,115}\). Parameters of interest include microvascular density (MVD), vascular permeability and the extravascular, extracellular space \(^\text{116}\). Notably, malignancy, stage, and prognosis have all been correlated with these enhancement parameters \(^\text{53-55,71,72}\). Several studies have shown that successful therapies also result in changes in DCE-MRI contrast enhancement parameters, which may prove a more accurate and earlier indication of response \(^\text{60,73-75}\). In animals, an antibody targeted against VEGF has been shown to rapidly reduce contrast enhancement, as measured by DCE-MRI \(^\text{61,117}\).

Dose-related changes in the contrast enhancement parameters on DCE-MRI were evaluated for its use as a biomarker to assess the pharmacological response of escalating dose levels of PTK/ZK in two ongoing phase 1 clinical studies, recruiting adult patients with advanced cancers in the subgroup with colorectal liver metastases. In addition, potential correlations of this parameter to pharmacokinetic endpoints (dose, drug plasma exposure (AUC) and trough plasma concentration \((C_{\text{min}})\)), and clinical endpoints (best tumour response and tumour shrinkage) were assessed. This chapter reports on the results of DCE-MRI as a potential biomarker for PTK/ZK, as defined by an objective measurement indicating a pharmacological response to a therapeutic intervention \(^\text{42}\).

**4.2 Patients and Methods:**

**4.2.1 Patient Selection**

As PTK/ZK is intended to treat solid tumours known to over-express VEGF and VEGF receptors, the patients enrolled on the two phase 1 studies were predominantly patients with advanced cancers, including colorectal cancers with liver metastases, renal cell, breast, lung, and prostate carcinoma. In order to assess DCE-MRI changes with relation to dose and clinical effect, the results were analysed by tumour sub-type. This chapter reports the DCE-MRI and pharmacokinetic results of patients with advanced colorectal cancers with liver metastases, the largest patient subpopulation that was treated with PTK/ZK on the dose escalation phase of both studies and who underwent the MRI protocol.
Patients with histologically confirmed advanced solid malignancies with no standard curative therapy were eligible for the studies. All patients were required to have at least one site of measurable or evaluable disease as determined by the South-western Oncology Group (SWOG). Inclusion was irrespective of stage of disease or extent of prior therapy. Patient entry criteria included: age ≥ 18 years old; WHO performance status of 0–2 (self caring, up for more than half the day but not necessarily able to work), adequate haematology (absolute neutrophil count ≥ 1.5 x 10⁹/L, haemoglobin ≥ 9 g/dL, platelets ≥ 100 x 10⁹/L); renal (serum creatinine ≤ 1.5 x Upper Limit of Normal [ULN], serum bilirubin ≤ 1.5 x ULN, 24 hour creatinine clearance ≥ 50 mL/min); and hepatic function (AST and ALT ≤ 2.5 x [ULN]), no known brain metastases; no recent prior chemotherapy or biologic therapies, radiotherapy or surgery; and a life expectancy of at least 12 weeks.

All patients were informed about the investigational nature of the study according to institutional and regional guidelines, and subsequently signed an approved informed consent form prior to start of the studies. Permission of local ethics regulatory bodies was obtained at each centre.

4.2.2 Drug Administration and Study Design

Two phase 1 dose escalation studies were conducted at centres involving the oncology and radiology departments at Leicester, UK and Freiburg, Germany. The objectives of the studies were to determine the dose-limiting toxicity, maximum-tolerated dose, safety, tolerability, pharmacokinetic profile, and biologic activity of oral PTK/ZK. PTK/ZK was administered orally on a continuous once daily schedule on 28-day cycles until patient discontinued from study due to intolerable toxicity or tumour progression. Three patients were enrolled per dose cohort onto each study, and an additional 3 patients were enrolled at the same dose level in the event that a dose-limiting toxicity (DLT) was observed. Additional patients were subsequently enrolled onto each study at the dose level defined as optimal, with respect to toxicity, pharmacokinetic and biomarker data. The starting dose was based on preclinical toxicity data with the subsequent dose escalation levels based on an approximated Fibonacci Series. PTK/ZK was given at doses of 50, 150, 300, 500, 750, 1000, 1200, 1500, and 2000 mg.
4.2.3 Pharmacokinetic Assessment

Full pharmacokinetic samples were obtained on days 1 and 28 in the first cycle of therapy at the following time points: pre dose (0), 0.25, 0.5, 1, 1.5, 2, 4, 6, 10, and 24 hours post dose. The exact methodology is described elsewhere.  

The pharmacokinetic parameters, AUC (area under the plasma concentration curve), $C_{\text{max}}$ (maximum plasma concentration) and $C_{\text{min}}$ (minimum plasma concentration) were determined for each individual. The AUC$_{0-24}$ was calculated on day 1 and day 28 using the linear trapezoidal rule up to 24 hours post dose.

4.2.4 DCE-MRI Methodology

MRI imaging was performed pre-treatment (baseline, within 1 week prior to treatment with PTK/ZK). Further studies were performed on day 2 and at the end of each 28 day cycle ("end of cycle 1" = EC1, "end of cycle 2" = EC2). Patients were imaged between 2 to 9 hours post dose on these days. All MRI images were acquired using a 1.5 Tesla whole body magnet equipped with 25 milliTesla/metre gradient coils and phased array surface coils (Siemens Magnetom Vision, Erlangen, Germany at Leicester or Siemens Magnetom Symphony at Freiburg). The DCE-MRI was performed as described in Chapter 3. At both sites the sequences used a matrix of 96 x 128, slice thickness 10 mm and an effective inversion time (TI) of 815 msec. (Freiburg TR 3.6 msec, TE 1.7 msec, TI 640 msec and FA 8, Leicester TR 3.3 msec, TE 1.4 msec, TI 655 msec and FA 8). An $S_0$ (proton density) sequence with no inversion pulse was performed at the end of the run to allow calculation of T$_1$. Low molecular weight Gadolinium-chelate 0.1 mmol/kg (Magnevist, Schering AG or Omniscan, Nycomed) was injected as a rapid bolus through an arm vein in less than 5 seconds. This injection speed was adhered to for all patients. Signal intensities were taken from a Region of Interest (ROI) drawn around the tumour and aorta separately using an image manipulation package, Analyze™ (Mayo clinic, Rochester, MN, USA) as described in chapter 3. The position of the ROI was corrected on a time point by time point basis for any movement over time. In all cases the profile of the tumour remained reasonably constant throughout the imaging sequence so the region of interest did not have to be altered in shape and volume. The bi-directional transfer constant ($K_t$, mls/100g/min) was then calculated using a two-compartment model. $K_t$ is linearly related to $K^{\text{trans}}$ and all results are presented as $K^{\text{trans}}$ for consistency in this thesis. As the imaging parameters are optimised for tumours rather than arterial enhancement a standard data set for aortic input was used as described in chapter 3. The timing of onset of the arterial input function
was measured from the artery imaged in the study. Imaging data from the Freiburg site was sent to Leicester (to the author's secure ftp site at the University of Leicester) for analysis by the author, so as to ensure uniformity and reproducibility of the data sets.

4.2.5 Tumour Assessments

Patients were evaluated for tumour response at the end of every 28-day cycle using the SWOG Solid Tumour Response Criteria using standard MRI sequences. Measurable, evaluable, and non-evaluable lesions were accounted for in the tumour assessment. Measurable lesions were quantified by using the product of the longest and its perpendicular diameters. Complete Response (CR) was defined as the complete disappearance of all measurable and evaluable disease, and with no new lesions and disease related symptoms. Partial Response (PR) was defined as at least a 50% decrease in the sum of the product of the perpendicular diameters of measurable lesions from pre-treatment and with no development of new lesions. Minor response (MR) was defined as at least 25% but not more than 50% decrease from pre-treatment in measurable lesions. Progressive Disease (PD) was defined as at least a 50% increase or an increase of 10 cm² (whichever is smaller) in measurable lesions, clear worsening from previous assessment of any evaluable disease, reappearance of any lesion which had disappeared, or appearance of any new lesion/site. Stable Disease (SD) was defined as the disease status where both the measurable lesions was less than the criterion to meet PR, but also not sufficient to meet the criterion for PD.

The best response criteria was used to categorise all evaluable patients as either non-progressors or progressors for the biomarker analysis in order to identify the differences in biological effects in response to PTK/ZK. The best response was determined from the sequence of tumour response: 1) CR, if two CR before progression, 2) PR, if two PR before progression, 3) SD, if two SD before progression, 4) PD, if one PD within the first two months of treatment. Non-progressors were defined as category 1), 2), and 3), while progressors were defined as category 4). No patient achieved a complete or partial response.

4.2.6 DCE-MRI Statistical Methods and Modelling

Due to anticipated variability in the pre-treatment enhancement of tumours, the relative rather than absolute changes of \( K_{trans} \) are more clinically meaningful. The variability in pre-treatment enhancement of tumours may be due to varying sizes or degree of necrosis of
liver metastases at the start of PTK/ZK treatment and should be accounted for by using an enhancement parameter expressed as a percentage of the pre-treatment value (\% of pre-treatment $K^{\text{trans}}$) as in chapter 3. The relationship between changes in $K^{\text{trans}}$ and pre-treatment $K^{\text{trans}}$ is studied.

Using non-parametric statistics, the degree of association between \% of pre-treatment $K^{\text{trans}}$ and dose, drug plasma exposure (AUC), and trough plasma concentration ($C_{\text{min}}$) were measured by the Spearman Rank correlation coefficient. The mean \% of pre-treatment $K^{\text{trans}}$ and standard error (SE) by time point (day 2 and EC1) for all doses and for doses of 1000 mg or greater were calculated. Mean \% of pre-treatment $K^{\text{trans}}$ and SE by time point for progressors vs. non-progressors were also calculated. Changes in $K^{\text{trans}}$ from pre-treatment use 'paired' non parametric Wilcoxon signed ranks test. The \% of pre-treatment $K^{\text{trans}}$ distributions was compared using p-values from the Mann-Whitney U Test. A test of significance of the degree of association between the \% of pre-treatment $K^{\text{trans}}$ and \% change in bi-dimensional product of liver metastases after 2 months on treatment (EC2) was conducted using the Spearman Rank Correlation coefficient.

Although a clear association exists between \% of pre-treatment $K^{\text{trans}}$ versus dose, AUC, and $C_{\text{min}}$, AUC is used in the modelling for the following reasons. Firstly, the intent is to characterise the pharmacodynamic relationship of DCE-MRI to systemic drug exposure (AUC) without the influence of the dose-exposure relationship (which appears non-linear and time dependent) and associated variability. Secondly, the systemic drug exposure is adequately estimated by AUC; whereas, $C_{\text{min}}$ is highly variable and does not accurately represent the plasma concentration at the time of DCE-MRI scan or the systemic drug exposure during a dose interval. In addition, to compensate for limited data points and the lower number of data points on EC1 at the higher range of AUC, the modelling was performed using pooled data from day 2 and EC1. This should be plausible since the \% of pre-treatment $K^{\text{trans}}$ is highly correlated to AUC.

4.3 Results:

4.3.1 Patient Characteristics

A total of 27 patients with advanced colorectal cancers were evaluated with MRI of their liver metastases. One patient treated at the 150 mg dose was excluded as a different MRI protocol was used. The demographics for the remaining 26 patients with advanced
colorectal cancers with liver metastases show 16 male, 10 female and a median and range of age of 62 (43-77) years.

All 26 patients were included in the pharmacokinetic analyses. Of these 26 patients, 4 patients did not complete cycle 1 treatment and were not evaluable for tumour response due to reasons other than intolerable toxicity or disease progression. For the 22 remaining patients with evaluable tumour response, 1 patient did not have the ‘day 2’ MRI scan performed and 1 patient did not have an evaluable EC1 MRI scan. Pharmacokinetic data was not available for 2 further patients at day 28.

