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Imaging Virus-Associated Cancer

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Abstract

Cancer remains an important and growing health problem. Researchers have made great progress in defining genetic and molecular alterations that contribute to cancer formation and progression. Molecular imaging can identify appropriate patients for targeted cancer therapy and may detect early biochemical changes in tumors during therapy, some of which may have important prognostic implications. Progress in this field continues largely due to a union between molecular genetics and advanced imaging technology. This review details uses of molecular-genetic imaging in the context of tumor-associated viruses. Under certain conditions, and particularly during pharmacologic stimulation, gammaherpesviruses will express genes that enable imaging and therapy *in vivo*. The techniques discussed are readily translatable to the clinic.

Keywords

EBV; molecular imaging; FIAU; bortezomib; thymidine kinase

INTRODUCTION

Significant progress in defining the molecular and genetic alterations that occur in cancer has been made during the past two decades. Concurrently, medical imaging has expanded toward more functional, rather than merely anatomic, techniques. Anatomic/functional imaging modalities, such as computed tomography (CT), magnetic resonance (MR) imaging and ultrasound (US) have been used to assess tumor size and structure. They can also provide valuable information on tumor perfusion, vessel density, vessel permeability, blood oxygenation, velocity, and resistance to flow. However, with the recent shift in cancer diagnosis and therapy toward more targeted paradigms, these conventional imaging modalities are often no longer adequate. Molecular imaging, which can be defined briefly as the remote sensing of cellular and molecular processes *in vivo*, can be used to monitor subtle changes in tissue, and can help in evaluating treatment efficacy at times much earlier than the conventional techniques [1, 2].

Although molecular imaging is a biology-driven enterprise, namely, one chooses a technique based on the underlying biology to be investigated, there are still a finite number of tools or modalities that enable such imaging to occur. The currently available modalities include: radiopharmaceutical-based (nuclear) imaging, advanced MR imaging techniques such as those applied to cell trafficking, MR spectroscopy, use of hyperpolarized species or the use of protein or peptide-selective pulse sequences, targeted US, bioluminescence, and

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fluorescence imaging. As suggested above, some of these techniques (MR and CT) are primarily anatomic, but are being applied increasingly to functional and more recently to molecular purposes. Others (bioluminescence and fluorescence) are basically outgrowths of *in vitro* methods of imaging that have been used for many years, however now are being employed with reporters that can be detected using imaging devices external to the living subject. Although several of these techniques can be used in the clinic, namely, MR and CT, the premier technique for clinical translation of molecular and molecular-genetic imaging are the radiopharmaceutical-based methods of positron emission tomography (PET) and single photon emission computed tomography (SPECT). That translatability is due primarily to the tracer principle, whereupon often $< 1 \mu\text{g}$ of radioactive material is delivered to the subject at a time – a dose that will almost always be subpharmacologic, thereby providing ready approval by the US Food and Drug Administration (FDA). More recently the largely anatomic methods of CT and MR have been fused with the molecular techniques of PET and SPECT to provide an even more precise and quantitative assessment of molecular events *in vivo*.

Molecular imaging has made significant inroads into the field – and medical practice – of oncology [3, 4]. Many new targets are being uncovered through study of gene and protein arrays and techniques such as phage display enable generation of novel imaging probes, a key aspect of molecular imaging research [5]. Of course the main benefit of molecular imaging, compared to standard methods of tissue interrogation, namely biopsy and *in vitro* analysis, is that no extracted sample is required, and the tissue can be studied in its intact microenvironment. In preclinical studies serial imaging allows animals to be used as their own controls in longitudinal studies.

New to imaging research is the ability to study molecular-genetic processes, i.e., signal transduction cascades, protein interaction networks and gene expression, noninvasively. Highly specific reporter systems have been developed to provide readouts on a variety of processes extending from hedgehog signaling to trafficking of neural progenitor cells [6, 7]. Several reviews of molecular-genetic imaging already exist [8–11] so this review will focus on the use of specific enzymes as targets for molecular-genetic imaging and will discuss strategies for overcoming certain difficulties in translating molecular-genetic imaging reporters to the clinic.

MOLECULAR IMAGING INSTRUMENTATION AND TECHNIQUES

Considerable efforts have been directed in recent years toward the development of noninvasive, high-resolution, small animal *in vivo* imaging technologies [12]. Molecular imaging requires two basic elements, namely, molecular probes, the concentration and/or spectral properties of which are altered by the specific biological process under investigation, and a means by which to monitor these probes. Imaging probes, like pharmacological agents, must target biological processes with high specificity. Devices that meet those demands have been designed and are now available, both for small animal research and for clinical studies. These detection devices include PET, SPECT, MRI, targeted US and optical imaging. Many fine reviews have been written discussing the various imaging modalities, however, the radiopharmaceutical-based techniques are most germane to our discussion of tumor-associated virus imaging.

Radiopharmaceutical-Based Imaging

PET and SPECT—PET records high-energy γ -rays emitted from within the subject. Natural biological molecules can be labeled with a positron-emitting isotope, e.g., ^{18}F or ^{11}C , which is capable of producing two γ -rays through emission of a positron from its nucleus, which eventually collides with a nearby electron to produce two 511 keV γ -

rays at $\sim 180^\circ$ apart. The γ -rays are detected in coincidence providing the raw data prior to iterative image reconstruction. Strictly γ -emitting isotopes (e.g., ^{99m}Tc , ^{111}In , ^{123}I) are also used for imaging living subjects using a gamma camera that, when sequentially placed around the subject, can be used to generate SPECT images. PET and SPECT are both fully quantitative techniques, with the former less common due to the need for an on-site cyclotron and dedicated radiochemists. Although PET has higher spatial resolution in practice, SPECT has a higher theoretical spatial resolution due to the absence of an annihilation event. SPECT is probably more useful for preclinical (small animal) radiopharmaceutical-based imaging because of its ability to resolve tiny structures. However, the need for physical collimation in SPECT decreases its sensitivity relative to PET, which has a sensitivity range of 10^{-11} – 10^{-12} M, so that it is about one to two orders of magnitude less sensitive. Nevertheless, the sensitivity is sufficient for most applications and the lower sensitivity relative to PET is offset by the far lower cost of radiopharmaceuticals and wider availability. Furthermore, SPECT data acquisition can be windowed to enable the use of two or more radiopharmaceuticals reporting on different events simultaneously – by virtue of the different energies of the emitted photons. PET is less versatile in that regard as all PET photons are 511 keV.

Molecular Imaging Strategies

Imaging strategies have been divided into three groups: direct, indirect and surrogate. Direct molecular imaging involves direct probe-target interaction, whereby the resultant image is directly related to probe localization and image intensity is relative to its interaction with the target epitope or enzyme. In direct imaging, the target (e.g., protein) is imaged directly and therefore requires a target-specific probe. Examples of such direct imaging strategies include but are not limited to imaging of cell-surface antigens or epitopes using radiolabeled antibodies or probing cell proliferation with [^{18}F]fluorothymidine (FLT), a substrate for thymidine kinase (TK), which is highly upregulated in cancer [13]. A major limitation of the direct imaging strategy is the need for a specific probe for each target, which is not always feasible. In addition, if the target is a receptor rather than an enzyme or transporter, the direct imaging technique may suffer from decreased sensitivity. Increased sensitivity from enzymatic reporters results from the fact that many substrates can react with the enzyme, which often leads to a product that is trapped and accumulated within the target cell. Receptor-based reporters may also benefit from signal amplification if the receptor is internalized with its ligand and can recycle back to the cell surface where it may interact with another. Molecular-genetic imaging, however, by focusing on specific signal transduction cascades, gene expression or protein-protein interaction, generally employs an indirect technique. Indirect imaging strategies have been developed and validated to be used in conjunction with gene therapy.

