Highly Specific PET Imaging of Prostate Tumors in Mice with an Iodine-124-Labeled Antibody Fragment That Targets Phosphatidylserine

Jason H. Stafford*, Guiyang Hao†, Anne M. Best1, Xiankai Sun2, Philip E. Thorpe††

1 Department of Pharmacology, The Simmons Cancer Center, University of Texas Southwestern Medical Center, Dallas, Texas, United States of America, 2 Department of Radiology, The Advanced Imaging Research Center, University of Texas Southwestern Medical Center, Dallas, Texas, United States of America

Abstract

Phosphatidylserine (PS) is an attractive target for imaging agents that identify tumors and assess their response to therapy. PS is absent from the surface of most cell types, but becomes exposed on tumor cells and tumor vasculature in response to oxidative stresses in the tumor microenvironment and increases in response to therapy. To image exposed PS, we used a fully human PS-targeting antibody fragment, PGN635 F(ab')2, that binds to complexes of PS and β2-glycoprotein I. PGN635 F(ab')2 was labeled with the positron-emitting isotope iodine-124 (124I) and the resulting probe was injected into nude mice bearing subcutaneous or orthotopic human PC3 prostate tumors. Biodistribution studies showed that 124I-PGN635 F(ab')2 localized with remarkable specificity to the tumors with little uptake in other organs, including the liver and kidneys. Clear delineation of the tumors was achieved by PET 48 hours after injection. Radiation of the tumors with 15 Gy or systemic treatment of the mice with 10 mg/kg docetaxel increased localization in the tumors. Tumor-to-normal (T/N) ratios were inversely correlated with tumor growth measured over 28 days. These data indicate that 124I-PGN635 F(ab')2 is a promising new imaging agent for predicting tumor response to therapy.

Introduction

Phosphatidylserine (PS) is an attractive target for imaging agents that can be used for disease diagnosis, staging and therapeutic planning. PS is a phospholipid that is generally not found on the surface of normal cells because lipid-specific transporters sequester it in the inner leaflet of the cell's plasma membrane [1,2]. When cells undergo apoptosis, as do tumor cells responding to chemotherapy, PS becomes exposed on their outer membrane surface through one or more calcium-dependent mechanisms [3,4]. PS exposure is also induced on the viable vascular endothelium in tumors by oxidative stresses within the tumor microenvironment [5-7] and increased PS exposure levels on the endothelium are consistently seen in tumors responding to therapy [8-11]. Since PS exposure on tumor endothelium and tumor cells correlates with tumor growth inhibition [8,9,12], it provides an excellent marker for predicting tumor response to therapy.

Several PS-targeting strategies have been employed to image tumors and determine their response to therapy. The PS binding protein, annexin V, has been radiolabeled with various positron emitters for positron emission tomography (PET) of tumors in several animal models [13-15]. Technetium-99m (99mTc)-labeled annexin V has been used for single photon emission computed tomography (SPECT) in humans and has shown prognostic value for head and neck cancer, late stage lung cancer and lymphoma [16,17]. Others have used the C2A domain of radiolabeled synaptotagmin I for PET and SPECT imaging of lung carcinomas in animals treated with paclitaxel [18,19]. Low molecular weight PS imaging probes, such as dipicolylamine-Zn2+ complexes [20], are also in development. While these probes have demonstrated diagnostic value, they...
Tumor Imaging with 124I-PGN635 F(ab')2

Materials and Methods

Radiochemistry

Iodine-124 (124I) was purchased from IBA Molecular, Inc. (Richmond, VA) and Iodine-125 (125I) was purchased from Perkin Elmer (Waltham, MA). Iodination tubes and Protein A agarose were from Pierce Biotechnology (Rockford, IL). Instant thin-layer chromatography plates (ITLC-SG) were from Pall Life Sciences (East Hills, NY). Horse-radish peroxidase (HRP) conjugated streptavidin was purchased from Jackson Immunoresearch Labs (West Grove, PA). Bio-Spin 6 gel filtration columns were purchased from Bio-Rad Laboratories (Hercules, CA). Recombinant human β2GP1 was obtained from Peregrine Pharmaceuticals, Inc. (Tustin, CA). 96-well Immunol 1B microtiter plates were purchased from Thermo LabSystems (Franklin, MA). L-α-phosphatidylserine (PS) was purchased from Avanti Polar Lipids (Alabaster, AL). Furosemide was purchased from Sigma-Aldrich (St. Louis, MO). Docetaxel was obtained from the UT Southwestern pharmacy (Dallas, TX).

