Vessel Size Imaging of the Shionogi Tumour Model using Contrast Enhanced MRI

by

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Abstract

Vessel size imaging is a relatively new technique in Magnetic Resonance Imaging. By measuring the change in the transverse relaxation constants $R_2$ and $R_2^*$ caused by the injection of a superparamagnetic contrast agent known as ferumoxtran, it is possible to create a map of vessel size index, which is a weighted average of the vessel radius. This technique was used on the Shionogi tumour model to monitor changes in vasculature as a result of androgen ablation by way of castration. A vessel size index map was created for androgen dependent, regressing and androgen independent tumours, and a statistically significant difference between the stages was found. Regressing tumours had the smallest vessel size index followed by androgen dependent and then androgen independent tumours. In addition, a qualitative comparison with Hoechst stained tumours was made.
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Chapter 1

Introduction

1.1 Motivation

Prostate cancer is expected to affect one in seven Canadian men in their lifetime. It is second only to skin cancer in the number of men diagnosed and is the second most deadly form of cancer after lung cancer for men [15]. Unfortunately, it is also one of the most poorly understood forms of human cancer, which is one of the primary reasons for this study.

As with most cancers, early detection is key to improving the chances of survival. With early detection there is a greater chance the tumour can be treated before it has metastasized. At this stage it can be treated quite effectively with surgery to remove the tumour, or radiation therapy (either external or brachytherapy). Once the tumour has metastasized, the chances for survival drop drastically and the only effective form of systemic therapy is androgen withdrawal. This treatment leads to a symptomatic and/or objective response in 80% of patients [12].

Prostate cancer is a hormone dependant tumour. It relies on male androgens to proliferate. If the supply of these is cut off, the tumour will regress, usually shrinking or at least slowing its growth. This treatment can be accomplished through castration, or less drastic chemical means. Luteinizing hormone-releasing hormone (LHRH) analogs reduce the amount of luteiniz-
ing hormone (LH) released from the pituitary gland and hence, decrease the production of testosterone. Within four to six months, this technique can be as effective as castration at reducing the supply of androgens. Anti-androgens act by inhibiting the body’s ability to use androgens and are often used in conjunction with LHRH analogs [15]. Unfortunately, in the majority of cases, the tumour progresses to an androgen independent state within a few years. At this point, chemotherapy may be used as a last resort but has a response rate of less than 10% and no survival benefit [12].

For the above reasons, it is particularly interesting to study the prostate tumour micro-environment as it progresses through the stages of androgen dependent growth, regression after androgen ablation, and subsequent relapse. It is already known that regression is linked with a decrease in vascular endothelial growth factor (VEGF), and relapse with a subsequent increase [8]. As tumour growth is largely restricted by oxygen and blood supply, a coordinated hormonal and anti-angiogenic treatment may prove more useful than either treatment alone. Vessel size imaging would provide an easy way to monitor the effect of this type of treatment.

1.2 Shiongi Model

There are very few animal models of prostate cancer. One of the main reasons for this is that prostate cancer rarely arises spontaneously in animals, and the human cancer is especially hard to grow in culture or as xenografts. The ideal animal model should model the human cancer as closely as possible. It should produce prostate-specific androgen (PSA) and be androgen sensitive. After castration the PSA levels should drop and the tumour should decrease in volume with accelerated apoptosis. Subsequently, enough
cells should survive to lead to regrowth of the tumour following castration. Eventually, there should be metastasis to bone and lymph nodes [17].

None of the existing models mimic the human strain perfectly or meet all the laboratory requirements for objective studies. Some of these include the Dunning rate model (and its sublines), the Shionogi model, the Noble rat prostate adenocarcinoma, Dr. Pollard’s spontaneous adenocarcinoma, and the ACI model. Of these, the Shionogi model is the most useful for the study of androgen ablation as the tumours reliably regress with significant tumour shrinkage and subsequently relapse [17].

The Shionogi carcinoma was developed from a spontaneously occurring hormone independent mammary carcinoma in 1961. Upon discovery, the tumour was transplanted into male host DD/S mice and carried forward by transplantation into male hosts only. After 19 generations, the tumour was again transplanted into both male and female mice, but no growth was seen in the female mice after 45 days. Further tests indicated the tumour would not grow in any female mice or castrated male mice, but growth could be initiated with testosterone. Androgen ablation in established tumours lead to complete tumour regression, but growth could be initiated again, even after 45 days, with testosterone. To differentiate the new tumour, it was named Shionogi Carcinoma 115 [13]. Subsequently, variant lines of Shionogi tumour were developed including strains that would progress to an androgen independent state [2]. A typical growth curve can be seen in Figure 1.1.

Fortuitously, some work has already been done on changes in vasculature in Shionogi tumours. Jain [8] used dorsal skin fold chambers to observe changes in Shionogi tumours from androgen dependent growth to the end of regression. He found that capillary density increased during androgen dependent growth and subsequently decreased to one-fifth of its pre-castration
value 2 weeks after castration. In addition, he found that the mean vessel diameter decreased from 38.3 μm to 9.4 μm. No data for relapsing tumours was given.

### 1.3 MRI Basics

Nuclear Magnetic Resonance Imaging (MRI - the N is omitted by convention) is a relatively new imaging modality having been developed in the 1970's and 80's. It is based on a phenomenon known as Nuclear Magnetic Resonance (NMR), which was discovered earlier by Bloch and Purcell in 1946 [14]. Today, its use is widely established, largely because, with the change of a few imaging parameters or the introduction of contrast agents, it can be used to measure a seemingly endless number of physiological and
biological parameters.

NMR is the phenomenon whereby a nucleus' magnetic moment will precess about an external field at a frequency known as the Larmor frequency:

\[ \omega = \gamma B \]  

(1.1)

where \( \gamma \) is known as the gyromagnetic ratio, which depends on the particular nucleus being studied. Hydrogen is the most commonly used nucleus for imaging largely because it is the most numerous in the body. For \(^1\text{H}\), \( \gamma = 26753 \times 10^4 \text{s}^{-1} \text{T}^{-1} \). In the absence of a magnetic field, the magnetic moments will be randomly oriented resulting in no macroscopic magnetic moment. The application of an external field causes the moments to align, producing a net magnetization, \( M \), which points along the direction of the main magnetic field of the magnet, \( B_0 \), which is conventionally defined as \( \hat{z} \).

An alternating radiofrequency field, \( B_1 \), tuned to the Larmor frequency can be used to rotate the magnetization down from \( \hat{z} \). Typically it is rotated down by 90° into the xy plane. Once here, several things will begin to happen as the magnetization “relaxes” back to its equilibrium state along \( \hat{z} \). These processes are described by the Bloch equation:

\[
\frac{dM}{dt} = M \times \gamma B - \frac{M_x \hat{x} + M_y \hat{y}}{T_2} - \frac{(M_z - M_0) \hat{z}}{T_1}
\]  

(1.2)

The slowest process is the longitudinal relaxation, the restoration of the magnetization along \( \hat{z} \). It is a result of exchanges in energy between nuclei and the surrounding lattice, which is enhanced by field fluctuations in the transverse direction. Hence, the constant governing the return to equilibrium, \( T_1 \), is known as the spin-lattice relaxation time constant.