Indirect imaging uses the reporter-probe concept. The idea has been used for many years by molecular biologists using green fluorescent protein (GFP) or β -galactosidase to report on genetic events *in vitro*. The detected event is stoichiometrically related to the event of interest, providing an indirect readout on the latter. But no “probe” is needed for the *in vitro* situation. For molecular imaging this concept has been extended to reporters that can be imaged using external imaging devices such as PET or SPECT scanners, MR or specially designed fluorescence or bioluminescence detection devices, when coupled, in general, to a probe. The indirect strategy is critical to molecular-genetic imaging because one cannot often image the gene expression event directly simply because a direct probe does not exist and/or would be prohibitively difficult to synthesize.

Surrogate imaging strategies reflect the downstream effects of one or more endogenous molecular-genetic or therapeutic processes. Considerable progress has been made toward targeted therapy. However, imaging is only now being employed as an adjunct to standard

therapeutic monitoring. Due to a lack of targeted imaging agents, it is rather difficult to follow new, targeted therapies or to predict the outcome of such therapies. One way to do so is to utilize existing metabolic markers, such as measurement of glucose metabolism with [^{18}F]fluorodeoxyglucose (FDG) or cell proliferation with FLT, to monitor the downstream effects of a targeted therapy. For example, one may use changes in TK levels observed after a thymidylate synthase (TS) inhibitor is used therapeutically. TS inhibitors deplete *in vivo* triphosphate pools. To compensate, cancer cells upregulate expression of TK1. Administration of TK1-specific imaging agents such as [^{11}C]thymidine or FLT therefore would result in an increase in radiopharmaceutical uptake and retention in a successful therapy [14, 15]. A classic example of clinically useful surrogate imaging for early assessment of treatment response is FDG PET imaging of gastrointestinal stromal tumors (GISTs) before and after STI571 treatment [16]. The surrogate marker approach is particularly attractive for translation into clinical studies in the near term, since direct and indirect molecular imaging studies are only beginning to be implemented in the clinic. However, the use of surrogate imaging techniques requires a clear understanding of the mechanistic aspects and downstream effects of a particular therapy and also is likely to be less specific than either the direct or even indirect approaches. Also, it remains to be shown as to whether there is sufficient correlation between the images obtained using the surrogate marker approach and direct molecular assays that reflect the activity of the intended pathway under study. Such validation studies are being intensely pursued and represent a necessary step before molecular imaging becomes accepted as a clinically viable tool in patient selection, prognostic determination or therapeutic monitoring.

MOLECULAR-GENETIC IMAGING: A BRIEF OVERVIEW FOCUSING ON HSV1-TK IMAGING

HSV1-TK and Reporter Gene Expression

Several molecular-genetic reporter systems have been developed to take advantage of the enzymatic signal amplification and trapping mechanism. A few examples include use of the herpes simplex virus type 1 TK (HSV1-TK) [17], *Escherichia coli* (*E. coli*) and yeast cytosine deaminases (CD and yCD) [18], and *E. coli* xanthine phosphoribosyltransferases (XPRT) [19]. Among these, the wild type and mutant *HSV1-tk* genes are the most frequently used PET reporter genes. Reporter genes have been used with imaging to study a variety of cellular processes including: initiation of transcription [20], transcriptional regulation [21] translation of mRNA [22] and protein-protein interaction [23–25]. In each case the event that is being studied is linked to production of a protein that can be imaged. For example, in the case of imaging translation of mRNA, pre-mRNAs are reprogrammed to encode imaging reporter proteins, which are assembled *in vivo* into the human *renilla* luciferase (hRLuc) reporter [22]. The enzymatic activity of HSV1-TK has been used for antiviral treatment and suicide gene therapy to treat cancer long before its use in molecular-genetic imaging [26].

The *HSV1-tk* reporter gene with radiolabeled versions of 5 -iodo -2 -deoxy- 2- fluoro - β -D-arabinofuranosyl)uracil (FIAU) as the corresponding reporter probe have been well established using gamma scintigraphy and PET with ^{131}I -, ^{124}I - or ^{18}F -labeled FIAU [27–29]. FIAU is phosphorylated by viral or mammalian cytosolic (TK1) or mitochondrial TKs (TK2) to FIAU-monophosphate (FIAUMP) (Fig. 1). FIAUMP is further phosphorylated and incorporated into DNA as FIAU-triphosphate (FIAUTP) or deiodinated and activated by TS to 1-(2'-deoxy-2'-fluoro- β -D-arabinofuranosyl)thymine (FMAU) monophosphate (FMAUMP). FMA- UMP is further phosphorylated and incorporated into DNA [30]. However, FIAU is preferentially phosphorylated by HSV-TK compared to mammalian TKs. In an effort develop highly specific and sensitive probes for HSV1-TK many pyrimidine

analogs such as IUdR, IVFRU [31, 32], FMAU [33, 34], FFAU [35], FCAU [36], FBAU [36], FEAU [37, 38], FFEAU [39, 40], and FIRU [32] have been investigated as reporter probes for HSV1-TK (Fig. 2). Of all the analogs tested to date, FIAU is still one of the most sensitive probes for wild type HSV1-TK. However, FIAU also demonstrates a certain amount of accumulation in cells that do not express HSV1-TK, contributing to nonspecific signal [40].

To improve the sensitivity of the *HSV1-tk* reporter gene system, mutants such as *HSV1-sr39tk* were developed using random sequence mutagenesis and were screened for high-affinity binding of specific acycloguanosine analogs [41]. Screening for a suitable combination of reporter gene/probe combination resulted in highly specific and sensitive acycloguanosine analogs such as FHBG [41]. Other acycloguanosines such as GCV, FGCV [42], PCV, FPCV [43] and FHPG and the pyrimidine analog FEAU have also been investigated as probes for *HSV1-tk* as well as for the mutant *HSV-sr39tk* [41, 44]. Currently [¹⁸F]FHBG is used for clinical studies as a substrate for *HSV1-sr39tk* [44]. The pyrimidine analog FEAU is perhaps the most sensitive and specific tracer for both wild type and mutant (*HSV1-sr39tk*) gene expression imaging [40, 45]. In a recent study, Likar *et al.*, developed an *HSV1-sr39tk* supermutant bearing an alanine-to-tyrosine substitution at position 167 (A167Y) [46]. The resultant *HSV1-A167Ysr39tk* was able to phosphorylate acycloguanosine analogs specifically and showed significantly reduced ability to phosphorylate pyrimidine-based nucleoside analogs, including clinically administered medications such as BVdU and thymine 1-β-D-arabinofuranoside (Ara-T). Subsequent imaging studies demonstrated a 2.5-fold increase in [¹⁸F]FHBG accumulation in the supermutant transduced tumors [46].