The fully human PS-targeting antibody, PGN635, was generated by Affitech A.S. (Oslo, Norway) in collaboration with Peregrine Pharmaceuticals, Inc. (Tustin, CA). For in vitro assays, PGN635 was mixed with an equal weight of human β2GP1 to enable binding to PS. Aurexis is a human monoclonal antibody that binds an irrelevant antigen (S. aureus clumping factor A) and was used as a negative control. PGN635 and Aurexis were produced under Good Manufacturing Practice (GMP) conditions. Goat anti-β2GP1 polyclonal antibody was purchased from Pierce Biotechnology (Rockford, IL). Horseradish peroxidase (HRP) conjugated donkey anti-goat IgG secondary antibody was purchased from Jackson Immunoresearch Labs (West Grove, PA).

Optimal images were obtained 72 h after injection, when concentrations of the probe in the blood had fallen to levels that did not obscure signal from the tumor [22].

Here we report the ability of 124I-labeled PGN635 F(ab')2 to image prostate tumors growing in mice by PET. 124I-PGN635 F(ab')2 produced clear PET images of subcutaneous and orthotopic prostate tumors in mice. Treatment with chemotherapy or radiotherapy increased tumor uptake of 124I-PGN635 F(ab')2, and the tumor to normal tissue (T/N) ratios correlated with the subsequent tumor growth inhibition. These data suggest that 124I-PGN635 F(ab')2 could be used as a diagnostic tumor imaging agent and for predicting tumor response to therapy.
purchased from HyClone (Thermo Scientific, Logan, UT) and was supplemented with 10% FBS and 2 mM L-glutamine.

**Tumor Models**

For subcutaneous (s.c.) tumors, 2 x 10^6 PC3-luc cells in matrigel/PBS (1:1) were injected into the upper right flank of male athymic nu/nu mice (Charles River, Frederick, MD). Tumor growth was monitored by measuring two perpendicular diameters and calculating tumor volume using the formula \( \pi/6 \times D \times d^2 \) where D is the larger diameter and d is the smaller diameter.

For orthotopic tumors, mice were anesthetized and a lower midline abdominal incision was made to expose the prostate capsule. The prostate capsule was opened and 10^6 PC3-luc cells in 50 µl matrigel/PBS (1:1) were injected into the dorsal prostate. The internal membrane was then sutured and the skin was clipped to close the incision. Tumor growth was monitored by bioluminescence imaging (BLI) with an IVIS Lumina imaging system (Xenogen, Alameda, CA).

This study was approved by the Institutional Animal Care and Use Committee at UT Southwestern Medical Center (protocol#: 2009-0152). The experiments were carried out in strict accordance with NIH guidelines including making all efforts to minimize animal suffering.

**In Vivo Biodistribution Studies**

Mice bearing s.c. or orthotopic PC3-luc tumors were injected i.v. with 50 µg/1.85 MBq of \(^{124}\)I-PGN635 F(ab')2, or \(^{124}\)I-Aurexis F(ab')2 (negative control) for 1 h. The cells were then washed with PBS and dissolved with 1N NaOH (30 min, RT). Activity from cell digests was measured using a γ-counter (Perkin Elmer, Waltham, MA).

Mice bearing s.c. PC3-luc tumors were injected i.v. with 50 µg/1.85 MBq of \(^{125}\)I-PGN635 F(ab')2. Animals were sacrificed 24 or 48 h after injection and tumors, blood, and other organs of interest were collected, weighed, and their radioactivity measured. \(^{125}\)I-PGN635 F(ab')2 uptake in each organ was expressed as the percentage injected dose per gram tissue (% ID/g) and percentage injected dose per organ (% ID/organ) (n = 3).

**PET Imaging of Tumors with \(^{124}\)I-PGN635 F(ab')2**

Mice bearing s.c. or orthotopic PC3-luc tumors were selected for imaging with a Siemens Inveon PET-CT Multimodality System (Siemens Medical Solutions Inc., Knoxville, TN). Thyroid uptake of \(^{124}\)I was blocked by adding 10 drops saturated KI per 100 ml of drinking water 24 h before injection of \(^{124}\)I-PGN635 F(ab')2. Stomach uptake was blocked by gastric lavage with 1.5 ml potassium perchlorate in 200 µl PBS 30 min before injection. The mice were injected into the tail vein with 50 µg/1.85 MBq of \(^{124}\)I-PGN635 F(ab')2 in 150 µl PBS. The mice were injected i.p. with 10 mg/kg furosemide and given 200 µl water by gastric lavage 1 h before imaging to clear residual activity from the bladder. For imaging, the mice were anesthetized with 3% isoflurane until stable vital signs were established and then placed on the imaging bed under 2% isoflurane for the duration of the procedure. CT images were acquired at 80 kV and 500 µA. CT images were reconstructed using COBRA Reconstruction Software. PET imaging was performed directly after acquisition of CT data, using the standard energy window of 350-650 keV. The scan time for PET images was between 10 and 20 min. The data were reconstructed using and the Fourier Rebinning and Ordered Subsets Expectation Maximization 3D (OSEM3D) algorithm provided by the Siemens Inveon Research Workplace (IRW) software. Reconstructed CT and PET images were also superimposed and analyzed using the IRW software. For quantification, tumor-margins were determined by CT morphology and regions of interest (ROIs) were defined manually.