The next fastest process is transverse relaxation. It is due to the same processes as longitudinal relaxation, but in addition, fluctuations in the
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$z$ component of the field due to interactions of the spins with each other makes this process faster than longitudinal relaxation, and the spin-spin time constant, $T_2$, is less than $T_1$. Solving equation 1.2 isolates these two processes:

$$M_z = M_0 \left(1 - e^{-t/T_1}\right) \quad (1.3)$$

$$M_{xy} = M_0 e^{-t/T_2} \quad (1.4)$$

Spin-lattice and spin-spin relaxation are irreversible. Once started, the only way to return to a transverse magnetization is to wait for $M_z$ to return to equilibrium and then flip it down into the transverse plane again. In contrast, the next process is reversible. It is due to inhomogeneities in the magnetic field from an imperfect magnet and susceptibility effects of tissue. These cause nuclei at different points in a volume to precess at slightly different rates, and thus loose phase coherence. This phase coherence can, however, be recovered by flipping the spins by $180^\circ$ in the transverse plane to create an echo. The $180^\circ$ pulse effectively reverses the spins so that any phase accumulated before the pulse is undone afterwards. If it is not recovered, the transverse magnetization decays according to:

$$M_{xy} = M_0 e^{-t/T_2^*} \quad (1.5)$$

This last process is a hint as to how imaging works. Rather than just having a constant external field, field gradients, $G_x, G_y$ and $G_z$, can be used to cause the magnetization to precess at different frequencies depending on their location. For example, if one wants to image a slice in the x-y plane, a $B_1$ pulse with a limited spectrum of frequencies applied at the same time as a gradient in the z-direction (slice gradient) will excite only those nuclei within a slice whose frequencies are part of the excitation range of the pulse.
Similarly, if the signal is read while a gradient in the x-direction is applied (read gradient), the x-location of each spin will be encoded by its frequency and spatial information can be returned by taking a Fourier transform. A third spatial direction can be encoded by introducing gradients in the y-direction (phase gradients) in between the slice selection and read gradients. This will introduce a phase shift in the spins that depends on the y location of each spin. Thus, the image is acquired in what is known as “k-space”, which can be converted to a spatial domain image by using a 2 dimensional Fourier transform.

The simplest imaging sequence used in MRI is the gradient echo (GE) sequence as seen in Figure 1.2(a). The read gradient is used to dephase and then rephase the spins so that the maximum signal is received in the centre of k-space. The time between the 90° pulse and the centre of the echo is known as the echo time ($T_E$). The sequence is repeated, each time with a different phase gradient, to fully map out k-space. The time between each 90° pulse is known as the repetition time ($T_R$). With a large $T_R$ and a moderate $T_E$, this sequence is primarily sensitive to $T_2^*$ effects.

The next sequence in Figure 1.2(b) is the spin echo (SE) sequence. This sequence, by virtue of the 180° pulse, rephases the spins that had been dephased by field inhomogeneities and thus produces an echo whose amplitude depends on $T_2$ and not $T_2^*$. By changing the parameters $T_E$ and $T_R$, the contrast of the images changes quite drastically. A long $T_R$ with a short $T_E$ produces an image whose contrast depends on proton density (PD weighted). A long $T_R$ with a long $T_E$ produces an image whose contrast depends primarily on $T_2$ differences in tissue ($T_2$ weighted). A short $T_R$ with a short $T_E$ produces an image whose contrast depends primarily on $T_1$ differences in tissue ($T_1$ weighted). Thus, with 2 different imaging sequences, four
Figure 1.2: The basic gradient echo and spin echo sequences used in MRI.
different parameters can be monitored with only slight changes (see Table 1.1).

The last sequence of significance to this study is the ‘Stejskal-Tanner’ diffusion weighted SE sequence shown in Figure 1.3. Perfectly stationary spins would ideally not be affected by the two symmetrical diffusion gradients since the phase introduced by the first gradient lobe would be undone by the second gradient lobe. As individual spins wander about on random trajectories in the presence of a gradient, due to diffusion, they will accumulate a phase that will also be random. Thus, the spins will not be brought back into phase by the symmetrical gradients and the signal that is measured is reduced by an amount [11]:

\[
\frac{S(G)}{S(G = 0)} = \exp \left( -\gamma^2 \delta^2 (\Delta - \delta/3) G^2 D \right)
\]

which is usually reduced to \( S(G) / S(G = 0) = e^{-bD} \) where \( b = \gamma^2 \delta^2 (\Delta - \delta/3) G^2 \). Plotting signal vs b-value will yield the diffusion coefficient.

The diffusion coefficient, \( D \), measured above is not the free diffusion co-

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Table 1.1: Standard images available with basic gradient echo and spin echo sequences.
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Figure 1.3: Stejskal-Tanner diffusion weighted spin-echo sequence with diffusion gradients in the slice direction.

efficient, which applies in water where molecules are free to diffuse in any direction, but rather, it is the apparent diffusion coefficient (ADC). The apparent diffusion coefficient differs from the free diffusion coefficient for two reasons. First, diffusion in tissue is not free. The movement of molecules is limited by biological structures such as cell membranes which leads restricted diffusion. Second, measurement of diffusion depends on the imaging sequence used to measure it. Because diffusion is not always isotropic, and structures such as membranes and fibres may cause diffusion to be greater in some directions than others, the diffusion coefficient is usually measured in three orthogonal directions (Figure 1.3 shows an imaging sequence for $D_{zz}$) so that the isotropic ADC = $1/3(D_{xx} + D_{yy} + D_{zz})$. 
1.4 Theory

In this section the equation for the Vessel Size Index (VSI) will be derived. This will begin with a brief background on the effect of susceptibility on magnetic fields and proceed to a calculation of the frequency shift of arbitrarily oriented infinite cylinders with a known susceptibility. To do this, the macroscopic $B$ field will be derived based on a continuous medium and then, the Lorentz correction will be applied to determine the field experienced by a local nucleus. [5]

1.4.1 Magnetic Susceptibility

The magnetic field, $B_0$, is the field that exists in the magnet in the absence of any material inside the magnet. Once a sample is placed inside the magnetic field, it will perturb the background magnetic field producing local variation. For simple geometries and many materials, the magnitude of this perturbation can be quantified.

For linear substances, the magnetization, $M$, induced in the material is proportional to the applied field, and the proportionality constant is the magnetic susceptibility, $\chi$:\(^1\)

$$M = \chi H$$  \hspace{1cm} (1.7)

There are three main types of magnetism defined by the relationship between $M$ and $H$. All molecules exhibit some form of diamagnetic shift. The applied field causes shifts in the electron orbits producing a moment opposing the applied field. Thus, if the molecules have small magnetic moments,

\(^1\)While $\chi$ is unit-less, its value depends on the system of coordinates being used. In this paper Système International (SI) units are used, but $\chi$ is frequently reported in Gaussian (cgs) units. The conversion relation is $\chi_{SI}/\chi_{cgs} = 4\pi$
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\( \chi < 0 \). Paramagnetic materials are made up of atoms or molecules with non-zero magnetic moments. In the presence of an external field these line up resulting in \( \chi > 0 \). Ferromagnets consist of permanent domains of aligned magnetic moments. The applied field causes these to shift and hence, the relationship between \( \mathbf{M} \) and \( \mathbf{H} \) traces out a hysteresis curve. Superparamagnetic materials consist of particles so small that each one is made up of only one ferromagnetic domain. Hence, as the name implies, they exhibit very strong paramagnetic behaviour.

