In vivo imaging of molecular-genetic targets for cancer therapy

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Molecular-genetic imaging provides visualization in space and time of normal as well as abnormal cellular processes at a molecular-genetic level. Molecular imaging has its roots in molecular biology and cell biology as well as in imaging technology and chemistry. Three different noninvasive in vivo imaging technologies have developed more or less in parallel: (1) magnetic resonance (MR) imaging (Ichikawa et al., 2002); (2) nuclear imaging (QAR, γ camera, and PET) (Blasberg and Gelovani Tjuvajev, 2002); (3) optical imaging of small animals (Edinger et al., 2002). There are some new and very exciting developments in in vivo optical imaging (Weissleder and Ntziachristos, 2003), including two-photon fluorescent imaging of viable cells, small organisms, and embryos (Hadjantonakis et al., 2002). It is the convergence of the imaging and molecular/cell biology disciplines that is at the heart of this success story and is the wellspring for further advances in this new field. The development of versatile and sensitive noninvasive assays that do not require tissue samples will be of considerable value for monitoring molecular-genetic and cellular processes in animal models of human disease, as well as for studies in human subjects in the future. Imaging molecular-genetic and cellular processes will compliment established molecular-biological assays that are invasive and require tissue sampling, and imaging can provide a spatial as well as temporal dimension to our understanding of various diseases.

We have identified three molecular imaging strategies—"direct," "indirect," and "surrogate"—and the reader is referred to the following reviews for greater details (Blasberg and Gelovani, 2003) and other perspectives on molecular imaging (Gambhir, 2002; Contag and Ross, 2002; Weissleder, 2002). Direct molecular imaging can be defined in terms of "molecular tracer"-target interactions, whereby the resultant image of probe localization and magnitude (image intensity) is directly related to its interaction with the target epitope or enzyme. This strategy has been established using MR, nuclear and optical imaging technology. Imaging cell surface-specific antigens or epitopes with radiolabeled antibodies and genetically engineered antibody fragments (e.g., minibodies) provides examples of direct molecular imaging that has evolved over the past 30 years. In vivo imaging of receptor density/occupancy using small radiolabeled molecular probes has also been widely used, particularly in neuroscience research, over the past two decades. These examples represent some of the first molecular imaging applications used in clinical nuclear medicine research. More recent research has focused on chemistry and the synthesis of small radiolabeled or florescent probes that target specific receptors (e.g., the estrogen or androgen receptors) and florescent probes that are activated by endogenous proteases (Jaffer et al., 2002). The $\alpha(v)\beta3$ integrin is highly expressed on tumor vasculature and plays an important role in metastasis and tumor-induced angiogenesis; initial studies on the targeting and imaging of the $\alpha(v)\beta3$ integrin with radiolabeled glycosylated RGD-containing peptides are very encouraging (Figure 1) (Haubner et al., 2001). Antisense and aptomer oligonucleotide probes that specifically hybridize to target mRNA or proteins have been constructed and studied in vivo. Although some effi-

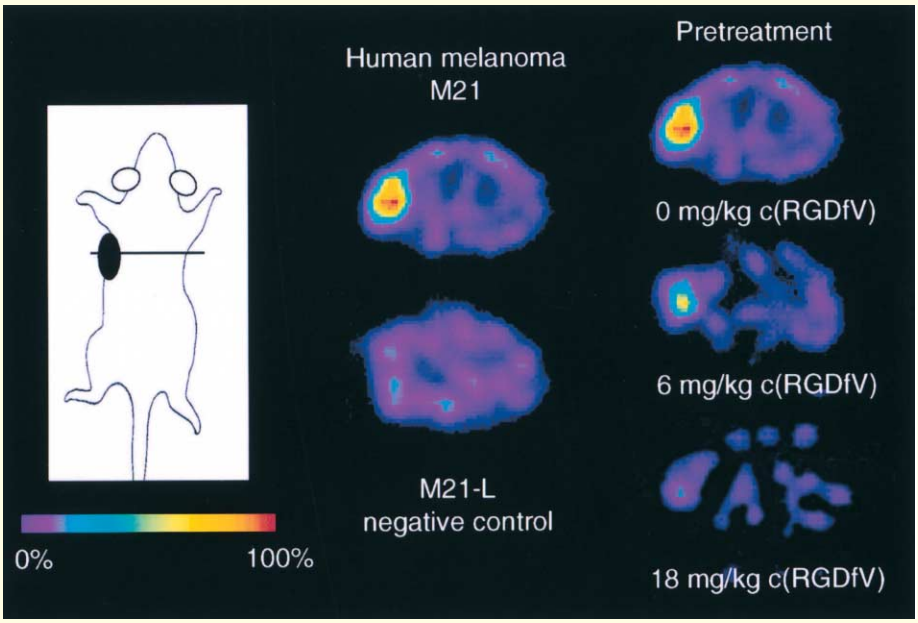

Figure 1. Noninvasive imaging of Avβ3 expression

Transaxial PET images of nude mice bearing human melanoma xenografts. Images were acquired 90 min after injection of approximately 5.5 MBq of (18F)Galacto-RGD. The *top left* image shows selective accumulation of the tracer in the avb3-positve (M21) tumor on the left flank. No focal tracer accumulation is visible in the avβ3-negative (*M21-L*) control tumor (*bottom left image*). The three images on the *right* were obtained from serial (18F)Galacto-RGD PET studies in one mouse. These images illustrate the dose-dependent blockade of tracer uptake by the avβ3-selective cyclic pentapeptide cyclo (-Arg-Gly-Asp-D-Phe-Val-). Figure adapted from Haubner et al., 2001, with permission from *Cancer Res.*