4.3.2 Pharmacokinetics

PTK/ZK was rapidly absorbed following oral administration with a peak plasma concentration ($C_{\text{max}}$) reached in 1 to 2.5 hours for all dose groups. At steady state, which was achieved by day 28, the systemic exposure (AUC) was approximately 30% lower than the exposure following a single dose for all doses above 150 mg (see figure 4.1).

![Figure 4.1](image_url)

**Figure 4.1**

PTK/ZK plasma concentrations (AUC) at Day 1 and day 28 of treatment. Bars represent standard error of the mean.
Metabolism via the CYP3A4 isoenzyme is the major elimination pathway, and autoinduction of this enzyme is likely the explanation for the observed decrease in AUC from day 1 to day 28. The half-life is approximately 3 to 6 hours for each dose group on day 2 and day 28. PTK/ZK exposure appears to be dose proportional up to 1000 mg on both day 2 and day 28; however, the exposure appears to be less than proportional above 1000 mg.

### 4.3.3 DCE-MRI Results

Absolute changes in $K_{\text{trans}}$ are proportional to the magnitude of the pre treatment $K_{\text{trans}}$ but not the percentage change. Also the pre-treatment $K_{\text{trans}}$ has a wide variability (CoV 61%) supporting the use of percentage change in $K_{\text{trans}}$ as the studied parameter. This is shown in figure 4.2.

**Figure 4.2**
The absolute change in $K_{\text{trans}}$ (a) on treatment increases in relation to the magnitude of pre-treatment $K_{\text{trans}}$, but the percentage change on treatment (b) does not.
The rapid reduction in enhancement within 26 to 33 hours post first dose in a liver metastasis from colorectal carcinoma is visibly demonstrated in Figure 4.3, with a relatively constant profile of the tumour, despite respiratory movement in the infero-superior plane.

As shown in Figure 4.4, this substantial reduction in enhancement is evident across all dose groups on day 2 with a mean reduction of $K^{\text{trans}}$ of 43% ($\pm$ 6.95%, $p=0.0002$). In the higher doses ($\geq 1000$ mg) where the maximum exposure is achieved, the reduction in enhancement is expectedly greater with a mean reduction of $K^{\text{trans}}$ of 58% ($\pm$ 5.2%, $p=0.001$) on day 2 and 60% ($\pm$ 5.3%, $p=0.005$) at EC1.
Figure 4.4
*Mean (± SE) % of pre-treatment $K_{\text{trans}}$ on day 2 and EC 1 for all doses (n=25), and for those who received at least 1000 mg dose (n=15) of PTK/ZK.*

Using non-parametric statistics, a significant negative relationship was found between increasing PTK/ZK dose, AUC, and $C_{\text{min}}$ with reducing enhancement on both day 2 and EC1. Spearman rank correlation coefficients and significance values are listed in Table 4.1.

<p>| Table 4.1 |
| Spearman Rank Correlation Coefficients for relationships between $K_{\text{trans}}$ with Dose, $C_{\text{min}}$, and AUC using Spearman Rank Correlation Coefficient. |</p>
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<tr>
<td>Trough plasma concentration ($C_{\text{min}}$)</td>
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<td>Area under plasma concentration curve, (AUC)</td>
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</table>

Of the 22 patients with evaluable tumour response 21 had DCE-MRI at day 2 and EC1. There were 12 non-progressors and 9 progressors. As shown in Figure 4.5, non-progressors had a significantly greater reduction in enhancement on day 2 and at EC1 compared to progressors (mean difference in % of pre-treatment $K_{\text{trans}}$ 87% minus 40% = 47%, p=0.004 (day 2); and 99% minus 48% = 51%, p=0.006 (EC1)).
In addition, Figure 4.6 shows the strong relationship between the percent of pre-treatment $K^{\text{trans}}$ on day 2 and EC1 compared with the change in liver disease size at the end of cycle 2 (EC2), as measured by the change in the sum of measurable liver lesions, calculated by the bi-dimensional product of disease on MRI. Correlation is obtained for day 2 $K^{\text{trans}}$ of 0.60 (p=0.004) and EC1 $K^{\text{trans}}$ of 0.75 (p=0.0001). In the case of progressive disease where the patient was 'off study' by EC2, the size change at EC1 was used.
Figure 4.6
Percent change in size of liver metastases at EC2 against % of pre-treatment $K^\text{trans}$ on day 2 (top; $R=0.61; p=0.004$) and EC1 (bottom; $R=0.75; p=0.0001$). Best response may involve presence of new lesions or growth in non-liver lesions.

As shown in table 4.1 there is a strong relation between % change in $K^\text{trans}$ and the plasma AUC pharmacokinetics. This can be shown for both day 2 and end cycle 1 (figure 4.7).
Using the support of professional statisticians an inhibitory $E_{\text{max}}$ model has been used to define the mathematical relationship between the DCE-MRI result and plasma AUC (Morgan et al 2003)\textsuperscript{100}.

$$\text{Effect} = E_0 - \left( E_0 - E_{\text{max}} \right) \times \left( \frac{AUC}{AUC + EAUC_{50}} \right)$$

where $\text{Effect} = \% \text{ of pre-treatment } K^{\text{trans}}$

$E_0 = \text{Approximated Baseline (~100%), expressed as } \% \text{ of pre-treatment } K^{\text{trans}}$

$EAUC_{50} = \text{AUC in which 50% of } E_{\text{max}} \text{ is achieved}$

$E_{\text{max}} = \text{Maximum Effect, expressed as } \% \text{ of pre-treatment } K^{\text{trans}}$

. The estimated pharmacodynamic parameters are : $E_0 = 108 \% \text{ of pre-treatment } K^{\text{trans}}$ (StdErr = 17.7, CV\% = 16.38), $EAUC_{50} = 39 \text{ hr}^*\mu\text{M}$ (StdErr = 25.24, CV\% = 64.47), and $E_{\text{max}} = 14.9 \% \text{ of pre-treatment } K^{\text{trans}}$ (StdErr = 9.97, CV\% = 66.76). The parameter $E_0$ is estimated to be slightly greater than 100% due to a few patients having very large $\% \text{ of pre-treatment } K^{\text{trans}}$ values. This system obeys a linear relationship in response between 20 to 80% of maximum response with smaller increments in response at higher exposure until a maximum response is achieved. In the case of PTK/ZK, the reduction in enhancement...
decreases linearly with exposure (on a log scale) until approximately 200 hr*μM is reached. Thereafter, a less than proportional reduction in enhancement is achieved with increasing exposure until the maximum effect is reached (~15% of pre-treatment $K^{\text{trans}}$) at an exposure of approximately 450 hr*μM. Most non-progressors on both day 2 and EC1 achieved an exposure of 50 hr*μM, an exposure corresponding to the lower limit of the standard deviation (SD) for the 1000 mg dose at EC1. This is consistent with the finding that most non-progressors received at least 1000 mg dose of PTK/ZK.

### 4.4 Discussion

PTK/ZK was shown to cause a significant reduction in DCE-MRI contrast enhancement parameters within 26 to 33 hours of administration of the first dose. The extent of reduction was dose dependent on both day 2 and EC1. There was a statistically significant relationship between reductions in contrast enhancement and disease response.

The exact mechanism of the reduction in enhancement is not clear. Gadolinium-chelate (Gd-chelate) uptake is multifactorial \(^{116}\) not only depending on vascularity but also vascular permeability and the extracellular space. Due to the relatively small size of Gd-chelate, permeability may be so high that enhancement is mainly related to vascularity (flow limited). Conversely, if permeability is low, enhancement will be permeability limited \(^{52}\). Sophisticated analysis measuring both vascularity and permeability is possible using macromolecular contrast media but these agents are not currently licensed for use in humans \(^{50,118}\). Gadolinium-chelate enhancement can therefore be considered to be related to a combination of microvascular density and permeability (product of both flow into the tissue vasculature, and extraction into the extravascular, extracellular space) \(^{97}\). In animal models, successful inhibition of VEGF receptors may result in a reduction in both permeability and vascularity within hours of treatment onset \(^{119,120}\). The rapid reduction of enhancement within 33 hours could therefore be attributed to either a reduction in endothelial permeability to Gd-chelate and/or to a reduction in vascularity due to removal of VEGF receptor signalling, as VEGF is a vascular survival factor. In this case the maximum reduction in enhancement achieved is 90%, which suggests a marked antivascular effect.

The data suggests that reduction in enhancement is associated with better clinical outcome and reductions of 40% or more are associated with lesion size reduction. However, these figures are based on this particular DCE MRI technique. If MRI is to be used to predict outcome in a wider sense, then standardised techniques are required.
Although all imaging slices include a section of the aorta, the arterial input function was not measured due to saturation of the MRI signal caused by the high arterial concentrations of Gd-chelate immediately after the bolus injection. A standard data set was used as described in the method section. Although this did not allow for variations of haemodynamics between patients, the study involved changes from pre-treatment enhancement, with each patient acting as their own control. The study does, however, assume that the pharmacokinetics of the bolus injection remain constant for individual patients throughout the study. The magnitude of the changes in $K_{\text{trans}}$ observed suggest that this was not a problem in this study.

This was one of the first human studies to show the potential use of DCE-MRI as a biomarker for the biological effect of an angiogenesis inhibitor targeting the VEGF receptor. It is known that such agents inhibit tumour growth, but do not necessarily induce tumour regression. Thus, it is increasingly important to identify biomarkers that demonstrate the required drug-target interaction and the desired downstream biological effects. In these 2 phase I studies, DCE-MRI was shown to be a reasonable predictor of clinical response as evidenced by statistically significant relationships between the DCE-MRI parameter, % pre-treatment $K_{\text{trans}}$, and a number of clinical parameters such as tumour response and the extent of tumour shrinkage. Statistically significant relationships between % pre-treatment $K_{\text{trans}}$ with PTK/ZK dose, concentration, and exposure strongly supports that the desired biological effect of PTK/ZK is likely the result of drug-receptor interaction (i.e. reversible binding of PTK/ZK to VEGF receptor). In addition, the inhibitory $E_{\text{max}}$ model sufficiently describes the relationship between % of pre-treatment $K_{\text{trans}}$ and exposure in mathematical terms. This model was useful in selecting the biologically active dose for further evaluation in phase II and III studies. This required the identification of a dose in which the lower limit (SD) of exposure is associated with at least 40% reduction in enhancement (60% of pre-treatment $K_{\text{trans}}$) and a level that is associated with non-progressive disease. At least a 1000 mg dose would be required to achieve this exposure and thereby, a sufficient reduction in enhancement for non-progressive disease.

The clinical evaluation of molecularly targeted therapeutic strategies can be facilitated and strengthened by the use of appropriate biomarkers that measure the pharmacologic response in humans. If further studies demonstrate the correlation of DCE-MRI (i.e. Day 2 response related effect) to the clinical endpoint, then DCE-MRI may have a wider impact allowing early assessment of efficacy and improved clinical management of such anti-angiogenesis therapies.
### 4.5 Appendices

#### 4.5.1 Data set derived from clinical studies described in chapter 4.

<table>
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<th>% pretreatment $K_{trans}$</th>
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Chapter 5: Clinical studies using DCE-MRI as a biomarker to compare once daily and twice daily dosing, and to select the optimal dosing strategy for ongoing trials

5.1 Introduction

In Chapter 4 dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) was demonstrated as a useful technique to help define the biological response and optimal dose of PTK/ZK in other phase I studies. These studies involved administering PTK/ZK on a once daily schedule when the drug half-life was shown to be 3-6 hours. We were interested in evaluating whether the administration of PTK/ZK on a twice-daily schedule would maintain blood levels above a threshold target known to interfere with VEGF signalling in preclinical models, and by providing more constant levels, potentially have greater efficacy. We therefore undertook a dose-finding study using PTK/ZK on a twice-daily schedule in patients with advanced malignancies. Tumour types that were known to generally over express VEGF were chosen. DCE-MRI was incorporated as a pharmacodynamic endpoint.