In Equation 1.7, \( \mathbf{H} \) is not the magnetic field, \( \mathbf{B} \), but rather it is related to it by:

\[
\mathbf{H} = \frac{1}{\mu_0} \mathbf{B} - \mathbf{M} \quad (1.8)
\]

where \( \mu_0 \) is the permeability of free space. Thus for linear media \( \mathbf{B} \) and \( \mathbf{H} \) are proportional [4]:

\[
\mathbf{B} = \mu_0 (\mathbf{H} + \mathbf{M}) = \mu_0 (1 + \chi) \mathbf{H} = \mu \mathbf{H} \quad (1.9)
\]

For simple geometries, it is possible to find an equation for the magnetic field that satisfies Maxwell’s equations:

\[
\nabla \cdot \mathbf{D} = \rho_f, \quad \nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t}
\]

\[
\nabla \cdot \mathbf{B} = 0, \quad \nabla \times \mathbf{H} = \mathbf{J}_f + \frac{\partial \mathbf{D}}{\partial t} \quad (1.10)
\]

where \( \rho_f \) and \( \mathbf{J}_f \) are the free charge and current densities and \( \mathbf{E} \) is the electric field. \( \mathbf{D} \) is the electric displacement, which is related to the electric field by the permittivity of the material, \( \varepsilon \):

\[
\mathbf{D} = \varepsilon \mathbf{E} \quad (1.11)
\]

For simple geometries, solutions to Maxwell’s equations can often be guessed by ensuring that the boundary conditions are met. In the absence
of free currents and varying fields, these are:

\[
\begin{align*}
H_i^{\parallel} &= H_c^{\parallel} \\
B_i^{\perp} &= B_c^{\perp}
\end{align*}
\]

where the subscripts \(i\) and \(e\) are used to indicate the field immediately internal or external to the boundary.

### 1.4.2 Macroscopic Field of a Spherical Body

Solutions for the macroscopic field inside and outside of a spherical body with susceptibility \(\chi\), placed in a background magnetic field \(B_o = B_o \hat{z}\), are available in various textbooks including [5], upon which much of the field derivation is based:

\[
\begin{align*}
B_e &= B_o \left( \hat{z} + \frac{\chi}{3 + \chi} \left( \frac{a}{r} \right)^3 (3\cos \theta \hat{r} - \hat{z}) \right) \\
B_i &= 3B_o \left( \frac{1 + \chi}{3 + \chi} \right) \hat{z}
\end{align*}
\]

where \(a\) is the radius of the sphere and \(r\) is the distance from the centre of the sphere to the point of interest.

The field shift is defined to be \(\Delta B \equiv B - B_o\) and has a magnitude given by \(\Delta B \equiv \Delta B \cdot B_o / B_o\). Thus, for a sphere the magnitude of the field shift is:

\[
\begin{align*}
\Delta B_e &= B_o \frac{\chi}{3 + \chi} \left( \frac{a}{r} \right)^3 (3\cos^2 \theta - 1) \\
\Delta B_i &= B_o \frac{2\chi}{3 + \chi}
\end{align*}
\]
1.4.3 Macroscopic Field of an Infinite Cylinder

For an infinite cylinder of continuous linear material with susceptibility $\chi$, aligned parallel with a magnetic field $B_o = B_o \hat{z}$, these solutions are [5]:

\[ B_e = B_o \hat{z} \]
\[ B_i = B_o (1 + \chi) \hat{z} \]  \hspace{1cm} (1.15)

and for an infinite cylinder aligned perpendicular to a magnetic field $B_o = B_o \hat{x}$, with the orientation shown in Figure 1.4(a), they are:

\[ B_e = B_o \left( \hat{x} + \frac{\chi/2}{1 + \chi/2} \left( \frac{a}{\rho} \right)^2 (\cos 2\theta \hat{x} + \sin 2\theta \hat{y}) \right) \]
\[ B_i = B_o \left( \frac{1 + \chi}{1 + \chi/2} \right) \hat{x} \]  \hspace{1cm} (1.16)

where $a$ is the radius of the cylinder and $\rho$ is the distance away from the centre of the cylinder. The magnetic field for an arbitrarily oriented cylinder may be found by taking the superposition of the above parallel and perpendicular orientations as shown in figure 1.4(b):

\[ B_e = B_o \cos \phi \hat{z} + B_o \sin \phi \left( \hat{x} + \frac{\chi/2}{1 + \chi/2} \left( \frac{a}{\rho} \right)^2 (\cos 2\theta \hat{x} + \sin 2\theta \hat{y}) \right) \]
\[ B_i = B_o \cos \phi (1 + \chi) \hat{z} + B_o \sin \phi \left( \frac{1 + \chi}{1 + \chi/2} \right) \hat{x} \]  \hspace{1cm} (1.17)

In the arbitrary orientation, $B_o = B_o (\sin \phi \hat{x} + \cos \phi \hat{z})$, thus the field shifts are:

\[ \Delta B_e = B_o \frac{\chi/2}{1 + \chi/2} \left( \frac{a}{\rho} \right)^2 \sin^2 \phi \cos 2\theta \]
\[ \Delta B_i = B_o \frac{\chi/2}{1 + \chi/2} (1 + (1 + \chi) \cos^2 \phi) \]  \hspace{1cm} (1.18)

taking the small susceptibility limit, $\chi \ll 1$, these become:

\[ \Delta B_e = B_o \frac{\chi}{2} \left( \frac{a}{\rho} \right)^2 \sin^2 \phi \cos 2\theta \]
\[ \Delta B_i = B_o \frac{\chi}{2} (1 + \cos^2 \phi) \]  \hspace{1cm} (1.19)
Figure 1.4: The orientation of an infinite cylinder of radius, $a$, perpendicular (a) to magnetic field $B_0 = B_0 \hat{x}$, and of an infinite cylinder oriented at an arbitrary angle, $\phi$, (b) to the magnetic field.

### 1.4.4 Lorentz Correction

While the above equations were derived for a continuous medium and hold on a macroscopic scale, the neighbourhood of a nuclei most definitely cannot be considered continuous. The neighbourhood of a nuclei consists of the host molecule, which induces a shift in the magnetic field known as the chemical shift, and surrounding molecules, which can each have an effect. To account for this discontinuity, a notion known as the Lorentz sphere is used. This is an imaginary sphere drawn around the host molecule such that all objects outside the sphere can be treated as a macroscopic continuum and those inside can be treated individually [3]. It can be shown that the field from surrounding molecules averages to zero [7]. Thus, the host molecule can be considered to reside in a hollow sphere surrounded by a constant, continuous magnetization, $M$. 

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**Figure 1.4:** Diagram showing the orientation of an infinite cylinder of radius $a$ perpendicular to the magnetic field $B_0 = B_0 \hat{x}$, along with a diagram of the Lorentz correction, showing the orientation at an arbitrary angle $\phi$. The diagrams illustrate the concept of treating objects outside the Lorentz sphere as a macroscopic continuum and those inside individually.
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To find the field shift associated with having a hollow sphere surrounding the host molecule, it helps to consider the superposition principle. That is, the field of a constant magnetization, $B_{\text{macro}}$, is the sum of the field due to a constant background magnetization with a spherical hole in it, $B_{\text{local}}$, and a sphere with the same constant magnetization, $B_{\text{sphere}}$. Recognizing that $B_{\text{macro}}$ is the field that has been calculated above for the spherical and cylindrical objects, and that the field at the centre of the hole in $B_{\text{local}}$ is the field experienced by the host molecule:

$$B_{\text{local}} = B_{\text{macro}} - B_{\text{sphere}}$$ (1.20)

The field at the centre of a uniformly magnetized sphere is available in most electrodynamics texts [4] and is:

$$B_{\text{sphere}} = \frac{2}{3} \mu_0 M$$ (1.21)

rewriting 1.21 using the relations in 1.8 and 1.9:

$$B_{\text{sphere}} = \frac{2}{3} \frac{\chi}{1 + \chi} B_{\text{macro}}$$ (1.22)