Noninvasive and quantitative imaging of transgene expression using either wild type or mutant *HSV1-tk*-based reporter gene systems has been evaluated in preclinical models for monitoring adenoviral gene delivery [47], oncolytic herpesvirus therapy [48], adoptive cell-based therapies [49], and antitumor immune response [50], to name a few. The use of a reporter gene strategy is particularly useful to determine the spatiotemporal distribution and magnitude of expression of transgene expression to direct gene therapy *in vivo*.

Yaghoubi *et al.* [51] monitored the treatment response of *HSV1-sr39tk*-expressing gliomas (C6sr39) to ganciclovir (GCV) noninvasively. PET imaging with [¹⁸F]FHBG was performed to image HSV1-sr39TK levels and with FDG to image tumor glucose metabolism and tumor volume. Following GCV treatment, the C6sr39 tumor volume, [¹⁸F]FHBG and FDG accumulation decreased whereas the wild type C6 tumor continued to grow. In this study, the authors noted regrowth of the C6sr39 tumors three weeks after completing GCV treatment. Further PET imaging studies revealed an increase in FDG accumulation in the tumors, whereas tumor [¹⁸F]FHBG concentrations remained at background levels. Variations in radiopharmaceutical uptake were attributed to a population of non-*HSV1-sr39tk*-expressing cells that survived GCV treatment and all the selective regrowth of tumors that did not express significant amounts of HSV1-sr39TK. These observations indicate that by choosing one or more appropriate probes, PET imaging can be used to monitor the effectiveness of GCV treatment of *HSV1-sr39tk*-expressing tumors.

In another study Freytag *et al.* [47] explored the efficacy and toxicity of using a second-generation adenovirus (Ad5-yCD/mutTKsr39rep-ADP) for combining replication-competent adenovirus-mediated suicide gene therapy with radiation therapy in an experimental dog model of pancreatic cancer. The authors demonstrated the feasibility of monitoring *HSV1-sr39tk* gene expression using [¹⁸F]FHBG during the therapeutic trial. Furthermore, the authors demonstrated that combining Ad5-yCD/mutTKsr39rep-ADP-mediated suicide gene

therapy with radiation significantly improved tumor control and survival beyond that of either modality alone.

Noninvasive imaging also allows for the characterization of the migration and survival of adoptively transferred lymphocytes or stem cells and can improve our understanding of the response of neoplastic and immune cells to therapeutics. Utilizing *HSV1-sr39tk* as a reporter gene, Dubey *et al.* [49] were able to show that adoptively transferred T cell antitumor responses could be quantified using small animal PET. Although the tumor cells are not transfected with the reporter in this instance, it is the behavior of the therapeutic agent, the T cells that are directed against the antigen-positive tumor, that is followed serially through imaging. Similarly, using bone marrow chimeric mice reconstituted with *hrl-egfp-tk*-expressing stem and progenitor cells and a strongly immunogenic, nonmetastasizing retrovirally induced rhabdomyosarcoma to initiate a potent antitumor immune response, Shu *et al.* [52] investigated the antitumor immune response by PET imaging. Both [¹⁸F]FHBG and FDG PET imaging were used to follow the immune cell response to tumor progression sequentially in the same animal. The authors demonstrated that alterations of the immune response after therapeutic manipulation with immunosuppressive drugs could be detected with PET.

To date there has only been a limited translation of transgene imaging studies into clinical trials to monitor gene therapy. The first noninvasive imaging of human gene therapy, using wild type *HSV1-tk*, was reported by Jacobs *et al.* in glioma patients undergoing stereotactic gene therapy. Imaging of *HSV-tk* expression was undertaken with [¹²⁴I]FIAU PET [48]. Only one out of the five patients studied showed an indication of radiotracer accumulation due to transgene expression. In another study, Peñuelas *et al.* monitored wild type *HSV1-tk* expression in patients with hepatocellular carcinoma after an intratumoral injection of a recombinant adenovirus expressing HSV1-TK. The authors demonstrated that gene expression levels monitored using [¹⁸F]FHBG showed a viral dose-dependent accumulation of the radiotracer [53]. In addition, the authors showed that *HSV1-tk* transgene expression monitoring by [¹⁸F]FHBG could be used to predict the response to the gene therapy procedure in cancer patients [53].

Both *HSV1-tk* and *HSV1-sr39tk* pose a risk of eliciting an immune response against cells and tissues transduced with these genes as they are not naturally expressed in humans [54]. To reduce that risk, endogenous human genes with severely restricted expression such as the human TK2 (*hTK2*), which is minimally expressed in the mitochondria of most human tissues, has been proposed as a reporter gene. The substrate specificity of DeltaTK2, which localizes in the cytoplasm, is similar to that of viral TK and has a less restricted expression pattern than in comparison to that of human (mammalian) hTK1. Recent studies have shown that hTK2 is capable of phosphorylating radiolabeled FIAU, FEAU or FMAU [55, 56]. These studies indicate the feasibility of a clinical translation in the near future, devoid of complications from immunogenicity.

TUMOR-ASSOCIATED IMAGING OF BACTERIAL INFECTION: EXPLOITING THE BACTERIAL TK

Solid tumors frequently contain hypoxic and necrotic regions in addition to their normoxic boundaries. Typically, it is only the normoxic regions that are sensitive to both chemo- and radiotherapy [57]. A means to eliminate the hypoxic compartments, which are refractory to radiotherapy, is needed to improve treatment outcome. Anaerobic bacteria were found to treat solid tumors as far back as 50 years ago [58], although the concept has only been recently revisited [59]. The objective was to select for a strain of spore-forming bacteria that no longer expresses its endogenous endotoxin while colonizing and growing only within the

hypoxic regions of solid tumors. The strain found to meet those criteria is *Clostridium novyi-NT* (*C. novyi-NT*). NT represents an engineered form of *C. novyi* in which the lethal toxin is no longer expressed. Extensive histological and radiochemical assays were performed to verify that bacterial spore germination occurred only within experimental, model tumors and not in normal tissues [60]. Germinated *C. novyi-NT* also naturally expresses a TK homologous to HSV1-TK, which proved critical to the imaging technique. FIAU can serve as the substrate for the bacterial TK, as in the case of the abovementioned virus. That allows for direct radiolabeled FIAU-based SPECT or PET imaging of *C. novyi-NT* or other bacterial infections caused by strains expressing a homologous TK [61]. Studies are currently under way to verify that radiolabeled FIAU does not detect inflammation in addition to bacteria expressing homologous TK. Nonspecific inflammatory tissue uptake along with infection is a drawback when using FDG PET, radiolabeled antibiotics or radiolabeled leukocytes, which may detect nontarget tissues or irrelevant biological processes [62–64].