After the studies recorded in chapter 4 a phase II study assessed the safety and preliminary response profile of oral PTK/ZK in combination with chemotherapy in 35 previously untreated patients with advanced colorectal carcinoma who were treated with escalating doses of PTK/ZK plus oxaliplatin/5-fluorouracil (5-FU)/leucovorin (FOLFOX4). The pharmacokinetics (PK) and toxicity profiles of both PTK/ZK and FOLFOX4 were unaffected by co administration. PTK/ZK was well tolerated at doses ≤ 1,250 mg/day; no DLTs occurred at 1,250 mg/day in the first 6 patients evaluated for MTD. A multinational phase III study then evaluated FOLFOX4 with PTK/ZK (1250 mg od) or placebo in advanced colorectal carcinoma. 1168 patients were randomised and although investigator review demonstrated a statistically significant improvement in progression free survival in patients receiving PTK/ZK (17% reduction in risk), central review (the primary analysis) did not achieve statistical significance. These results where disappointing in the light of promise from the phase I and II trials. It has been suggested that efficacy may have been lost due to the choice of a once daily over twice daily dosing schedule. Reasons given include the relatively short half life of PTK/ZK (2-3 hours) and the fact that the exact mechanism of action of the early DCE-MRI changes and their
relation to efficacy is unknown. It is possible that the early reductions in enhancement are due to inhibition of VEGF induced nitric oxide formation. This has many possible outcomes in cancer but the immediate effect would be a vasoconstriction. It is possible that if the early effects of PTK/ZK are mediated by vasoconstriction then the effect would be optimal if the vasoconstriction persists uninterrupted. Imaging however was mainly performed 2-4 hours after dosing (range 2-9 hours). This would therefore only relate to maximum concentrations of drug. During this time there was no relation of DCE-MRI effect to time of scanning but there is no information about whether the effect persists throughout the day. The results of once daily and twice daily dosing are therefore compared to see if there are any differences in DCE-MRI effect, which may help in the decision of optimal dose strategy.

This study therefore has 2 objectives. The first is to expand the experience of the pharmacokinetics (PK) and toxicity of this agent in a twice daily dosing regime and to establish further promise of efficacy. The DCE-MRI is analysed as in chapter 4 but the initial area under the contrast enhancement curve (IAUC) is also calculated and compared with values obtained with quantitative measures of $K_{\text{trans}}$. The second objective is to compare the results for once and twice daily dosing to help decide on the optimal dosing schedule.

5.2 Patients and Methods

5.2.1 Patient selection

Patient selection is the same as for chapter 4, recruiting advanced cancer. The recruitment favoured patients with liver metastases, and particularly from a colorectal primary. The local medical ethics committee approved the protocol, and patients gave their written informed consent according to ICH/EU-GCP regulation before being registered.

5.2.2 Study design

This phase I trial was designed as an open label, nonrandomised dose-escalation study. Patients were recruited in cohorts of 3 to 6 and received sequentially increasing doses of PTK/ZK until dose-limiting toxicity (DLT) was observed. Four dose levels were scheduled: 150 mg, 250 mg, 500 mg and 1000 mg twice daily. The maximum tolerated dose and/or biologically active dose cohort was expanded to include 15-25 additional patients to further evaluate safety, tolerability, pharmacokinetics and biological activity.
During the study, dose escalation decisions were based on data from patients treated for at least 4 weeks. No intra-patient dose escalation was permitted. Adverse events were assessed weekly during the first 8 weeks of treatment and every 2 weeks thereafter.

Treatment was administered until progression of disease, unacceptable toxicity or patient refusal to continue. There was a planned expansion of the cohort at MTD to further evaluate safety, tolerability, biologic activity and pharmacokinetics.

Within 14 days prior to treatment, patients had their medical history taken, physical examination with clinical tumour measurement, assessment of performance status, full haematological status and blood chemistry and assessment of tumour parameters using DCE-MRI. During treatment, haematological and biochemical tests were repeated weekly for the first 8 weeks and every 2 weeks subsequently.

5.2.3 Drug supply

PTK/ZK (Novartis, New Jersey, US, and Schering AG, Berlin, Germany) was provided in 50 mg, 100 mg and 250 mg capsules and given orally, with a 2 hour fast prior to and after the administration of the capsules. The second dose was administered approximately 8 hours after the morning dose. Patients were dosed continuously with a treatment cycle defined as 28 days.

5.2.4 Analytical assay & Pharmacokinetics

Method described in chapter 4 and in more detail elsewhere (Thomas et al 2005).104

5.2.5 DCE-MRI Imaging and Tumour Assessments

The DCE-MRI protocol and tumour assessment is as for chapter 4 except that all scans were performed at Leicester.

5.2.6 DCE-MRI Statistical Methods and Modelling

Analyses were performed on all cancer types, and in certain cases, performed for a homogeneous sub-patient population with liver metastases. As seen in chapter 4, the relative rather than absolute changes of $K^{\text{trans}}$ are more clinically meaningful due to variability in pre-treatment enhancement of tumours.

The overall effect of PTK/ZK on MRI $K^{\text{trans}}$ was evaluated. The mean % of pre-treatment MRI $K^{\text{trans}}$ and standard error (SE) by time point (day 2, EC1 (end cycle 1)) for all doses
and doses at least 1000 mg or greater were calculated. Mean % of pre-treatment MRI $K_{\text{trans}}$ and SE by time point (day 2, EC1) for progressors vs. nonprogressors were calculated and statistically assessed. Where means are compared statistically the non-parametric Wilcoxon signed ranks test is used for paired data and the Mann-Whitney U test for independent data. Where percentages are compared the Chi-Squared test is used. The degree of association between % of pre-treatment MRI $K_{\text{trans}}$ and tumour size by day 56, dose, AUC $C_{\text{max}}$ and $C_{\text{min}}$ were measured by the Spearman Rank correlation coefficient. (SPSS 13.0 for windows, Chicago, IL). The % change in tumour size by day 56 was plotted against MRI $K_{\text{trans}}$ on day 2.

5.3 Results

5.3.1 Patient Characteristics

Forty-three patients with advanced cancer were enrolled onto the study; 26 patients in the dose-escalation phase and 17 patients in the expansion phase. Patient demographics included 25 males and 18 females with median (and range) of age 57 (39-76) years.

Three patients had liver metastases on the DCE-MRI imaging that were too small to measure (<3cm) and could not be evaluated in this part of the study. One patient’s pre-treatment DCE-MRI examination was not of sufficient quality to allow comparison of subsequent scans. A further 3 cases had failed pre-treatment DCE-MRI scans. Thus, a total of 7 patients were not eligible for DCE-MRI evaluations. The remaining 36 patients form the data set (appendix 5.5) for this chapter. Thirty five had successful day 2 DCE-MRI and 29 successful DCE-MRI at end of cycle one (EC1).

Primary tumour site included 21 colorectal, 1 breast, 1 mesothelioma, 5 neuroendocrine, 2 renal, 3 sarcoma and 2 gastric and 1 ovarian tumour. Of the 3 patients with sarcomas, 2 had gastrointestinal stromal tumours (GIST) expressing c-kit. Site of measured lesion included 26 liver metastases, 4 lymph node, 3 lung metastases and 3 with other sites.

Of those patients having successful DCE-MRI the following patients were considered not evaluable for assessment of efficacy: 3 patients whose therapy was interrupted due to a ‘clinical hold’ in the early stages of the trial (two patients did not complete cycle 1 and the third patient was just starting cycle 2), 1 patient who discontinued due to adverse event before completing cycle 1 and 1 patient with breast cancer who took concurrent tamoxifen. Thus 31 patients were assessable for clinical response. The data set for once daily dosing is
also listed in appendix 5.5 but with the plasma PK given as ng/L rather than micromolar for consistency with the twice daily study.

5.3.2 Toxicities and Tolerability

The toxicity and tolerability data is covered in greater detail elsewhere \(^{104}\). The major dose limiting toxicity was ‘light headedness’ which was severe in \(\frac{1}{3}\) patients at the maximum dose 1000 mg bid and was associated with ataxia. Light headedness occurred to some extent at all doses, it was dose related, generally worse in the 2-3 hours post dose and tended to resolve after the first week of treatment. Other toxicities included nausea (61%), vomiting (54%) and diarrhoea. All these symptoms were mild and controllable with standard medications. Hypertension was seen in 7 patients, which was pronounced in 4 patients. A skin rash was also seen in one patient.

5.3.3 Pharmacokinetics

The pharmacokinetic data is covered in greater detail elsewhere \(^{104}\). Thirty-three (day 1) and 29 (day 28) patient profiles are available for pharmacokinetic analysis. PTK/ZK was rapidly absorbed following oral administration with a peak plasma concentration (\(C_{\text{max}}\)) reached in approximately 2 hours. At steady state, which was achieved by day 28, the systemic exposure (AUC) was approximately 40% lower than the exposure following a single dose for all doses (Figure 5.1).
Metabolism via the CYP3A4 isoenzyme is the major elimination pathway, and autoinduction of this enzyme is likely to be the explanation for the observed decrease in AUC from day 1 to day 28. The half-life was estimated from other PTK/ZK daily dosing studies and was determined to be approximately 3 to 6 hours on both day 2 and day 28. PTK/ZK exposure increases with increasing dose up to 500 mg bid (1000 mg/day) and appears to be plateau above this dose level. In general, the dose and time dependent pharmacokinetic findings for twice daily dosing are similar to those observed for once daily dosing. At equivalent total daily doses, the twice-daily dosing achieved comparable exposure on days 1 and 28; however, as expected the trough concentration achieved at steady state was higher for twice daily dosing (figure 5.8a, see below).
5.3.4 Anti tumour Activity

While efficacy was not the primary end point in this phase I study, anti-tumour activity was evaluated. No patients achieved CR. Of the 31 patients evaluable for response, 10 patients had PD. Although survival was not a primary end point of this study, the median survival to date is >8 months, which is beyond what would be expected for a group of patients with advanced, previously treated disease.

5.3.5 Pharmacodynamic Results of DCE-MRI

For the analysed cases, the difference in $K^{\text{trans}}$ correlated with the pre-treatment $K^{\text{trans}}$ value, but, similar to this study, there was no correlation of the percentage difference to the pre-treatment $K^{\text{trans}}$ further supporting the conclusions of chapters 3 & 4 to use the relative rather than absolute changes in $K^{\text{trans}}$.

Therefore 36 patients had DCE-MRI scans that were available for assessment, 35 patients on day 2 and 29 on EC1. Of these 35 patients, 31 were evaluable for tumour response. A rapid reduction in enhancement was observed by day 2 post PTK/ZK treatment in all patients. Respectively, on day 2 and EC1 for all dose groups, the mean (±SE) % of pre-treatment MRI $K^{\text{trans}}$ was 60.2 (±5.5, p<0.0001) and 66.2 (±6.9, p=0.0002). As shown in Figure 5.2, the reduction is more pronounced with the higher dose group (>1000 mg/day) on both days 2 and EC1. Respectively, on day 2 and EC1, the mean (±SE) % of pre-treatment MRI $K^{\text{trans}}$ was 53.8 (±6.5, p<0.0001) and 60.4 (±8.1, p=0.001) for the ≥1000 mg/day dose group.
As expected from chapter 4, patients who achieved non-progressive disease status responded to PTK/ZK treatment with a greater reduction in enhancement on day 2 (n=30). This reduction in MRI $K^{\text{trans}}$ is greater in a patient subpopulation with liver metastases (n=22) (Figure 5.3). The difference in % of pre-treatment (pre-treatment) $K^{\text{trans}}$ was statistically significant on day 2 of treatment (mean difference of 17.3%; p=0.04) and on day 2 for the liver metastases group (mean difference of 22.4%; p=0.047).
As suggested in chapter 4, greater than 40% reduction of pre-treatment MRI $K^{\text{trans}}$ on day 2 of treatment is a good predictor for disease status (MRI $K^{\text{trans}}$ of less than 60% of pre-treatment value). Supporting this finding is Figure 5.4 which demonstrates that changes in % of pre-treatment MRI $K^{\text{trans}}$ is positively associated to % change in tumour size by 2 months (EC2) of PTK/ZK treatment (Spearman rank correlation coefficient 0.43, p=0.023) with greater than 40% reduction in $K^{\text{trans}}$ resulting in target lesion shrinkage. In the cases where patients were off study by day 56, the % change in tumour size at EC1 was used.
A significant negative relationship was found between increasing PTK/ZK Dose, AUC, \(C_{\text{min}}\), and \(C_{\text{max}}\) with reducing enhancement on day 2 of treatment, and between PTK/ZK AUC and \(C_{\text{min}}\) with reducing enhancement at EC1 (Table 5.1).