In the most general sense, the spherical or cylindrical objects that have been considered will not exist in free space, rather, they may be blood vessels in the body or doped capillaries in a water phantom. These external surroundings will in general have a susceptibility $\chi_e$, and the internal susceptibility will be $\chi_i$ such that $\Delta \chi \equiv \chi_i - \chi_e$. Thus, the field shift due to a spherical body, Equation 1.14, becomes, in the limit of small susceptibility:

$$\Delta B_c = B_o \frac{\Delta \chi}{3} \left( \frac{a}{r} \right)^3 \left( 3 \cos^2 \theta - 1 \right) + B_o \frac{\chi_e}{3}$$

$$\Delta B_i = B_o \frac{\chi_e}{3}$$ (1.23)
and the field shift due to an infinite cylinder, equation 1.19, becomes:

$$\Delta B_e = B_o \frac{\Delta \chi}{2} \left( \frac{a}{\rho} \right)^2 \sin^2 \phi \cos 2\theta + B_o \frac{\chi_e}{3}$$

$$\Delta B_i = B_o \frac{\Delta \chi}{6} \left( 3 \cos^2 \phi - 1 \right) + B_o \frac{\chi_e}{3}$$  \hspace{1cm} (1.24)

In consideration of the fact that MRI is always measuring frequencies, the spread in Larmor frequency around the circumference is quoted instead of $\Delta \chi$:

$$\delta \omega = \frac{1}{2} \gamma \Delta \chi B_o$$  \hspace{1cm} (1.25)

### 1.4.5 VSI Equation

**Gradient-Echo Imaging**

The signal in a gradient echo image has been derived, using the static dephasing approximation, by Yablonksiy and Haacke [24], and is summarized in Appendix A.1.2. Using the approximation $\zeta_o \ll 1$, it is:

$$S_{GE} (T_E) \approx (1 - \zeta_o) \exp \left( -\frac{2}{3} \delta \omega \zeta_o T_E \right) \exp \left( \frac{T_E}{T_2^*} \right)$$  \hspace{1cm} (1.26)

where $T_2^*$ accounts for all $T_2^*$ effects other than those caused by the vessels and $\zeta_o$ is the total blood volume fraction.

Using the above and the result in Equation A.8, it is clear that the change in signal due to the contrast agent, $\Delta R_{2*}^*$, is:

$$\Delta R_{2*}^* \approx \frac{1}{3} \gamma B_o \zeta_o \Delta \chi$$  \hspace{1cm} (1.27)

where $\Delta \chi$ is the change in blood susceptibility due to the introduction of the contrast agent.
Chapter 1. Introduction

Static Dephasing Regime

The static dephasing regime holds if the characteristic diffusion time of the vessel, \( \tau_D = R^2/4D \), is much greater than the characteristic time of the vessel, \( \tau_c = \delta \omega^{-1} \), that is:

\[
R \gg \sqrt{\frac{2D}{\gamma \Delta \chi B_0}} \tag{1.28}
\]

Spin-Echo Imaging

The signal for a spin echo experiment cannot be determined by the Yablon-skiiy and Haacke model alone but needs to consider the diffusion of water molecules. This was done in an analytical model developed by Kiselev and Posse [10] and is summarized in Appendix A.1.2. Assuming a low blood volume, the signal is:

\[
S^{SE} (T_E) \approx (1 - \zeta_0) \exp \left( -0.694 \delta \omega^{2/3} D^{1/3} \zeta_0 R^{-2/3} T_e \right) \exp \left( -\frac{T_E}{T_2} \right) \tag{1.29}
\]

where \( R \) is the weighted mean of vessel radii, which is defined as the Vessel Size Index (VSI):

\[
VSI = R = \left( \int_0^{\infty} R^{-2/3} f (R_v) dR_v \right)^{-\frac{3}{2}} \tag{1.30}
\]

In the above equation, \( \zeta_0 f (R_v) \) is the volume fraction of vessels with radius, \( R_v \), such that \( \int_0^{\infty} f (R_v) dR_v = 1 \).

\( \Delta R_2 \) can be extracted from Equation 1.29 or more easily from Equation A.17 by assuming that the susceptibility difference between blood and tissue, is much less than the change induced by the contrast agent, \( \Delta \chi_{\text{blood}} \ll \Delta \chi \):

\[
\Delta R_2 \approx 0.694 D^{1/3} \left( \frac{1}{2} \gamma B_0 \Delta \chi \right)^{2/3} \zeta_0 R^{-2/3} \tag{1.31}
\]
Slow Diffusion Approximation

The slow diffusion approximation applies if the diffusion length, \( l_D = \sqrt{Dt} \), is much shorter than the radius of the vessels causing the magnetic field perturbations, that is:

\[
R \gg \sqrt{DT_E} \tag{1.32}
\]

Vessel Size Index

Combining Equations 1.27 and 1.31 results in a relationship linking the vessel size index \( R \), to the diffusion coefficient \( D \), the total blood volume fraction, and the change in the relaxation rate constants \( \Delta R_2 \) and \( \Delta R_2^* \):

\[
\Delta R_2 \approx 0.909 D^{1/3} \zeta_0^{1/3} R^{-2/3} (\Delta R_2^*)^{2/3} \tag{1.33}
\]

All the above parameters are easily measured using MRI except for the blood volume fraction, which can be derived from Equations 1.25 and 1.27:

\[
\zeta_0 = \frac{3}{2} \frac{\Delta R_2^*}{\gamma \Delta \chi B_o} \tag{1.34}
\]

and thus, the vessel size index is:

\[
VSI = R = 1.507 \left( \frac{D}{\gamma \Delta \chi B_o} \right)^{1/2} \left( \frac{\Delta R_2^*}{\Delta R_2} \right)^{3/2} \tag{1.35}
\]

Consequently, measurement of the vessel size index requires knowledge only of the diffusion coefficient, the increase in the susceptibility of blood due to the contrast agent, and the increase in the transverse relaxation rates, \( \Delta R_2 \) and \( \Delta R_2^* \).

1.4.6 Range of Validity

The lower limit of the range of validity for equation 1.35 is given by equations 1.32 and 1.28. Assuming typical values of \( D = 10^{-9} \text{ m}^2\text{s}^{-1}, \ B_o = 2.35 \text{ T}, \)
\( \Delta \chi = 5 \text{ ppm} \) and a \( T_E \) of 60 ms, it is clear that in order for Equation 1.35 to be valid, \( R > 7 \mu m \), and Monte Carlo simulations performed by Troprès [20] indicate that this is a good lower limit.

The upper limit of the range of validity for equation 1.35 is based on the nature of the model. The derivation of equation 1.35 is based on a statistical average assuming a large number of vessels in a particular voxel. Clearly, as the vessel sizes approach the size of the voxel this will no longer be possible [10]. The approximate number of vessels in a voxel, \( N \), can be found by calculating the number of vessels if they were all running perpendicular to a surface:

\[
N \approx \frac{\zeta(R) A}{\pi R^2} \tag{1.36}
\]

Assuming \( \zeta(R) = 0.02 \) for 25 \( \mu m \) vessels, and a 128 x 128 pixel, 40 mm field of view image with 2 mm slice thickness, \( N \approx 2 \). This clearly will not provide sufficient statistical averaging to accurately measure \( R \). If the volume of interest is increased to include the entire tumour slice (assuming 1 cm diameter), then \( N \) improves to 50, which should provide sufficient averaging.