We have taken advantage of the natural expression of TK within most strains of bacteria, including within *C. novyi-NT*, a therapeutic, hypoxic tumor targeting strain, to image tumor-mediated bacterial infection and treatment by gamma scintigraphy [61] as well as by PET. We demonstrated the specificity of radiolabeled FIAU uptake in TK-homologous vs that of bacteria that do not express homologous TK (Fig. 3). The exploitation of FIAU as a suicide substrate in endogenously expressed TK in therapeutic *C. novyi-NT* treatment may have use in the clinic to follow the course of treatment or possibly as a radiotherapeutic agent. A bystander effect may allow for both hypoxic core elimination (*via* the bacterial infection) as well as destruction of the normoxic rim by the emitted radiation.

VIRUS-ASSOCIATED TUMORS

Over the past 30 years increasing evidence suggests a role for several infectious agents in human cancer. It has been estimated that nearly 20% of human cancers have an infectious etiology [65, 66]. Most of these tumors are of viral origin. Virus-associated cancer refers to a cancer where viral infection results in the malignant transformation of the host's infected cells. Those human viruses that have been identified as causes of cancer are shown in Table 1. They include hepatitis B virus (HBV) and hepatitis C virus (HCV), which are associated with hepatocellular carcinoma, human papilloma virus (HPV), which is associated with cancers of the cervix, anus, vulva and vagina, Epstein-Barr virus (EBV), which is associated with lymphoma, nasopharyngeal carcinoma and gastric carcinoma, human T lymphotropic virus type 1/2 (HTLV-1/2), which is associated with adult T cell leukemia/lymphoma, human herpesvirus 8 (KSHV/HHV-8), which is associated with Kaposi sarcoma and lymphoma and human immunodeficiency virus (HIV), which is associated Kaposi sarcoma and lymphoma [67–73]. In combination these virus-associated tumors represent a burden of approximately 1.3 million cases of cancer each year, with HBV/HCV-associated liver cancer accounting for 523,000 cases, and HPV-associated tumors accounting for 561,000 cases [72]. Hence, virus-associated cancer represents a large group of heterogeneous tumors that have in common a viral etiology. Methods of virus-associated tumor treatment include surgery, chemotherapy, and external beam radiation therapy. Radioimmunotherapy (RIT), an alternative modality for the treatment of cancer that combines radiation and immunotherapy, started nearly three decades ago. RIT utilizes the specificity of the antigen-antibody interaction to deliver radionuclides producing cytotoxic doses of particulate radiation to tumor cells and provides a valuable alternative to chemotherapy [74, 75]. RIT has been successful in the treatment of lymphoma, with two FDA-approved radiolabeled monoclonal antibodies (mAbs), Zevalin^{TM} and Bexxar^{TM} (anti-CD20 mAbs labeled with ⁹⁰Y and ¹³¹I, respectively), in clinical use. It seems likely that RIT will find a niche as a first-line treatment for follicular lymphoma [76]. Recently we developed an new approach for

imaging and therapy for gammaherpesvirus-associated tumors [77]. That approach is bortezomib-induced enzyme-targeted imaging and radiotherapy in tumors, detailed below.

BORTEZOMIB-INDUCED ENZYME-TARGETED IMAGING

As mentioned above, the gammaherpesvirus family includes EBV and KSHV. EBV and KSHV are associated with a variety of tumors, most prominently lymphoma. The viral genome serves as a nearly tumor-specific target [78]. Thus virus-associated metabolic pathways can be used to approximate tumor-specific metabolic pathways.

Mechanism of Bortezomib Induction of TK

Bortezomib is known to have anti-nuclear factor-kappa B (anti-NF- κ B) activity by inhibition of the degradation of inhibitory protein kappa B (I κ B). One of the effects of bortezomib that is well recognized is stabilization of I κ B and resultant inhibition of NF- κ B [79]. In an effort to determine whether this pathway might also be important in induction of lytic gene expression, we investigated the effects of an I κ B super-repressor [I κ B (sr)] in transfection experiments in a Burkitt's lymphoma cell line. As shown in Fig. (4), EBV viral load increased by approximately 12-fold following transfection. Moreover, when EBV(+) Akata cells were incubated in the presence of 20 nM bortezomib for 48 h, 67% of cells entered the lytic cycle, and the induction proved to be dose-dependent [76]. Others and we have shown that inhibition of the NF- κ B pathway induces the EBV lytic cycle [76, 80]. Our results may uncover a link between anti-NF- κ B activity and lytic induction, providing a mechanism for the result observed in the EBV-associated lymphomas studied. These findings suggest the possibility that there may be ways to exploit the viral association of some tumors for imaging. Notably, several therapeutic strategies have been explored that involve modulation of viral gene expression in tumors [78, 81–84].

Imaging of Engineered EBV-TK *In Vivo*

As detailed above, viral genes have been engineered to serve as reporters in a variety of gene therapy models. The HSV1-TK has seen particularly wide use [17]. A homolog of the HSV1-TK is encoded by EBV. The EBV-TK will selectively phosphorylate FIAU. In order to be sure that the ability to image EBV-associated tumors following bortezomib treatment did not reflect induction of a cellular kinase leading to FIAU accumulation, we also imaged osteosarcoma xenografts. No tumor uptake was evident, with or without bortezomib. However, the same cell line engineered to express the EBV-TK constitutively was readily imaged (Fig. 5). Those results suggest that the effects of pharmacologic induction that are relevant to imaging are mediated by the viral TK rather than activation of a cellular kinase.

Bortezomib Induction of EBV TK *In Vitro*

Direct imaging of EBV-associated tumors with FIAU is not possible because the enzyme is not expressed or is expressed only at very low levels in EBV-associated tumors. In a screen of clinically approved drugs to identify those that induce EBV lytic infection, bortezomib was identified as active (J. Chen, in preparation). As shown in Fig. (6), bortezomib leads to EBV-TK expression as shown by immunoblot and time-dependent accumulation of [^{14} C]FIAU in EBV(+) Burkitt's cell lines but not in the isogenic EBV(–) Burkitt's cell line.

Imaging of EBV-TK and KSHV-TK Activity by Bortezomib Induction *In Vivo*

We demonstrated [^{125} I]FIAU imaging of EBV-TK in murine xenografts of EBV(+) Burkitt's lymphoma in concert with treatment with bortezomib. Mice bearing EBV(+) Burkitt's lymphoma xenografts were treated with bortezomib or phosphate-buffered saline (PBS) and imaged at various times after injection of [^{125} I]FIAU (Fig. 7). Images obtained at early time points revealed only cardiac blood pool, liver, urinary bladder and thyroid,

consistent with the usual biodistribution of [^{125} I]FIAU. By one day postinjection, the tumor in the animal treated with bortezomib can be visualized and by four days postinjection, the tumor in the treated animal is clearly visible, despite abundant radioactivity within the bladder. Experiments with EBV(+) gastric cancer (KT tumors) and KSHV(+) primary effusion lymphoma (BCBL1) provided similar results (*Fig. 8A and B*, respectively) [85].