**Table 5.1**  
Spearman rank correlation coefficients and significance values for the relationship of \(K^{\text{trans}}\) to dose and PK.

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<th>(K^{\text{trans}}) Day 2</th>
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An $E_{\text{max}}$ model was applied to the PTK/ZK exposure (AUC) as described in chapter 4 (Fig 5.5). The estimated pharmacodynamic parameters are (AUC): $E_0 = 99.6\%$ of pre-treatment MRI $K^{\text{trans}}$ ($CV\% = 108.2$), $EAUC_{50} = 28046 \text{ hr*ng/mL} (CV\% = 414.0)$, and $E_{\text{max}} = 28.1\%$ of pre-treatment MRI $K^{\text{trans}}$ ($CV\% = 43.7$). Please note plasma concentrations are given in ng/ml which has a relation for PTK/ZK of ng/ml units = 365* micromolar units (used in chapter 4).

5.3.6 Comparison of IAUC and $K^{\text{trans}}$

The data was re-evaluated and IAUC[60] and IAUC[180] were calculated in the manner described in chapter 3. There was good correlation between percent changes on treatment seen in IAUC and $K^{\text{trans}}$ (Fig. 5.6) calculated from $R_1$ values.
After one day (3 doses) of treatment with PTK/ZK, there were similar significant changes in enhancement parameters, with $K^{\text{trans}}$ at 60.2% +/- 5.5% (mean +/- standard error of the mean (SEM)) and IAUC[60] at 62.2% +/- 5.6% of pre-treatment value (n=35). For colorectal liver metastases receiving 1000mg or more of PTK/ZK per day (n=19) the effect was increased with $K^{\text{trans}}$ at 48.5% +/- 5.0% and IAUC[60] at 49.3% +/- 4.5% of pre-treatment value, again both showing a similar treatment effect.

5.3.7 **Comparison of once and twice daily dosing, what is the optimum biological dose?**

All comparisons for DCE-MRI derived changes in $K^{\text{trans}}$ are made for liver metastases for uniformity. Figure 5.7 shows percent change in $K^{\text{trans}}$ plotted against plasma AUC for both once and twice daily dosing. The bold line (twice daily) and the dotted line (once daily) represent logarithmic curve fits. The data shows little difference in the spread of plasma AUC values or DCE-MRI parameters, nor in their relation for the two dosing regimes.
Figure 5.7
Percent change in $K^{\text{trans}}$ plotted against plasma AUC for both once and twice daily dosing. The bold line (twice daily) and the dotted line (once daily) represent logarithmic curve fits.

![Graph showing percent change in $K^{\text{trans}}$ plotted against plasma AUC for both dosing regimes.]

Figure 5.8 shows that the efficacy of both regimes appears similar as shown by the range of size reduction seen in liver metastases (as previously described in chapter 4). Both regimes show a similar relation of liver metastases size change to DCE-MRI derived $K^{\text{trans}}$. Both clinical studies in chapters 4 & 5 suggest that a greater than 40% reduction in $K^{\text{trans}}$ is clinically significant. Combining both studies, 42 patients with liver metastases where available for analysis with 27 patients having a DCE-MRI response >40% and 15 patients <40%. The >40% response group has a mean reduction in liver tumour size by bi-dimensional product of 12.5% as opposed to a mean increase of 19.1 percent in the poor DCE-MRI response group ($p=0.0002$, Mann Whitney U test). Figure 5.8 b shows this data plotted independently for once and twice daily dosing and that there is no obvious difference between the dosing regimes.
Figure 5.8 a& b

a. Percent change in tumour size at day 56 plotted against % change $K^{\text{trans}}$ for both once and twice daily dosing. b. Mean % change in tumour size for groups selected by achieving greater or less than a 40% DCE-MRI response again for once (OD) and twice (BID) daily dosing.

As stated in the introduction to this chapter DCE-MRI was performed mainly at 2-4 hours post dose and therefore mainly at $C_{\text{max}}$. DCE-MRI correlates well with $C_{\text{min}}$ however (table 4.1 & 5.1).
Figure 5.9 a & b.
Mean +/-SE Plasma average $C_{min}$ (a) and mean percent pre-treatment $\chi^{tran}$ on day 2 (b) plotted for the two dose regimes for less than and equal or greater than 1000mg per day.

(a)

Plasma Mean $C_{min}$ (ng/l)

\begin{center}
\begin{tabular}{c | c | c}
Oral Dose (mg) & $<1000mg$ & $\geq1000mg$ \\
\hline
Once Daily & n=11 & n=7 \\
Twice Daily & n=15 & n=23 \\
\end{tabular}
\end{center}

(b)

Mean % Pre treatment $\chi^{tran}$

\begin{center}
\begin{tabular}{c | c | c}
Oral Dose (mg) & $<1000mg$ & $\geq1000mg$ \\
\hline
Once Daily & n=10 & n=15 \\
Twice Daily & n=7 & n=19 \\
\end{tabular}
\end{center}
Figure 5.9a shows that $C_{\text{min}}$ is greater for the twice daily dose regime as would be expected ($p=0.004$) (correspondingly the average $C_{\text{max}}$ is greater for once daily dosing for the same daily dose). Figure 5.9b shows that, for colorectal liver metastases, there is no significant effect of dosing regime on $k_{\text{trans}}$ for the higher dose group but that for doses less than 1000mg, the BID group has a greater reduction in $k_{\text{trans}}$, but this is not statistically significant. This suggests that although the $C_{\text{min}}$ value may be important at lower doses, for higher doses a threshold is reached above which no improvement is seen in the DCE-MRI derived $k_{\text{trans}}$ performed at $C_{\text{max}}$.

Reviewing the PK for all cases in both once and twice daily studies shows a strong relation of $k_{\text{trans}}$ but also PK with tumour size at two months. This relation is shown in table 5.2. It can be seen that the lowest plasma PTK/ZK concentration ($C_{\text{min}}$) shows the strongest correlation, again suggesting that $C_{\text{min}}$ may be an important factor. However the correlation with $C_{\text{min}}$ does not stand controlling for plasma exposure. The fact that DCE-MRI is often performed at $C_{\text{max}}$, and that this is not as strongly correlated to tumour size response as $C_{\text{min}}$, suggests however that DCE-MRI performed at $C_{\text{min}}$ may provide important data.

<p>| Table 5.2 |</p>
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### 5.4 Discussion

When this study was designed, it was considered that the biologically active dose of PTK/ZK would not necessarily be the same as the MTD. Indeed, it was not clear from the preclinical data whether any toxicity would be evident. Dose limiting toxicity was, however, seen in this trial using PTK/ZK on a continuous twice daily basis in patients with advanced cancer. The DLT was grade 3 light headedness observed at 1000 mg bid, and to a lesser degree at the lower dose levels. A variety of symptoms accompanied the light headedness. In the worst case, ataxia and dysarthria with light headedness caused the patients to be bed bound. In the mild cases, dizziness was not at all incapacitating. For the
majority of patients that experienced light headedness, the symptom subsided over several days, with no dose adjustment of PTK/ZK necessary. The aetiology of this adverse event is not clear; it may occur less than 1 hour after ingestion of the capsules but usually appears by day 2. Thus, the light headedness may be related to early high exposure.

Findings from this study further support the use of DCE-MRI as a biomarker for assessment of biological activity with anti-angiogenic agents. The reduction in MRI $K^{\text{trans}}$ was greater in non progressors compared to progressors. A reduction in MRI $K^{\text{trans}}$ of greater than 40% is associated with a significant increase in response rate. The change in DCE-MRI was correlated with PTK/ZK exposure including AUC, $C_{\text{max}}$ and $C_{\text{min}}$ and to a lesser extent with oral dose, especially on day 2. Therefore, relationship between AUC and MRI $K^{\text{trans}}$ was characterised by an inhibitory $E_{\text{max}}$ model on day 2 and EC1 to explore PK/PD relationship on DCE-MRI in early treatment and at steady-state with a twice daily regimen. As with the OD regimen, this suggests a biologic dose of 1000 mg/day or greater (500 mg bid) is achieved with a PTK/ZK twice daily oral regimen. Furthermore, statistical analysis indicates that MRI $K^{\text{trans}}$ (day 2) is a strong predictive marker for disease stabilization, thus supporting the use of DCE-MRI as a biomarker for early assessment of tumour response.

In chapter 4 most non progressors received a single daily dose of $\geq 1000$ mg. All but 10 patients in this BID study received total daily doses of $\geq 1000$ mg daily. Thus, 33 of the 43 patients were treated at a biologically active dose and only 5 experienced rapid disease progression. This study confirms that a total daily dose $\geq 1000$ mg has anti-tumour activity, particularly in colorectal disease and neuroendocrine tumours. Twice daily dosing of PTK/ZK produces prolonged periods of disease stabilization and tumour responses similar to those seen in patients receiving PTK/ZK on a daily schedule.

PTK/ZK has been studied in combination with FOLFOX as first-line treatment of patients with advanced colorectal cancer. As stated in section 5.1, phase III trials in combination have been disappointing and issues have been raised concerning optimal dosing strategy. Review of these results suggests that 1000mg or greater is the optimal dose. However there are still issues concerning dosing schedule. Firstly, as toxicity appears to be related to peak concentrations of drug, and that peak concentration falls by day 15 along with symptoms of toxicity, there is a good rational for starting at a relatively lower dose and increasing this by day 15. The second issue is that although efficacy is strongly related to PTK/ZK plasma AUC and DCE-MRI $K^{\text{trans}}$ there is also a strong relation to plasma $C_{\text{min}}$. 

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and DCE-MRI is not performed at the time when $C_{\text{min}}$ occurs. The fact that DCE-MRI is often performed at $C_{\text{max}}$, and that this is not as strongly correlated to tumour size response as $C_{\text{min}}$, suggests that DCE-MRI performed at $C_{\text{min}}$ may provide important data. The data presented here shows that twice daily dosing may be more efficacious at lower doses, possibly due to higher $C_{\text{min}}$ levels but that at higher doses a threshold is reached making once and twice daily dosing equivalent. It is possible however due to the toxicity issues related to $C_{\text{max}}$ and the fact that DCE-MRI is performed at $C_{\text{max}}$ we have not properly explored the potential to increase overall drug exposure.

The author’s overall impression is that twice daily dosing should be the preferred option starting with a relatively low dose and increasing up to 750mg BID by day 15. This will substantially increase $C_{\text{min}}$ and AUC and may have increased efficacy with less side effects. This would be particularly important if the early effect of PTK/ZK is by inhibiting nitric oxide induced vasodilatation, as this effect would be sub-optimal if not consistently achieved throughout the treatment cycle. Further, it would be advisable to do a DCE-MRI study with scans performed at $C_{\text{max}}$ and $C_{\text{min}}$ to further explore this relationship.
### 5.5 Appendices

#### 5.5.1 Data set derived from studies described in chapter 4 & 5.

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Chapter 6:

Qualitative visual analysis of enhancement changes seen using DCE-MRI

6.1 Introduction

As reviewed in chapter 1 and 3 there are many potential ways of analysing the enhancement curves generated by DCE-MRI. The ideal method is to derive physiologically relevant components and to relate these to underlying biological processes. However, the more complex the model used for analysis is; the better the quality of data that is required. There is a balance between the reproducibility and sensitivity of a test against accuracy and specificity. For these reasons it is unlikely that the same techniques would be used to study cerebral gliomas, where tumours can be held still for long periods of time, as opposed to liver and lung tumours undergoing bulk motion due to respiration. In chapter 3 a variety of methods of analysing the signal intensities from DCE-MRI were discussed. The best performing parameters relating to reproducibility where IAUC, maximum enhancement and $K_{\text{trans}}$ based on data converted to $R_1$. As stated earlier it may be advisable to avoid maximum enhancement as it may be sensitive to changes in a single data point. IAUC was shown to be equivalent to $K_{\text{trans}}$ in the study of the activity of PTK/ZK on liver tumours in chapter 5.