### 1.5 Blood Susceptibility

In order to get a quantitative measurement of the Vessel Size Index, the increase in blood susceptibility due to the introduction of the contrast agent \( \Delta \chi \), must be known. Fortunately, much work has already been done on blood susceptibility due to Blood Oxygen Level Dependant (BOLD) imaging and functional imaging.

These studies are based on the knowledge that the susceptibilities of oxygenated and deoxygenated red blood cells are quite different [23]: \( \Delta \chi_{oxy} = \)
\(-0.33 \pm 0.09 \times 10^{-6}\) and \(\Delta \chi_{\text{deoxy}} = 1.97 \pm 0.09 \times 10^{-7}\). Thus, knowing the hematocrit \(Hct\), and oxygen saturation of hemoglobin \(Y\), the susceptibility of blood may be deduced [23]:

\[
\chi_{\text{blood}} = Hct \left( Y \chi_{\text{oxy}} (1 - Y) \chi_{\text{deoxy}} \right) + (1 - Hct) \chi_{\text{plasma}}
\]

(1.37)

The introduction of a contrast agent, also changes the susceptibility of blood [20]:

\[
\chi_{\text{blood+agent}} = Hct \left( Y \chi_{\text{oxy}} (1 - Y) \chi_{\text{deoxy}} \right) + (1 - Hct) \chi_{\text{plasma}} + (1 - Hct) \chi_{\text{agent}}
\]

(1.38)

Most often, though, measurements are made with respect to water so that \(\Delta \chi_{\text{blood+agent}} = \chi_{\text{blood+agent}} - \chi_{\text{water}}\) is measured instead of \(\chi_{\text{blood+agent}}\). Also, the susceptibility of plasma (and tissue) is very close to that of water [23] so that \(\Delta \chi_{\text{plasma}} = \chi_{\text{plasma}} - \chi_{\text{water}} = 0\).

\[
\Delta \chi_{\text{blood+agent}} = Hct \left( Y \Delta \chi_{\text{oxy}} (1 - Y) \Delta \chi_{\text{deoxy}} \right) + (1 - Hct) \Delta \chi_{\text{agent}}
\]

(1.39)

so that the change in susceptibility of interest is \(\Delta \chi = (1 - Hct) \Delta \chi_{\text{agent}}\) and thus:

\[
\Delta \chi = \Delta \chi_{\text{blood+agent}} - Hct \left( Y \Delta \chi_{\text{oxy}} + (1 - Y) \Delta \chi_{\text{deoxy}} \right)
\]

(1.40)

### 1.6 Contrast Agent

The contrast agent used in this study is part of a class of agents known as ultra-small superparamagnetic iron-oxide (USPIO) particles. It was obtained from Advanced Magnetics, Inc. (Cambridge MA, USA) but is also
being developed by Laboratoires Guerbet (Aulnay-sous-Bois, France). It is known by the generic name ferumoxtran-10 and by the Advanced Magnetics code 7227 (also AMI-227). It is also known by the trade names Combidx and Sinerem in the United States and Europe respectively. For the purposes of this paper, the contrast agent will be referred to as ferumoxtran or simply as the contrast agent.

Ferumoxtran consists of an iron oxide core 4-6 nm in diameter coated in an 8-12 nm thick dextran shell to form a relatively inert particle. The thick dextran layer reduces the reaction of the particle with plasma proteins and reduces the rate at which the particles are eliminated by phagocytosis [9]. This yields a particle contrast agent with a relatively long half life (4.5 hours in rats [20]) which makes it ideal for this particular study.

1.7 Objectives

There are a multitude of techniques for measuring vasculature. Perfusion imaging, using either Positron Emission Tomography (PET), Single Proton Emission Computed Tomography (SPECT), or MRI all have established techniques for this type of imaging. While these techniques are useful for monitoring blood flow they do not provide physical parameters that could be used to monitor angiogenesis. Vessel size imaging promises to do just that. First, it must be established that the technique works not only in the brain, but also in subcutaneous tumours and that it can be used to make serial measurements. Once this has been established, vessel size imaging can be used to study angiogenesis in the Shionogi tumour model, and in particular, any changes in vessel size as a result of hormone changes in the tumour. This would be particularly useful for designing anti-angiogenic therapies to
complement existing techniques for treating hormone dependent tumours.
Chapter 2

Experimental Methods

2.1 Apparatus

All experiments were performed on an imaging spectrometer equipped with a 2.35 T, 40 cm bore magnet (Bruker) attached to a SMIS console. Imaging was performed using a 2.5 cm diameter, 3 turn solenoid coil. Spectroscopy experiments were performed using a 3 cm diameter, single turn surface coil.

2.2 Animal Preparation

This study was conducted using 17 DD/S mice with Shionogi tumours implanted in the lower back. This location was more prone to ulceration than the upper back but imaging the upper back often led to severe motion artifacts.

In preparation for imaging, the mice were anaesthetized using 4 % isoflurane in air and then maintained at 1.5 %. The mouse was placed on its back on the imaging bed with the tumour centered in the coil. The body temperature was maintained at 35 to 37 °C using a hot water heating pad and monitored using a rectal probe. A 27 gauge × 1/2 inch long needle attached to 0.38 mm inner diameter polyethylene tubing was inserted into the tail vein to facilitate remote injection of the contrast agent. A 200 μmol Fe/kg dose of the contrast agent was prepared by drawing a measured length of
the contrast agent into a separate length of tubing. After proper positioning of the needle was confirmed using saline, the line was switched from saline to the measured dose of contrast agent. Once set up on the bed, the mouse was placed in the magnet and manually shimmed on the $^1$H signal.

Layup sequences were then used to ensure the tumour was centred in the magnet and to select a transverse slice.

2.3 Imaging Parameters

Vessel size imaging relies on measurement of $T_2$ and $T_2^*$ as can be seen in Equation 1.35, as well as knowledge of the apparent diffusion coefficient. These parameters were measured using standard gradient-echo, spin-echo and diffusion weighted spin-echo sequences as discussed in Section 1.3. In addition to the images necessary for obtaining a Vessel Size Index map, a multi slice, proton density image was obtained for determination of tumour size.

All images were obtained in the transverse plane with a 2 mm thick slice. Except for the proton density anatomical image, which covered the entire tumour, all the images were acquired only on a central slice. All sequences used a $T_R$ of 2500 ms in order to minimize any $T_1$ bias in equation 1.35. The proton density map was acquired with a $T_E$ of 10 ms, the $T_2^*$ weighted gradient-echo sequence with a $T_E$ of 10 ms, and the $T_2$ and diffusion weighted spin-echo sequences with a $T_E$ of 60 ms. The diffusion weighted sequence was performed four times, once with no diffusion gradients and then once each with a diffusion gradient in three perpendicular directions (read, phase and slice). All except the proton density image were acquired with three averages. The diffusion imaging sequence had $\Delta = 35$ ms, $\delta = 15$ ms and
G = 45.5 mT/m for a b value of 1000 s/mm².

After acquisition of diffusion, proton density, $T_2^*$, and $T_2$ weighted images, the contrast agent was injected followed by approximately a 0.05 mL saline flush. The agent was given 5 minutes to perfuse and then the $T_2^*$ and $T_2$ weighted images were acquired again so that $\Delta R_2^*$ and $\Delta R_2$ could be calculated:

$$(\Delta R_2 \text{ or } \Delta R_2^*) = \frac{1}{T_E} \ln \left( \frac{S_{\text{pre}}}{S_{\text{post}}} \right)$$  \hspace{1cm} (2.1)

### 2.4 Susceptibility Measurement

For susceptibility measurements, 5 mice were injected with a 200 μmol/kg dose of AMI-227 as described in Section 2.2. After allowing the agent to perfuse for 10 minutes, blood was drawn out of the heart. The blood from the five mice was mixed together and with heparin to prevent clotting. A sample was then taken for analysis on an Instrumentation Laboratory Micro 13 pH/Blood Gas analyzer for $pO_2$, $pCO_2$ and blood pH, and to be centrifuged for Hematocrit (Hct) measurement.