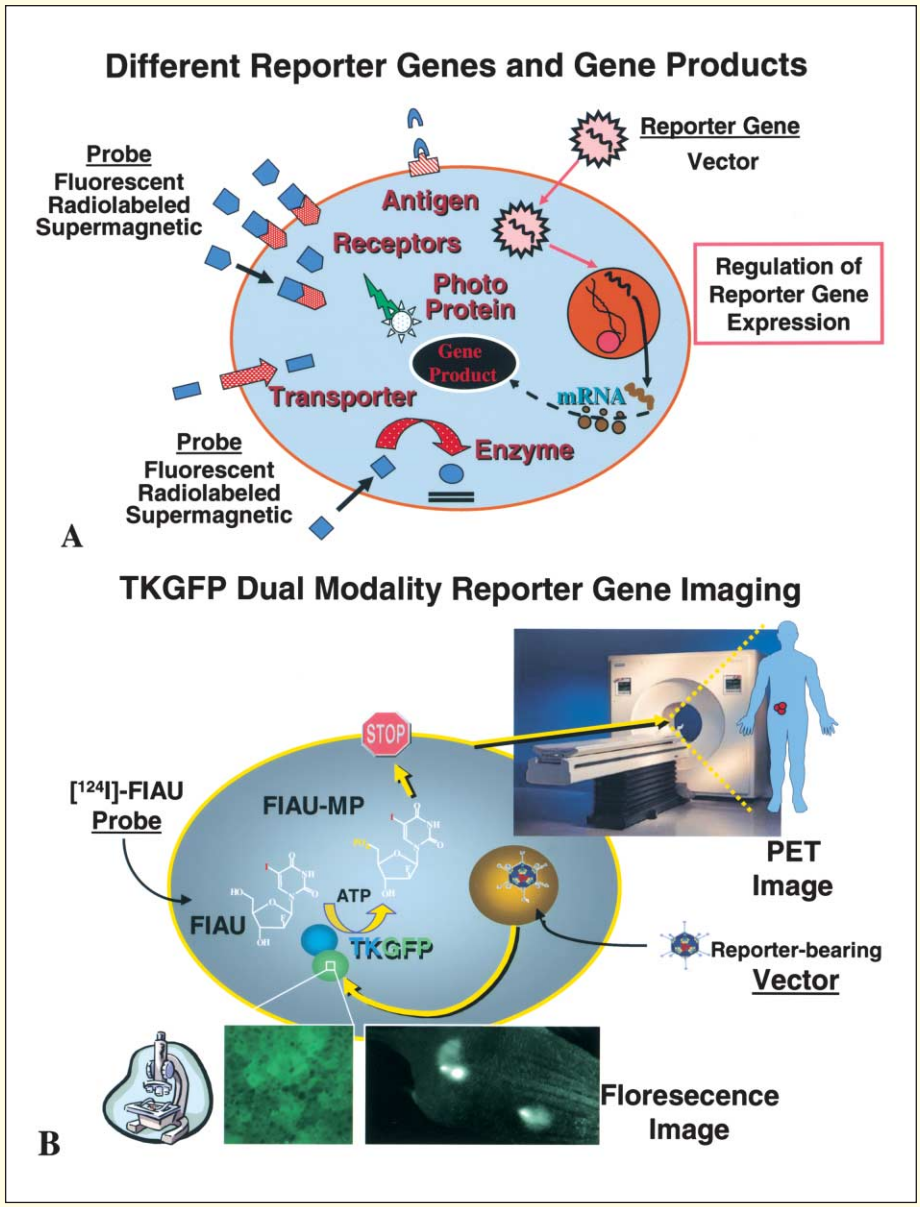

Figure 2. Schematic of different reporter genes and gene products, and dual modality imaging of HSV1-tk/GFP reporter gene expression

A illustrates that different reporter genes have been developed, and the reporter gene products include enzymes (HSV1-tk, HSV1-sr39tk, firefly and Renilla luciferases), transporters (hNIS), receptors (hSSTR2, hD2R), cell surface antigens and proteins, and fluorescent proteins (eGFP, mRFP). Different reporter probes corresponding to specific reporter genes have also been developed and used for nuclear (PET), magnetic resonance (MR), and optical (fluorescence and bioluminescence) imaging as described and referenced in the text.

