

# Molecular Imaging in Experimental Therapeutics of Cancer

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The increasing development of novel gene and targeted therapies for treating tumors has necessitated the development of imaging approaches to determine their efficacy in preclinical animal models. This overview will provide selected examples of using imaging for quantitating tumor cell kill (anatomical and diffusion MRI), early treatment-induced changes in tumor cellularity/metabolism (diffusion and sodium MRI, PET), gene expression levels ( $^{19}\text{F}$  MRS) and vascular effects (perfusion MRI).

The use of *in vitro* screening assays to quantify the effectiveness of anticancer agents are widely used to rapidly evaluate a wide variety of agents and doses. Drugs which show therapeutic activity are then evaluated against animal tumor models. For orthotopic tumor models, animal survival, colony-forming efficiency (CFE) assays of cells disaggregated from solid tumors, and measurements of excised tumor weights have been used to quantify efficacy [1] since serial measurements of tumor growth rates are difficult to obtain. The myriad of new antineoplastic agents on the horizon requiring *in vivo* testing underscores the need for improved high-throughput surrogate markers for preclinical evaluation of therapeutic efficacy. The application of non-invasive imaging methods for quantitating the effects of experimental treatments could accelerate the process of drug development for translation to clinical trials [2-3].

## Overview

Imaging cancer patients is an essential aspect of clinical care. However, the significant advances in molecular biology along with the imaging sciences have provided additional unique opportunities for interrelated applications between these two research areas. The combination of molecular biology and the imaging sciences has melded into a new research field termed 'molecular imaging' which crosses into all imaging modalities used in cancer including magnetic resonance imaging (MRI) [4-18], radionuclide imaging [19-27], X-ray computed tomography (CT) [28, 29] and optical imaging methods [30-42]. This overview will cover selected examples of recent work using imaging to assess therapeutic efficacy by non-invasive quantitation of cell kill, early detection of therapeutic response, detection of spatial heterogeneity of tumor response and quantitation of transgene expression. Examples of the use of bioluminescence imaging (BLI) and PET for *in vivo* evaluation of treatment and for detection of transgene expression will

also presented. The successful application of these imaging technologies to assess experimental interventions of *in vivo* tumor models can provide unique insights related to therapeutic efficacy.

### **Quantitation of Tumor Cell Kill using Imaging**

Measurements of orthotopic tumor volumes in individual animals over time is not possible without the use of imaging technologies, hence the majority of studies evaluate therapeutic response using enhancement of animal survival time. This approach has proved valuable for *in vivo* testing of therapeutic approaches, but requires large numbers of animals due to variations in tumor growth kinetics between animals. MRI has been reported [2] for non-invasively monitoring the growth kinetics and therapeutic response of the intracranial rat 9L brain tumor model [1]. This approach allows for quantitation of tumor cell kill in individual animals [2]. In brief,  $\log(\text{cell kill}) = \log_{10}(V_{\text{pre}}/V_{\text{post}})$  where  $V$  represents the tumor volume obtained from MRI measurements before (pre) and following (post) therapeutic intervention. As each animal serves as their own pre-treatment control, the use of MRI provides for a very sensitive approach (quantitation of  $>0.1$  log kill can be detected). The use of anatomical MRI in this fashion is applicable to a wide variety of therapeutic interventions and tumor models for facilitating evaluation of experimental interventions.

Application of optical-based methods for *in vivo* tumor detection and evaluation of treatments is an active area of research. Fluorescence and bioluminescence optical imaging approaches for cancer detection and monitoring treatment are very promising. The use of BLI necessitates detection of light emitted from expression of the bioluminescent enzyme firefly luciferase (Luc) from tumor cells [10, 39]. BLI has been shown to allow for quantification of therapeutic efficacy in orthotopic 9L brain tumors in rats [10]. Expression of Luc in 9L tumor cells (9L<sup>luc</sup>) was accomplished in this study and implantation of 9L<sup>luc</sup> cells into rats resulted in tumors which could be visualized by MRI as well as BLI. Quantitative plots of MRI volumetric data and BLI photon counts over time was found useful for calculating the  $\log(\text{cell kill})$  as described above. Comparison of log kill values using these two imaging techniques revealed similar results [10].

### **Diffusion MRI**

The clinical value of conventional MRI stems from the ability to non-invasively observe the gross tumor morphology and follow changes over time and/or treatment. There remains large untapped potential for using MR technology to provide significant functional, structural and molecular information. Such information may be derived from quantitation of tissue properties which reflect, for example, perfusion dynamics, oxygenation levels, biochemistry/metabolism, cellularity and levels of gene expression.