There are several ways to measure the susceptibility of blood [23] [20]. One of the most basic is to use NMR spectroscopy. Two 1.1 mm inner-diameter heperinized glass tubes filled with distilled water were placed in a 1.5 mL plastic vial perpendicular to each other. The drawn blood was placed in the vial and then the vial in the magnet, with one of the water filled capillaries aligned parallel with $B_0$ and the other perpendicular to it as shown in Figure 2.1.

Using Equation 1.24, the difference in the fields of the two water filled capillaries can be calculated:

$$B^\parallel - B^\bot = \frac{1}{2} B_0 \Delta \chi_{\text{blood+AMI-227}}$$  \hspace{1cm} (2.2)
Figure 2.1: Susceptibility measurement using two perpendicular water filled capillaries as a reference for an unknown substance in a test tube.
Rearranging and multiplying by the gyromagnetic ratio yields the frequency:

$$\Delta \chi_{\text{blood}+AM1-227} = \frac{2}{\gamma B_0} (\omega^\parallel - \omega^\perp)$$  \hspace{1cm} (2.3)

from which $\Delta \chi$ can be calculated using Equation 1.40.

### 2.5 Animal Care

All mice were cared for in accordance with UBC animal care committee guidelines. Mice were castrated when tumours reached approximately 1.5 to 2.0 cm$^3$. Mice that developed ulcerating tumours were euthanized using CO$_2$. Androgen independent tumours did not appear to be prone to ulceration and these mice were euthanized when their tumours reached approximately 2.0 cm$^3$. At least two VSI measurements were attempted for each stage of the tumour progression on each mouse. Due to the number of mice and time required for imaging, and the build up of scar tissue around the tail veins, this was the upper limit for androgen dependent and relapsing tumours. An extra measurement was possible for relapsing tumours.

Four tumours were harvested for histology (protocol provided by Dr. Yapp from the BC Cancer Agency). This involved intravenously injecting 100 $\mu$L of Hoechest 33342 dye before the mice were euthanized using CO$_2$. The tumours were then harvested, cut in half and placed cut side down in plastic holders filled with OCT. The tumours were then frozen on dry ice and sent to the BC Cancer Agency to be scanned and digitized.

Blood for susceptibility measurements was drawn by direct cardiac puncture from mice under isoflurane sedation. These mice were subsequently euthanized by cervical separation.
Chapter 3

Results

3.1 Introduction

It is now clear that there is a way to calculate all of the parameters in Equation 1.35, and thus to calculate a vessel size index for each tumour. The pre-contrast $T_2^*$ weighted image was used as a base so that the tumour could be outlined to create a region of interest (ROI). The mean signal from the tumour for both the pre- and post-contrast $T_2^*$ and $T_2$ weighted images was used in Equation 2.1 to calculate $A_i^{R_2}$ and $A_7^{R_2}$ for the tumour. The mean apparent diffusion coefficient was also calculated for the entire tumour using Equation 1.6. The VSI measurements for which the uncertainty in $\Delta R_2$ exceeded $\Delta R_2$ were not included in the following analysis.

In addition to tumour VSI measurements it is also possible to construct a VSI map of the tumour by calculating the VSI for each pixel. While the statistical limits in Section 1.4.6 are not satisfied by each pixel, the map gives valuable information on the distribution of blood vessels [21]. Figure 3.1 shows the changes in $R_2$ and $R_2^*$ calculated using Equation 2.1. For aesthetic purposes, the images in Figure 3.1 have had the background noise removed using a mask created from the pre-contrast $T_2$ weighted image. The mask was created using 10% of the maximum value in the image as a threshold.
Figure 3.1: The derived images used to produce a VSI map of a sample tumour.
3.1.1 Tumour Numbers

Of the 17 mice used in this study, 2 were euthanized pre-castration due to ulcerating tumours, 2 died from complications from castration, and one died in the magnet during imaging for the second regression time point. Of the 12 remaining mice, only 6 had relapsed after over 15 weeks. This is an unusually low fraction since typically at least 80% of tumours are expected to relapse. The fact that the mice were castrated at lower tumour sizes to avoid problems with ulceration likely contributed to this low relapse rate. Four of the relapsed tumours were harvested for staining with Hoechst dye. The remaining 6 mice were sacrificed for blood-susceptibility measurements.

3.2 Blood Susceptibility

The blood gasses were measured to be $pO_2 = 66.0 \pm 5$ mmHg and $pCO_2 = 28.1 \pm 5$ mmHg at $pH = 7.47 \pm 0.05$. Due to problems calibrating the blood gas machine, fairly generous errors were assigned. Regardless, these values have little impact on the calculation of $\Delta X$. After the blood was centrifuged, the Hematocrit was measured to be $Hct = 38 \pm 1\%$. In Equation 1.40, the oxygen saturation $Y$, is required rather than the partial pressure. This may be found from the oxygen dissociation curve, which is often characterized by the Hill equation [18]:

$$ Y = \frac{(pO_2)^n}{(pO_2)^n + (p50)^n} $$

(3.1)

The exponent, $n$, is typically taken to be 2.8 and p50, the partial pressure of oxygen at which half of the hemoglobin binding sites are bound to oxygen, for a typical mouse is 52 mmHg [6]. Using these values, $Y = 66 \pm 5\%$.

The frequency shift measured in the spectroscopy experiment was $3.27 \pm$
0.07 ppm. Using Equation 1.40, the susceptibility shift due to the contrast agent is $\Delta \chi = 6.37 \pm 0.15$ ppm. This compares favourably with the value reported by Troprès [20] of $7.18 \pm 0.4$ ppm for a rat model at a dose of 200 $\mu$mol Fe/kg. The difference is likely due to the fact that the susceptibility of the Ferumoxtran depends strongly on the size of the iron oxide core and varies from one lot to the next [20].

3.3 Noise in the Images

The vessel size index map is derived from several other images and depends on small variations between them to be accurate. Thus, while the Signal to Noise Ratio (SNR) may be quite good in the original $T_2$ and $T_2^*$ weighted images, it is not adequate to provide a suitable SNR in the VSI map. For instance, in Figure 3.1 the $T_2$ weighted images used to produce 3.1(a) have a SNR of 50, yet due to the propagation of errors, the SNR of the $\Delta R_2$ map is only 5. Similarly, the $T_2^*$ weighted images with a SNR of 95, lead to a SNR in figure 3.1(b) of 25 but when it comes to calculating the VSI images, a SNR of 3.3 can be expected. This is clearly not adequate; a value of at least 5 is desirable [22].

The scan time is already quite significant at 1.5 hours for a full set of images including diffusion weighted so the best alternative is to apply a filter. A Gaussian filter performs this task well and by varying its width, the degree of filtering can be easily controlled and as much detail retained as possible. The Gaussian mask for convolving with the image can be calculated using:

$$G(x, y) = \frac{1}{2\pi\sigma^2} \exp\left(-\frac{x^2 + y^2}{2\sigma^2}\right)$$

(3.2)

where the width of the mask should be at least $3\sigma$ [1].
Chapter 3. Results

A 7 x 7 mask was applied to each of the raw images before the VSI map was derived and a \( \sigma \) of 0.7 pixels was found to be adequate to improve the SNR in the VSI map to 7.5 and still maintain as much detail as possible. The results of this filtering for the images in Section 3.1 can be seen in figure 3.2.