B illustrates the steps involved for dual modality imaging of HSV1-tk/GFP reporter gene expression. The HSV1-*tk*/*GFP* fusion gene is transfected into target cells by a vector. Inside the transfected cell, the gene is transcribed to mRNA and then translated to a fusion protein, HSV1-TK/GFP, that retains both HSV1-TK enzymatic activity and GFP fluorescence. After administration of a radiolabeled probe (e.g., (124I)-FIAU or (18F)-FHBG) and its transport into the cell, the probe is phosphorylated by the HSV1-TK component of the reporter gene product. The phosphorylated radiolabeled probe does not readily cross the cell membrane and is "trapped" within the cell. Thus, the magnitude of probe accumulation in the cell (level of radioactivity) reflects the level of HSV1-TK enzyme activity and level of HSV1-tk gene expression. The GFP component of the reporter gene product retains fluorescence and can be used for in vivo and in situ imaging as well as FACS analysis of transduced tumor cells.

cacy for γ camera and PET imaging endogenous gene expression using RASONs has been reported, RASON imaging has several serious limitations (Blasberg and Gelovani Tjuvajev, 2002).

Indirect imaging strategies are more complex because they involve pretargeting components that function as molecular-genetic sensors. One example of indirect imaging that is now being widely used is reporter gene imaging. In vivo reporter gene imaging is currently most widely used in radionuclide-based and optical-based studies, and to a lesser degree with MR imaging. Most reporter imaging paradigms couple a reporter gene with a complimentary reporter probe. Imaging the level of reporter gene product activity through the level of probe accumulation or level of emitted light provides indirect information that reflects the level of reporter gene expression, and the level of endogenous signaling and transcription factors that drive reporter gene expression. Reporter transgenes can encode for an enzyme (e.g., *HSV1-tk*), a receptor (e.g., *hD2R* and *hSSTR2*), a transporter (e.g., *hNIS*), or an antigen as shown in Figure 2A. A general paradigm for gene imaging using radiolabeled probes was initially described in 1995 (Tjuvajev et al., 1995) and is shown diagrammatically for the HSV1-*tk*/*GFP* dual modality (nuclear + optical) reporter in Figure 1B. Wild-type *HSV1-tk* (Tjuvajev et al., 1998) or a mutant *HSV1-tk* gene, *HSV1-sr39tk* (Gambhir et al., 2000), are the reporter genes, and the complementary radiolabeled probes, (124I)-FIAU and (18F)-FHBG, are most commonly used in current molecular imaging studies.

Imaging endogenous biological processes

Imaging the transcriptional regulation of endogenous genes or the activity of specific signaling pathways in living animals (and potentially in human subjects) using noninvasive imaging techniques can provide a better understanding of normal and disease-related biological processes. Two recent papers from our group showed that p53-dependent gene expression and activated NFAT (nuclear factor of activated T cells) can be imaged in vivo with PET, and by in situ and in vivo fluorescence (Doubrovin et al., 2001; Ponomarev et al., 2001). In these studies, the transcription of the reporter gene (a viral thymidine kinase and green fluorescent fusion gene, HSV1-*tk*/*GFP*) was inducible and regulated by upstream response elements sensitive to endogenous p53 and NFAT (activated transcription factors), respectively. However, imaging endogenous gene

