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14. ABSTRACT: We will generate a novel approach for detection and therapy of advanced breast cancer based on three fundamentally novel discoveries and concepts. The first critical component exploits a novel antibody, which targets phosphatidylserine (PS), expressed on tumor vasculature. In collaboration with Peregrine Pharmaceuticals, this agent has been chimerized and is being developed for clinical trials as bavituximab (formerly TarvacinTM). Normally, PS exclusively resides on the cytosolic leaflet of the plasma membrane, but in tumors PS becomes externalized providing a viable target. The agent not only targets various tumors, but also induces vascular damage and tumor regression with minimal accompanying toxicity. The second component is the identification of diverse arsenic radionuclides suitable for imaging based on PET and radio immunotherapy together with new means of isolating the radionuclides. Importantly, the antibodies can be effectively labeled forming viable products of high specific radiochemical and biological activity. Thirdly, our program brings together pharmacological and radiochemical expertise to facilitate collaboration, progress, and synergy. This project will develop a single agent (bavituximab) for tumor detection, dosimetry, and therapy based on differential properties of arsenic radionuclides, but exploiting a single chemistry.

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Introduction

We proposed to develop and evaluate a novel therapeutic approach to breast cancer, which may be effective at any stage of tumor development, but which would be most relevant to advanced disseminated disease. We will combine a novel tumor vascular target with new radioarsenic chemistry to generate a platform technology encompassing tumor detection, dosimetry, and radio immunotherapy (RIT). Recently, Thorpe *et al.* identified phosphatidylserine (PS) as a new vascular target and generated an antibody (3G4) (1-3). It is thought that PS is exposed on tumor vasculature due to stress conditions in the tumor microenvironment. Externalized PS is a desirable marker for tumor targeting for several reasons: 1) PS is specifically and abundantly expressed on tumor vascular endothelium; 2) it is present on a high percentage of tumor endothelial cells in various solid tumors; 3) it is absent from normal vessels; 4) it is on the luminal side of tumor endothelium, which is readily accessible for targeting drugs. Since this target should be ubiquitous among tumors, we believe PS can form the foundation for a potent new anti breast cancer therapy. 3G4 has been developed as Tarvacin (a human chimerized monoclonal antibody, which has recently been given the USAN name bavituximab) by Peregrine Pharmaceuticals, who now have an IND to initiate Phase 1 clinical trials based on direct therapeutic efficacy (4). However, at much lower doses, we believe Tarvacin may be used to detect breast tumors using positron emission tomography (PET) and treat them by radio immunotherapy based on arsenic radionuclide ligands. Such systemic therapy should effectively target disseminated disease, yet provide highly localized tumor specific toxicity.

To efficiently apply bavituximab to breast cancer, we will use radioisotopes of arsenic. Multiple isotopes are available with diverse characteristics making them suitable for nuclear medicine imaging and radio immunotherapy. Arsenic-74 has a long half-life (~18 days) and positron emitting activity making it suitable for PET in animal models, while ^{72}As would be suitable in the clinic (5-7). Meanwhile, ^{77}As is a pure β^-emitter, potentially suitable for radio immunotherapy (RIT). Jennewein and Rösch (consultants) have developed novel chemistry to effectively isolate arsenic radionuclides

from irradiated germanium targets and generate ligands of radio arsenic on antibodies to target breast tumors. To date dosimetry has been a major obstacle to effective RIT, since the pharmacokinetics and accumulation in tumors of mAbs can be highly variable. We believe we can establish a novel approach for tumor detection (both primary and metastases), which will allow analogous chemistry for targeted radio immunotherapy.

It should be noted that the antibody Tarvacin has now been formally named bavituximab.

Body and Progress

Phase 1 Optimize Tarvacin labeling with arsenic radionuclides for imaging, biodistribution, and radio immunotherapy.

Task 1 Months 1-4

a) Tarvacin (a human chimeric anti phosphatidylserine (PS) monoclonal antibody (available by collaboration with Peregrine Pharmaceuticals) will be derivatized with N-succinimidyl S-acetylthioacetate (SATA), labeled with the radionuclide ^{77}As, and evaluated for activity. SATA derivatization will be performed under various reaction conditions to achieve different levels of labeling. ^{77}As (obtained from Dr. O'Kelly, UT Austin) will be attached and the radiolabeled Tarvacin will be evaluated: parameters to be assessed will include radiochemical yield, specific activity, molecular weight, and biological activity (ELISA). Biodistribution will be assessed after sacrifice 48 h hours post IV infusion of ^{77}As-SATA-Tarvacin into nude mice bearing small orthotopic MDA-MB-231 tumors (0.5 cm diameter). The level of arsenic labeling providing highest tumor delivery, with acceptable normal tissue activity will be used for subsequent Tasks. (6 mice at each of 4 optimized arsenic loading levels = 24 mice).

b) Preliminary data suggest that labeling is straightforward and highly effective with no apparent perturbation of antibody activity. However, should radiochemical or biological activity appear sub optimal, we could examine alternative labeling procedures such as use of dimercaptoarsenicals.

We have received several shipments of ^{77}As from UT Austin and ^{77}As has been isolated for radiochemical labeling. Since the isolated activity of the ^{77}As obtained from natural abundance germanium oxide targets was lower than anticipated, we have also purchased and tested isotopically enriched $^{76}GeO_2$.

