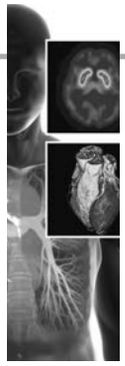

BOLD MRI: a tool for predicting tumor therapy outcome based on tumor blood oxygenation and vascular function

"Whichever oxygen-sensitive MRI method is ultimately found to be most useful, it has important implications for the practice of radiation oncology … The development of highly focused beams and stereotactic body radiation therapy allows higher doses with a few fractions to be considered…"

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We believe that oxygen-sensitive MRI can be used to evaluate tumor hypoxia so that therapy may be individualized and optimized for the characteristics of an individual patient. Specifically, we foresee a simple test based on breathing hyperoxic gas to characterize tumor hypoxia and the potential for modulating tumor hypoxia and hence therapeutic response.

Radiology has become the mainstay for clinical diagnosis and together with pathology underpins clinical medicine. Imaging has come a long way from the revolutionary, but crude x-rays of Roentgen at the turn of the 20th century to the exquisite temporal and spatial revolution offered by modern computed tomography, providing multislice (or 3D) sub-millimeter spatial and sub-second temporal resolution. Beyond x-rays, MRI has revolutionized soft-tissue analysis by providing tissue contrast based on multiple parameters identified by varying repetition and echo times and gradients to reveal T_1-, T_2-, T_2*-, diffusion- and flow-weighted images. Nonetheless, the discipline hitherto remained essentially an anatomical analysis providing tumor location, size and spread.

A new vision foresees prognostic radiology, whereby tumors are not only identified with high sensitivity and specificity, but there is stratification of tumors according to expected response to therapy, as well as early evaluation of therapeutic efficacy. Clinically, we are privileged to have a wide range of therapeutic choices, but this presents the challenge of selecting the right therapy. Societally, we are demanding greater efficacy at reduced cost. Increasingly, it has been realized that tumors that may outwardly appear quite similar may exhibit very different responses to therapy. As a result, there is increasing emphasis on tumor characterization using genomic, proteomic and metabolic techniques with the promise of assessing tumor aggressiveness and predicting response to therapy: generally, they require biopsy. A notable example is the evaluation of Her2-neu expression for efficacy of Herceptin®. Noninvasive assessments are even more attractive.

One tumor characteristic that has come to the forefront of attention is tumor hypoxia and, more generally, oxygenation. Since the fundamental studies of Gray *et al.* [1] 50 years ago, it has been accepted that cellular response to radiation is oxygen dependant with a threefold increase in radiation resistance when pO_2 declines from approximately 10 mmHg to hypoxia (<1 mmHg). The original work used cultured cells *in vitro*, but noting the inefficient tortuous nature of tumor vasculature hypoxia was expected *in vivo*. A meta-analysis of some 10,000 patients indicated a marginal clinical benefit for manipulating tumor hypoxia [2]. However, the overall conclusion was that there was a pressing need to identify those patients (*viz*. tumors) who would actually benefit from the added cost and complexity of addressing hypoxia. The importance and relevance of hypoxia and feasibility of imaging hypoxia in tumors are drawing increasing interest. There is a consensus that while many methods have been proposed there is currently no gold standard available for assessing tumor hypoxia in patients. Criteria considered important for a useful technique include invasiveness, radiation exposure, resolution, safety, availability, imaging time and lead time to effective implementation in the clinic [3].

The development of the Eppendorf electrode system permitted measurement of pO_2 (mean, median, hypoxic percentiles and distributions) *in vivo* and several studies have now shown distinctly poorer prognosis for patients with large hypoxic tumors in the cervix and head and neck [4,5]. Moreover, hypoxia has

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been reported in the brain, lung and prostate. However, the needle electrode was sufficiently invasive to discourage widespread use and it is no longer manufactured. Fluoromisonidazole and similar agents such as EF5 based on ^{18}F as well as ^{64}Cu-ATSM have been applied in clinical trials and promise insight into identifying tumor hypoxia using PET [6]. However, the associated radioactivity and inability to undertake rapid dynamic studies are limiting factors. ^{19}F MRI efficiently measures pO_2 in preclinical studies based on perfluorocarbon-reporter molecules such as hexafluorobenzene and fluorocarbon relaxometry using echo planar imaging for dynamic oxygen mapping (FREDOM) has been successfully used to assess tumor hypoxia and interventions to modulate radiotherapy in rats [7]. Based on FREDOM, we have found three categories of tumor: category I hypoxic tumors, which do not respond to hyperoxic gas intervention; category II hypoxic tumors, which do respond to hyperoxic gas intervention; and category III, well-oxygenated tumors. In every case examined to date, well-oxygenated tumors became even better oxygenated with hyperoxic gas breathing. However, the lack of ^{19}F MRI in human scanners has stymied development.