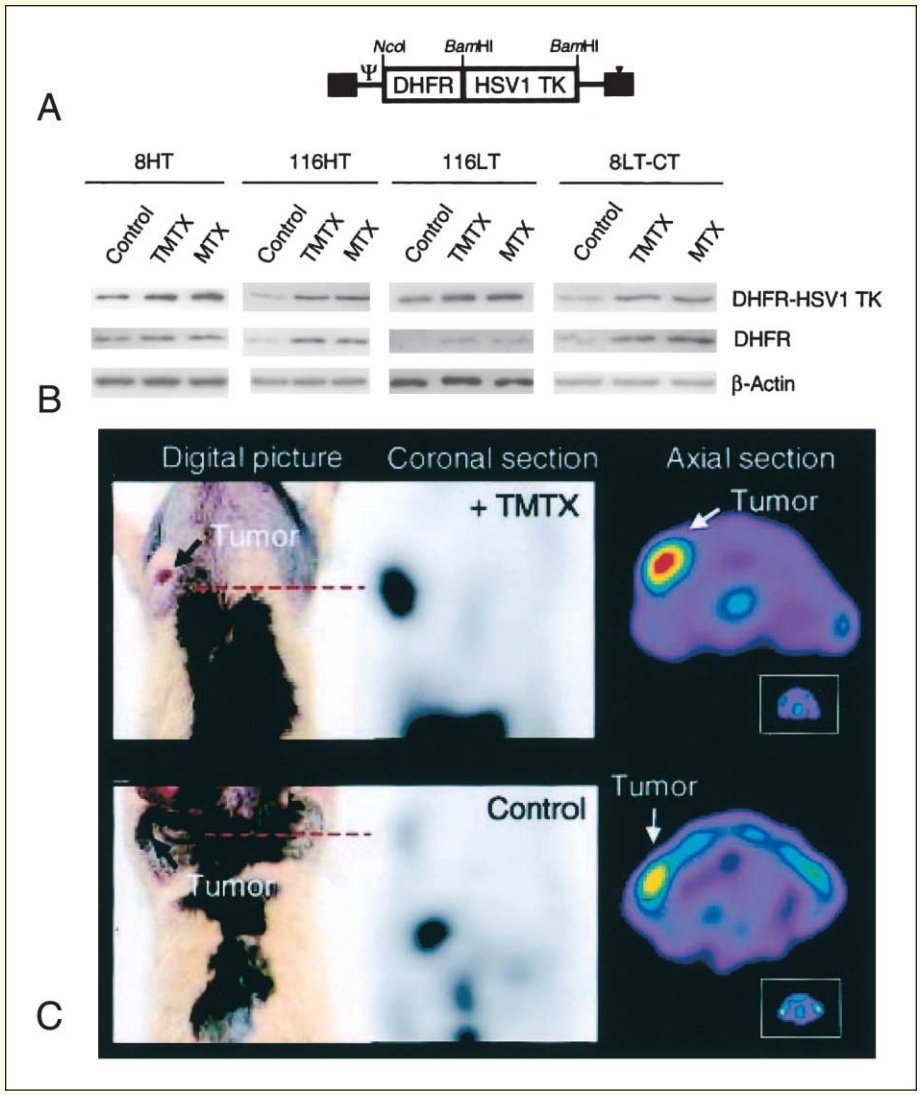

Figure 3. Posttranscriptional regulation of gene expression

Schematic representation of the retroviral vector used for imaging; the retroviral vector codes for the DHFR-HSV1TK fusion protein (**A**). Western blot analysis using an anti-DHFR antibody shows increased DHFR-HSV1TK fusion protein levels in various transduced and parental colon cancer cells exposed to antifolate (1 μM TMTX or MTX) for 24 hr (**B**). PET images obtained in *DHFR-HSV1tk* transduced xenograft-bearing rats, 24 hr after i.v. (124I) FIAU administration (260 μCi/rat) following treatment with TMTX (10 mg/kg × 3 days or 100 mg/kg single dose) or saline (**C**). A 1.5- to 4-fold increase in reporter expression was observed in the antifolate-treated animals, demonstrating a significant level of posttranscriptional regulation DHFR-HSV1TK. Figure adapted from Mayer-Kuckuk et al., 2002, with permission from *Proc. Natl. Acad. Sci. USA*.

expression may be limited when weak promoters in their usual *Cis*-configuration are activated and used to induce reporter gene expression. This can result in low transcriptional activity of the reporter gene and a low or nondetectable level of probe accumulation. To address this issue and enhance the transcriptional activity of the androgen-responsive prostate-specific antigen promoter (PSE), a two-step transcriptional amplification (TSTA) approach was developed to amplify expression of firefly luciferase and mutant herpes simplex virus type 1 thymidine kinase (HSV1-sr39tk) in a prostate cancer cell line (LNCaP) (Iyer et al., 2001). In another study, methods to enhance the transcriptional activity of the carcinoembryonic antigen (CEA) promoter using a *trans*-system (similar to the TSTA described above) were used to selectively treat and image CEA-expressing tumors (Qiao et al., 2002). Gene expression levels are also regulated by posttranscriptional modulation, including the translation of mRNA. A recent study demonstrated that imaging posttranscriptional regulation of gene expression is feasible (Figure 3) (Mayer-Kuckuk et al., 2002). This was shown by exposing cells to antifolates and inducing a rapid increase in the levels of the enzyme dihydrofolate reductase (DHFR). The DHFR enzyme binds to its own mRNA in the coding region, and inhibition of DHFR by antifolate treatment releases the DHFR enzyme from the mRNA; consequently, this release results in an increase in translation of DHFR protein. This adaptive cellular response mechanism could be used to determine whether posttranscriptional regulation of gene expression could be monitored by reporter-PET imaging. Several recent studies have shown that it is possible to image protein-protein interactions in vivo using PET and optical techniques (Ray et al., 2002, Luker et al., 2002), as well as MR (Perez et al., 2002). These initial results are very encouraging, but at the same time they demonstrate only constitutive interactions of the known pairs of interacting proteins. Further studies will be required to validate the approach with respect to the sensitivity and dynamic range of these reporter systems for monitoring the induction and inhibition of endogenous protein-protein interactions.