Materials and Methods. All reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted and used as received. Natural and enriched GeO_2 (85.3% ^{76}Ge) powder targets were purchased from Strem (Newburyport, MA) and Campro Scientific (Berlin, Germany), respectively. Milli-Q water (18 MΩ-cm) was obtained from a Millipore Gradient Milli-Q water system (Billerica, MA). Centricon filters (YM-30: MWCO 30 KDa) were purchased from Millipore, dialysis tubing from Spectrum Laboratories (Rancho Dominguez, CA). BupH™ phosphate buffered saline (PBS) packs (0.1M PBS + 0.15 M NaCl, pH 7.2 – 7.5), D-Salt excellulose desalting column (5 mL), hydroxylamine, and dimethylsulfoxide were purchased from Pierce (Rockford, IL). BOND ELUT ENV cartridges with a sorbent mass of 25 mg were purchased from Varian (Palo Alto, CA). High performance liquid chromatography (HPLC) and radio-HPLC were performed on a Waters 600 Multisolvent Delivery System equipped with a Waters 2996 Photodiode Array (PDA) detector and an in-line Shell Jr. 2000 radio-detector (Fredericksburg, VA) on a size exclusion column.

Isotope production. Arsenic-77 ($t_{1/2}$ = 1.6 d) was generated from the beta decay of ^{77}Ge ($t_{1/2}$ = 11.3 h) produced by nuclear reaction of $^{76}Ge(n,\gamma)^{77}Ge$ at the TRIGA reactor ($\phi = 4.0 \times 10^{13}$ n/cm^2 ·s) of the Nuclear Engineering Teaching Laboratory of the University of Texas at Austin. The thermal neutron irradiation was carried out on either natural GeO_2 (*ca.* 2 grams) or 85.3% enriched GeO_2 powder (150 mg). The natural abundance of ^{76}Ge is 7.84%. ^{77}As activity at the peak time (~ 24 h post irradiation) was expected to be around 1.2 mCi., however currently it appears that the actual activity of ^{77}As is around 200 μCi per irradiation. We are investigating the discrepancy. Given the transport time from Austin to Dallas the maximal ^{77}As activity prior to our target processing is approximately 130 μCi.

Our collaborator, Sean O'Kelly, the Associate Director of the Nuclear Engineering Teaching Laboratory at The University of Texas at Austin believes that he can increase the production efficiency of ^{77}As. One approach will be longer target irradiation. He believes this should give a 25% increase in yield. A second approach will be use of higher neutron flux, increasing from $2x10^{12}$ NV about 5 fold, which could double the yield of ^{77}As. This will cause additional heating requiring a quartz target container as opposed to polyethylene and is being tested.

Separation of ^{77}As from irradiated GeO_2 targets. The GeO_2 target processing was carried out using the method described by Jennewein *et al.* (7). Briefly, the irradiated GeO_2 was dissolved in 48% hydrofluoric acid (150-mg enriched GeO_2 in 2-mL or 2-g natural GeO_2 in 5-mL). To facilitate and expedite the target dissolution, sonication (2 min each time) was applied to the mixture in a water-bath. In six trials of the target processing during the first year of this grant, no complete dissolution was obtained even after constant stirring for six hours plus frequent sonication. Generally, the solubility of natural GeO_2 is better than the enriched targets. Due to radioisotope decay, two hours are usually allowed for the target dissolution in 48% hydrofluoric acid. **CAUTION**: *All procedures involving the use of hydrofluoric acid must be carried out in a hood fume, all containers and reaction vials are made of polypropylene. The HF safety is closely monitored by the UT Southwestern Environmental Health Safety Office*. The dissolved target in 48%HF is white milky. The mixture was centrifuged at 15,000 rpm for 5-min. The supernatant was carefully removed for further processing. By weight, the enriched target was about 2/3 dissolved, while the natural target was over 80% dissolved. To the supernatant, KI pellets were added to give a KI concentration of 10-mg/mL in the HF solution. The reaction between I^- and AsF_5^{2-} gives AsI_3, a compound with limited solubility in 48%HF. The reaction was allowed to proceed for 1 h at room temperature. While waiting for the reaction to complete, a BOND ELUT ENV cartridge was pre-condition by passing 5-mL of methanol, followed by 5-mL of Milli-Q water, and 5-mL of 5-mg/mL KI solution in 48%HF by gravity. Preconditioning by using syringe to elute the column gave poorer yields of ^{77}As recovery. The reaction mixture (yellowish) was loaded on to the conditioned cartridge and allowed to pass through

by gravity. The eluate was passed again through the cartridge to improve the recovery of ^{77}As activity. Then a constant nitrogen flow was used to remove HF and dry the cartridge over 2-h. Prior experience indicated that removal of HF from ^{77}As activity is critically important to avoid potential danger to animas. The dried cartridge was eluted with two portions of 500 μL EtOH. Around 80% of radioactivity could be recovered from the cartridge as determined by a Capintec dose calibrator at a setting of either 481*10 or 481*100. As shown in Figure 1, the radioactivity rinsed off the cartridge is nearly 100% ^{77}As. The characteristic gamma peaks of ^{77}As: 239 keV (1.6%), 521 keV (0.43%); those of ^{77}Ge: 211 keV (29%), 215 keV (27%), 264 keV (51%).