**Assessing Tumor Response to Therapy with \(^{124}\)I-PGN635 F(ab')2**

Treatment (n=3) was initiated in mice bearing subcutaneous PC3-luc tumors when the tumor volumes reached 0.3-0.7 cm³. Mice treated with chemotherapy (CTx) were injected i.p. with docetaxel (10 mg/kg). Mice treated with x-irradiation (xRT) received a single dose of 15 Gy to their tumors delivered with an XRAD 320 biological irradiator (Precision X-Ray, North Branford, CT). PET imaging of tumors with \(^{124}\)I-PGN635 F(ab')2 was performed 24 h later. Tumor/normal (T/N) ratios were calculated as the % ID/g tumor/% ID/g muscle (left forelimb) at 48 h after injection of \(^{124}\)I-PGN635 F(ab')2. Tumor growth was monitored for 28 days after imaging. The tumor volume immediately before treatment (Vol0) and 28 days after treatment (Vol28) were recorded for each individual animal. The tumor growth index for each animal was calculated as Vol28/ Vol0. The correlation coefficient (Pearson’s r) between the T/N ratio and the tumor growth index was calculated using log values.
Results

\textbf{\textsuperscript{124}I-PGN635 F(ab')\textsubscript{2} Binding and Stability}

PGN635 F(ab')\textsubscript{2} was radioiodinated with labeling efficiencies ranging between 14.1% and 19.5%. Radioiodination did not affect the ability of the antibody fragment to bind PS immobilized on plastic. Figure \textsuperscript{1}A shows that a 10-fold excess of \textsuperscript{124}I-PGN635 F(ab')\textsubscript{2} or unlabeled F(ab')\textsubscript{2} inhibited the binding of PGN635-biotin to PS by 55.5% and 57.3%, respectively. Radioiodination also did not affect the ability of PGN635 F(ab')\textsubscript{2} to bind PS exposed on the surface of irradiated endothelial cells or irradiated prostate tumor cells (Figure \textsuperscript{1}B).

To determine the stability of \textsuperscript{124}I-PGN635 F(ab')\textsubscript{2} \textit{in vivo}, serum collected from mice injected with \textsuperscript{124}I-PGN635 F(ab')\textsubscript{2} was analyzed by HPLC size exclusion chromatography. There was no evidence of lower molecular weight \textsuperscript{124}I-labeled degradation products or free \textsuperscript{124}I in the circulation 48 h after injection of the probe (Figure \textsuperscript{S1}A). The majority of the \textsuperscript{124}I-PGN635 F(ab')\textsubscript{2} eluted earlier than the control \textsuperscript{124}I-PGN635 F(ab')\textsubscript{2}, suggesting that the antibody had bound to a plasma protein. To determine if the increased molecular weight of the \textsuperscript{124}I-PGN635 F(ab')\textsubscript{2} was due to binding to mouse β2GP1 \textit{in vivo}, mice were injected with full-length PGN635 and 24 h later the antibody was recovered from serum with protein A agarose. Western blotting with antibodies to β2GP1 revealed that the PGN635 co-purified with mouse β2GP1 (Figure \textsuperscript{S1}B). Mouse β2GP1 is more highly glycosylated than human β2GP1 \cite{29} and therefore, migrated slightly slower during gel electrophoresis.

\textbf{Biodistribution of \textsuperscript{124}I-PGN635 F(ab')\textsubscript{2}}

Biodistribution studies were conducted in male nu/nu athymic mice bearing subcutaneous PC3 tumors to evaluate uptake of \textsuperscript{124}I-PGN635 F(ab')\textsubscript{2} in specific tissues. 24 h after i.v. administration of \textsuperscript{124}I-PGN635 F(ab')\textsubscript{2}, activity in the blood was 6.7% ID/g (10.9% ID/organ) whereas uptake in all other tissues, including tumor, was <3% ID/g (<2% ID/organ) (Figure \textsuperscript{S2}). \textsuperscript{125}I-Aurexis F(ab')\textsubscript{2} (control) displayed low uptake in all tissues with an activity in the blood of only 0.2% ID/g (0.4% ID/organ) after 24 h. After 48 h, \textsuperscript{125}I-PGN635 F(ab')\textsubscript{2} was predominantly localized to the tumor (1.2% ID/g) and blood (1.4% ID/g) (Figure \textsuperscript{2}). The tumor:blood ratio was 0.9:1 whereas the tumor:liver ratio was 3.3:1.