For the pixel VSI analysis in Section 3.5.2, the raw images were filtered with a 7 x 7 pixel Gaussian with \( \sigma = 0.7 \) before any of the other images were derived.

3.4 Tumour Size Dependence

Before any detailed analysis can be conducted on vessel size changes as a tumour progresses through growth, regression and relapse, it is necessary to determine if there is any relationship between vessel size and tumour size.

Tumour sizes were calculated from multi-slice, proton-density weighted, spin-echo images (\( T_R = 2500 \) ms, \( T_E = 10 \) ms). The slices were 2 mm thick with no gap and taken in the same plane as the VSI images (axial). The border of the tumour was outlined on each of the images and the tumour size found by taking the sum of the areas in each slice. The tumour VSI was calculated using the mean signal from the tumour as described in section 3.1.

A plot of the VSI for each tumour against the tumour's size appears to have little, if any, dependence on the size of the tumour (see Figure 3.3). The Spearman rank-order correlation coefficient \( r_s \) [16], was used to test for any correlation between VSI and tumour size.

Most of the Spearman \( r_s \) values seen in Table 3.1 are quite low indicating that VSI and tumour size are uncorrelated. The exception is the relapsing
Figure 3.2: Result of filtering raw images with a Gaussian filter, $\sigma = 0.7$. 
Figure 3.3: The VSI appears to be independent of tumour size in each of the three stages of tumour growth.
Table 3.1: Spearman rank-order correlation coefficient for tumour size dependence.

<table>
<thead>
<tr>
<th>Phase</th>
<th>( r_s )</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growing</td>
<td>0.391</td>
<td>0.032</td>
</tr>
<tr>
<td>Regressing</td>
<td>0.049</td>
<td>0.828</td>
</tr>
<tr>
<td>Relapsing</td>
<td>0.561</td>
<td>0.067</td>
</tr>
<tr>
<td>All tumours</td>
<td>0.386</td>
<td>0.003</td>
</tr>
</tbody>
</table>

tumour, but the P-value is still too high to reject the null hypothesis that there is no correlation.

### 3.5 Vessel Size during Tumour Progression

#### 3.5.1 Individual Tumour Trends

In Section 3.4, it was shown that vessel size index is independent of tumour size. It is now safe to assume that there are only two independent variables: the stage of tumour growth and inter-mouse variability. The mean tumour VSI for each group of tumours was calculated and there appears to be a fairly large inter-mouse variability (see Table 3.2), but also a significant change in VSI between the different stages of growth.

Advantage can be taken of the longitudinal nature of this study to observe what happens as the tumours progress through androgen dependent growth, regression and relapse. The six tumours that went through the full progression are the most interesting. The data was grouped into three time points for each mouse (androgen dependent, regressing, and androgen independent tumour), and then trends of the tumour vessel size index were
Table 3.2: Mean vessel size index for the different stages of tumour development, the standard deviation and number of tumour VSI measurements that met the conditions of Section 3.1.

<table>
<thead>
<tr>
<th></th>
<th>mean VSI (µm)</th>
<th>σ</th>
<th># of measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>androgen dependent</td>
<td>35.2</td>
<td>25.5</td>
<td>30</td>
</tr>
<tr>
<td>regressing</td>
<td>15.1</td>
<td>6.56</td>
<td>22</td>
</tr>
<tr>
<td>androgen independent</td>
<td>45.4</td>
<td>41.8</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 3.3: Paired Student’s t test statistic for the difference in the means between the different stages of growth.

<table>
<thead>
<tr>
<th></th>
<th>Paired t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androgen dependent v Regressing</td>
<td>3.205</td>
<td>0.0076</td>
</tr>
<tr>
<td>Androgen dependent v Androgen independent</td>
<td>-0.215</td>
<td>0.838</td>
</tr>
<tr>
<td>Regressing v Androgen independent</td>
<td>-2.417</td>
<td>0.067</td>
</tr>
</tbody>
</table>

In the 13 mice that lived long enough to collect data on regressing tumours, all but two saw the mean VSI decrease after castration and, as can be seen in Figure 3.4, all but one of the relapsing tumours saw its VSI increase from its value during regression. By taking advantage of the trends for each mouse the paired Student’s t test can be used to accurately assess the changes during the tumour progression.

As can be seen in Table 3.3, the low P-value between androgen dependent and regressing tumours indicates a significant decrease in vessel size index. The evidence for a subsequent increase isn’t nearly as conclusive, and suffers from the low number of relapsing tumours.
Figure 3.4: The changes in tumour vessel size index for 6 tumours as they progress from androgen dependence to androgen independence.
Figure 3.5: Histogram of pixel Vessel Size Index for all tumours grouped into growing, regressing and relapsing tumours.

3.5.2 Pixel VSI Results

In Section 3.4, it was shown that vessel size index is independent of tumour size, and if the inter-mouse variability is ignored, it is possible to produce an approximate histogram of the individual pixel VSI's. To limit the errors introduced by the limits in Section 1.4.6 only those pixel with a VSI between 7 and 100 μm were included in Figure 3.5.

When compared to the initially growing tumour, the relapsing tumour vessel sizes seem to be smaller and the regressing tumours' vessel sizes ap-
Chapter 3. Results

Table 3.4: Kolmogorov-Smirnov statistic $D$ for the difference between distributions and associated significance values.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>$D$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>androgen dependent v regressing</td>
<td>0.087</td>
<td>≪ 0.01</td>
</tr>
<tr>
<td>androgen dependent v androgen independent</td>
<td>0.109</td>
<td>≪ 0.01</td>
</tr>
<tr>
<td>regressing v androgen independent</td>
<td>0.182</td>
<td>≪ 0.01</td>
</tr>
</tbody>
</table>

PEAR to be larger. The difference between the distributions can be tested by using the Kolmogorov-Smirnov (K-S) test. The K-S test works on unbinned distributions that are functions of a single variable and does not depend on the type of distribution (i.e. it does not assume a normal distribution), nor on the scaling of the x axis in Figure 3.5. The K-S statistic $D$, is the maximum value of the difference between the cumulative distribution function of two distributions [16]. When the cumulative distribution functions of the VSI sizes are plotted for the three different groups, it becomes more obvious that there is a difference between them (See Figure 3.6).

More importantly, the significance values of the K-S statistic $D$, as a disproof of the null hypothesis that the distributions are the same, can be calculated and are shown in Table 3.4. The very small P-values are a good indication that the distributions are in fact different and thus, the hypothesis that the distributions are the same can be rejected at a very high significance level.
Figure 3.6: Cumulative distribution function of Growing, Regressing and Relapsing tumours lumped together.
3.6 Histology

Hoechst dye 33342 stains the epithelial cells of active vessels so that they can be observed under microscope with light walls and dark lumen. Unfortunately, no direct comparison can be made between the Hoechst stained slides and vessel size index. It was not possible to match a histological slice to an MRI slice so for the histological slices, effort was made to take a central slice. Also, the histological images were not calibrated and lacked sufficient resolution to measure blood vessel diameters.

With this in mind, an attempt was made to perform a qualitative analysis of the tumours for which slides were available. As can be seen if Figure 3.7, the vessel distribution in the histological slides is not homogeneous. The tumours appear to have vascularized sections primarily on the periphery, combined with some necrotic (dark) tissue in the centre of the tumour. This agrees with the findings of the VSI maps, that most of the vascularization occurs in sections of the tumour, with the centre of the tumour being hypoxic or necrotic.