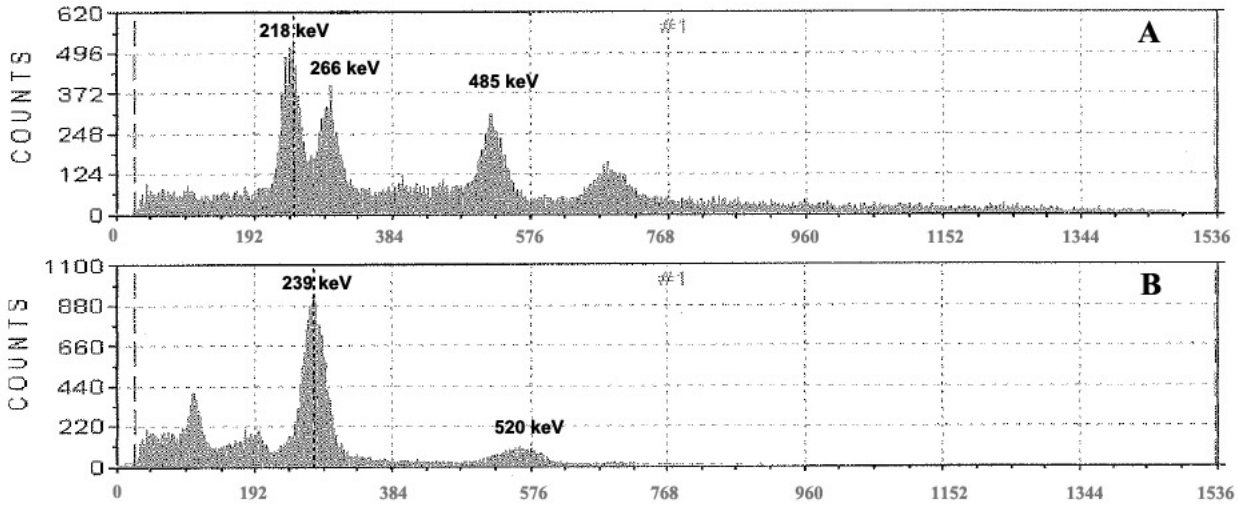

Figure 1. Gamma spectra of (A) GeO_2 target in 48%HF solution before processing and (B) $^{77}AsI_3$ in ethanol eluted from a BOND ELUT ENV. It is evident that isotopically pure ^{77}As has been separated from the irradiated GeO_2 target.

The ethanol solution of $^{77}AsI_3$ was evaporated to dryness (dark yellow) at room temperature under a constant nitrogen flow. This process typically takes more than one hour. Because $^{77}AsI_3$ is a little volatile, strong nitrogen flow (**Caution**: *Possible loss of $^{77}AsI_3$ due to spillover*) and prolonged evaporation time can result in the loss of $^{77}AsI_3$. Normally the loss of $^{77}AsI_3$ is less than 10% in this process.

Conjugation of Tarvacin with SATA. The conjugation was conducted according to the protocol provided by Pierce. The stock solution of bavituximab (~ 6 mg) was provided by Dr. Thorpe's laboratory. The bavituximab solution was dialyzed twice against 0.1M PBS buffer overnight to remove small molecules that may interfere with the following procedures. Then bavituximab solution was divided into six aliquots, which were kept at 4°C in a refrigerator. The conjugation was normally performed two days prior to the above target processing. Briefly, 2.0 mg of SATA (N-Succinimidyl S-Acetylthioacetate) was dissolved in 500 μL of DMSO, giving a 13.4 mM solution. To an aliquot of bavituximab, 6 μL of the SATA solution in DMSO was added. The molar ratio of the conjugation is 9:1 of SATA to bavituximab. The resulting solution was gently mixed on a rotating mixer at room temperature for two hours, after which the SATA- bavituximab conjugate was separated on a 5-mL Pierce D-Salt™ Excellulose™ Desalting column that had been preconditioned by passing two portions of 5-mL 0.1M PBS. Three 1-mL fractions were collected and the SATA- bavituximab concentration was determined by UV. The conjugate was presented in fractions 2 (~ 25%) and 3 (~75%), nearly nothing was in fraction 1. By comparing the UV absorbance of the solution before and after the column separation, it was determined that approximately 85% of SATA- bavituximab was recovered.

Deacetylation of SATA-bavituximab conjugate. Prior to radiolabeling, the thiol group in SATA-bavituximab conjugate was deprotected by reaction with hydroxylamine according to the Pierce protocol. Briefly, the deacetylation solution was prepared by dissolving 1.73 g of hydroxylamine and 0.359 g of EDTA in 40 mL 0.1 M PBS. After the pH was adjusted to 7.2 by addition of 10 N NaOH, the deacetylation solution was constituted with 0.1M PBS to 50 mL. To 1-mL of SATA- bavituximab conjugate (~ 0.7 mg bavituximab) solution, 100 μL of the deacetylation solution was added. The resulting solution was gently mixed on a rotating mixer at room temperature for three hours. Then the deprotected SATA- bavituximab conjugate was separated by either passing a desalting column, as above, or using Millipore centricon filters (MWCO: 30 KDa). In the latter approach, the centricon tube containing the conjugate solution was centrifuged at 6,000 rpm for 30 min at 4°C. Four portions of

0.1M PBS with 10 mM EDTA were used to rinse off the low molecular weight reagents under centrifuge. Both methods gave similar recovery rate of the deprotected conjugates (over 80%).

Radiolabeling of SATA- bavituximab Conjugate with $^{77}AsI_3$. To the above deprotected conjugate solution, all $^{77}AsI_3$ radioactivity (typically activity range at the Capintec suggested calibrator setting: 481*100: 100 – 300 μCi) was added. The resulting solution was incubated at 37 °C for one hour, after which the $^{77}AsI_3$-labeled was separated by either passing a desalting column or using Millipore centricon filters (MWCO: 30 KDa), as above. The radioactivity recovery rate in the former method (~ 40%) was a little lower than in the latter (~ 50%). The centricon method offers an advantage in that it gives more concentrated product, typically in 200 μL.