\textbf{MicroPET Imaging of PC3-luc Tumors with \textsuperscript{124}I-PGN635 F(ab')\textsubscript{2}}

Mice bearing subcutaneous PC3-luc tumors growing in their right flank were injected i.v. with \textsuperscript{124}I-PGN635 F(ab')\textsubscript{2} and imaged at 24 and 48 h. Tumor-bearing mice injected i.v. with \textsuperscript{124}I-Aurexis F(ab')\textsubscript{2} were used as negative controls. After 24 h, the high background signal from normal tissues did not allow for specific imaging of the tumor. However, 48 h after injection of \textsuperscript{124}I-PGN635 F(ab')\textsubscript{2} uptake in the tumor was significantly higher than background allowing clear delineation of the tumor (Figure \textsuperscript{3}, Figure \textsuperscript{S3}). Average tumor uptake of \textsuperscript{124}I-PGN635 F(ab')\textsubscript{2} determined by PET signal quantitation was 1.2% ID/g. Whereas average uptake of the probe in the heart, liver and muscle was 0.9, 0.7, and 0.2 %ID/g respectively. \textsuperscript{124}I-Aurexis F(ab')\textsubscript{2} (control) did not show significant tumor uptake (0.03 %ID/g).

\textsuperscript{124}I-PGN635 F(ab')\textsubscript{2} also imaged orthotopically-implanted PC3-luc tumors (Figure \textsuperscript{4}, Figure \textsuperscript{S4}). Average uptake of \textsuperscript{124}I-PGN635 F(ab')\textsubscript{2} in the prostate tumors 48 h after injection was 1.6% ID/g. Localization of the probe to the prostate was confirmed by coincidental bioluminescence imaging. Tumor size and localization of \textsuperscript{124}I-PGN635 F(ab')\textsubscript{2} correlated to the size and location of the BLI signal. \textsuperscript{124}I-Aurexis F(ab')\textsubscript{2} (control) did not localize to orthotopic tumors (0.08 %ID/g).
Figure 2. \( ^{124}\text{I}-\text{PGN635 F(ab')2} \) biodistribution. Mice (n =3) bearing s.c. PC3-luc tumors were injected with 1.85 MBq (50 \( \mu \text{g} \)) of \( ^{125}\text{I}-\text{PGN635 F(ab')2} \) or \( ^{125}\text{I}-\text{control F(ab')2} \). Antibody distribution to the indicated organs was determined after 48 h by counting the radioactivity with a gamma counter. A) Biodistribution by percent injected dose per gram (%ID/g) of tissue. B) Biodistribution by percent injected dose per organ (%ID/organ). The % ID in the blood was calculated assuming a blood volume of 2.18ml/25 g body weight.

doi: 10.1371/journal.pone.0084864.g002

Assessing Tumor Response to Therapy with \( ^{124}\text{I}-\text{PGN635 F(ab')2} \)

Since chemo- and radiotherapy increase the exposure of PS on apoptotic tumor cells [30,31] and the tumor vasculature [8-10], we determined whether therapy would increase the localization of \( ^{124}\text{I}-\text{PGN635 F(ab')2} \). Mice bearing PC3-luc tumors were treated with 10 mg/kg docetaxel or with 15 Gy X-irradiation and were injected with \( ^{124}\text{I}-\text{PGN635 F(ab')2} \) 24 h later. Forty-eight hours later, the mice were imaged by PET. Figure 5A shows that while the tumors in all groups were similar sizes at the time of imaging, both treatments increased tumor localization of \( ^{124}\text{I}-\text{PGN635 F(ab')2} \). In untreated control mice the average tumor to normal tissue (muscle) ratio (T/N) was 2.1 (Figure 5C). Chemotherapy (CTx) increased the T/N ratios to an average of 3.9, while radiotherapy (RTx) increased the T/N ratios to an average of 6.7 (Figure 5C). Figure 5D shows that the T/N ratios were inversely correlated with the subsequent tumor growth measured over 28 days (Pearson’s \( r = -0.85; P<0.01 \)). Untreated tumors increased in volume by an average of 11.5-fold whereas the volume of tumors treated with CTx increased on average by only 2.4-fold (Figure 5B, Figure 5S). RTx was more effective at inhibiting tumor growth than CTx with the volume of irradiated tumors decreasing on average by 10% (Figure 5B, Figure 5S). These data indicate that imaging with \( ^{124}\text{I}-\text{PGN635 F(ab')2} \) may be useful for predicting tumor response to therapy.