Monitoring vector targeting and gene therapy

A noninvasive, clinically applicable method for imaging the expression of successful gene transduction in target tissue or specific organs of the body would be of considerable value for monitoring and evaluating gene therapy in human subjects (Jacobs et al., 2001; Blasberg and Gelovani Tjuvajev, 2002). Noninvasive imaging techniques using selected reporter gene and reporter probe combinations can provide a practical and clinically useful way to identify successful gene transduction and to monitor the level of gene expression over time in patients undergoing gene therapy. Several vector reporter gene paradigms have been developed for optical and nuclear imaging. Many of these paradigms provide the ability to repeatedly assess gene expression over time, especially when multiple sequential biopsies are not feasible. The absence of any perturbation of the underlying tissue which occurs with biopsy procedures, and the ability to obtain 3D spatial information in the entire body as well as target organs and tumors repeatedly over time, will be of considerable value when addressing toxicity

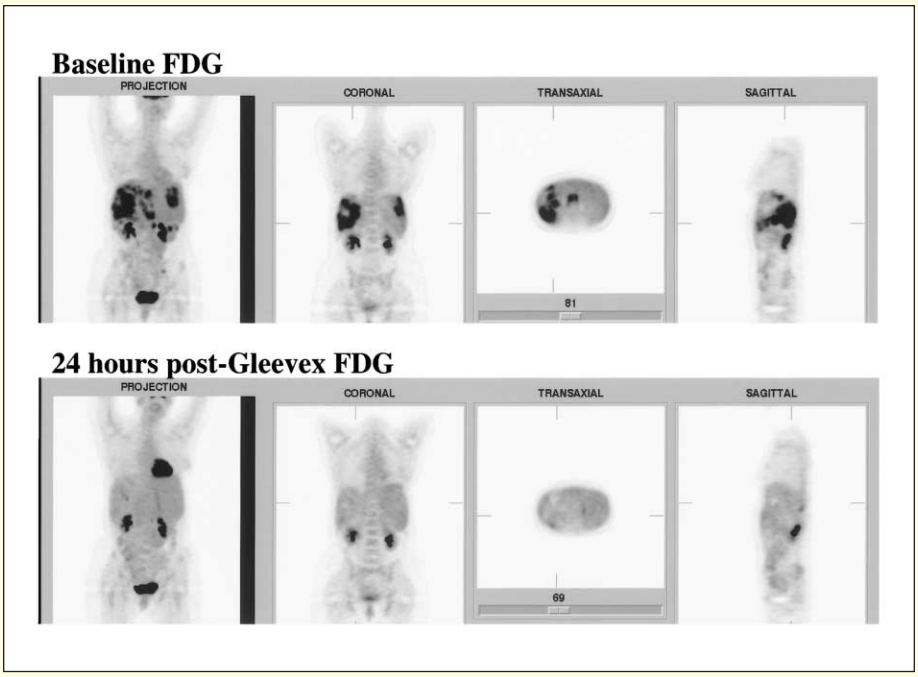

Figure 4. FDG PET imaging before and 24 hr after treatment

In patients with gastrointestinal stromal tumors (GIST), tumor glucose utilization is very high and these tumors can be readily visualized by (18F)-fluorodeoxyglucose (FDG) positron emission tomography (PET). A striking observation in patients with GIST who are treated with STI571 (Gleevec) is the rapid and sustained decrease in fluorodeoxyglucose (FDG) uptake determined by PET scan. For the patient shown here, a 55% decrease was seen as early as 24 hr following one dose of STI571, and this was sustained over 15 months. The high FDG levels seen in the heart, kidney, and bladder are normal in both the pre- and post-Gleevec images. Figure adapted from Demetri et al., 2002, with permission from N. Engl. J. Med.

issues. Noninvasive imaging will become more important in the assessment and validation of new tissue-specific and pretargeted viral and nonviral gene delivery vectors following systemic as well as local routes of administration. The ability to repeatedly image over time will provide information on the biodistribution and pharmacokinetics of these novel vectors, and will facilitate the translation and application of these vectors in clinical studies.

Imaging cell trafficking, adoptive therapy, and tumor development

Reporter-gene imaging is also being used to visualize adoptively transferred cells (e.g., T lymphocytes and stem/progenitor cells) as well as the growth and metastasis of orthotopic and transgenic tumors. Genetically labeled cells have been used previously for monitoring adoptive therapy, and this dates back to the late 1980's, where reporter gene expression was detected by PCR or FACS analysis of peripheral blood. However, visualization and quantification of the in vivo distribution of infused cells in the body over time cannot be addressed by this approach. Several elegant imaging studies of T cell trafficking have used MR (Zhao et al., 2002), optical (Mandl et al., 2002), and nuclear (Dubey et al., 2003; Koehne et al., 2003) imaging technology. MR achieves better resolution, PET is better for quantitation, and optical is most cost effective in small animals. Similar approaches have been used to image orthotopic tumor growth and metastases and transduced cardiac tissue, as well as the endogenous development of tumors in transgenically altered mice (Vooijs et al., 2002).