HPLC Quality Control of ^{77}As-SATA-Tarvacin High performance liquid chromatography (HPLC) and radio-HPLC were performed on a Waters 600 Multisolvent Delivery System equipped with a Waters 2996 Photodiode Array (PDA) detector and a radio-detector (Shell-USA, Fredericksburg, VA). The radiochemical purity of the radiolabeled antibody was determined by radio-HPLC. Briefly, a volume of 30 μL of radiolabeled antibody was injected into a BioSuite 450 SEC column (separation range: 20 K – 7 M Da), which was eluted with 20 mM HEPES and 150 mM NaCl buffer at an isocratic flow rate of 1.0 mL/min. The UV wavelength was 280 for the radiolabeled antibody detection and the radioactivity was determined by an in-line Shell-USA radiodetector. Since the very low gamma energy of ^{77}As, the signal from radio-channel was so weak that a fraction collector was used, and then the fractions were counted by a gamma counter.

HPLC Quality Analysis The fraction was collected every 30 seconds. After gamma counting, the data were plotted as counts/each fraction vs. time. The peak plotted and the one detected from UV channel overlaid very well (as shown in Fig. 2), with a retention time at 9.546 min. The radiochemical purity was over 95%.

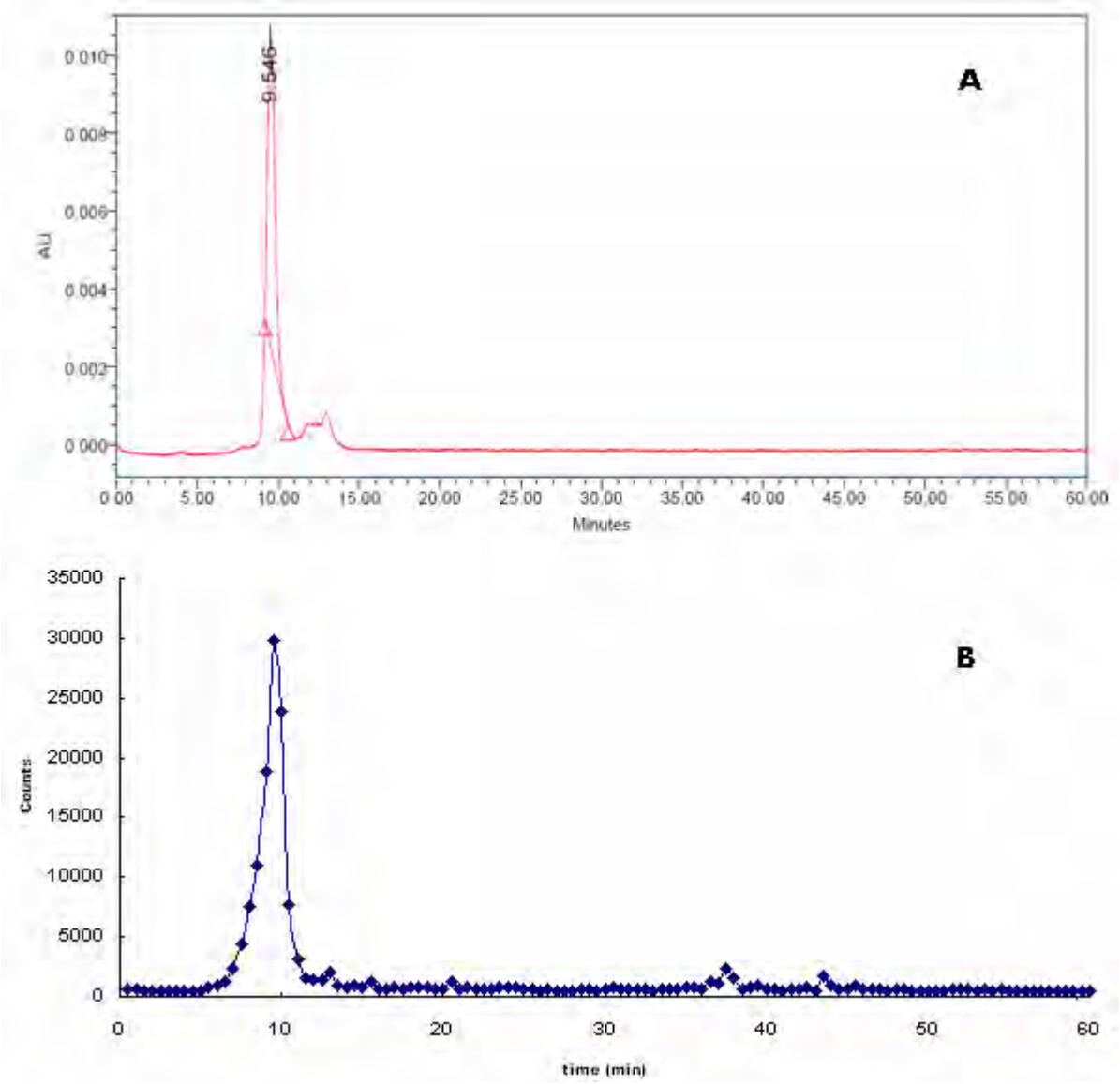

Figure 2 HPLC quality analysis of ^{77}As-SATA-Tarvacin by SEC column. A) Peak detected from UV channel at 280 nm, B) peak plotted based on fractional collections counting data.

Biodistribution in Tumor-Bearing mice. About 150 µL ^{77}As labeled antibody solution in 10 mM PBS was administered intravenously via the tail vein to 10-week-old breast cancer bearing nude female mice. The mice were sacrificed at 24 or 48 h post injection for biodistribution analyses. Before dissection, cardiac perfusion was performed to minimize the blood background. The blood, heart, lung, liver, stomach, spleen, intestines, kidney, muscle, fat, and tumor were collected, weighed, and the amount of radioactivity present in each organ was quantified via gamma- counter and scintillation counter respectively. Before the scintillation counting, tissues were processed by two steps:

dissolving the tissue in 6 M sodium hydroxide first, and then decolorizing the tissue by using 35% hydrogen peroxide.