To confirm that the radioactivity ostensibly shown within tumor in the images in *Fig. (7)* was indeed within tumor and to quantify the amount of uptake, we performed *ex vivo* biodistribution assays in animals bearing EBV(+) xenografts in additional experiments. Although increased [^{125} I]FIAU uptake was evident earlier, it peaked at 96 h postinjection in tumors of animals pretreated with bortezomib. *Fig. (7A)* shows a lack of tumor uptake at 96 h (0.039% ID/g) in mice pretreated with PBS, whereas *Fig. (7B)* shows the time course of uptake in the tumors of bortezomib pretreated animals. Tumor radiopharmaceutical uptake increased from 0.039% ID/g to 0.85% ID/g, a nearly 22-fold increase over baseline values after treatment with bortezomib (*Table 2*). The radioactivity detected within other tissues (i.e., liver, spleen, kidney, and muscle) diminished between the 24- and 96-h time points.

We have shown that EBV Burkitt's lymphoma xenografts can be imaged with [^{125} I]FIAU and a dedicated gamma camera after pharmacologic induction of viral TK expression. Although radiolabeled nucleoside analogs have been previously used as reporters for cells that were genetically engineered to express herpesvirus TK genes, no such engineering was required here. Tumor-associated virus itself provided the reporter gene to image tumor directly. These results suggest the possibility that there may be ways to exploit the viral association of some tumors for imaging. Several therapeutic strategies that involve modulation of viral gene expression in tumors might be monitored by imaging noninvasively [81, 85–89].

MOLECULAR RADIOTHERAPY

The most familiar form of this type of therapy is radioimmunotherapy (RIT). As stated above, RIT has proved successful in the treatment of lymphoma, however, it has proved less successful for solid tumor type carcinomas. Bexxar^{TM} and Zevalin^{TM} are administered to patients suffering from CD-20-expressing, B-cell non-Hodgkin's lymphoma. Patients so treated have achieved long-term favorable response rates of nearly 90% [90]. Currently, monoclonal antibodies directed against a variety of targets are under investigation for use in clinically approved RIT [91, 92] (*Table 3*). To be successful, the targeting antibody must fulfill several criteria, each of which requires optimization. The most important criterion is the ability to access the chosen molecular target. Prostate-specific membrane antigen (PSMA) is been a molecular target sought for diagnostic purposes in hormone refractory prostate cancer [93]. Due to the tumor-specific and tissue-restricted expression of PSMA [88], several PSMA-specific monoclonal antibodies have been cultivated, with only one clinically approved for diagnostic use. [^{111} In]-ProstaScint^{TM}, however, targets an intracellular epitope of PSMA and therefore displays limited target uptake as well as significant background radioactivity [94]. A suitable target must be expressed in high levels on cancer cells, on the order of 10^6 or more per cell, or the radiolabeled antibody should experience target-mediated tumor internalization so that fewer targets per cell are required to concentrate the antibody's radioactive payload. Other criteria requiring optimization include use of a radionuclide with an appropriate physical half-life to reflect long antibody circulation times and use of a non-immunogenic or humanized antibody. A pre-targeting strategy utilizing unlabeled primary antibodies first seeks and marks the chosen molecular target. This is followed by washout of excess antibody, leaving nontarget tissues free of the primary antibody. Once the excess unlabeled primary antibody has cleared from nontarget sites, primary, specific secondary vehicle, loaded with the radiotherapeutic payload, is then

introduced to deliver cytotoxic radiation only to the cells expressing the chosen molecular target of interest [91, 92]. Lack of optimization of any of these criteria necessary for effective RIT may lead to excess nontarget tissue cytotoxicity and ineffective cell kill.

The choice of radionuclide must reflect the physical characteristics of the end target. There are three types of isotope decay that are useful for RIT. They include β -particle emission, α -particle emission and Auger electron emission. β -Particle emitters are typically used for solid tumor radiotherapy due to the relatively long distance of emission particle flight before coming to rest. That means that the radiotherapeutic payload can effect cell kill over a greater distance. That becomes necessary in solid tumors where blood flow and antibody delivery to the whole tumor are poor. The most commonly used β -emitters for RIT are ^{131}I , having a 193 h physical half-life, 182 keV β -particle decays as well as high energy γ -emissions, and ^{90}Y , a pure β -emitter, which experiences a 64 h half-life and 2.2 MeV β -particle decays. Ninety percent of the emitted β -particle energy from ^{131}I is deposited within 0.36 mm of the decay particle while the ^{90}Y emission particles traverse 3.78 mm of tissue before coming to rest [95]. α -Particle emitters, such as ^{213}Bi or ^{211}At , are ideal for disseminated and micrometastatic disease. The particle emission is deposited within 100 μm of the decay event [96]. ^{213}Bi has a 46 min half-life making its use challenging, but provides other benefits such as relatively lower radiation doses to nontarget tissues. α -Particle emission includes high linear energy transfer, resulting in the need for relatively few decay events per cell to kill that cell, possibly as few as one. Auger electron emitters such as ^{125}I or ^{67}Ga , are best used for individual cell kill. Those radionuclides need to be internalized within the cell and brought adjacent to the nucleus or integrated into the DNA for lethality [97]. Antibodies tethered to α -particle-emitting nuclides have not been used extensively in clinical trials. Use of targeted α -emitting radionuclides has advantages and disadvantages above and beyond the uses outlined above. For example, ^{131}I has a long radiological half-life and the resulting γ -emissions require patient isolation. Determining radiological dose for patients receiving ^{131}I -bearing radiopharmaceuticals must take into account both the biological half-life of the RIT antibody as well as the extent of dehalogenation based on the length of time the targeting antibody resides in the body [92]. ^{90}Y -Labeled antibody therapy does not require patient isolation and is more easily retained within cells, possibly providing an added therapeutic benefit over radioiodinated compounds. Nevertheless, compounds labeled with ^{131}I actually demonstrate little deiodination *in vivo* and the small presence of thyroid uptake is due to antibody-associated metabolism rather than loss of free ^{131}I -mediated from chemical dehalogenation. The validity of radioactive iodine use for signal amplification or as a therapeutic agent in monoclonal antibodies has been established over many years, culminating in the use of ^{131}I as a radionuclide to treat lymphoma and other types of cancer. Incidentally, the CD20 molecular target to which Bexxar^{TM} binds is internalized [98], providing further rationale for the continued development of radioiodinated and ^{90}Y -chelated therapeutic agents. Advantages of radionuclide-based therapy in general over other targeted therapies include the fact that these therapies can be effective even in the context of tumor hypoxia. Due to decay particle path length properties, a bystander (cross-fire) phenomenon occurs enabling a increased cell kill effect with only a small number of RIT antibodies bound. That bystander effect also applies in the case of cytotoxic drug laden antibodies, which deposit their payload to the target sites.

We have taken advantage of the trapping mechanism of HSV1-TK that is induced in bortezomib-treated, gammaherpesvirus-associated tumors to provide a new type of molecular radiotherapy. The method is in analogy with the abovementioned FIAU-based imaging, but we merely switch to [^{131}I]FIAU. Bortezomib-induced enzyme targeted radionuclide therapy (BETR) has proved effective in experimental models of epithelial tumors as well as lymphoma.