Discussion

The present study demonstrates that the \( ^{124}\text{I}-\text{labeled F(ab')2} \) fragment of the fully human PS-binding antibody PGN635 provides clear PET images of subcutaneously and orthotopically-implanted PC3 prostate tumors in mice. Tumor localization also correlated with inhibition of tumor growth in animals treated with docetaxel or irradiation suggesting that \( ^{124}\text{I}-\text{PGN635 F(ab')2} \) could be useful for predicting tumor response to therapy in patients. In addition to its in vivo activity, the radioiodinated antibody fragment fully retained its structural integrity in vivo and its ability to bind to immobilized PS and to PS-expressing irradiated cells in vitro.

We previously labeled the full-length chimeric IgG bavituximab with 74As and obtained clear images of solid tumors in rats by PET [22]. Recently, Ogasawara et al. demonstrated that full-length PGN635 labeled with 99Zr could image apoptotic tumors in mice [32]. In the present study, the F(ab')2 fragment of PGN635 was used instead of full length IgG because its faster blood clearance should yield greater T/N ratios at earlier time points [33,34]. Preliminary imaging of tumor-bearing mice with F(ab')2 fragments labeled with 64Cu showed high PET signals from the liver (16.3% ID/g) and kidneys (23.5% ID/g) (unpublished data). We hypothesized that high uptake in these organs was due to transchelation of 64Cu from the 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) chelator required for radiolabeling to copper-binding proteins such as Cu\(^{2+}\)/Zn\(^{2+}\) super oxide dismutase (SOD1)[35]. Therefore, we chose to use iodine-124 as our reporter nuclide because it allowed us to directly label the PGN635 F(ab')2 without the use of a chelator/linker molecule.

\( ^{124}\text{I}-\text{PGN635 F(ab')2} \) did indeed allow for more rapid imaging of tumors than 74As-bavituximab [22], but the blood half-life was longer than is typical of a F(ab')2 fragment. We have previously reported that PGN635 F(ab')2, labeled with a near-infrared dye (800CW-PGN635 F(ab')2) was cleared from blood with a half-life of approximately 6 hours [36]. \( ^{124}\text{I}-\text{PGN635 F(ab')2} \) was observed to have a similar half-life with 6.5% ID/g of remaining in the blood at 24 h, as compared to 0.2% ID/g for the control Aurexis F(ab')2. The longer than expected blood half-life of \( ^{124}\text{I}-\text{PGN635 F(ab')2} \) is most likely due to the generation of 210 kDa F(ab')2/\( \beta2\text{GP1} \)complexes that are cleared at a rate that is
significantly slower than that of the 110 kDa Aurexis F(ab')2 control. For this reason, optimal imaging with 124I-PGN635 F(ab')2 was not obtained until approximately 48 h after injection when blood levels of the isotope had fallen to levels where they did not obscure antibody localization in the tumor.

The 48 hour lag time between 124I-PGN635 F(ab')2 injection and tumor imaging along with the higher radiation burden associated with 124I decay may be identified as significant drawbacks when compared to fludeoxyglucose (18F-FDG), the current standard for tumor imaging by PET. 18F-FDG allows imaging within 2 hours of injection, rapidly decays ($t_{1/2} = 110$ min), and is excreted within 24 hours [37]. However, the biodistribution of 124I-PGN635 F(ab')2 may be preferable to that of 18F-FDG for imaging some malignancies. 18F-FDG labels tumors because they take up and metabolize high amounts of glucose, but normal tissues also metabolize glucose. Thus, 18F-FDG imaging can suffer from relatively high background throughout the body and in particular, the brain and kidneys [38]. Furthermore, 18F-FDG is not effective for diagnosing prostate cancer since well-differentiated, androgen-dependent prostate carcinomas do not metabolize high amounts of glucose [39]. By comparison, the clarity of prostate tumor imaging with 124I-PGN635 F(ab')2 was remarkable. At 48 h after injection there was no significant signal from normal tissue, with the exception of the heart because of the relatively large pooling of blood.

PET quantification with 124I can be difficult due its high energy positrons and complex decay scheme that results in single photon emission in the same energy window as the annihilation photons used for image reconstruction [40]. Various methods of data correction have been implemented to address these problems [41], but the background signal from 124I-PGN635 F(ab')2 in normal tissues was low enough to allow us to use standard PET quantification protocols. Importantly, we observed no significant difference between the biodistribution of 124I-PGN635 F(ab')2 and quantification of 124I-PGN635 F(ab')2 PET in PC3-luc tumors at 48 h after injection.