For a mouse sacrificed 24 h after administration of ^{77}As-bavituximab IV, the biodistribution showed predominant activity in the kidney and tumor (Figure 3). Sacrifice after 48 h showed activity in tumor to be 50% greater than kidney, but considerable activity in spleen (50% greater than tumor) and liver (3 times higher) (Table 1 and Figure 4). We will compare future batches of labeled antibody to verify relative uptake in different organs.

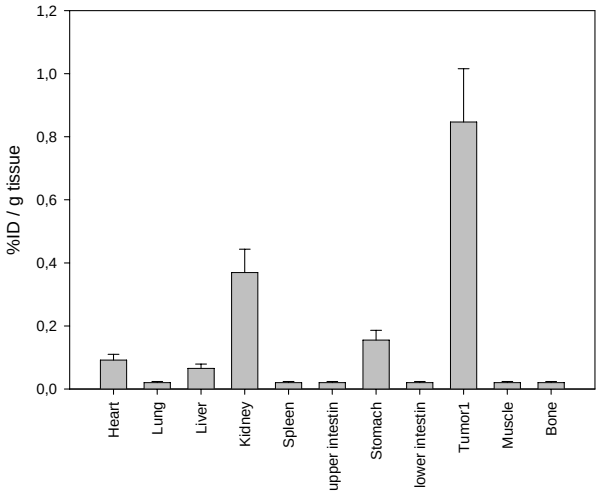

Fig. 3: biodistribution of [^{77}As]SATA-ch3G4, 24 h p.i., orthotopic breast cancer in mice.

Table 1 Tumor-to-muscle and tumor-to-blood based on gamma and beta counting

		Gamma counting	Beta counting
Tumor / Muscle	Mouse 1	4.94	2.61
	Mouse 2	26.39	6.81
Tumor / Blood	Mouse 1	1.23	N/A
	Mouse 2	6.67	N/A

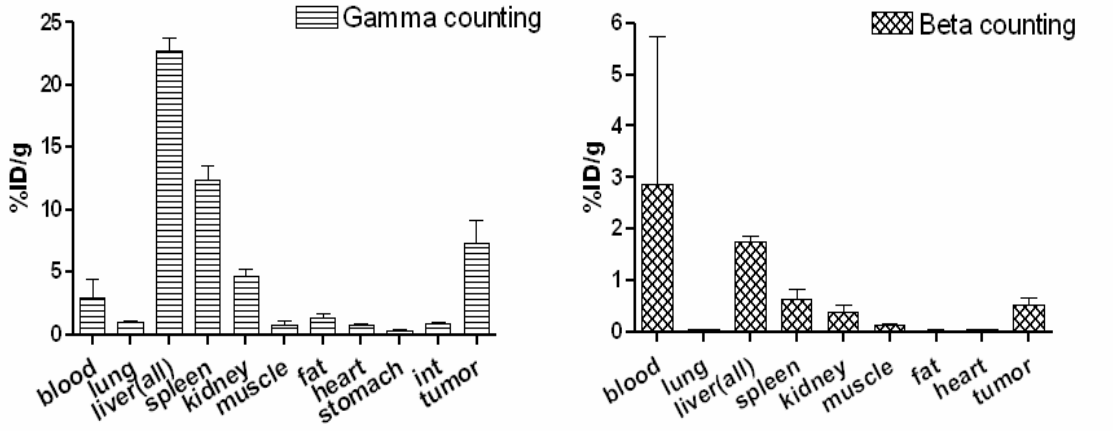

Figure 4. Biodistribution data obtained by gamma and beta counting of ^{77}As-SATA-Tarvacin in each organ and tumor at 48 h. Data are present as percent injected dose per gram tissue (%ID/g) ± standard deviation.

Dr. Thorpe's original anti-PS monoclonal antibody showed cross reactivity across all species. As the antibody has been further refined and chimerized for human clinical administration it has lost reactivity with mouse tissue. However, Dr Thorpe has discovered that this can be restored by co-administering ß2-glycoprotein I. Reactivity has been retained in rats and humans. While optimizing the isolation and ^{77}As and labeling procedures, we tested the uptake of the antibody in mice using a near infrared fluorescent label. Labeling was found to be effective with localization in an orthotropic breast tumor (MDA-MB 231) and maximum target to background after about 3 days, consistent with preliminary data in other animals and tumors prior to this grant funding.

Task 2 Months 5-6

Tarvacin derivatization with ^{74}As. We anticipate that the chemistry of any arsenic radionuclide will be identical. Our initial tests will use ^{77}As, which is readily available locally (Austin). ^{77}As will also be the isotope of choice for radio immunotherapy in Phase 3. Imaging based on Positron Emission Tomography (PET) will require ^{72}As or ^{74}As. Ultimately, ^{72}As would be the isotope of choice for clinical applications, but ^{74}As has a much longer half-life making it ideal for investigations in animals. Since

the only source of ^{72}As or ^{74}As is currently in Europe, the long half-life of ^{74}As ($t_{1/2}$ ~ 18 days) is particularly valuable to ensure efficient use following transportation. We will verify that methods developed with ^{77}As apply to ^{74}As. Biodistribution will be assessed in each of six tumor bearing mice at 2 different times to confirm consistent behavior with ^{77}As labeled Tarvacin. (12 mice).