Drug discovery and monitoring response

Increasing insights into the genetics and molecular biology of cancer have resulted in the identification of a large number of potential molecular targets for anticancer drug discovery and development. These targets can be approached through exploitation of emerging structural biology, "rational" drug design, screening of chemical libraries, or a combination of these methods. Based on analyses of structures of key signaling proteins, secondary structure peptidomimetic approach is a rational way to develop novel therapeutic agents. The identification of biologically significant peptidomimetic structures has led to several nonpeptide pharmaceuticals. In recent years, the pharmaceutical industry has become adept at developing effective inhibitors of protein kinases. A most impressive example of such inhibitors in the clinic has been imatinib (Gleevec)—the inhibitor of BCR–ABL that has provided a great leap forward in the treatment of chronic myeloid leukaemia and gastrointestinal stromal tumors. Inhibitors of other growth factor receptor (GFR) kinases that are involved in several signaling pathways have been under development for some time, and many have now entered clinical trials. At least six small molecule inhibitors of EGFR tyrosine kinase activity are now in clinical trials. The two drugs in this class that are at the most advanced stage of development are ZD1839 (Iressa) from AstraZeneca and OSI-774 (Tarceva) from OSI/Genentech.

Recent attempts to develop several radiolabeled 4-(anilino)quinazoline derivatives as molecular tracers for imaging EGFR kinase occupancy/activity have been reported (Ortu et al., 2002) and illustrate the general failure of approaches that attempt to radiolabel therapeutic compounds in order to obtain "imageable" EGFR kinase-specific tracers. The failure to obtain meaningful images of EGFR expression/activity are related to (1) the high lipophilicity of the compounds (LogP > 3.5), which results in rapid clearance of these radiotracers from blood and redistribution in the body (depot effect); (2) the predominantly hepato-biliary, rather than renal clearance, of these lipophylic tracers; (3) the absence of a specific signal and high background activity during image acquisition (the relatively short half-life of the radionuclides used for labeling of these tracers, ^{11}C–20 min and ^{18}F–110 min, precludes the use of a "washout strategy" [Tjuvajev et al., 1994]); (4) the position of the radionuclide in the chemical structure of the tracer is important with respect to the clearance of radiolabeled metabolites. Derivatization and radiolabeling of therapeutic compounds with more suitable pharmacokinetics for targeting EGFR kinase and PET imaging should consider the following: (1) compound lipophilicity (e.g., 0.8 < LogP <1.5); (2) resistance to metabolic degradation; (3) predominantly renal clearance; (4) "irreversible"

binding to the EGFR kinase ATP-binging domain to ensure that the half-life of probe-target complex is substantially longer than that for elimination of nonspecifically accumulated radioactivity. Successful imaging of EGFR or other GFR kinase expression/activity should be possible following optimization of EGFR kinase-specific tracers.

The development of molecular imaging approaches to assess the level of expression of specific signaling proteins and, most importantly, the level of signaling activity would have significant clinical impact. The pre-selection of patients based on high levels of drug-target expression, suggesting a higher potential sensitivity to the given drug, is feasible. In addition, repetitive imaging during the course of therapy would allow for monitoring target activity during dose escalation studies and could provide unique information to determine the optimum dose of drug for target inhibition in individual patients. Variants of these approaches have been investigated with all three imaging modalities.

Alternatively, "surrogate" imaging can reflect the downstream effects of one or more endogenous molecular/genetic processes. This is particularly attractive for potential translation into clinical studies in the near term, since direct and indirect molecular imaging studies are only beginning to be implemented in the clinic (Jacobs et al., 2001). Existing radiopharmaceuticals and imaging paradigms are gaining increasing attention, particularly as they relate to the development and testing of new pathway-specific drugs. The translation of surrogate-marker imaging paradigms into patients will be far easier than either the reporter transgene or direct imaging paradigms outlined above. Nevertheless, it remains to be shown whether there is a sufficiently high correlation between surrogate imaging and direct molecular assays that reflect the activity of a particular molecular/genetic pathway of interest. The assessment of noncytotoxic, cytostatic drugs, such as the antiangiogenic class of drugs, pose particular problems for treatment assessments that are based on imaging tumor volume. Imaging of tumor vascularity is being proposed as a surrogate assessment of antiangiogenesis treatment response. Another example of a clinically useful surrogate is FDG imaging for the assessment of early treatment response, prior to tumor volume changes. This was shown dramatically for gastrointestinal stromal tumors (GIST) pre- and 24 hr post-STI571 (Gleevec) treatment (Figure 4) (Demetri et al., 2002); similar pre- and post-treatment FDG images have been shown for other solid tumors (e.g., lymphomas). Whether imaging surrogate markers will be of value for assessing treatment directed at other molecular/genetic abnormalities in tumors (EGFR, p53, c-Met, HIF-1, etc.) remains to be demonstrated.