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Ideally, tumor oxygenation would be characterized noninvasively generating prognostic criteria for therapy without the need for exogenous reporter molecules. The fundamental reports of Thulborn [8] and Ogawa [9] suggested that blood could reveal vascular oxygenation based on T_2*-weighted MRI. Deoxyhemoglobin is paramagnetic and induces signal loss in T_2*-weighted images and blood-oxygen-level-dependant (BOLD) contrast proton nuclear magnetic resonance forms the basis of so-called functional MRI in brain activation studies, where it is thought to reflect changes in perfusion. In tumors, a BOLD response is more difficult to interpret, since it may also be influenced by blood flow, as investigated extensively by Howe *et al.* [10], who coined the term flow-and-oxygen-level-dependant (FLOOD) contrast. In addition, variation in vascular volume can introduce signal perturbation. In tumors, BOLD effects are often evaluated in response to a challenge such as hyperoxic gas breathing, and several studies have indicated a correlation with relative pO_2 [11–13]. There was always a positive correlation, but a 10% change in relative signal intensity in the BOLD experiment could correspond to an increase in pO_2 of less than 25 torr or more than 100 torr. We contend that either change would be radiobiologically pertinent. Once pO_2 exceeds 10 torr there is relatively little further oxygen enhancement achieved by increasing pO_2 further. It has been suggested that BOLD measurements are only useful in well-vascularized tumors since the changes depend on deoxyhemoglobin concentration. However, we believe that the ability to oxygenate a tumor depends strongly on vascular supply and that a small BOLD response due to low vascularization will itself be indicative of hypoxic tumors.

Nonetheless, BOLD changes alone may not be definitive for tumor hypoxia. Recently, Matsumoto *et al.* showed that a T_1 response accompanied breathing hyperbaric oxygen for squamous cell carcinoma xenografts in mice [14]. The so-called tissue-oxygen-level-dependant (TOLD) response is sensitive to changes in tissue pO_2, as opposed to vascular oxygenation. Since many additional factors such as protein concentrations and ions may influence the absolute T_1 value, it is not a reliable quantitative assay of absolute pO_2, but we are finding it to be a robust surrogate of changes in pO_2 in response to interventions.

We propose that combined assessment of T_1 and T_2* changes in tumors accompanying breathing hyperoxic gas will allow noninvasive characterization of tumor hypoxia. In preliminary investigations we have identified distinct characteristics for tumor oxygenation and dynamics: category I tumors show essentially no signal response to breathing hyperoxic gas; category II tumors show modest response; and category III show large BOLD and TOLD responses. As expected, BOLD responses precede TOLD since vascular oxygen delivery is required before changes in tissue pO_2.

We are already able to examine patients and have preliminary data in breast, cervix, head and neck and lung tumors. Lung tumors are most challenging owing to respiratory and cardiac motion. We should note that others are also pursuing such measurements, notably Padhani *et al.* (Mount Vernon, London, UK) in prostate based on T_2* [15] and O'Connor *et al.* (Manchester, UK) characterizing TOLD in multiple organs and tissues [16]. We note that Rodrigues *et al.* reported relationships between baseline T_2* and response to radiation [17].

Whichever oxygen-sensitive MRI method is ultimately found to be most useful, it has important implications for the practice of radiation oncology. Current radiotherapy uses extended fractionation regimens requiring patients to attend daily for many weeks. This is predicated on the need to avoid normal tissue damage. The development of highly focused beams and stereotactic body radiation therapy allows higher doses with a few fractions to be considered again. Hypoxia is likely to play a greater role for small numbers of high-dose irradiations, since there is less opportunity for the interfraction reoxygenation encountered between daily low doses. Each method of evaluating hypoxia presents new opportunities for individualized treatment exploiting 'dose painting' or intensity-modulated radiotherapy or a hypoxia-selective cell cytotoxin.

In terms of hypoxia in human tumors, we perceive twin needs: to develop an effective method of detecting hypoxia noninvasively prior to therapy, and to assess the ability to modulate tumor hypoxia in order to overcome resistance to therapy. We believe we are at an historic juncture where we not only have technologies for identifying hypoxia, but, more importantly, methods of tailoring therapy successfully to accommodate or exploit the hypoxia. In conclusion, BOLD can provide insight into tumor hypoxia, but we believe dynamic oxygen challenge evaluated by nuclear magnetic resonance T_1 and T_2* (DOCENT), which exploits BOLD and TOLD contrast to noninvasively detect changes in tumor oxygenation using proton MRI, will provide a robust prognostic test to reveal tumor hypoxia.

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