One major factor in the two clinical studies (chapter 4 & 5) of PTK/ZK in liver tumours is the obvious visible changes in contrast enhancement (figures 1.5 and 4.3). Radiologists who are aware of these potentially large changes in contrast enhancement are now seeing these changes in CT scan studies of anti cancer agents such as Bevacizumab even when the study has not been designed to pick up enhancement changes. It is possible that imaging protocols could be devised, and radiologists trained, to be able to discern significant contrast enhancement changes on treatment in a visual and qualitative fashion. In simple terms; ‘if the reduction in enhancement cannot be seen, the drug is not working’.

This chapter involves a ‘visual’ assessment of enhancement parameters using DCE-MRI cases in patients with liver metastases from the dose escalating cohorts of the phase I studies described in chapters 4 and 5. As this study was performed before the finish of the phase I clinical studies, and liver metastases rather than colorectal liver metastases are used from the phase I trial in chapter 4, there is a different group of recruited cases;
however the same data set for $K^{\text{trans}}$, pharmacokinetics and response is available for most patients. Although qualitative studies of liver tumour enhancement have been performed in both clinical practice and trials $^{87,131}$ the author is aware of no studies in this context. If visual analysis of DCE-MRI day 2 can be shown to correlate with clinical outcome this could have advantages, as it could aid quick decision making without the need for potentially time consuming creation of parameter maps from the images.

6.2 Methods

28 patients with liver metastases from advanced carcinoma were treated with PTK/ZK on phase 1 dose escalating studies using DCE-MRI at pre-treatment, day 2 and at end of cycle (EC1). DCE-MRI methodology and details on patient selection, pharmacokinetics and assessment of clinical response are as detailed in chapters 3, 4 & 5. The value for $K^{\text{trans}}$ of a representative liver tumour for each case was then derived as in chapter 3.

The image sets of the DCE-MRI sequence where summarised to an image at each of the time points $t=0$ (pre-contrast) and 18, 45, 90 and 180 seconds post contrast. The images where randomised for scan date and depicted in random order by A, B, and C (figure 6.1) and printed at high resolution on A4 size ‘photo quality’ paper using an ink jet printer.

![Figure 6.1](image)

_A sequence of 3 DCE-MRI studies on the same patient randomised for study date, with time points post contrast injection. In this study B=pre-treatment, A=day 2 and C=EC1._

Three observers graded tumour enhancement independently on a visual analogue scale blinded to dose and imaging date. Their aim is to visually assess 3 parameters as shown in figure 6.2:
1. Overall enhancement – area under the curve of the enhancing part of the tumour
2. Peak enhancement – The maximal enhancement seen of the enhancing part of the tumour
3. Rate of enhancement of the enhancing part of the tumour

The results where recorded on a visual analogue scale for the three parameters. The distance along the scale was then recorded for cases A, B and C. After completion of the study the data was un-randomised for scan date, and the difference in recorded enhancement for the pre-treatment, day 2 and EC1 scans was then measured as the difference in distance between the data points presented as a percentage of the total length of the scale. The data was reviewed for the individual reviewers and their averaged response. As the primary purpose of assessing response visually would be to predict clinical response at an early stage the analysis concentrated on the day 2 DCE-MRI data. Statistical analysis is as for chapter 4 except receiver operating curve analysis is done to compare prediction of efficacy, as this is a useful way to compare tests without specifying the test result required (cut off point) to predict efficacy.

6.3 Results

Twenty-eight image sets where evaluated. The patient demographics are similar to the study in chapter 4. All cases were of liver metastases secondary to colorectal cancer (22),
breast (2), lung (1), thyroid (1) and urothelial (1) cancer. One case was a primary liver
tumour. DCE-MRI imaging was not available at day 2 for one case and for end of cycle 1
(EC1) in 6 cases. The data set therefore includes 27 and 22 patients at ‘day 2’ and ‘EC1’
respectively (21 ‘day 2’ and 17 ‘EC1’ for colorectal liver metastases).

On analysis of the ‘average’ visual response scores, as expected from previous results in
chapters 4 & 5, there was a significant reduction in contrast enhancement after treatment
with PTK/ZK on day 2 of 13.6 % (SE +/- 3.6, p=0.002), 10.9% (SE +/- 3.3, p=0.005), 12.3
% (SE +/- 3.5, p=0.004) for overall, rate and peak scores respectively (p values from paired
Wilcoxon rank sum test). For patients on ≥1000mg of PTK/ZK (n=12) this is increased to
21.1 % (SE +/- 4.6, p=0.005), 19.0% (SE +/- 4.7, p=0.006), 19.4 % (SE +/- 4.5, p=0.008)
for overall, rate and peak scores respectively. There was no significant difference in the
type of visual observation used. Although these results are statistically significant they
show smaller changes with lower significance than for the computer derived parameters
from chapters 4 & 5.

There is variable inter observer agreement as shown graphically in figure 6.3. Analysed for
showing either increase or decrease in enhancement, Cohen’s Kappa scores for rate of
enhancement show substantial agreement for reviewers 2 & 3 (Kappa score 0.77) and
moderate agreement for reviewer 1 with 2 & 3 (Kappa scores 0.42 and 0.48 respectively).
The following data concern the averaged data from the three reviewers. There is strong correlation between visually derived and computer derived enhancement parameters. Although it may be expected that the correlations for visually derived and computer derived parameters, such as visual peak Vs peak enhancement, overall Vs IAUC and visual rate Vs $K^{\text{trans}}$ enhancement, would be better this was not the case (Table 6.1).

Table 6.1

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<th>Parameter</th>
<th>IAUC[180]</th>
<th>'p' value</th>
<th>$K^{\text{trans}}$</th>
<th>'p' value</th>
<th>Peak Enhancement</th>
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Table 6.2 shows correlations for both visually derived and computer derived parameters against pharmacokinetics (plasma AUC and $C_{mm}$) and tumour response (size change at 2 months). Correlation exist for the visually derived parameters but are not as strong as for
the computer derived $K_{\text{trans}}$, IAUC[180] and peak enhancement. This is shown for average visual assessment of rate plotted against plasma AUC in figure 6.4.

Table 6.2

| Spearman Rank Correlation Coefficients for visually derived and computer derived parameters on day 2 with Plasma AUC, $C_{\text{min}}$ (both n=27) and response (n=15). |
|---|---|---|---|
| **Correlation coefficient and Statistical Significance** |
| Parameter | $K_{\text{trans}}$ | 'p' value | IAUC[180] | 'p' value |
| Plasma AUC | -0.683 | <0.0001 | -0.626 | 0.0004 |
| Trough plasma concentration ($C_{\text{min}}$) | -0.754 | <0.0001 | -0.641 | 0.0003 |
| Tumour size at 2 months | -0.729 | 0.002 | -0.757 | 0.001 |
| Visual Overall |  |  | Visual Rate |  |
| Plasma AUC | -0.374 | 0.055 | -0.432 | 0.025 |
| Trough plasma concentration ($C_{\text{min}}$) | -0.371 | 0.057 | -0.476 | 0.012 |
| Tumour size at 2 months | -0.438 | 0.1 | -0.561 | 0.030 |

Figure 6.4

*Change in enhancement as from visual assessment and computer derived $K_{\text{trans}}$ plotted against plasma AUC. The solid line ($K_{\text{trans}}$) and the dotted line (Visual) represent curve fits.*
Table 6.2 shows that there is correlation with the visually derived assessment of rate of enhancement and tumour size at 2 months. In the previous 2 chapters day 2 $K^{\text{trans}}$ was shown to correlate well with response. In order to compare visual assessment with $K^{\text{trans}}$ for the prediction of response, the subgroup of liver metastases from colorectal primary cancer is chosen (n=21). Day 2 results are compared with best response as defined by SWOG criteria (as described in chapter 4). Figure 6.5 shows receiver operating curves (ROC) for the two parameters for prediction of clinical response (a best response of stable disease). Receiver operating curve analysis is done as this is a useful way to compare tests without specifying the test result required (cut off point) to predict efficacy. Both parameters show a statistically significant relation but $K^{\text{trans}}$ analysis is superior to visual analysis with areas under the ROC curves of 0.91 (p=0.008) for $K^{\text{trans}}$ and 0.82 (p=0.04) for visual assessment.

![ROC analysis for visual assessment of the rate of enhancement and $K^{\text{trans}}$ predicting clinical response](image)

### 6.4 Discussion

These data show that visual analysis does provide relevant information relating to the biological activity of the anti-angiogenic agent. Although currently computer derived quantitative analysis provides improved information the ROC data is encouraging. This is particularly so, as although the blinded review was performed by consultant radiologists experienced in MRI, it was their first exercise in trying to quantify enhancement on a
sliding scale, with no training being given. Problems also include difficulties in quantifying signal to a reference and that the instructions for analysis could be refined, and a better standardised model developed. With this in mind, this data provides encouragement that if agents such as PTK/ZK become clinically important in cancer therapy, visual analysis of contrast enhancement may become part of the radiological report.
### 6.5 Appendices

#### 6.5.1 Data from clinical studies in chapter 6.

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Chapter 7:

Discussion

7.1 Clinical Trials using DCE-MRI

Chapter 4 & 5 concern phase 1 studies, conducted in Leicester and Freiburg, that have shown that DCE-MRI can provide useful information in the clinical study of an angiogenesis inhibitor\(^{100,104}\). The agent (PTK787/ZK222584) is a small molecule inhibitor of VEGF tyrosine kinases. We showed significant reductions in enhancement rates as early as day 2 after start of treatment, which may persist for months. These changes correlated significantly with increasing dose and plasma levels, as dose was escalated. Furthermore, the degree of enhancement reduction correlated well with changes in tumour size and clinical response. Similar changes have been seen in Glioblastoma Multiforme with the same agent\(^{111}\). A similar trial using an anti-VEGF antibody HuMV833 shows a similar magnitude of changes in contrast enhancement parameters\(^{132}\). These studies have helped in the selection of the dose and tumour types to be used in phase II and III studies.

Although there is a clear relationship between the measured enhancement parameters and treatment with the drug, the exact mechanism is not clear. The mechanism therefore has to be inferred, based on our knowledge of pre-clinical data and knowledge of the various factors that can affect the imaging test\(^ {124}\). In pre-clinical studies with other anti-VEGF agents, changes in endothelial cell survival and vessel density may not occur for some days after initiation of therapy\(^ {133}\). However, some studies show more rapid reductions in the density of immature vessels\(^ {120}\). \(K^{\text{trans}}\) reflects not only tumour vascularity and blood flow, but also vascular permeability. The effects observed may therefore be due to acute changes in permeability, vascularity or even other factors such as the action of VEGF on nitric oxide production\(^ {125}\). It seems likely however, that specific targeting of immature vessels contributes to rapid enhancement changes and the ‘normalisation’ of the vasculature as proposed by Jain\(^ {134}\).

Similar results have been obtained with the vascular targeting agent, Combretastatin A4 phosphate (CA4P)\(^ {135,136}\). DCE-MRI studies were performed to examine changes in parameters related to blood flow and vascular permeability (\(K^{\text{trans}}\)) and the initial area under the contrast medium concentration-time curve (IAUC) during a 24 hour period after...
treatment with CA4P. Eighteen patients in a phase I trial received escalating doses, and significant reductions in tumour $K^{\text{trans}}$ after treatment were seen.