In neither the present, nor the earlier study of 800CW-PGN635 F(ab')2 [36], was there accumulation of antibody in the liver and kidneys. Uptake in these organs has been problematic for imaging with 99Tc-labeled annexin V and the C2 domain of synaptotagmin [18,42]. Different linkers such as hydrazinonicotinamide (HYNIC) and mercaptoacetyltriglycine (MAG3) have been used to limit transchelation of 99Tc, but the resulting annexin-based probes exhibited only a modest improvement in biodistribution [43,44]. Localization of annexin V and synaptotagmin to the liver and kidneys is not precisely understood, but is probably due, at least in part, to nonspecific uptake systems in these organs that capture and metabolize low molecular weight proteins [45,46]. It is also possible that annexin V binds molecules other than PS in these organs as it has been shown to bind anionic polysaccharides such as heparan sulfate [47]. Finally, 124I-PGN635 F(ab')2 PET did not

Figure 3. PET imaging of subcutaneous PC3 tumors with 124I-PGN635 F(ab')2. Mice bearing s.c. PC3-luc tumors were injected with 124I-PGN635 F(ab')2 or 124I-control F(ab')2. The animals were imaged by PET/CT 48 h later. Reconstructed PET and CT images were fused and analyzed. The images clearly show preferential labeling of the tumor by PET (arrow). Signal from the heart is due to 124I-PGN635 F(ab')2 in the blood. 124I-control F(ab')2 did not label tumors and was rapidly cleared from the circulation. Representative mice from groups of 4 mice are shown.

doi: 10.1371/journal.pone.0084864.g003
label the skeletons of the mice compared to 89Zr-PGN635 which releases a small amount of free 89Zr that reacts non-specifically with mineral bone [32].

The data showing a relationship between the level of tumor localization and therapeutic efficacy suggests that an important clinical use for 124I-PGN635 F(ab')2 may be assessing tumor response to therapy. PS is constitutively expressed at low levels on several types of viable tumor cells [48], but large increases in PS exposure occur in response to therapy as a result of apoptotic and necrotic changes [49]. Prior work supports this possible clinical application. We previously demonstrated that PGN635 binds specifically to PS exposed on tumor cells and tumor endothelial cells and that binding is increased in tumors treated with radiotherapy [36]. Gong et al. showed that tumor localization of 800CW-PGN635 F(ab')2 in mice was increased 4-fold 24 h after administration of docetaxel (unpublished data). Osagawara et al., showed that non-small cell lung cancer tumors exhibited more than a 6-fold increase in uptake of 89Zr-PGN635 in mice treated with an agonistic death receptor antibody that activates extrinsic apoptosis and a 2.5-fold increase after administration of paclitaxel that induces intrinsic apoptosis [32]. SPECT imaging studies with 99mTc-labeled annexin V have demonstrated a correlation between apoptosis imaging and tumor response in cancer patients as early as 24 h after treatment initiation [17,50]. PET imaging studies with 18F-labeled annexin V have also shown a correlation between imaging and tumor response in mice [15]. Consistent with these prior reports, we found that prostate tumors in mice treated with chemotherapy or radiotherapy showed a 2.5 to 5-fold higher uptake of 124I-PGN635 F(ab')2 relative to untreated tumor-bearing control mice.

Although the T/N ratios in treated and untreated tumors were predictive of the subsequent tumor response, we found that total tumor uptake (%ID/g) of 124I-PGN635 F(ab')2 was less than that reported for 89Zr-PGN635 [32]. The difference between the two probes may be attributed to the FcRn receptors on endothelium maintaining serum levels of the full-length IgG construct and allowing it more time to bind the wave of PS exposure induced by treatment. Alternatively, the difference may be due to the different tumor models and induction of apoptosis by the extrinsic versus intrinsic pathways.

Conclusion

Our findings indicate that 124I-PGN635 F(ab')2 is a highly specific tumor imaging agent. Images obtained with this probe are substantially clearer than other PS-targeting probes because of very low uptake in normal tissues. The 48 h lag time between probe injection and tumor imaging may complicate clinical translation, but the data suggest 124I-PGN635 F(ab')2 could still be a valuable tool for predicting how a tumor will respond to therapy. Ideally, the patient’s tumor would be imaged before initiation of therapy and again early in the course of treatment. Increases in tumor signal would be indicative of effective therapy. Conversely, lack of increase in tumor signal would indicate a change of treatment strategy could be required. Since exposed PS appears to be a universal marker of tumor vasculature and is universally exposed on tumors responding to therapy, 124I-PGN635 F(ab')2 could have broad application for tumor detection and prediction of response to treatment in cancer patients.
Figure 5. $^{124}$I-PGN635 F(ab')$_2$ localization predicts tumor response to therapy. A) Mice bearing PC3-luc tumors were treated with 10 mg/kg docetaxel (CTx) or their tumors were irradiated with 15 Gy (RTx). After 24 h, the mice were injected with $^{124}$I-PGN635 F(ab')$_2$ and imaged by PET 48 h later. B) CTx and RTx significantly inhibited tumor growth (one-way ANOVA, P < 0.01). C) $^{124}$I-PGN635 F(ab')$_2$ uptake was significantly higher in treated tumors (one-way ANOVA, P < 0.05). D) The tumor-to-normal (T/N) ratio inversely correlated with the tumor growth over the next 28 days (Pearson’s r = -0.85, P < 0.01). The tumor volume on day 28 was divided by the volume on the day of treatment to calculate the fold change in tumor volume. The T/N ratio at the time of imaging was predictive of the tumor response.

