of specific molecular/genetic pathways in diseases such as cancer (e.g., FDG PET). Biomarker imaging is very likely to be less specific and more limited with respect to the number of molecular-genetic processes that can be imaged. Nevertheless, it benefits from the use of radiopharmaceuticals that have already been developed and are currently being used in human subjects. This application strategy is most dramatically illustrated by the use of [18F]FDG PET to image the response, recurrence and progression of particular tumors (e.g., Gleevac treatment of GIST). The translation and application of biomarker imaging paradigms into patient studies, using clinically-approved radiopharmaceuticals or contrast agents, will be far easier than either the direct imaging or reporter transgene imaging paradigms.

Reporter gene imaging studies will be more limited in patients compared to that in animals, due to the necessity of transducing the target tissue or cells with specific reporter constructs, or the production of transgenic animals bearing the reporter constructs. Ideal vectors for targeting specific organs or tissue (tumors) do not exist at this time, although this is a very active area of human gene therapy research. Each new vector requires extensive and time-consuming safety testing prior to regulatory approval for human administration. Nevertheless, reporter gene imaging, particularly the genetic labeling of cells with reporter constructs, has several advantages. There are now three well-defined human genes (*hNIS, hNET and hSSTR2*) with complimentary, clinically approved, radiopharmaceuticals for PET or gamma camera imaging in patients. These complimentary pairs (gene + probe) are excellent candidates for future reporter gene imaging in patients. Importantly, these human genes are less likely to be immunogenic compared to the reporter genes currently used in animals (e.g., viral thymidine kinases, luciferases, fluorescent proteins). It should also be noted that a single reporter gene – reporter probe pair can be used in different reporter constructs to image many different biological and molecular-genetic processes. Once a complimentary reporter-pair (gene + probe) has been approved for human studies, regulatory issues will focus will shift to the particular backbone and regulatory sequence of the reporter construct.

The major factor limiting translation of reporter gene imaging studies to patients is the "transduction requirement"; target tissue or adoptively administered cells must be transduced (usually with viral vectors to achieve high transduction efficiency) with reporter constructs for reporter gene imaging studies. At least *two* different reporter constructs will be required in most future applications of reporter gene imaging. One will be a "constitutive" reporter that will be used to identify the site, extent and duration of vector delivery and tissue transduction or for identifying the distribution/trafficking, homing/targeting and persistence of adoptively administered cells (the "normalizing" or denominator term). The second one will be an "inducible" reporter that is sensitive to endogenous transcription factors, signaling pathways or protein-protein interactions that monitor the biological activity and function of the transduced cells (the "sensor" or numerator term). The initial application of such double-reporter systems in patients will most likely be performed as part of a gene therapy protocol or an adoptive therapy protocol where the patients own cells are harvested (e.g., lymphocytes, T cells or blood-derived progenitor cells), transduced with the reporter systems and expanded *ex vivo*, and then adoptively re-administered to the patient. For example, adoptive T cell therapy could provide a venue for imaging T cell trafficking, targeting, activation, proliferation and persistence. These issues could be addressed in a quantitative manner by repetitive PET imaging of the double-reporter system described above in the same subject over time.

Once in place, Cancer Clinical Trials and Personalized Medicine will be able to benefit from the noninvasive imaging paradigms described above; similar to the benefits of sequential FDG PET scans performed today in order to monitor GIST tumor response and recurrence. The ability to visualize transcriptional and post-transcriptional regulation of endogenous target gene expression, as well as specific intracellular protein-protein interactions in patients will provide the opportunity for new experimental venues in patients. They include the potential to image the malignant phenotype (e.g., signal pathway activity) of an individual patient's tumor at a molecular level and to monitor changes in the phenotype over time. The potential to image a drug's effect on a specific target molecule or signal transduction pathway in an individual patient's tumor provides the opportunity for monitoring treatment response at the molecular level.

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Causes and consequences of glycolysis and acid pH in tumors

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Over the last decade, we have developed and improved MR-based techniques with which to measure the pH of tumors. These have included 31-P MRS and 1-H MRSI approaches and, most recently, pH-dependent relaxometry using pH-dependent contrast agents. This latter approach has been particularly challenging as it required simultaneous and independent quantification of relaxation rates and contrast agent concentration. All of these approaches have shown that the extracellular-interstitial pH (pHe) of tumors is unequivocally acidic, reaching as low as pH 6.7. This low pHe is caused by high glucose metabolism in tumors coupled with poor perfusion. The high glucose metabolism occurs in the presence or absence of oxygen, also known as the Warburg Effect., WE. There is evidence, by us and others, that the WE is hardwired in the most aggressive tumors, and that this can occur through the oncogenic activation of at least 6 different pathways. Darwinian evolution selects for phenotype, not genotype and thus, we have proposed that the glycolytic phenotype is evolutionarily selected early during the in situ stage of carcinogenesis, when it is an avascular disease. This does not explain, however, why this phenotype continues to be selected later in carcinogenesis, when invasive and metastatic cells have access to the vasculature. Examining the sequelae of glucose catabolism yields a finite number of consequences that could lead to further selection, including acid production. Acid production could be selected because it has been shown to induce invasion and exacerbate metastasis. We have proposed that this occurs by induction of cathepsin release and export of H^+ from growing tumors into surrounding parenchyma, thus facilitating their ability to invade host tissue. Notably, tumor acidity can be inhibited with oral buffers, such as bicarbonate, and we have shown that this inhibits spontaneous metastasis in some, but not all, animal tumor models.

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MR imaging of angiogenesis

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Angiogenesis has been validated as a target in multiple randomized clinical trials that tested the advantage of adding VEGF inhibitors to conventional treatment. There remains a clear need to identify the patients who most benefit from this class of drug as the data demonstrate only a modest improvement in overall survival if all patients in a defined disease population are treated; some pre-clinical and clinical data suggest that maintenance therapy is required; the drugs can be toxic; and because the development of combination regimens that include VEGF inhibitors can only occur once we have learned how to identify the patients who most benefit from this class of drug.

Biomarker science is evolving to address the issue of treatment individualisation. Imaging offers the advantage of allowing serial measurements of tumour vascular pathophysiology and has been implemented throughout the development of anti-angiogenic agents. To date multiple clinical trials have evaluated VEGF inhibitors with Dynamic Contrast Enhanced Magnetic Resonance Imaging (DCE-MRI) and have demonstrated a relationship between drug dose and reductions in DCE-MRI parameters and secondly between the reduction in DCE-MRI parameters and patient benefit. These relationships are confounded by heterogeneity. However, histogram analysis of imaging data, to examine vascular heterogeneity in greater detail demonstrates clinically useful information that is otherwise overlooked.

One of the parameters that evolved from the analysis of heterogeneity was the enhancing fraction, which reflects the vascularity of the tumour. In several clinical trials using MR or CT, in patients treated with anti-vascular agents, cytotoxic drugs or radiotherapy we have demonstrated the clinical value of measuring the vascular enhancing fraction and have shown that this parameter augments traditional prognostic factors. These data led to further clinical trials which demonstrated that VEGF inhibitors reduce the