We have postponed ^{74}As studies temporarily. There is also a possibility of obtaining ^{74}As from a linear accelerator in Denton TX and we are pursuing this possibility, since it would be far more convenient than importing radioisotope from Europe,

Phase 2 Detection of diverse primary breast tumors and evaluation of metastatic spread using radio arsenic labeled Tarvacin.

Task 3 Months 7-12

Determine ability to detect human breast tumor xenografts in nude mice. MDA-MB-231-Luc (luciferase expressing) and MDA-MB-435-Luc cells will be implanted in mammary fat pad (MFP) of mice and allowed to grow. Growth will be monitored using calipers and bioluminescence imaging (BLI). When tumors reach 0.5 cm diameter ^{74}As-SATA-Tarvacin (100 μCi) will be administered IV to half the mice and distribution of agent will be assessed every 12 h using planar γ-scintigraphy. After 60 h small animal PET will be used to quantify biodistribution *in vivo*. At this stage, the dosed mice will be sacrificed to assess biodistribution *ex vivo*. The remaining animals will be further monitored by BLI and are expected to develop lung metastases, which will be visualized by BLI. Once metastatic spread is apparent (typically, when primary tumors reach ~ 1 cc) ^{74}As-SATA-Tarvacin will again be administered to assess the ability to detect metastases using PET. Following PET, mice will be sacrificed to determine biodistribution *ex vivo* and examine extent of metastases histologically. We will also label Rituximab, a commercial non-vascular targeting chimeric antibody, as a control (12 tumors x 2 tumor types x 2 different antibodies = 48 nude mice)

We have generated and tested highly expressing MDA-MB-231-Luc tumor cells, which will be required for the radio tracing studies and PET.

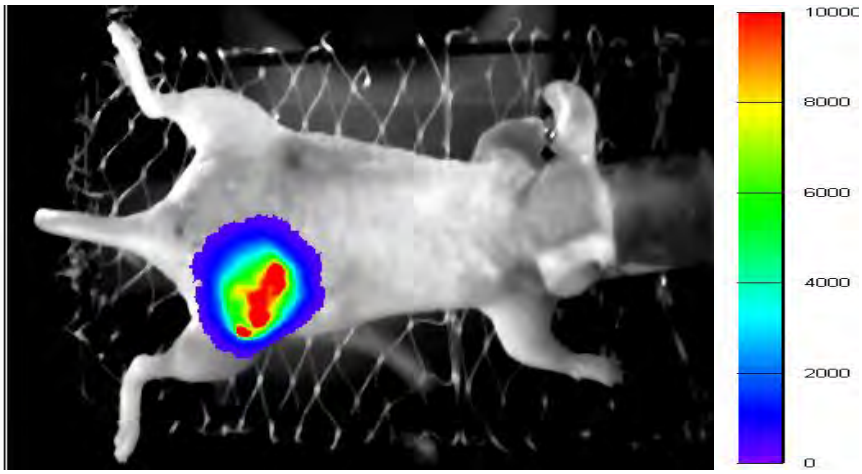

Figure 5 Bioluminescent image of MDA-MB 231 tumor growing in nude mouse

Task 4 Month 12

Prepare annual report and manuscript.

Report provided herewith.

KEY RESEARCH ACCOMPLISHMENTS:

1 We have successfully isolated ^{77}As and derivatized the antibody bavituximab (formerly named Tarvacin)

2 We have successfully detected ^{77}As- labeled bavituximab in mice bearing MDA-MB-231 orthotopic tumors and performed biodistribution after 48 h. The tumor showed higher uptake (7 % injected dose per gram) than any organ expect liver and spleen.

REPORTABLE OUTCOMES: Accepted Abstract for conference presentation (appended):

1 Optical Imaging of Exposed Phosphatidylserine in Tumors" W. Cui, R. Levenson, P. E, Thorpe, R. P. Mason", *Proc SMI*, Waikoloa Village, Hawai'i, August 2006

2 U24 CA126608A – SAIRP The diversity of small animal cancer imaging investigations at UT Southwestern organized by Dr. Mason has resulted in a new NCI U24 Small animal Imaging research program beginning April 2007. This provides substantial infrastructure and expertise to facilitate future imaging investigations. The current IDEA breast cancer award to develop arsenic radionuclides for novel imaging methods contributed to our success.

CONCLUSION: We have verified the ability to generate and isolate ^{77}As based on activation of germanium oxide in a nuclear reactor. The current yield is lower than anticipated and we are examining methods of increasing the yield. We have successfully labeled the antibody and determined biodistribution in breast tumor bearing mice 48 h after injection. These new results provide a foundation indicating that the project will be successful.

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APPENDICES:

Optical Imaging of Exposed Phosphatidylserine in Tumors" W. Cui, R. Levenson, P. E, Thorpe, R. P. Mason", *Proc SMI*, Waikoloa Village, Hawai'i, August 2006

http://www.utsouthwestern.edu/vgn/images/portal/cit_56417/21/1/317888weina-Optical_Imaging.pdf

Optical Imaging of Exposed Phosphatidylserine in Tumors

Weina Cui[1], Richard Levenson[2], Philip E. Thorpe[3], Jennifer McAnally[1], Ralph P. Mason[1]

[1]Cancer Imaging Program, Department of Radiology and [3]Department of Pharmacology,
University of Texas Southwestern Medical Center, Dallas, Texas and [2]Biomedical Systems, CRI, Inc.