A similar study of sixteen patients treated with 5,6-Dimethylxanthenone-4-acetic acid (DMXAA), an agent that causes vascular shutdown in pre-clinical models, in a dose escalating trial \(^{137}\) showed 9 of 16 patients had significant reductions in IAUC 24 hours after treatment.

In the last two studies, although there was some evidence of a dose-related response, the correlations were not clear-cut, presumably due to variation in response in each dose cohort. The PTK/ZK trials only studied the dose response for a similar tumour type (colorectal liver metastases) and although the dose-response exists for all tumour types, it is not as strong \(^{112}\). Furthermore, establishing a strong dose correlation requires starting with a dose that has little efficacy!

Effects on blood flow have also been observed with classical chemotherapy agents. DCE-MRI measurements using $K^{\text{trans}}$ have been made in sixteen patients receiving preoperative chemo-radiotherapy \(^{138}\). $K^{\text{trans}}$ and VEGF levels correlated before treatment. Eight responsive tumours had higher pre-treatment $K^{\text{trans}}$ values than non-responsive tumours, and showed a marked reduction in $K^{\text{trans}}$ at the end of treatment suggesting the possibility of anti-angiogenic action. In a separate, similar trial from the same centre, Taxane-based chemotherapy showed no effect \(^{139}\). However, in separate trials of preoperative chemotherapy, reductions in enhancement parameters have been seen in a variety of tumours with Docetaxol, with some relation to efficacy \(^{140}\). These trials concentrated on high temporal resolution scans with analysis of the ‘first pass’ gradient of the enhancement curve rather than $K^{\text{trans}}$ or IAUC.

It is important to know if chemotherapy regimes affect vascularity as seen on DCE-MRI, not only to judge DCE-MRI results from chemotherapy in combination with anti-angiogenesis agents \(^{141}\), but also to assess possible anti-angiogenic effects of low dose metronomic chemotherapy.

Initial DCE-MRI results may also predict response to treatment, generally with increased enhancement parameters predicting a good response \(^{66,138}\). This may be related to many factors, including tumour oxygenation, access of chemotherapy, and potential correlation with angiogenesis.
Cerebral contrast-enhanced T$_2^*$-weighted imaging has also proved useful in the brain in 24 patients undergoing treatment with carboplatin and thalidomide for malignant gliomas. Cerebral blood volume (CBV) maps created for the tumours before and after treatment showed marked reduction in patients treated with thalidomide and carboplatin in comparison to carboplatin alone. These changes correlated with efficacy after one year $^{141}$.

### 7.2 Other imaging methods measuring blood flow

#### 7.2.1 MRI measures of tumour blood flow without contrast media

For some time, MRI angiography methods have been available that can measure flow velocity in large blood vessels, but these are of no use in the tumour microvasculature. A newer technique, MRI arterial spin labelling (ASL) is a perfusion imaging technique that involves exciting protons (spin tagging) in a well-defined vessel that feeds the organ, and then recording the consequent signal change in that organ / tumour. This effectively measures tissue perfusion using arterial water as the probe. ASL has shown correlation with contrast-enhanced methods of cerebral blood flow in brain tumours $^{142}$. Although this technique has the advantage of using no exogenous contrast agent, allowing multiple studies to be performed sequentially, the signal to noise is considerably poorer than contrast-enhanced methods. The available signal to noise has been improved with the introduction of clinical MRI scanners operating at a higher magnetic field of 3 Tesla (compared with 1.5 Tesla in more common usage) $^{131}$. In investigational studies, the ability to do multiple sequential measurements in the same day may allow assessment of the perfusion changes over time, particularly in combination with pharmacokinetic measurements. The author is aware of a clinical study where serial ASL MRI is performed to predict the time of maximum pharmacodynamic effect in order to optimise DCE-MRI.

A less direct method of assessing vasculature uses intrinsic blood oxygenation level dependent (BOLD) contrast MRI. The BOLD technique uses a heavily T$_2^*$-weighted image that can depict changes in blood oxygenation. As an isolated study it is difficult to quantify, but changes in oxygenation can be detected by comparison with baseline. Signal intensities can be affected by vasodilatation by pharmacologic agents or even mental activity (functional brain imaging) $^{143}$. These techniques may be useful for mapping vascular maturity, since immature vessels do not have smooth muscle activity and do not respond to vasodilators. Bold contrast MRI has been used for mapping vascular maturation using the response of mature vessels to hypercapnia (inhalation of air vs. air and 5% CO$_2$) and the response of all vessels to hyperoxia (air and 5% CO$_2$ vs. oxygen and 5% CO$_2$
(carbogen))\textsuperscript{144-146}. This may help predict response to anti-vascular therapy\textsuperscript{147}. These techniques can be used in the clinic, although breathing carbogen can be unpleasant\textsuperscript{145}.

7.2.2 Computed tomography

CT has the advantage of being widely available and, like T\textsubscript{1}-weighted MRI, shows enhancement of the image with increasing concentration of contrast agent, although the volumes of contrast agent used are much higher with CT. Iodinated contrast agents in CT show similar distribution to MRI media, i.e. they are extravascular, extracellular agents with no specific uptake mechanism. However, the mechanism of contrast in CT is different: the image brightness depends on the degree of attenuation of the X-ray beam, and is related to the density of the tissue or contrast agent. With iodinated compounds, the degree of enhancement (i.e. increase in tissue-attenuation) is proportional to the concentration of iodine, making quantification straightforward, particularly when measuring arterial contrast enhancement during bolus injections\textsuperscript{148,149}. As for DCE-MRI, 'functional' contrast-enhanced CT techniques have shown increases in tissue perfusion that may reflect malignancy and stage\textsuperscript{150}. Furthermore, in a trial of 35 patients contrast-enhanced CT parameters were shown to correlate with microvascular density and VEGF expression in lung adenocarcinoma\textsuperscript{151}. CT is a faster and easier procedure to perform than MRI, with fewer potential artefacts and a higher spatial resolution (typically around 0.5 mm). Clinical MRI has a resolution in the order of 1-2 millimetres, although pre-clinical MRI can achieve resolutions of a few tens of microns. This makes CT a more robust technique that potentially allows automated analysis. Indeed, in one example, a retrospective trial was possible in 130 patients with primary lung carcinoma showing correlations with VEGF expression and microvascular density based on relatively straightforward acquisition parameters\textsuperscript{152}.

CT has the weakness of potentially poor anatomical coverage, which is being solved to some extent by multi-slice spiral technology\textsuperscript{153}. Generally, CT contrast media are safe, but have a worse side effect profile than standard MRI contrast agents. In particular, the 'hot flush' that many patients experience can make reliable multiple breath holding protocols for dynamic enhancement studies problematic.

The chief weakness of CT compared to MRI, and MRI compared to nuclear medicine, is the low sensitivity for detecting current clinical contrast agents or labelled probe. While CT contrast agents are often used in the millimolar concentration range, and MRI agents range from millimolar to micromolar, nuclear medicine agents are down to true "tracer"
picomolar concentrations. One must therefore be careful not to overwhelm the system under investigation, and the development of new, more targeted CT agents is difficult due to potential toxicity.

Theoretically, since CT and MRI standard contrast agents have similar pharmacokinetics, any DCE-MRI findings should be translatable to CT. This was demonstrated in a study of the VEGF-specific antibody Bevacizumab in human rectal cancer, where a rapid antivascular effect was shown by dynamic contrast-enhanced CT.

Despite the ease of using CT in clinical applications, there is the concern about the associated radiation exposure. The increased risks of radiation from a CT scan may seem trivial for many cancer patients; however, there are now strict regulations in Europe concerning techniques used for research that involve radiation, but are not of direct benefit to the patient. Currently, tumour perfusion studies are not of proven benefit to the patient, so these regulations make MRI easier to organise in clinical trials, unless CT studies can be linked with standard clinical CT protocols.

7.2.3 Radionuclide Imaging.

Single photon emission computed tomography (SPECT) and positron emission tomography (PET) utilise compounds labelled with radioisotopes as molecular probes. Both techniques have considerably poorer spatial resolution in comparison to CT and MRI, but much better sensitivity to low concentrations of tracer. While SPECT uses gamma ray emitters, PET uses positron emitters, with the annihilation of the emitted positron and an electron producing two photons (gamma rays of 512 KeV energy) that travel in almost exactly opposite directions. Detection of both these photons allows the location of the original positron emission to be determined to within a few millimetres. PET has advantages over SPECT: it has better spatial resolution, greater sensitivity to radiopharmaceuticals, easier quantification of tissue radiopharmaceutical concentration, and biologically important radiopharmaceuticals are easier to manufacture. Since most SPECT applications (such as labelled Annexin to image apoptosis) can be adapted to PET imaging techniques, this article will concentrate on PET applications.

A wide variety of simple positron-emitting atoms can be created, such as isotopes of oxygen, nitrogen, carbon and fluorine (chemically similar to hydrogen) and numerous others, without changing their chemical and biological properties. Since very low concentrations of probe are required, pharmacological effect is not usually a concern.
Unfortunately, PET is not widely available since, in addition to the scanner, on-site (or nearby) radiochemistry facilities and a cyclotron are needed to generate the short-lived isotopes, making it an expensive technique. Quantification of the tracer concentration is difficult, due to variable attenuation of photons from the deep structures, which can make follow up studies difficult.

Despite the expense, PET imaging in oncology is becoming standard in some areas. Its main applications use the probe 18-Fluro deoxyglucose (\(^{18}\text{FDG}\)) as an indirect marker of metabolically active cancer cells. This is a glucose analogue, which is transported into cells and undergoes hexokinase mediated phosphorylation. The end product, FDG-6-PO4, is not a significant substrate for subsequent reactions and is retained in the cell in proportion to rate of glycolysis. Increased metabolism is a biomarker for the presence of a tumour, since many tumours have high levels of glucose utilization via glycolysis rather than oxidative metabolism (Warburg effect). The relative specificity for FDG uptake by tumours has lead to PET becoming a standard tool in the staging of lung cancer, particularly in combination with CT scanning which improves spatial localization. Figure 7.1 shows an example of fused CT / PET imaging in a case of non small cell lung cancer, with no evidence of distant spread.

**Figure 7.1**

A Fused CT/PET study showing: (A) a coronal CT image showing a lung mass, (B) a coronal PET image showing increased \(^{18}\text{FDG}\) uptake in lung and (C) a fused CT/PET image showing the increased \(^{18}\text{FDG}\) uptake is directly related to the lung mass.

\(^{18}\text{FDG}\) -PET is often used during treatment of tumours such as lymphoma, and early work has shown changes related to prognosis after one cycle of chemotherapy. Dramatic responses have been seen in patients with advanced gastrointestinal stromal tumours within days of the first dose of the signal transduction inhibitor Imatinib (Gleevec). In a study of fifty-seven patients with non-small-cell lung cancer, reduction of metabolic activity after one cycle of chemotherapy as shown by \(^{18}\text{FDG}\) -PET was closely correlated with final outcome of therapy.
Using radiotracers, such as H$_2^{15}$O, $^{11}$CO or C$^{15}$O, and dynamic phase (monitoring the concentration over time) $^{18}$FDG-PET, blood flow and blood volume estimations can be made. Water provides perfusion information and CO, which binds to hemoglobin, gives blood volume information. In order to calculate flow; tissue and arterial tracer concentration measurements need to be made. Methodology (and problems encountered) are similar to that described for quantifying DCE-MRI enhancement and are largely based on continuing developments to the Fick and Kety principles in 1870 and 1951. Measurement of arterial tracer concentration is difficult for both PET and DCE-MRI. Unlike DCE-MRI, however, where arterial tracer concentrations can be estimated based on injection rate, injected volume and patient body weight, radionuclide studies require that the tracer activity is measured during the scan, since it changes over time due to rapid radioactive decay. Measurement of arterial tracer activity can be determined directly by arterial sampling, or by measuring the signal from a region of interest over the left ventricle or larger arteries, such as the aorta.