BORTEZOMIB-INDUCED ENZYME TARGETED RADIONUCLIDE (BETR) THERAPY

Radioimmunoconjugates may be limited by the target level of expression, antibody affinity, or the physical characteristics of the antibody that may impede delivery to large tumors or protected compartments [99]. Targeting metabolic pathways such as those involved in concentrating iodine in thyroid tissue is an alternative approach, the more general application of which has been limited by the ability to identify appropriate tumor-specific, substrate-concentrating pathways. We demonstrated that naturally occurring tumor cells harboring EBV could be imaged with a radiolabeled nucleoside analog, if a pharmacologic inducer of the viral TK was utilized [77]. We extended that observation to demonstrate that an inducing agent with a radiotherapeutic nucleoside analog allows targeted therapy of virus harboring tumor cells in xenograft models.

Engineered Constitutive EBV TK Expression and Therapeutic Effect

As described above, to evaluate specificity with regard to the concentration of FIAU in tumor tissue, we used a human osteosarcoma cell line engineered to express the EBV-TK [81]. Tumor cells were engrafted on the flanks of SCID mice. After tumor was palpable, [^{125} I]FIAU was administered intravenously and mice were sacrificed (Fig. 9). Selective concentration of radioactivity in tumor was apparent by two hours postinjection and radioactivity levels in the tumor remained constant until the last time point at 96 h, whereas levels in nontarget tissues decreased, consistent with previous investigators' reports in a similar murine model with tumor constitutively expressing the HSV1-TK [100]. To determine whether concentration of radioisotope in tumor tissue was adequate to achieve a therapeutic effect, mice were treated with [^{131} I]FIAU or buffered saline (Fig. 10a). Control and TK tumors in mice injected with buffered saline, and control tumors in mice injected with [^{131} I]FIAU, showed similar growth curves. However, the growth slope of TK tumors in mice injected with [^{131} I]FIAU flattened. In separate experiments, increasing doses of [^{131} I]FIAU were associated with increasing effects on tumor growth (Fig. 10b). When chimeric tumors were generated from admixtures of TK and control tumor cells, increasing percentages of TK-expressing tumor cells in the admixture were also associated with increasing effects on tumor growth (Fig. 10c).

BETR for Gammaherpesvirus-Associated Tumor

Can a therapeutic effect be achieved in a naturally infected tumor? In order to evaluate the specificity in naturally infected tumor tissue, EBV(+) Burkitt's lymphoma cells were engrafted subcutaneously on the flanks of SCID mice. After tumor was palpable, [^{125} I]FIAU was administered intravenously 24 hr after bortezomib and mice were sacrificed. Selective concentration of radioactivity was apparent in these tumors (Table 4). Therefore, we proceeded with an evaluation of a therapeutic isotope. As above, mice with Burkitt's lymphoma xenografts received either buffer or bortezomib followed the next day by treatment with [^{131} I]FIAU or no treatment. As can be seen in Fig. (11a), tumor growth curves in mice injected with buffer followed by [^{131} I]FIAU were similar to tumor growth curves in mice injected with buffer alone. Bortezomib without [^{131} I]FIAU slowed tumor growth. Bortezomib followed by [^{131} I]FIAU stopped tumor growth. Although EBV is associated with Burkitt's, AIDS-related, Hodgkin's lymphoma, NK and other lymphomas, the numerically most important associations are with nasopharyngeal cancer (~80,000 new cases/year) and approximately 10% of gastric carcinoma (~93,000 new cases/year) [72, 101, 102]. In order to determine whether the bortezomib induction strategy might also be applicable to epithelial malignancies, we studied a transplantable human EBV-associated gastric cancer (KT) xenograft [103]. Following engraftment, mice were induced with bortezomib followed by [^{125} I]FIAU. Tissue distribution studies showed selective

concentration of [^{125} I]FIAU in tumor, although the ratios in tumor to other tissues were not as dramatic as with the Burkitt's lines (Table 4). When induction was followed by [^{131} I]FIAU, tumor growth slowed (Fig. 11b). Could the approach be extended to KSHV-associated tumors? We studied two primary effusion lymphoma (PEL) cell lines that harbor KSHV. In a SCID xenograft model, bortezomib allowed imaging of both BCBL1 and BC3 with [^{125} I]FIAU (Fig. 8b). Tissue distribution studies showed selective concentration in tumor tissue (Table 4), and when induction was followed by treatment with [^{131} I]FIAU tumor growth slowed (Fig. 11c). Thus, bortezomib allowed targeting of [^{131} I]FIAU with therapeutic effect.

The HSV1-TK has been used as a tool in gene therapy [26]. Even the notion that a viral TK might be used to target radiation therapy has been explored by others [104]. Similarly, induction of the EBV-TK with a histone deacetylase inhibitor followed by treatment with ganciclovir has been reported to induce remissions in some patients [105]. Here, we model a new approach: BETR. We show that the BETR approach yields promising results in lymphoid and epithelial malignancies in murine xenografts of human tumors. FIAU was previously investigated as an antiviral agent [106]. Chronic dosing of FIAU was associated with hepatic damage and in some cases fatality [107]. In those trials, treatment for less than four weeks with a cumulative dose less than 200 mg was not associated with either clinical or biochemical evidence of toxicity. Thus, the "no-effect dose" of FIAU might be conservatively estimated to be on the order of 0.1 mg/kg per administration. [^{124} I]FIAU has been administered to patients for imaging without any adverse effect on hepatic function [108]. Extrapolating from our xenograft experiments, a radiotherapeutic effect is likely to be achieved at doses of FIAU that are orders of magnitude times less than the no-effect FIAU dose in humans.

BETR therapy offers possible advantages over other lytic induction-suicide prodrug approaches. All lytic induction approaches are limited by the tendency of gammaherpesviruses to latency. When ganciclovir or a similar agent mediates cell killing, a therapeutic effect will likely require that a large fraction of tumor cells express viral enzymes. Although the phenomenon of bystander killing attributed to the exchange of phosphorylated nucleotides and nucleotide analogs between cells *via* gap junctions has been recognized [109], such killing is limited. In the gene therapy setting attempts have been made to engineer increased expression of the connexin protein in the gene therapy vector so as to increase such killing [110]. However, this approach to increasing bystander killing is not readily applicable in approaches that do not involve gene therapy. Indeed, some of the therapeutic agents used to treat tumors may interfere with such bystander killing [111]. [^{131} I]FIAU bystander killing is not limited by connexin expression or gap junctions but is a function of β -emissions with a maximum energy of 0.61 MeV that deposit 90% of their energy in a sphere of radius 0.7 mm [112]. That cross-fire effect is likely an important contributor to the efficacy of radioimmunotherapy in lymphoma [113]. BETR therapy may also offer advantages over radioimmunotherapeutic approaches. Internalization of radioimmunoconjugates may lead to degradation and release of free isotope with consequent loss of specificity. In contrast, we observed that following injection of FIAU, isotope is stably associated with EBV-TK expressing tumor. Enzymatic phosphorylation and incorporation into DNA (as has been reported in HSV1-TK expressing cells) offers the possibility of achieving high concentrations in tumor tissue not limited by concentration gradients. Finally, both FIAU and bortezomib are small molecules and thus better tumor penetration would be anticipated in comparison with antibody conjugates. FIAU does not penetrate the blood-brain barrier but does reach brain tumors reflecting disruption of the blood brain barrier [89]. More broadly, the use of small molecules to activate tissue or tumor-specific metabolic pathways suggests the possibility that metabolic targeting of radiation may have applications beyond thyroid cancer.