doi: 10.1371/journal.pone.0084864.g005
Supporting Information

Figure S1. 124I-PGN635 F(ab’)2 is stable in vivo and binds serum β2GP1. A) HPLC analysis of serum from mice injected i.v. with 124I-PGN635 F(ab’)2 48 h earlier. HPLC analysis showed an increase in the molecular weight of 124I-PGN635 F(ab’)2 with no evidence of lower molecular weight proteolytic fragments. B) Western blot analysis for β2GP1. PGN635 complexes were retrieved with protein A agarose from mouse serum 24 h after i.v. injection, and were probed with antibodies to β2GP1. The data show that PGN635 bound to circulating mouse β2GP1. Control antibody (Aurexis) collected from mouse serum 24 h after injection did not bind β2GP1. (TIF)

Figure S2. 125I-PGN635 F(ab’)2 biodistribution at 24h. Mice (n=3) bearing s.c. PC3-luc tumors were injected with 1.85 MBq (50 µg) of 125I-PGN635 F(ab’)2 or 125I-control F(ab’)2. Antibody distribution to the indicated organs was determined after 24 h by counting the radioactivity with a gamma counter. A) Biodistribution by percent injected dose per gram (%ID/g) of tissue. B) Biodistribution by percent injected dose per organ (%ID/organ). The % ID in the blood was calculated assuming a blood volume of 2.18 ml/25 g body weight. (TIF)

Figure S3. Transverse images of subcutaneous PC3 tumors imaged with 124I-PGN635 F(ab’)2. Transverse images also clearly show preferential labeling of the tumor by PET and relatively low uptake in normal tissues at 48 h post-injection. 124I-control F(ab’)2 did not label tumors. (TIF)

Figure S4. Sagittal images of orthotopic PC3-luc tumors imaged with 124I-PGN635 F(ab’)2. Sagittal PET images clearly show preferential labeling of the orthotopic prostate tumor at 48 h post-injection. 124I-control F(ab’)2 did not label the tumors. (TIF)

Figure S5. Effects of CTx and xRT on the growth of subcutaneous PC3-luc tumors. A) Growth curves for untreated PC3-luc tumors (n=3). Tumor-to-normal (T/N) ratios for 124I-PGN635 F(ab’)2 uptake were determined by PET imaging at 24 days after implantation (dashed arrow). Tumor volume increased by an average of 11.5-fold between day 21 and day 49. B) Growth curves for PC3-luc tumors treated with 10 mg/kg docetaxel (CTx) at 21 days after implantation (n=3). 124I-PGN635 F(ab’)2 PET imaging (dashed arrow) was performed 72 h after treatment. Tumor volume increased by an average of 2.4-fold 28 days after treatment. C) Growth curves for PC3-luc tumors irradiated with 15 Gy (xRT) at 21 days after implantation (n=3). Again, 124I-PGN635 F(ab’)2 PET imaging (dashed arrow) was performed 72 h after treatment. Tumor volume decreased by an average of 10% at 28 days after treatment. (TIF)

Materials S1. (DOCX)

Acknowledgements

This report is dedicated to the memory of Dr. Philip E. Thorpe (1951-2013), who passed away in March 2013. Phil made significant contributions to cancer research over the course of his career and was instrumental in designing and implementing this study and drafting the manuscript. We also thank Patrick Thomas for assistance with imaging and Alan Schroit and Ralph Mason for reviewing the manuscript.

Author Contributions

Conceived and designed the experiments: JHS GH XS PET. Performed the experiments: AMB. Analyzed the data: JHS GH XS PET. Contributed reagents/materials/analysis tools: JHS GH XS PET. Wrote the manuscript: JHS PET.