PET perfusion studies are complex and may take as long as three hours. The half life of 'non'-$^{18}$FDG tracers is short: for $^{15}$O it is 123 seconds, requiring onsite cyclotron facility. Like CT and MRI, the image is made of voxels - cuboid volumes each of which has signal intensity - and smaller voxels imply better spatial resolution. Partial volume effects occur when voxels are too large to capture the details of signal intensities that change frequently in small imaged volumes. These partial volume effects may be significant if the tumour size is of in the order of (or less than) the resolution of the scanner (~ 2cm). Partial volume effects are compounded by a phenomenon called "spill over" or "spill in" of signal counts from surrounding structures with high blood flow, such as the heart and aorta, or within areas of relatively high flow, such as liver.

As for MRI, PET has been used to measure the effects of Combretastatin A4 phosphate (CA4P) on tumour and normal tissue perfusion and blood volume in humans. Significant dose-dependent reductions were seen in tumour perfusion and tumour blood volume within 30 minutes after dosing, although by 24 hours there was evidence of tumour vascular recovery. Interestingly, the two-fold decrease seen in humans was not nearly as dramatic as the eight-fold reduction in tumour perfusion seen in rats at one hour, or the 100-fold decrease at six hours. This again emphasises the need to confirm pre-clinical findings in the clinic.
Herbst et al. imaged primary and metastatic lesions serially using $H_2^{15}$O-PET and $^{18}$FDG-PET to assess changes in tumour blood flow and metabolism during treatment with human recombinant Endostatin. They showed measurable effects on tumour blood flow and metabolism even in the absence of demonstrable anticancer effects. The data suggest that there is a complex, possibly nonlinear, relationship between tumour blood flow, tumour metabolism, and Endostatin dose. In a study of 35 patients with locally advanced breast cancer, $^{18}$FDG and $^{15}$O-water PET imaging before and after 2 months of chemotherapy were used to assess metabolism and perfusion. Although both resistant and responsive tumours had an average decline in metabolic rate over the course of chemotherapy, resistant tumours had an average increase in blood flow. Patients whose tumours failed to show a decline in blood flow after 2 months of therapy had poorer disease-free and overall survival. These studies clearly show that measured perfusion is not necessarily coupled with metabolism or response.

### 7.2.4 Ultrasound

Ultrasound imaging is inexpensive, quick to perform and a mainstay in obstetrics and the diagnosis of disease. Ultrasound uses pulses of high frequency sound waves (usually between 3 and 20 MHz) that are transmitted into the body, and reflected by the different structures. These echoes are detected by a piezoelectric crystal, which can turn the reflected sound waves into an electrical voltage. The resolution of traditional ultrasound depends on the frequency used, with higher frequency giving better resolution, but poorer depth penetration, a problem for high-resolution clinical imaging. High frequency ultrasound may be useful in accessible human tumours such as ocular melanoma and skin tumours. Imaging deep structures is also compromised by poor accessibility to certain anatomical regions (for example, those that are behind bone) and operator dependence. Blood flow can be measured by using the Doppler shift in the echo frequencies caused by movement of the blood. Using pulsed Doppler, this information can be displayed as a waveform of vascular flow velocity at a certain position, while colour Doppler gives an image of mean blood flow velocities (Figure 7.2). Power Doppler, on the other hand, shows a map of blood flow amplitude, which is useful for assessing flow in small vessels. Ultrasound therefore has the potential to provide effective, low cost, sequential monitoring of vascular changes associated with malignant tumours and their response to treatment.
Figure 7.2

A: Colour Doppler image of the renal vessels. Red represents flow towards, and blue away from the ultrasound probe. Colour Intensity is related to flow velocity.

B. Pulsed Doppler trace showing flow velocity patterns with systolic (large arrowhead) and diastolic (small arrowhead) flow. The upper trace shows a high resistance pattern with relatively low diastolic flow compared to the lower trace. Changes in vascular resistance may be of use in monitoring treatment.

Non-invasive monitoring of anti-angiogenic therapy has been performed by serial power Doppler and colour Doppler ultrasound imaging of pre-clinical tumours, showing reduction in vascularity with treatment by anti-vascular and anti-VEGF therapies. Colour flow Doppler has also been used to characterise superficial solid tumours in patients. In a study of sixty-seven patients with melanomas before surgical excision, high-frequency sonography and colour Doppler sonography parameters correlated with tumour aggressiveness. In a further study, tumour vascularity index was evaluated with power Doppler US in 44 patients with advanced hepatocellular carcinoma treated with 200-300 mg/d thalidomide. The pre-treatment vascularity index was significantly higher in responders than in non-responders.

The development of ultrasound contrast agents relies on one of the main disadvantages of ultrasound: the fact that ultrasound waves do not travel well through air. Any air / soft tissue interface causes strong echogenicity, and deeper structures cannot be seen. Ultrasound cannot therefore be used to ‘see’ though lung, and gas-filled bowel loops can
prevent successful abdominal imaging. Ultrasound contrast media use ‘microbubbles’ of air surrounded by a polymer shell, which are intensely echogenic. This improves the image of any vascular structure and enhances Doppler studies allowing smaller vessel sizes down to 40 μm to be discriminated. Increasing the energy of the ultrasound pulse, or selecting a particular ‘harmonic’ frequency can also destroy these microbubbles. This allows imaging the reappearance of the microbubbles, reflecting flow into the imaged area, and quantification of perfusion 169.

Correlations between ultrasound-derived enhancement parameters and microvascular density have been demonstrated in animals 169. In human breast tumours, enhancement parameters have been shown to be different in carcinomas and benign lesions after intravenous injection of microbubbles 170. Contrast-enhanced power Doppler ultrasonography (PDUS) has also been used in determining the angiogenic status of 21 patients with renal cell carcinoma. The colour pixel ratios of selected images were calculated as the ratio of the number of pixels showing power Doppler signals to the total number of pixels within the lesion. A significant correlation was found between colour pixel ratio and microvascular density 171.

Thirty-five consecutive patients with pathologically confirmed, non-resectable pancreatic carcinoma were examined with contrast-enhanced US before systemic chemotherapy. The median time to progression and median survival was longer in patients who had avascular tumours compared with patients who had vascular tumours 172. In 15 patients, follow-up examinations after stereotactic, single-dose radiotherapy were performed using contrast-enhanced ultrasound showing a significant reduction of the arterial vascularisation in treated tumours (p<0.05) 173.

7.2.5 Discussion of blood flow imaging

The results described above have caused great excitement in the field of drug development, because they offer the hope not only of establishing a ‘proof of concept’ of drug activity with relatively few patients, but also of aiding dose selection for phase II trials without relying on dose-limiting toxicity. There is a problem, however, in that although a positive result is reassuring, many promising agents have not revealed positive results using PET and MRI 174. Also, initial encouraging findings using these tests are no guarantee of later success. Positive results in combination therapy in the presence of toxicity may give encouragement for other regimes to be explored. In evaluating all biological agents, it must be recognised that they affect not only their primary target but also the activity of other
kinases, some known and some possibly unknown. The exact mechanism of enhancement reduction is also unclear and may be different for different agents and at different times. A positive result from an indirect test, therefore, may not relate to the expected activity of the agent. Further there is a danger that efficacious treatments could be dismissed because DCE-MRI with standard contrast media is insensitive to their mode of action, or their onset of action is too slow. The development of new contrast media and isotope probes will considerably aid understanding of the mechanisms of enhancement reductions. Furthermore, the fact that Endostatin has produced measurable effects on tumour blood flow using PET but not MRI in the absence of tumour regression, and that, as previously stated, measures of metabolism do not always couple with measures of perfusion, provides evidence that different tumour imaging methods may be required as end points in different situations.

There is also no consensus about how MRI or PET scans should be performed and how the data should be analysed. Although there is a wealth of experience in animal models, these sometimes do not help in the planning of human trials, since different tumour types are often studied, with imaging protocols that are not feasible for clinical trials due to potentially toxic, unlicensed agents or clinically impractical imaging protocols. Translational imaging studies comparing effects in animals and humans using similar regimes do, however, provide information helpful in planning and interpreting clinical studies. Reproducibility studies such as in chapter 3 are required to judge the numbers required to obtain significant results in trials but also, possibly more importantly, to judge the significance of changes in the individual patient. Reproducibility varies depending on methods employed, but often shows coefficient of variation of approximately 14 - 20%. This implies that such studies should be sensitive to treatment changes of approximately 15 - 20% if cohort studies of 10 patients are used. The intra-patient repeatability, which is an indicator of the significance on an individual patient's response, is generally higher, of the order of 30-40% . Technique refinement should improve these values in future.

Due to the dynamic nature of MRI contrast enhancement and PET studies, care must be taken not to interpret 'reductions in enhancement' as an indication that the drug will be delivered less effectively to the tissues. In both PET and MRI studies, a reduction in enhancement may simply relate to a delay in achieving maximum tissue concentrations of the tracer, due to reducing the perfusion of a tumour or vascular permeability. The potential maximum concentration of the tracer in the extravascular space may never be
achieved due to limited clinical imaging times, ranging from 5 to 20 minutes, and the fact that the tracer concentration declines, either by renal excretion or the short half-life of isotopes, during this time. This is important since, during treatment, ‘steady state’ plasma levels of a pharmaceutical compound should be achieved, and delays in achieving peak tissue concentration, even of several hours, should not be significant. Also, it has been suggested that blocking VEGF signalling “normalises” the tumour vasculature by selective destruction of immature blood vessels. A further treatment effect includes lowering the interstitial fluid pressure creating a hydrostatic pressure gradient across the vascular wall. This induced pressure gradient may actually lead to better delivery of molecules into tumours. Thus, anti-VEGF therapy may paradoxically improve the access of therapeutic agents to cancer cells.

7.3 Indirect tests not measuring blood flow

7.3.1 MR Spectroscopy

By altering the way in which the signal from hydrogen is measured, the slightly different resonance frequency of some common metabolites allows their concentration to be estimated (\(^1\)H MR Spectroscopy). With more specialist MRI equipment, metabolites containing phosphorus, such as adenosine triphosphate, can also be measured (\(^31\)P MR spectroscopy).

Measuring the levels of different molecules in vivo has considerable appeal, although progress has been slow due to the poor sensitivity of the technique and therefore the limited range of molecules that can be studied. Considerable improvement in clinical results has been possible with use of increasing magnetic field strength in commercially available MRI platforms. Elevated Choline levels are detectable by \(^1\)H-MRS in cancer, and correlate with malignancy and cell proliferation in brain tumours. This can help diagnose malignancy, and has particular clinical value in distinguishing radiation necrosis from recurrent tumour in the brain (figure 7.3).
Figure 7.3 A,B & C

A: A $T_2$ weighted MR image of a brain lesion after radiotherapy for a brain tumor. Black box represents a region of interest over a brain lesion for study by $^1$H-MRI spectroscopy.

B. The expected spectrum for a malignancy with high Choline (Cho) level in comparison to Creatine (Cr). N-Acetyl Aspartate (NAA), an axonal marker, is low in concentration.

C. The spectrum for radiation necrosis returned in this case. Both the Choline and N-Acetyl Aspartate are low compared to Creatine.

Although not specifically related to angiogenesis, any technique that shows a measurable difference between benign and malignant tissue could be adapted as a potential test for response to treatment. Garwood and colleagues have shown that $^1$H-MRS can demonstrate changes as early as 1 day in neo-adjuvant breast cancer therapy, which are correlated with response after 6 weeks $^{180,181}$.