Introduction

Phosphatidylserine (PS) is tightly segregated to the internal surface of the plasma membrane in most cell types, including the vascular endothelium. Loss of the phosphatidylserine asymmetry occurs during apoptosis, necrosis, cell activation, and transformation, resulting in the exposure of phosphatidylserine on the external surface of the cells. The monoclonal antibodies, 3G4, 2aG4 and the chimeric version, bavituximab, directly bind anionic phospholipids. Bavituximab localizes specifically to vascular endothelial cells in tumors [S. Ran and P. E. Thorpe, Int. J. Radiat. Oncol. Biol. Phys. 2002, 54, 1479-1484.]. 3G4 and 2aG4 recognize PS on necrotic cells and on certain viable tumor cells. We now report the first optical imaging investigation of 3G4 and 2aG4 in breast tumors and show similar pharmacokinectics to those found in prostate tumors previously using radiolabels.

Materials and Methods

Antibodies 3G4, 2aG4, C44 and ß2-glycoprotein I were generated in house and provided by Dr. Philip E. Thorpe. SAIVI™ Alexa Fluor 680 Antibody/protein labeling kit was obtained from Invitrogen and used to conjugate with anti PS antibodies 3G4, 2aG4 and control antibody C44. Fluorescence imaging was performed using a CRi Maestro™ system with excitation filter range 575-605 nm, and emission filter range 645 nm (longpass).

Fluorescence imaging of tumor cells in vitro:

Human breast cancer MDA-MB-231 cells were incubated with H_2O_2 for one hour, washed and cultured in serum free DMEM with buffer or 3G4-SAIVI-680 for 1 hr. Cells collected after washing with PBS were used for fluorescence imaging.

Fluorescence imaging in vivo:

a) MCF7 breast cancer cells were implanted in both flanks of a nude mouse and allowed to grow about 1 cm^3. For proof of principle, 3G4-SAIVI 680 was injected directly into the left tumor.

b) MDA-MB 231 tumor cells were implanted in the flank of nude mouse and allowed to grow about 1 cm^3. 100 ug 2aG4-Saivi 680 or C44-Saivi 680 respectively were injected with 100 ug ß2-glycoprotein I intravenously. Fluorescence imaging was performed before infusion and after 1 min, 30 min, 1 hr, 2 hr, 1 day, 2 days, 6 days and 12 days.

Results

Both isolated MDA-MB 231 cells labeled with 3G4-SAIVI 680 and tumor following direct injection of 3G4-SAIVI 680 showed strong emission peak at 710 nm. Considerable autofluorescence was observed at 670 nm (Figure 1).

The SAIVI 680 labeled antibodies C44 and 2aG4 were found to circulate throughout the mice for several days (Figures 2 and 3). The emission peaks for both 2aG4-SAIVI 680 and C44-SAIVI 680 in vivo occur at 710 nm. Food has strong autofluorescence at 690 nm, which can be separated easily from the emission spectrum of tumor autofluorescence and SAIVI 680 labeled 2aG4 and C44.

Although both 2aG4-SAIVI 680 and C44-SAIVI 680 were detected in the tumors, the clearance rates from body and tumor are different for these two antibodies. Prior to antibody injection, tumor and body had similar fluorescent light emission intensity for both mice. Two days after infusion, 2aG4-SAIVI 680 showed much stronger fluorescence in tumor than the body, whereas C44-SAIVI 680 showed similar intensity in tumor and body (Figure 3).

Conclusion

We have demonstrated that fluorescence imaging is a powerful tool for detecting the activity of phosphatidylserine antibodies 3G4 and 2aG4 in tumor cells and tumor. In particular, fluorescence imaging could distinguish the distributional difference of 2aG4 and its control antibody C44 between tumor and mouse body. The vascular targeting agent, Bavituximab, the chimeric version of 3G4 and 2aG4, has entered Phase I clinical trials with applications to diverse tumor types. We believe optical imaging with Bavituximab holds great promise for assessing PS expression, and therapeutic activity in vivo and optimizing treatment protocols.

Acknowledgments

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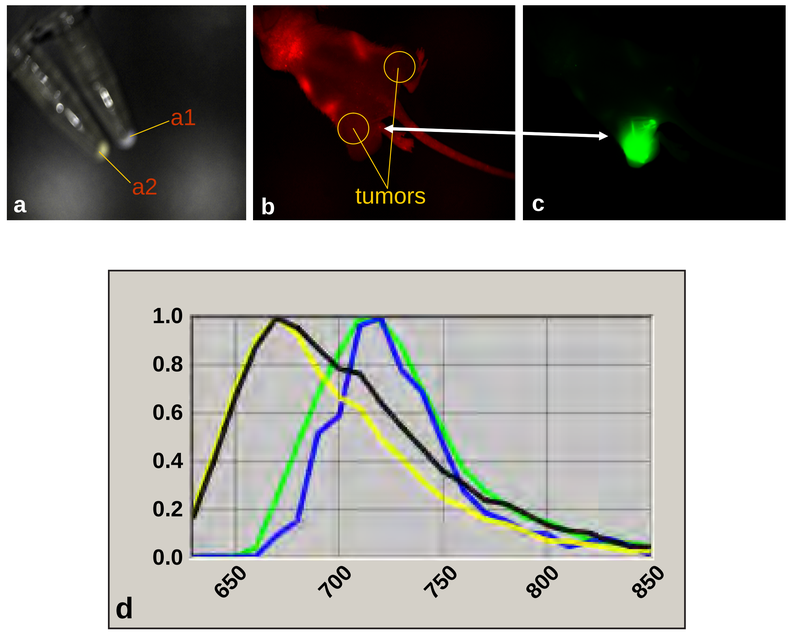


Figure 1.

a) Fluorescence imaging of MDA-MB 231 cells cultured with 3G4-SAIVI 680 (a1) and buffer (a2).
b) Fluorescence imaging of nude mouse with MCF7 tumor implanted on both flanks.
c) 100 µg 3G4-SAIVI-680 was injected directly into the left tumor and provided intense fluorescence (green).
d) Normalized emission spectra: Yellow: control cells; Blue: cells cultured with 3G4-SAIVI 680; Black: control tumor; Green: tumor injected 3G4-SAIVI 680.