One very interesting application of MRI is its use to follow therapeutic-induced macroscopic changes in tumors. Since molecular and cellular changes typically precede observable macroscopic changes in gross tumor size, the use of MRI to quantify therapeutic-induced changes in tumor cellularity using a surrogate marker (i.e. water diffusion) has been reported [4, 7, 43-50]. The use of water diffusion as a surrogate marker to probe tissue is compelling since this parameter is strongly affected by viscosity and membrane permeability between intra- and extracellular compartments, active transport and flow, and directionality of tissue/cellular structures that impede or enhance mobility. Successful treatment of tumors can

result in significant damage and/or killing of cells thus altering cell membrane integrity. This has a net effect of increasing the fractional volume of the interstitial space due to apoptotic body formation and cell loss resulting in an increase in the mobility (diffusion) of water within the damaged tumor tissue. Diffusibility of tissue water *in vivo* can be non-invasively quantified as an apparent diffusion coefficient (ADC) using diffusion MRI where the MR signal intensity is made dependent on water mobility by application of additional pulsed magnetic field gradients to the MR sequence [43]. Quantitation of ADC values can be obtained since individual nuclear spins of water molecules within the tumor tissue accumulate a phase shift proportional to their spatial position within the magnetic field gradient. These water molecules are then given an evolution time to diffuse followed by application of an identical, but inverse, pulse which results in a complete refocusing of stationary spins, while the mobile spins (those undergoing movement due to diffusion) are refocused incompletely. The net result is that the paired gradient pulses attenuate the signal in proportion to the local water mobility within that region of tissue. Quantitative measurements of diffusion reported as an ADC are obtained by measuring signal attenuation as a function of varying gradient strength and evolution time. The use of diffusion MRI for monitoring early events in tumor treatment in a variety of rodent tumor models [4, 7, 13, 43-48] has been reported along with clinical translation to patients [48-50]. The use of diffusion MRI has potential for monitoring early changes in tumors which may be reflective of treatment response. It is envisioned that imaging approaches such as this may assist physicians in tailoring treatments for individual patients and allow for alternative therapies to be attempted in a more timely fashion if a tumor is found to be resistant. This approach also provides the significant potential of assessing the regional/spatial heterogeneity of therapeutic response within a tumor. The heterogeneity of response may be accentuated in applications involving direct intratumoral administration of the therapeutic agent as is done in certain therapeutic protocols involving cancer gene therapy.

### **Imaging of Cancer Gene Therapy**

The goal of cancer gene therapy is to overcome the dose-limiting systemic toxicity of chemotherapy by introducing a gene into tumor cells which encodes for an enzyme that converts low-toxicity prodrugs into potent cytotoxic agents. The effectiveness of this approach depends on sufficient transgene expression and localized conversion of a prodrug to the cytotoxic compound, the relative sensitivity of the tumor to this agent and finally the ability of the cytotoxic agent to reach a majority of the tumor cells. Non-invasive assessment of therapeutic response and correlation of the location, magnitude and duration of transgene expression would be useful in facilitating optimization of gene transfer protocols, vector development and prodrug dosing schedules.

#### *Imaging gene therapy response*

Diffusion MRI has been shown to detect treatment-induced changes to the adenoviral delivered yeast cytosine deaminase (*yCD*) gene therapy paradigm [13, 46]. The prodrug used in these studies was Flucytosine (5-fluorocytosine, 5-FC). 5-FC itself does not elicit cytotoxicity, and its efficacy depends on the ability of the microbial enzyme CD to convert 5-FC to the antimetabolite 5-fluorouracil (5-FU). *yCD* is not found in mammals, thus providing 5-FC with a favorable therapeutic index. Expression of the *yCD* gene specifically in tumor cells, followed by systemic administration of 5-FC resulted in the generation of 5-FU within the tumor. This localized production of 5-FU chemotherapy avoids the systemic toxicity associated with