3.7 Discussion

The mean vessel size index calculated in this study appears to vary somewhat from the values reported by Jain [8]. They found a mean vessel radius of 19.2 μm as opposed to the mean VSI of 35.2 μm calculated in this paper. This is not terribly disconcerting as the lower limit of 7 μm used in calculating the mean VSI cuts off a large fraction of the vessels, and the imaging technique leads to an underestimation of ΔR² [20]. It is worth noting that, like Jain, the vessel size dropped significantly post-castration in regressing
Figure 3.7: Comparison of perfused tissue as seen by Hoechst staining to VSI maps of tumours.
tumours, but again, the lower bound on the VSI index using vessel size imaging obscures the full effect. The relapsing tumours show a subsequent increase in mean VSI above even the androgen dependent tumour. The slower growth during this phase may allow more significant vessels to form during this phase.
Chapter 4

Conclusions

Vessel size imaging has proved to be a viable method of monitoring angiogenesis during the progression of a Shionogi tumour from androgen dependence through to androgen independence. The mean VSI measured in the tumour is not an accurate assessment of the true mean vessel radius because the limits on the theoretical basis for this technique fail below 7 µm and overestimate vessel radii. Nevertheless, pixels whose VSI is large enough to be significant stand out clearly in VSI maps, and appear to show the same trends in vascularization as histological examination using Hoechst stain.

Most significantly, vessel size imaging has proved to be a viable technique for monitoring the growth and size of blood vessels for tumours progressing from androgen dependence through to androgen independence. The mean VSI for androgen dependent tumours is 35.2±4.7 µm, for regressing tumours is 15.1 ± 1.4 µm, and for relapsing tumours is 45.4 ± 12.6 µm. These results are sufficient to show a statistically significant drop in vessel size in regressing tumours followed by an equally significant increase in vessel size in androgen independent relapsing tumours.
Bibliography


Appendix A

A.1 Derivation of Signal Equations

The MRI signal equations for gradient-echo and spin-echo experiments are derived by Yablonskiy [24] and Kiselev [10] respectively. They are summarized here.

A.1.1 Gradient-Echo Experiments

Using the static dephasing approximation and ignoring diffusion effects the signal equation for a gradient-echo experiment has been derived. The signal from a gradient echo experiment has already been reported in Equation 1.5:

\[ S(T_E) \propto \exp(-R^*_2 \cdot T_E) \]  

where

\[ R^*_2 = \frac{1}{T^*_2} = \frac{1}{T_2} + R'_2 \]  

\( R'_2 \) is used to model the signal relaxation rate from local field inhomogeneities so that \( R'_2 = \gamma \Delta B \). In this way, the signal can be separated into \( T_2 \) and non-\( T_2 \) components:

\[ S(t) \propto \exp(-R_2 t) s^{GE}(t) \]  

In the most general sense, the signal from a volume of interest (voxel) is:

\[ s^{GE}(t) \propto \frac{1}{V} \int \exp(-i \omega(\mathbf{r}) t) d^3 \mathbf{r} \]  

where $\omega(r)$ is the local Larmour frequency at position $r$, and $V$ is the volume of the voxel of interest.

In the case of a number of objects perturbing the magnetic field, the contribution from each (ignoring the signal inside the objects themselves) can be taken into account through the superposition principle:

$$s^{GE}(t) \propto (1 - s) \prod_{n=1}^{N} \frac{1}{V - v_n} \int \exp(-i\omega_n(r)t) d^3r$$

(A.5)

where $v_n$ and $\omega_n(r)$ are the volume occupied by and frequency perturbation from the $n^{th}$ object, and $s$ is the volume fraction occupied by the $N$ objects.

For the purpose of this paper, the objects of interest are blood vessels which can be modelled as randomly oriented infinite cylinders so that $s = \zeta_0$ and $\omega_n(r, \theta)$ is just Equation 1.24 multiplied by $\gamma$. To account for the randomly oriented cylinders, averaging over the available angles can be performed:

$$\omega_n(r) = \int \omega_n(r, \theta) P(\theta) d\theta$$

(A.6)

where $P(\theta) = \sin \theta / 2$.

Performing the angular and spatial averaging as well as the product in Equation A.5, the signal asymptotically ($\delta \omega T_E \gg 1$) becomes:

$$s^{GE}(t) \propto (1 - s) \exp(-R'_2 t)$$

(A.7)

with

$$R'_2 = \frac{1}{3} s\gamma B_0 (\chi_i - \chi_e)$$

(A.8)

where $\chi_i$ and $\chi_e$ are the susceptibility internal and external to the objects.

A.1.2 Spin-Echo Experiments

The derivation of the spin-echo signal begins in a similar fashion to the gradient-echo signal, by separating the equation into $T_2$ and non $T_2$ related
Appendix A

effects:

\[ S(t) \propto \exp(-R_2 t) s^{SE}(t) \]  \hspace{1cm} (A.9)

In this situation, however, diffusion is the main non-\( T_2 \) related effect and cannot be ignored; rather, the slow diffusion approximation is used. The phase accumulated by a spin is no longer just \( \phi = \omega(r) t \), but the phase accumulated by a spin will depend on its path:

\[
\phi = \int_0^t \omega(r(t')) dt'
\]  \hspace{1cm} (A.10)

so that:

\[
s^{SE}(t) = \frac{1}{V} \langle \int \exp(-i\phi) d^3r \rangle
\]  \hspace{1cm} (A.11)

where \( \langle \rangle \) indicates averaging over both proton diffusion paths and the vascular network.

While equation A.10 describes the phase of one migrating spin, the evolution of the magnetization of a signal spin packet, \( \psi(r, r_0, t) \), initially at \( r_0 \), is governed by the Bloch-Torrey equation [19]:

\[
\frac{\partial \psi}{\partial t} = \nabla^2 D \psi - i\omega_1(r) \psi
\]  \hspace{1cm} (A.12)

The total magnetization, \( \Psi(r_0, t) \), is found by integrating \( \psi \) over \( r \). Thus, the signal attenuation factor can be calculated:

\[
s^{SE}(t) = \int \frac{dr_0}{V} \int dr \psi(r, r_0, t) = \int \frac{dr_0}{V} \Psi(r_0, t)
\]  \hspace{1cm} (A.13)

In the static dephasing regime and for the case of slow diffusion, the magnetization can be calculated directly [10]:

\[
\Psi(r_0, t) = \exp \left( -i\omega(r_0) t - \frac{D}{3} \left( \nabla \omega(r_0) \right)^2 t^3 \right)
\]  \hspace{1cm} (A.14)
Again, superposition may be used as in Equation A.5 to account for all of the objects perturbing the magnetic field:

\[ s^{SE}(t) \propto (1 - s) \prod_{n=1}^{N} \int \Psi(0, t) d^3rdR_n d\phi \]  

(A.15)

After the integrations and averages are performed over the vascular network, the signal asymptotically \((\delta \omega T_E \gg 1)\) becomes:

\[ s^{SE}(t) \propto (1 - s) \exp(-\Delta R_2 t) \]  

(A.16)

with

\[ \Delta R_2 \approx 0.695 D^{1/3} (\gamma B_0 (\chi_i - \chi_e))^{2/3} s \int_0^{\infty} R_v^{-2/3} f(R_v) dR_v \]  

(A.17)

where \(\zeta_0 f(R_v)\) is the volume fraction of vessels with radius \(R_v\), such that \(\int_0^{\infty} f(R_v) dR_v = 1\). In the Equation A.17, the integral over vessel radii in the volume of interest is really just the same as the integral in Equation 1.30 for the vessel size index.