PERSPECTIVE

Molecular-genetic imaging generally requires transfection of a reporter gene that provides information about the expression of a gene or signal transduction event of interest. We have exploited the homology of the EBV-TK (and bacterial TKs) to the HSV1-TK in imaging these organisms without the need for a transfection step. As infection is becoming increasingly associated with cancer, this method may provide an indirect readout on the tumors themselves. In the case of EBV and other gammaherpesviruses we can take this a step further by imaging tumors only that are pharmacologically activated. That has important implications in two ways: first, it provides a way to test whether patients will be responsive to bortezomib therapy for virus-associated lymphoma and second, the integrated viral genome provides a tumor specificity-conferring mechanism for enzymatic, molecular radiotherapy (BETR). The former implication suggests that molecular imaging can provide a personalized approach to patient management, relying on the phenotype of the patient's tumor, while the latter therapy should be readily translatable due to absence of the need for introducing foreign DNA (transfection) to the patient. Activation and imaging of endogenous genes may provide a rapid and feasible way for molecular imaging to reach its full clinical potential.

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ABBREVIATIONS

FIAU	(1-(2'-Deoxy-2'-fluoro-1-β-D-arabinofuranosyl)-5-iodouracil)
FMAU	(1-(2'-Deoxy-2'-fluoro-1-β-D-arabinofuranosyl)-5-methyluracil)
FHBG	9-(4-Fluoro-3-(hydroxymethylbutyl)guanine
HSV1-TK	Herpes simplex virus type 1 thymidine kinase
PET	Positron emission tomography
SPECT	Single photon emission computed tomography
FLT	[¹⁸ F]Fluorothymidine
FDG	[¹⁸ F]Fluorodeoxyglucose
BETR	Bortezomib-induced enzyme targeted radionuclide therapy

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Fig. 1. Reporter gene expression imaging with radiolabeled FIAU

Description of herpes simplex virus thymidine kinase (*HSV1-TK*) type genes as an imaging reporter gene with nucleosides as reporter probes. FIAU and other nucleosides are preferentially phosphorylated by *HSV1-TK* type reporter genes rather than by mammalian TKs.

Name	R1	R2	R3
IUDR	I	H	H
IVFRU	CH ₂ =CH ₂ -I	H	F
FIRU	I	H	F
FAU	H	F	H
FMAU	CH ₃	F	H
FEAU	CH ₂ CH ₃	F	H
FFEAU	CH ₂ CH ₂ F	F	H
FFAU	F	F	H
FCAU	Cl	F	H
FBAU	Br	F	H
FIAU	I	F	H

Name	R1	R2	R3
GCV	O	OH	H
PCV	CH ₂	OH	H
FGCV	O	OH	F
FPCV	CH ₂	OH	F
FHPG	O	F	H
FHBG	CH ₂	F	H

Fig. 2. Nucleosides tested as reporter probes for imaging gene expression

IUDR, (1-(2'-deoxy-5-iodo-1-β-D-ribofuranosyl)-uracil); **IVFRU**, (1-(2'-deoxy-2'-fluoro-5-iodovinyl-1-β-D-ribofuranosyl)-uracil); **FIRU**, (1-(2'-deoxy-2'-fluoro-5-iodo-1-β-D-ribofuranosyl)-uracil); **FAU**, (1-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-uracil); **FMAU**, (1-(2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl)-5-methyluracil); **FEAU**, (1-(2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl)-5-ethyluracil); **FFEAU**, (1-(2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl)-5-fluoroethyluracil); **FFAU**, (1-(2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl)-5-fluoroluracil); **FCAU**, (1-(2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl)-5-chlorouracil); **FBAU**, (1-(2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl)-5-bromouracil); **FIAU**, (1-(2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl)-5-iodouracil); **GCV**, 9-[(2-hydroxy-1-hydroxymethyl)ethoxy)methyl]guanine; **PCV**, 9-(4-Hydroxy-3-(hydroxymethyl)butyl)guanine; **FGCV**, 8-fluoro-9-[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl]guanine; **FPCV**, 8-fluoro-9-[4-hydroxy-3-(hydroxymethyl)butyl]guanine; **FHPG**, 9-[(3-fluoro-1-hydroxy-2-propoxy)methyl]guanine; **FHBG**, 9-(4-fluoro-3-(hydroxymethyl)butyl)guanine.

Fig. 3. [^{124}I]FIAU PET of TK(+) and TK(-) bacterial strains

[^{124}I]FIAU PET of a Balb/c mouse with a TK- *S. aureus* abscess (left thigh, SA) and TK+ *E. coli* abscess (right thigh, EC) imaged 3h postinjection of radiopharmaceutical. Two coronal slices are shown, the image in the left panel approximately 3 mm superior to that on the right. PET images were acquired using a GE Vista eXplore small animal PET instrument. **T**: thyroid; **GB**: gallbladder; **S**: stomach; **D**: duodenum; **B**: urinary bladder; **EC**: *E. coli*; **SA**: *S. aureus*.

Fig. 4. EBV suppression in Rael cells

EBV(+) Rael cells were transfected with a construct carrying the IκB super-repressor [IκB (sr)] and a control vector. After 48 h, viral load was measured by real-time PCR.

Fig. 5. Imaging EBV-TK(+) tumors *in vivo*

Two animals at different time points are depicted, one in (A) and (B) and one in (C) and (D). (A) and (B), imaging in tumors (osteosarcoma 143b) engineered to express constitutively the EBV-TK (TK143b). (C) and (D), osteosarcoma 143b tumors that were sham engineered with an empty vector (V143b). Two tumors are present in each animal (large arrows). The dark area [(A), small arrow] represents lead shielding of bladder to improve the dynamic range of the images. Animal in (C) and (D) was pretreated with bortezomib (2 $\mu\text{g/g}$) to determine whether the agent led to up-regulation of a cellular kinase that might account for FIAU phosphorylation.

Fig. 6. Bortezomib induces EBV-TK and Zta expression

Immunoblot showing EBV-TK (**A**) and Zta (**B**) expression following treatment of an EBV(+) Burkitt's cell line (Rael) with bortezomib. Total cellular protein was isolated and 10 μ g of protein per lane were separated by 12% SDS-PAGE. AGS-HC13 cells expressing Zta promoter were treated with bortezomib, and luciferase activity was measured (**C**). Accumulation of [^{14} C]FIAU in EBV(+) Burkitt's [EBV(+) Akata, EBV(+) Rael] but not EBV(-) Burkitt's [EBV(-) Akata] following treatment with bortezomib (**D**). BL, Burkitt's lymphoma.

Fig. 7. Time course of uptake of [^{125} I]FIAU by Burkitt's lymphoma xenografts [EBV(+) Akata] following treatment with bortezomib as assessed by planar gamma scintigraphy *in vivo*. Large arrows, tumors. The dark area (A, small arrow) represents lead shielding of the bladder to improve the dynamic range of the images. Each animal has one tumor placed in the hind limb. A, no tumor uptake is evident in animals pretreated with PBS only (control). B, tumors are visualized at later time points in the pretreated animals (2 μ g/g bortezomib).