References

1. Williamson P, Schlegel RA (1994) Back and forth: the regulation and function of transbilayer phospholipid movement in eukaryotic cells. Mol Membr Biol 11: 199-216. doi:10.1016/09687689(94)90043-0. PubMed: 7711830.
2. Marconescu A, Thorpe PE (2008) Coincident exposure of phosphatidylethanolamine and anionic phospholipids on the surface of irradiated cells. Biochim Biophys Acta 1778: 2217-2224. doi:10.1016/j.bbamem.2008.05.006. PubMed: 18570887.
3. Bitbol M, Fellmann P, Zachowski A, Devaux PF (1987) Ion regulation of translocation in human erythrocytes. Biochim Biophys Acta 904: 269-282. doi:10.1016/0005-2736(87)90376-2. PubMed: 3117114.
4. Balasubramanian K, Mirnikjoo B, Schroit AJ (2007) Regulated externalization of phosphatidylserine at the cell surface: implications for apoptosis. J Biol Chem 282: 18357-18364. doi:10.1074/jbc.M700202200. PubMed: 17470427.
5. Storz P (2005) Reactive oxygen species in tumor progression. Front Biosci 10: 1881-1896. doi:10.2741/1667. PubMed: 15769673.
6. Ran S, Downes A, Thorpe PE (2002) Increased exposure of anionic phospholipids on the surface of tumor blood vessels. Cancer Res 62: 6132-6140. PubMed: 12446386.
7. Zulueta JJ, Yu FS, Hertig IA, Thannickal VJ, Hassoun PM (1995) Release of hydrogen peroxide in response to hypoxia-reoxygenation: role of an NAD(P)H oxidase-like enzyme in endothelial cell plasma membrane. Am J Respir Cell Mol Biol 12: 41-49. doi:10.1165/ajrcmb.12.1.7529030. PubMed: 7529030.
8. Huang X, Bennett M, Thorpe PE (2005) A monoclonal antibody that binds anionic phospholipids on tumor blood vessels enhances the antitumor effect of docetaxel on human breast tumors in mice. Cancer Res 65: 4408-4416. doi:10.1158/0008-5472.CAN-05-0031. PubMed: 15899833.
9. He J, Luster TA, Thorpe PE (2007) Radiation-enhanced vascular targeting of human lung cancers in mice with a monoclonal antibody that binds anionic phospholipids. Clin Cancer Res 13: 5211-5218. doi:10.1158/1078-0432.CCR-07-0793. PubMed: 17765577.
10. He J, Yin Y, Luster TA, Watkins L, Thorpe PE (2009) Antiphosphatidylserine antibody combined with irradiation damages tumor blood vessels and induces tumor immunity in a rat model of glioblastoma. Clin Cancer Res 15: 6871-6880. doi:10.1158/1078-0432.CCR-09-1499. PubMed: 19887462.
11. Stafford JH, Thorpe PE (2011) Increased exposure of phosphatidylethanolamine on the surface of tumor vascular endothelium. Neoplasia 13: 299-308. PubMed: 21472134.
12. Kartachova M, van Zandwijk N, Burgers S, van Tinteren H, Verheij M et al. (2007) Prognostic significance of 99mTc Hynic-rh-annexin V scintigraphy during platinum-based chemotherapy in advanced lung cancer. J Clin Oncol 25: 2534-2539. doi:10.1200/JCO.2006.10.1337. PubMed: 17577031.
Zanzonico P et al. (2011) Evaluation of 18F-annexin V as a PET imaging agent in an animal model of apoptosis. J Nucl Med 52: 1173-1180. doi: 10.2967/jnumed.110.086165. PubMed: 21764796.

Wu AM (2009) Antibodies and antimatter: the resurgence of immunoPET. J Nucl Med 50: 2-5. PubMed: 19091888.

Wall JS, Kennel SJ, Paulus M, Gregor J, Richey T et al. (2006) Radiolabeling of light chain amyloid with a fibril-reactive monoclonal antibody. J Nucl Med 47: 2016-2024. PubMed: 17138745.

Orlova A, Wallberg H, Stone-Elander S, Tolmachev V (2009) On the structural diversity of human annexin A5 at its binding site with cell membranes. FEBS Lett 583: 918-926. doi: 10.1016/j.febslet.2009.03.025. PubMed: 19333469.

Klauaas CD, Alekseenas LM (2010) Xenobiotic, bile acid, and cholesterol transporters: function and regulation. Pharmacol Rev 62: 1-96. doi: 10.1124.pr.109.002014. PubMed: 20103563.

Capilla I, Hernaiz MJ, Mo YD, Mealy TR, Campos B et al. (2001) Annexin V–heparin oligosaccharide complex suggests heparan sulfate–mediated assembly on cell surfaces. Structure 9: 57-64. doi:10.1124/pr.109.002014. PubMed: 20103563.

Riedl S, Rinner B, Asslaber M, Schaider H, Walzer S et al. (2011) In vivo imaging of apoptosis in patients with head and neck cancer treated with chemoradiotherapy. Eur J Nucl Med Mol Imaging 35: 509-518. doi: 10.1007/s00259-007-0624-z. PubMed: 17994297.