intravenous 5-FU therapy and may improve outcomes by achieving higher intratumoral 5-FU concentrations. Changes in diffusion values occurred prior to shrinkage of the tumor volume indicating that this imaging approach can detect early changes in the tumor mass following initiation of gene therapy. Moreover, a symmetrical shift towards higher diffusion values for the entire tumor mass was observed which indicated that the entire tumor mass was affected by this therapy. Tumor images revealed a relatively uniform pattern of diffusion values indicating a uniform (untreated or non-necrotic) distribution of cellular structures (e.g. intra- and extracellular space). In contrast, animals treated identically except that the *yCD* gene was administered to the established intracerebral 9L tumor through a direct intratumoral injection of an adenoviral vector yielded an entirely different effect on the diffusion MR image. The effects of 5-FC treatment on observed tumor diffusion changes were a heterogeneous distribution of bright voxels located throughout the tumor mass. The interpretation of these images was that the bright areas (high diffusion) represented regions of *yCD* expression leading to conversion of 5-FC to the cytotoxic product, 5-FU, with subsequent cell death leading to regions of focal necrosis. In contrast to what was observed for untreated animals, histograms for the 5-FC-treated animals broadened with a fraction of the histogram area moving to the right (higher diffusion). The fraction of tumor tissue that exhibited an increase in diffusion was interpreted as the relative fraction of the tumor that is undergoing a significant therapeutic response. The region of the histogram in the treated animal that did not increase was reported to reflect the cell fraction which was not exposed to sufficient 5-FU concentrations. Overall, these studies revealed that diffusion MRI could provide an early, spatial indicator of animal tumor response.

### *Imaging of transgene expression*

The use of non-invasive imaging technologies including radionuclide or optical reporters for evaluation and quantitation of transgene expression is an exciting area of research and the subject of reviews [15, 19, 20, 22, 39]. BLI has been shown to be useful for repetitive measurements of transgene expression for assessing gene expression levels following adenoviral-mediated delivery of *yCD* [13]. These types of imaging applications provide kinetic information related to temporal changes associated with *in vivo* gene expression to be non-invasively probed over time. For example, BLI was used to monitor the longevity of cells which produced the cytotoxic product, 5-FU (e.g. ‘factory’ cells) following intratumoral administration of an adenoviral vector containing both the *yCD* and *luciferase* transgenes. Direct intratumoral injection of the *yCD* adenoviral vector was found to produce a heterogeneous distribution of *yCD*-positive cells within the 9L tumor mass. The ability to follow transgene expression levels provides invaluable information related to the efficiency and kinetics of transgene expression which will assist in, for example, the optimization of prodrug dosing schedules. These types of surrogate markers for gene expression using BLI and correlation with spatial heterogeneity and magnitude of therapeutic response using diffusion MRI facilitate preclinical optimization of gene therapy paradigms prior to translation into the clinical setting.

### *Non-invasive Detection of Transgene Activity*

While optical and radionuclide imaging modalities are useful for evaluating transgene expression levels in living organisms, it is not feasible to specifically monitor individual metabolites using these techniques. The use of fluorine-19 (<sup>19</sup>F) magnetic resonance spectroscopy (MRS) for quantitatively evaluating the *yCD*-catalyzed conversion of 5-FC to 5-FU has recently been reported [47]. This approach is viable since the prodrug used in this

therapeutic paradigm contains a fluorine atom and is administered at concentrations which can be observed using *in vivo*  $^{19}\text{F}$  MRS. In this study, mice with subcutaneous HT29 or HT29/yCD carcinomas were injected with 5-FC. The presence of 5-FC could be non-invasively detected in HT29 tumors. When tumors expressed yCD, enzymatic conversion of 5-FC to 5-FU could be observed dynamically over time with subsequent cellular conversion to additional metabolites. This approach is also quantitative since the area under the individual peaks can be converted to absolute metabolite concentration with appropriate calibration. This allowed for pharmacokinetic modeling of 5-FC conversion to 5-FU by endogeneous enzymes based upon the dynamic data provided by  $^{19}\text{F}$  MRS which yielded measurements of yCD gene expression levels and the rate of fluoronucleoside synthesis in individual animals [47].

## Summary

Non-invasive imaging of anatomy coupled with improved imaging of function (biochemistry, physiology and cellularity) and molecular events will yield significant improvement in our understanding of the biology and pathophysiology of neoplastic diseases. The current imaging technologies and reporter genes for investigating gene expression and molecular events in living tissue are propelling the burgeoning field of molecular imaging rapidly forward into vitally important areas of research in biology and medicine. It is anticipated that molecular imaging will provide specific and complementary information which, when combined with anatomical imaging, will revolutionize our current applications of imaging in both drug discovery and clinical care.

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