Translation of reporter imaging paradigms to patients

The major factor limiting translation of reporter gene imaging studies to patients is the transduction requirement; target tissue must be transduced with the reporter constructs for reporter gene imaging studies. At least two different reporter constructs will be required in most cases; one will be a "constitutive" reporter that will be used to identify the site, extent and duration of vector delivery, and tissue transduction (the normalizing or denominator term), and one will be an "inducible" reporter that is sensitive to endogenous transcription factors, signaling pathways, or protein-protein interactions, as described above. The initial application of such double-reporter systems in patients will most likely be performed as part of a gene therapy protocol (Jacobs et al., 2001) or an adoptive therapy protocol where the patients own cells are harvested (e.g., lymphocytes, T cells, or blood-derived progenitor cells) and can be transduced and expanded ex vivo, and then adoptively readministered to the patient. This scenario couples reporter gene imaging with existing adoptive therapies and allows for ex vivo transduction and expansion of harvested cells. For example, adoptive T cell therapy could provide a venue for imaging T cell trafficking, targeting, activation, proliferation, and persistence (Ponomarev et al., 2001; Koehne et al., 2003). This imaging paradigm could address several important questions related to adoptive T cell therapies. For example, is there substantial proliferation of adoptively transferred T cells at the target site or does activation and proliferation occur at other sites (e.g., specific lymphoid organ sites), followed by migration and localization to the target site? This question could potentially be addressed in a quantitative manner by repetitive PET imaging of the double-reporter system described above in the same animal or subject over time.

References

Blasberg, R., and Gelovani Tjuvajev, J. (2002). Molecular imaging: A nuclear based perspective. Mol. Imaging *1*, 160–180.

Blasberg, R., and Gelovani, J. (2003). Molecular-Genetic Imaging: Current and Future Prespectives. J. Clin. Invest., in press.

Contag, C.H., and Ross, B.D. (2002). It's not just about anatomy: in vivo bioluminescence imaging as an eyepiece into biology. J. Magn. Reson. Imaging *16*, 378–387.

Demetri, G.D., von Mehren, M., Blanke, C.D., Van den Abbeele, A.D., Eisenberg, B., Roberts, P.J., Heinrich, M.C., Tuveson, D.A., Singer, S., Janicek, M., et al. (2002). Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N. Engl. J. Med. *347*, 472–480.

Doubrovin, M., Ponomarev, V., Beresten, T., Balatoni, J., Bornmann, W., Finn, R., Humm, J., Larson, S., Sadelain, M., Blasberg, R., and Gelovani Tjuvajev, J. (2001). Imaging transcriptional regulation of p53-dependent genes with positron emission tomography in vivo. Proc. Natl. Acad. Sci. USA *98*, 9300–9305.

Dubey, P., Su, H., Adonai, N., Du, S., Rosato, A., Braun, J., Gambhir, S.S., and Witte, O.N. (2003). Quantitative imaging of the T cell antitumor response by positron-emission tomography. Proc. Natl. Acad. Sci. USA *100*, 1232–1237.

Edinger, M., Cao, Y.A., Hornig, Y.S., Jenkins, D.E., Verneris, M.R., Bachmann, M.H., Negrin, R.S., and Contag, C.H. (2002). Advancing animal models of neoplasia through in vivo bioluminescence imaging. Eur. J. Cancer *38*, 2128–2136.

Gambhir, S.S., Bauer, E., Black, M.E., Liang, Q., Kokoris, M.S., Barrio, J.R., Iyer, M., Namavari, M., Phelps, M.E., and Herschman, H.R. (2000). A mutant herpes simplex virus type 1 thymidine kinase reporter gene shows improved sensitivity for imaging reporter gene expression with positron emission tomography. Proc. Natl. Acad. Sci. USA *97*, 2785–2790.

Gambhir, S.S. (2002). Molecular imaging of cancer with positron emission tomography. Nat. Rev. Cancer *2*, 683–693.

Hadjantonakis, A.K., Macmaster, S., and Nagy, A. (2002). Embryonic stem cells and mice expressing different GFP variants for multiple non-invasive reporter usage within a single animal. BMC Biotechnol. *2*, 11–20.

Haubner, R., Wester, H.J., Weber, W.A., Mang, C., Ziegler, S.I., Goodman, S.L., Senekowitsch-Schmidtke, R., Kessler, H., and Schwaiger, M. (2001). Noninvasive imaging of alpha(v)beta3 integrin expression using 18F-labeled RGD-containing glycopeptide and positron emission tomography. Cancer Res. *61*, 1781–1785.

Ichikawa, T., Hogemann, D., Saeki, Y., Tyminski, E., Terada, K., Weissleder, R., Chiocca, E.A., and Basilion, J.P. (2002). MRI of transgene expression: correlation to therapeutic gene expression. Neoplasia *4*, 523–530.

Iyer, M., Wu, L., Carey, M., Wang, Y., Smallwood, A., and Gambhir, S.S. (2001). Two-step transcriptional amplification as a method for imaging

reporter gene expression using weak promoters. Proc. Natl. Acad. Sci. USA *98*, 14595–14600.

Jacobs, A., Voges, J., Reszka, R., Lercher, M., Gossmann, A., Kracht, L., Kaestle, C., Wagner, R., Wienhard, K., and Heiss, W.D. (2001). Positron-emission tomography of vector-mediated gene expression in gene therapy for gliomas. Lancet *358*, 727–729.