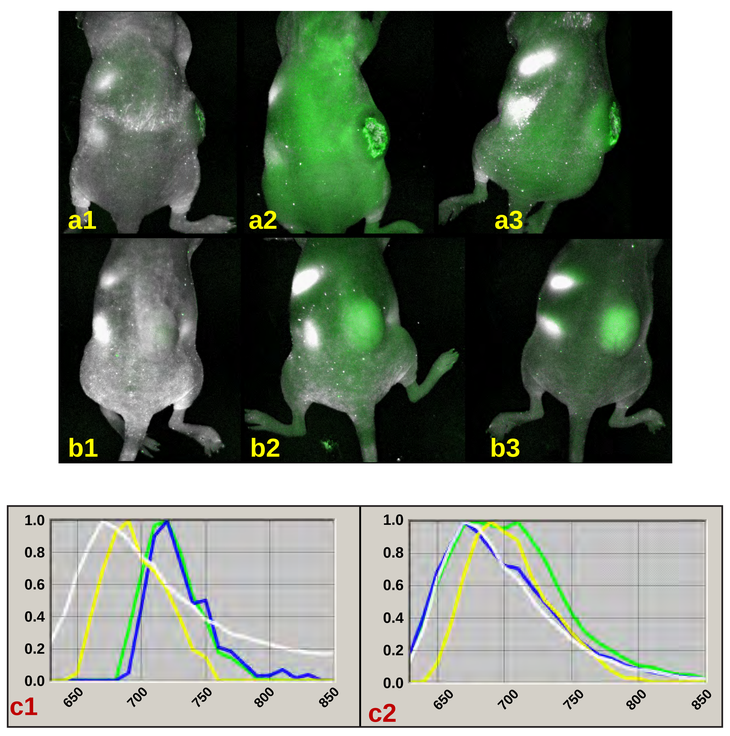
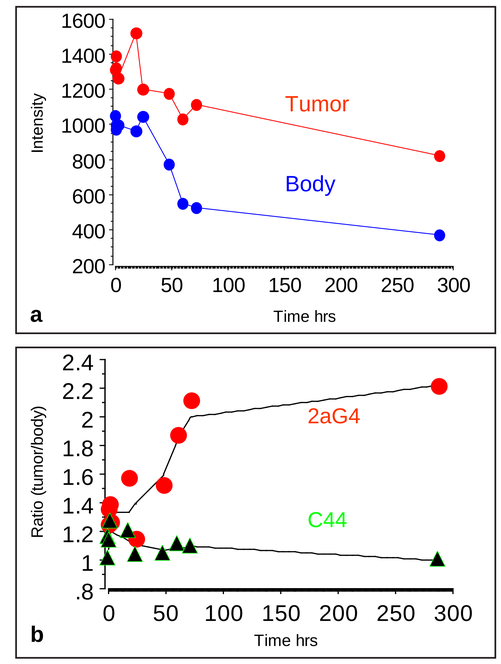


Figure 2. In vivo fluorescence imaging.

a) Unmixed images of the nude mouse injected with control C44-SAIVI 680 and B2GP1 (a1, pre injection; a2, 2 days after IV injection; a3, 6 days after IV injection).
b) Unmixed images of the nude mouse injected with 2aG4-SAIVI 680 and B2GP1 (b1, pre injection; b2, 2 days after IV injection; b3, 6 days after IV injection).
Note: the images were processed by 4-way unmixing (food, skin, and tumor signals at days 2 and 6) and then adding the food and skin together, and adding the two tumor images together.
c) Normalized emission spectra for A(C1) and B (C2). White: skin; Yellow: food; Green: 2 day tumor; Blue: 6 day tumor.



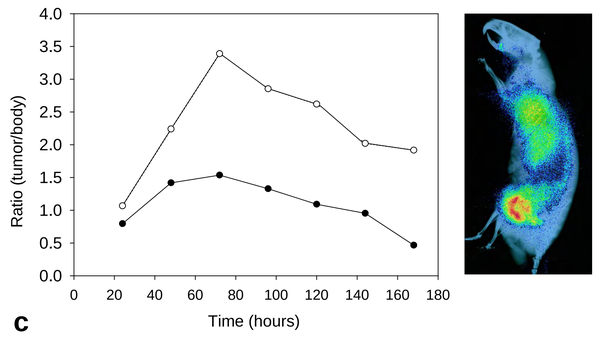


Figure 3. Pharmacokinetics of antibody uptake and clearance

a) Relative fluorescent signal intensity detected at 710 nm for 2aG4-SAIVI 680 in the tumor and body respectively showing greater signal from the tumor at all times and more rapid clearance from the body than tumor.
b) Ratio of relative fluorescent signal intensity detected at 710 nm for tumor and body for each of the labeled mAbs 2aG4-SAIVI 680 and C44-SAIVI 680. 2aG4 shows specificity for the tumor.
c) We previously showed distribution of radiolabeled [^{74}As]SATA-ch3G4 in Dunning prostate R3327-AT1 tumor bearing Copenhagen rat over 7 days based on in vivo storage phosphor technology. (● tumor: upper body ratio; ○ tumor rim: upper body ratio) The pharmacokinetics and relative uptake of tumor to normal body tissue using these two techniques appear quite similar (data previously presented at AACR in collaboration with Drs. Marc Jennewein and Frank Rösch of the University of Mainz, Germany)

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