7.3.2 Diffusion-weighted MRI

By using the magnetic field gradients, the MRI signal can be made sensitive to water motion at the microscopic level. Images that are sensitised in this way are used extensively
in the clinical investigation of stroke, being extremely sensitive to the changes in water mobility that occur in acute ischemia because of cytotoxic oedema due to membrane pump failure. There is also increasing interest in this technique as a method of monitoring apoptosis, which is a demonstrated result of anti-angiogenesis treatment 182.

While water mobility decreases in acute ischemia, it increases in the case of extracellular oedema. This is consistent with the observation that treatment of tumours can cause an initial decrease in measured water diffusion with a subsequent increase 183. These techniques have been shown to be of value in a combination trial demonstrating the value of adding Taxol to radiation therapy 184.

7.3.3 Radionuclide Imaging.

Although 18FDG-PET scans are those most commonly used in clinical oncology, there is increasing use of other agents with indirect mechanisms of measurement but acting as more specific indicators. Agents are available that are sensitive to programmed cell death (apoptosis), due to affinity to phosphatidyl serine which is externalised on the cell wall early in the apoptosis pathway (99mTc-labeled annexin) 185 and proliferation by 18F-fluorothymidine (18FLT-PET) 186. These agents can also show some perfusion information in the dynamic phase. Detection of programmed cell death (apoptosis) by imaging is potentially interesting for assessing malignant and benign disorders, since apoptosis mediates tumour cell and angiogenic vascular endothelial cell regression.

Hypoxia in tumour tissue is also an important prognostic indicator of response to either chemotherapy or radiation therapy. Therefore, detection of hypoxia in advance of such interventions is of importance in optimising the use and outcome of different therapeutic modalities. Furthermore, many anti-angiogenic therapies alter oxygen levels in tumours. Misonidazole molecules bind in inverse proportion to oxygen levels and [18F]-fluoromisonidazole (18FMISO) or more recently 60Copper diacetyl-bis(N-methylthiosemicarbazone) (60Cu-ATSM), can be used to study hypoxia and changes in oxygen status 187. 18FMISO has been used to quantify hypoxia in the rat glioma by PET, and may provide functional information about the results of anti-angiogenic therapy 188. In fourteen patients with biopsy-proved cervical cancer, 60Cu-ATSM-PET, before initiation of radiotherapy and chemotherapy, showed the frequency of loco-regional nodal metastasis was greater in hypoxic tumours. Tumour 18FDG uptake did not correlate with 60Cu-ATSM PET uptake showing measurement of hypoxia is independent of metabolism as measured by 18FDG-PET 189. Similar correlations have been found in lung cancer 190.
[18F]-fluorothymidine (18FLT) acts as a marker for proliferation and has the potential to be used as a specific agent for assessing disease activity in various stages of different malignancies. As cytotoxic chemotherapeutic agents affect cell division earlier and more prominently than glucose metabolism, 18FLT-PET may prove to be superior to 18FDG-PET for assessing response to treatment 191.

### 7.4 Specific Angiogenesis Imaging in Development

As well as observing downstream effects of successful treatment, whether specific to angiogenesis (blood flow) or simply related to successful treatment at a cellular level, there is interest in imaging specifically to document the effect of treatments on their intended site of action. This section will rely mainly on pre-clinical in vivo data to speculate about what may be achieved in human trials in the future. Imaging will almost certainly rely on contrast media or other probes which, to be successful in humans, will need to be imaged at very low concentrations. Because of this, PET imaging is at the forefront. Due to the ability to label molecules with isotopes such as oxygen and carbon for PET imaging, it is possible to label just about any specific marker. PET studies also have the potential advantage that the treatment agent can be directly labelled. This allows direct imaging of drug delivery by ‘microdosing’, and chemotherapeutic agents, such as 18F-fluorouracil, have been synthesised to assess their pharmacokinetics and metabolism. The concentration of 18F-fluorouracil in metastatic colorectal cancer has been correlated with patient survival 192. Labelled VEGF and other mediators of angiogenesis can also be used to predict response to anti-VEGF treatment 193. For studying angiogenesis, there has been some work in labelling integrins, specific to endothelial markers in angiogenesis, and endothelial growth factor receptors including a Her2/neu agent 194,195. Direct labelling of the actual therapeutic agent can provide crucial information necessary for trial design and optimal dosing 196. In a study of twenty patients with progressive solid tumours treated with various doses of the anti-VEGF antibody HuMV833, the agent was labelled with 124Iodine. PET showed antibody distribution and clearance were markedly heterogeneous between and within patients and between and within individual tumours 132. This suggests future trial designs for this type of agent that use defined tumour types and potentially intra-patient dose escalation.

Molecular imaging by MRI has been thoroughly reviewed elsewhere 26. The main problem is developing a contrast agent, which can be ‘seen’ by MRI at nanomolar concentrations and that can be linked to specific probes. What works in animals may not be helpful in
humans due to long development times and potential toxicity. Pre-clinical imaging with MRI scanners with much smaller access bores allows much higher magnet strengths to be achieved (typically six times that of a standard clinical scanner) giving greater sensitivity to low concentrations, or better spatial resolution in the range of 10-100 microns rather than millimetres.

Nanoparticles composed of a perfluorocarbon emulsion coated with a layer of lipid have been developed. Linked to the lipid layer of each nanoparticle are up to 90,000 molecules of gadolinium-DTPA, enough to enable detection at low concentrations. Into the lipid outer layer, hundreds of homing molecules can be added, such as antibodies, peptides, or peptidomimetics. By targeting a protein alpha v beta 3-integrin, it is possible to detect the immature blood vessels that characterise angiogenesis in vivo in pre-clinical models.

An exciting property of MRI contrast media is that they are not imaged directly but by their effect on surrounding water. This means they have the potential to be activated by chemical reactions in the body, an effect has been used in imaging gene expression in vivo in pre-clinical models. Where a gene transfer is attempted by a vector, a technique that may be used to modify angiogenesis in the future, transduction efficiency of the vector can be tested by the inclusion of a marker enzyme with the vector. The marker enzyme's effect could be to activate the MRI contrast agent. Such systems and further different approaches have been designed in pre-clinical models.

The high sensitivity of ultrasound to microbubble contrast means that high frequency ultrasound systems can be designed to be sensitive to a single microbubble. As well as microbubbles of air, perfluorocarbon nanospheres, similar to that used in MRI, have been developed. Vectorisation of these contrast agents, in particular with a specific alpha v beta 3-integrin monoclonal antibody, directed at endothelium in tumour vessels, has already been accomplished in pre-clinical models. Since it is possible to focus ultrasound energy to destroy these spheres, targeted drug delivery under ultrasound guidance may also be possible.

Optical imaging is based on the use of molecules that may affect or emit radiation in the visible or near visible spectrum in a variety of ways including scattering, absorption, and fluorescence. These 'chromophores' or 'fluorophores' may be intrinsic to the tissue, or may be administered. Optical imaging is currently limited to research, but with endoscopic imaging technology, fluorescent and bioluminescent probes could be seen in clinically relevant sites in humans. The inability of light to pass from deep tissues is the
biggest stumbling block, although the use of near infrared (NIR) light emitters and recent advances in laser technology and photon detection has improved this. The main intrinsic mechanisms of NIR light attenuation in tissue are scattering due to variations of the cellular organelles, and absorption mainly due to oxy- and deoxyhaemoglobin and some lipids and water. The combination of multiple NIR light measurements through tissue at several projections, allows tomographic techniques to be used. The interpretation of these types of image may be helped by registration with CT or MRI images.

Administered contrast agents may be specific to anatomical, physiological, biochemical or molecular function. Optical tomography, using intrinsic hemoglobin concentration, and separately with extrinsic indocyanine green, a light absorber, has successfully been used to detect breast lesions in a clinical setting.

Although these techniques do not immediately lend themselves to human studies, the approach may be useful. Whether they are translatable to humans remains to be seen, and depends on the toxicity of the agents and the ability to achieve satisfactory imaging resolution and signal-to-noise.

7.5 Conclusion

This is a continually evolving field and it is difficult to know what the future brings. What is clear is that imaging tests are available that can give useful information to aid development of anti-angiogenesis strategies in humans. The good news is that when changes are seen in the clinic, they are almost always rapid and there are few cases where imaging ‘too early’ has failed to see a response. Many studies either show or suggest a relationship between dose and response or efficacy, although human trials are always confounded by heterogeneity in patients and tumour types. It is clear that positive results may not always show correlation with each other or with clinical outcome, and there should not be over-reliance on the accuracy of any one technique. Although perfusion and glucose metabolism are sometimes ‘coupled’ in untreated tumours, studies in which both parameters have been measured before and after treatment show that perfusion and glucose metabolism may not change in parallel in response to therapy.

However, when imaging is ‘successful’, there is a danger of putting too much weight on cases where imaging is positive, ignoring tumour types where there is no imaging response. This is particularly true in comparisons of MRI enhancement effects in angiogenesis inhibition. It is reasonable to assume that treatment will have a bigger (or
more rapid) effect on metastatic lesions, with high proportions of immature strongly-
angiogenic blood vessels, than a primary tumour. This, however, may take attention away
from a more subtle but clinically significant response in the primary tumour with its larger
proportion of mature vessels and better perfusion. Variations in the effect of angiogenesis
treatments may also be due to differing levels of natural anti-angiogenic agents. In one
case, removal of a primary colorectal tumour resulted in an increase in metabolic activity
in its liver metastasis with a concomitant drop in levels of angiostatin and Endostatin in
urine and plasma, respectively. These circulating inhibitors of angiogenesis have been
shown to affect the growth of distant micro metastatic disease in patients with cancer, and
the level of these factors may well affect the degree of response to be expected from
biomarker studies. Variations in effect could also be caused by several other confounding
factors. The old adage therefore applies: treat the patient, not the images.

Many advances in the past have been made because of the observation that something
works, without the need to discover the mechanism. Now, drugs are being designed to
have an effect on specific mechanisms. Unfortunately, understanding of these mechanisms
is incomplete, and designing drugs to work perfectly in the test tube is no guarantee of
ultimate success. Furthermore, lack of understanding of the specific mechanism is no
guarantee that it will not work for other reasons. Whilst it will always be important to
progress understanding of mechanisms of action for both imaging and treatment, a more
pragmatic approach is needed in the interim. Whether an imaging test is valuable depends
on whether it can be established as a surrogate endpoint or biomarker for the desired effect,
and therefore answer key questions for drug development rather than simply providing
interesting data. These questions include:

Did imaging help to assess whether the mechanistic goals were achieved?

Did imaging assist dose selection for phase II?

Did imaging provide assistance for schedule selection for phase II?

Can imaging select subpopulations enriched for response?

This approach has been used in the summary of a recent encouraging publication of the
efficacy of a novel oral angiogenesis inhibitor AG-013736.

To achieve further progress, a huge multidisciplinary effort is required. Radiologists
clearly have to learn about molecular biology, but also clinical oncologists, molecular
biologists, scientists and particularly the pharmaceutical companies need to understand imaging. A multidisciplinary approach is essential to achieve validation and standardization of imaging methodology and to draw up guidelines to ensure consistent and standardized reporting on findings. Comparison studies to determine which imaging methods work best (alone or in combination) should be instituted. The pharmaceutical companies could play a key role in developing advanced contrast agents whose main clinical role may be in the assessment of novel anti-cancer agents. Pharmaceutical companies must also take a translational 'bench to bedside' approach to imaging: pre-clinical development of angiogenesis inhibitors should include developing imaging approaches suitable for use in subsequent clinical trials.

In Leicester our strategy is to work with the international community, developing imaging protocols specific to different organ systems, refining analysis protocols and particularly taking the best available imaging and analysis protocols and applying them to large clinical trials. We are currently involved in several multicentre trials including advanced cancer, liver metastatic disease and prostate cancer with a variety of novel cancer therapies including potential chemo preventative strategies.

With further cooperation and progress, these imaging techniques, as surrogate endpoints for efficacy of biological agents, may become as commonplace as CT scans for drug development, and may even become standard imaging tests for all oncology patients.
Chapter 8: References

Acknowledgements:
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