Jaffer, F.A., Tung, C.H., Gerszten, R.E., and Weissleder, R. (2002). In vivo imaging of thrombin activity in experimental thrombi with thrombin-sensitive near-infrared molecular probe. Arterioscler. Thromb. Vasc. Biol. *22*, 1929–1935.

Koehne, G., Doubrovin, M., Doubrovina, E., Zanzonico, P., Gallardo, H., Ivanova, A., Balatoni, J., Teruya-Feldstein, J., Heller, G., May, C., et al. (2003). Short and long-term in vivo imaging of targeted migration of HSV-TK transduced human antigen-specific lymphocyets. Nat. Biotechnol. *21*, 405–413.

Luker, G.D., Sharma, V., Pica, C.M., Dahlheimer, J.L., Li, W., Ochesky, J., Ryan, C.E., Piwnica-Worms, H., and Piwnica-Worms, D. (2002). Noninvasive imaging of protein-protein interactions in living animals. Proc. Natl. Acad. Sci. USA *99*, 6961–6966.

Mandl, S., Schimmelpfennig, C., Edinger, M., Negrin, R.S., and Contag, C.H. (2002). Understanding immune cell trafficking patterns via in vivo bioluminescence imaging. J. Cell. Biochem. Suppl. *39*, 239–248.

Mayer-Kuckuk, P., Banerjee, D., Malhotra, S., Doubrovin, M., Iwamoto, M., Akhurst, T., Balatoni, J., Bornmann, W., Finn, R., Larson, S., et al. (2002). Cells exposed to antifolates show increased cellular levels of proteins fused to dihydrofolate reductase: a method to modulate gene expression. Proc. Natl. Acad. Sci. USA *99*, 3400–3405.

Ortu, G., Ben-David, I., Rozen, Y., Freedman, N.M., Chisin, R., Levitzki, A., and Mishani, E. (2002). Labeled EGFr-TK irreversible inhibitor (ML03): in vitro and in vivo properties, potential as PET biomarker for cancer and feasibility as anticancer drug. Int. J. Cancer *101*, 360–370.

Perez, J.M., Josephson, L., O'Loughlin, T., Hogemann, D., and Weissleder, R. (2002). Magnetic relaxation switches capable of sensing molecular interactions. Nat. Biotechnol. *20*, 816–820.

Ponomarev, V., Doubrovin, M., Lyddane, C., Beresten, T., Balatoni, J., Bornman, W., Finn, R., Akhurst, T., Larson, S., Blasberg, R., et al. (2001). Imaging TCR-Dependent NFAT-Mediated T-Cell Activation with Positron Emission Tomography In Vivo. Neoplasia *3*, 480–488.

Qiao, J., Doubrovin, M., Sauter, M.V., Huang, Y., Guo, Z.S., Balatoni, J., Akhurst, T., Blasberg, R., Gelovani Tjuvajev, J., Chen, S.H., and Woo, S.L.C. (2002). Tumor-Specific Transcriptional Targeting of Suicide Gene Therapy. Gene Ther. *9*, 168–175.

Ray, P., Pimenta, H., Paulmurugan, R., Berger, F., Phelps, M.E., Iyer, M., and Gambhir, S.S. (2002). Non-invasive quantitative imaging of protein-protein interactions in living subjects. Proc. Natl. Acad. Sci. USA *99*, 3105–3110.

Tjuvajev, J., Macapinlac, H., Scott, A., Daghighian, F., Ginos, J., Finn, R., Kothari, P., Desai, R., Zhang, J., Beattie, B., et al. (1994). Imaging of the tumor proliferative activity with [^{131}I]-iododeoxyuridine. J. Nucl. Med. *35*, 1407–1417.

Tjuvajev, J.G., Stockhammer, G., Desai, R., Uehara, H., Watanabe, H., Gansbacher, B., and Blasberg, R.G. (1995). Imaging the expression of transfected genes *in vivo*. Cancer Res. *55*, 6126–6132.

Tjuvajev, J.G., Avril, N., Oku, T., Sasajima, T., Miyagawa, T., Joshi, R., Safer, M., Beattie, B., DiResta, G., Daghighian, F., et al. (1998). Imaging herpes virus thymidine kinase gene transfer and expression by positron emission tomography. Cancer Res. *58*, 4333–4341.

Vooijs, M., Jonkers, J., Lyons, S., and Berns, A. (2002). Noninvasive imaging of spontaneous retinoblastoma pathway-dependent tumors in mice. Cancer Res. *62*, 1862–1867.

Weissleder, R. (2002). Scaling down imaging: molecular mapping of cancer in mice. Nat. Rev. Cancer *2*, 11–18.

Weissleder, R., and Ntziachristos, V. (2003). Shedding light onto live molecular targets. Nat. Med. *9*, 123–128.

Zhao, M., Kircher, M.F., Josephson, L., and Weissleder, R. (2002). Differential conjugation of tat peptide to superparamagnetic nanoparticles and its effect on cellular uptake. Bioconjug. Chem. *13*, 840–844.