Fig. 8. [^{125}I]FIAU SPECT-CT imaging of tumors following bortezomib treatment (A) EBV(+) gastric carcinoma (KT) tumor at 72 h postinjection (p.i.). (B) KSHV(+) lymphoma (BCBL1) at 48 h p.i. Arrows indicate tumor location. Side bars indicate the range of [^{125}I]FIAU uptake as percentage injected dose per gram of tissue (ID/g) [0.68% in (A) and 1.53% in (B)].

Fig. 9. [^{125}I]FIAU tissue distribution in a murine xenograft model

[^{125}I]FIAU (5 μCi) was administered intravenously to SCID mice engrafted with EBV-TK(+) tumors. Animals (3–4 at each time point) were sacrificed and tissue distribution was measured. The percent of the injected dose per gram tissue (%ID/g) is shown in the various organs. Note that after the initial time point, [^{125}I]FIAU remains sequestered solely within the tumor suggesting significant exposure of tumor to β -particle emission with [^{131}I]FIAU.

Fig. 10. Tumor growth curves in murine xenografts

(a) Mice with control tumors (human osteosarcoma 143B cells with empty vector) or TK expressing tumors (human osteosarcoma 143B cells expressing EBV-TK) were treated intravenously with 1.6 mCi [¹³¹I]FIAU or buffered saline. (b) Dose response. Mice with EBV-TK expressing tumors were treated with buffered saline, 1 mCi [¹³¹I]FIAU or 3 mCi [¹³¹I]FIAU. (c) Mice with tumors resulting from engraftment of admixtures of EBV-TK expressing and control tumor cells were treated with 1.7 mCi [¹³¹I]FIAU. Each time point corresponds to 3 animals. The mean, SEM, and least squares linear regression are plotted. The confidence intervals (CI, 95%) of the slopes of the best fit linear regression of tumor growth curves are shown in parentheses in the legend.

Fig. 11. Tumor growth curves in murine xenografts: comparison to epithelial tumors (a) EBV(+) Burkitt's lymphoma (Rael), (b) EBV(+) gastric adenocarcinoma (KT), and (c) KSHV(+) primary effusion lymphoma (BCBL1) were injected intravenously with buffered saline, buffered saline followed 24 hours later by [¹³¹I]FIAU, bortezomib alone, or bortezomib followed 24 hours later by [¹³¹I]FIAU. Each time point corresponds to 3 animals. Mice were treated with 1.6 mCi (a,c) or 1.7 mCi (b). The mean, SEM, and least squares linear regression are plotted. The confidence intervals (CI, 95%) of the slopes of best fit linear regression of tumor growth curves are shown in parentheses in the legend.

Table 1**Major Human Virus-Associated Cancers**

Virus	Malignancy
Epstein-Barr virus (EBV)	Lymphoma, nasopharyngeal carcinoma, gastric carcinoma
Hepatitis B virus (HBV)	Hepatocellular carcinoma
Hepatitis C virus (HCV)	Hepatocellular carcinoma
Human papilloma virus (HPV)	Cancer of the cervix, head and neck
Human T lymphotropic virus type-1 (HTLV-1)	Adult T-cell leukemia
Human T lymphotropic virus type-2 (HTLV-2)	Hairy-cell leukemia
Human herpes virus 8 (HHV-8)	Kaposi sarcoma and lymphoma
Human immunodeficiency virus (HIV)	Kaposi sarcoma and lymphoma

Table 2 Tissue Distribution [^{125} I]FIAU in SCID Mice Bearing Human EBV Tumor

Tissue	%ID/g $\pm$ SD (n=3, 96 hr) PBS Treatment	Bortezomib Treatment
Tumor	0.038 $\pm$ 0.010	0.850 $\pm$ 0.102
Liver	0.029 $\pm$ 0.011	0.016 $\pm$ 0.002
Spleen	0.014 $\pm$ 0.006	0.060 $\pm$ 0.011
Kidney	0.068 $\pm$ 0.014	0.070 $\pm$ 0.010
Muscle	0.030 $\pm$ 0.010	0.040 $\pm$ 0.005
Blood	0.033 $\pm$ 0.009	0.022 $\pm$ 0.010

Table 3

Current FDA-Approved Antibodies for Parenteral Use in the Detection and Treatment of Cancer. Adopted from [92]

Generic Name	Trade Name	Agent/Target	Cancer Indication	Approval
Unconjugated				
Rituximab	Rituxan	Chimeric anti-CD20 IgG1	B-cell lymphoma	1997
Trastuzumab	Herceptin	Humanized anti-HER2 IgG1	Breast	1998
Alemtuzumab	CamPath	Humanized anti-CD52	Chronic lymphocytic leukemia	2001
Cetuximab	Erbix	Chimeric anti-EGFR	Colorectal Head/neck	2004 2006
Bevacizumab Radioconjugates	Avastin	Chimeric anti-VEGF	Colorectal	2004
Satumomab pendetide	OncoScint	[¹¹¹ In]Murine anti-TAG-72 IgG	Colorectal, ovarian	1992
Nofetumomab merpentan	Verluma	[^{99m} Tc]Murine anti-EGP-1 Fab'	Small cell lung	1996
Arcitumomab	CEA-Scan	[^{99m} Tc]Murine anti-CEA Fab'	Colorectal	1996
Capromab pendetide	ProstaScint	[¹¹¹ In]Murine anti-PSMA	Prostate	1996
Ibritumomab tiuxetan	Zevalin	[⁹⁰ Y]Murine anti-CD20 IgG+rituximab	B-cell lymphoma	2002
Tositumomab "anti-B1"	Bexxar	[¹³¹ I]Murine anti-CD20 IgG+unlabeled tositumomab	B-cell lymphoma	2003
Drug Conjugates				
Gemtuzumab ozogamicin	Mylotarg	Humanized anti-CD33 IgG4 conjugated to colicheamicin	Acute myelogenous leukemia	2000

Table 4 Tissue Distribution of [^{125} I]FIAU (5 mCi) in Mice Bearing Human Tumors

Tumor	TK143b Osteosarcoma	Rael Burkitt's Lymphoma	KT Gastric Cancer	BC3 Lymphoma	BCBL1 Lymphoma
Days postinjection	4	4	4	5	4
N	3	4	3	3	5
Virus	EBV TK	EBV			KSHV
TK expression	<-constitutive->	<-----bortezomib induction----->			
Tissue	%ID/g±SD				
Tumor	1.667±0.681	1.200±0.356	0.598±0.472	1.213±0.214	1.100±0.361
Liver	0.013±0.006	0.052±0.027	0.050±0.017	0.009±0.002	0.0617±0.022
Spleen	0.027±0.015	0.064±0.011	0.080±0.035	0.008±0.002	0.064±0.014
Kidney	0.033±0.058	0.146±0.054	0.090±0.030	0.027±0.003	0.167±0.038
Muscle	0.015±0.014	0.084±0.012	0.080±0.062	0.008±0.004	0.078±0.005