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Award Number: DAMD17-02-1-0034

TITLE: Image Guidance and Assessment of Radiation Induced Gene
Therapy

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REPORT DATE: February 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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20040720 029

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE February 2004	3. REPORT TYPE AND DATES COVERED Annual (7 Jan 2003 - 6 Jan 2004)	
4. TITLE AND SUBTITLE Image Guidance and Assessment of Radiation Induced Gene Therapy			5. FUNDING NUMBERS DAMD17-02-1-0034	
6. AUTHOR(S) Charles A. Pelizzari, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The University of Chicago Chicago, Illinois 60637 <i>E-Mail:</i> c-pelizzari@uchicago.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES Original contains color plates: All DTIC reproductions will be in black and white.				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) Image guidance and assessment techniques are being developed for combined radiation/gene therapy, which utilizes a radiation-inducible gene promoter to cause expression of tumor necrosis factor alpha in irradiated tissues. TNF attacks vasculature, increasing the tumor killing effect of radiation. The radiation confines TNF toxicity to the irradiated region. This therapy has proven effective in several animal tumor models and phase I clinical trials. This project is developing imaging to visualize the effects of the combined modality therapy, by combining electron paramagnetic resonance imaging (EPRI), mapping oxygen concentration in 3D in vivo, and nuclear magnetic resonance imaging (MRI), measuring quantities such as vascular permeability and perfusion rate that reflect the status of vasculature. EPRI and MRI of prostate tumors in mice and rats will be conducted. Fusing the two sets of images from the same tumor before, during and after therapy, an image-derived signature will be developed which identifies regions responding well to therapy. In the final stage, this information will be used as the basis of an adaptive treatment regimen where regions that have responded less well will be given a boost, via image guided injection of additional gene vector prior to the next fraction of radiation.				
14. SUBJECT TERMS Radiation therapy; gene therapy; angiogenesis inhibitors; magnetic resonance imaging; electron paramagnetic resonance imaging; adaptive therapy planning; image guided therapy;			15. NUMBER OF PAGES 59	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

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INTRODUCTION

Combined radiation and gene therapy is a promising modality for cancer treatment. At the University of Chicago, a combined therapy utilizing a radiation-inducible tumor necrosis factor alpha (TNF α) gene vector has been studied extensively. In this treatment modality, vascular destructive effects of TNF α amplify the tumoricidal effect of radiation therapy, while the gene expression is confined to the irradiated region, limiting TNF-induced complications. This research project is intended to test the hypothesis that a combination of magnetic resonance imaging (MRI) and electron paramagnetic resonance imaging (EPRI) can detect, characterize and quantify the distribution of tissue changes resulting from combined radiation and TNF α gene therapy, as validated by comparison with histology. EPRI using trityl spin probes and existing imagers can provide 3D image maps of oxygen concentration *in vivo*, with spatial resolution approximately 1mm and oxygen resolution approximately 2 torr in small animals. Dynamic MRI using suitable contrast agents can provide high spatial resolution (down to approximately 0.05 mm in our laboratory) images which can yield local measures of blood volume, capillary permeability, and blood flow. The characteristic size and density distribution of these image-defined changes is to be used as a "damage kernel" in a superposition based treatment planning system. Finally, an image-guided injection scheme utilizing such a treatment planning paradigm will be developed which can be used in an adaptive fractionated treatment course to achieve spatially uniform tumor response, in large prostate tumors grown in rats.

BODY

Progress has been made in each task which was programmed to be addressed in months 12-24. Problems with critical major resources have prevented full accomplishment of our goals, however. These problems and their impact on the progress of our work are detailed here.

1) The animal MRI imaging facility, which is an essential part of the experimental protocol, was to undergo a major upgrade beginning approximately 1 July 2003 and lasting for approximately 6-8 weeks. This is the only MRI facility available for our use. In mid May 2003 a problem developed with the existing MRI console and the decision was made not to attempt to repair the older console, but to wait for the upgrade to occur. The upgrade also unfortunately was not accomplished in a timely manner. Due to various problems with the new console and new superconducting magnet, the expected 6-8 week upgrade instead took 6 months, and the MRI facility only came back online in January 2004. We are now beginning to image mice again, but there is a learning curve associated with the new MRI system, associated animal monitoring systems, adaptation of immobilization devices and resonators to the new magnet, so we are not yet ready to resume our full treatment and imaging protocol.

2) When it became clear that the MRI facility would be down for a considerable time, we decided that EPR imaging alone on some treated mice would be worthwhile in order to both continue improvement of EPR image quality, which has been problematic, and to continue work on identification of the EPR imaging "signature" of treatment. As discussed in last year's report, a number of innovations in animal management have been made in order to help the quality of EPR images as well as to allow survival of the animals through multiple imaging sessions requiring anesthesia of several hours each time. However, this effort was also frustrated when in late September, the animal irradiation facility used to deliver the radiation treatments to the mice went offline due to a failed X-ray tube. This facility is owned by the Department of Radiation Oncology, which reached the decision that the existing machine should be replaced instead of repaired, since it had failed numerous times in the past several years and simply replacing the X-ray tube would not necessarily result in a durable solution. A refurbished medical X-ray therapy unit was ordered from a vendor in Connecticut, and the inevitable delays occurred in purchasing, refurbishing, delivering and installing that replacement machine. In early February 2004, the irradiation facility finally came on line for animal use.

The result of these two failures in major experimental resources is that we have been unable to treat and image animals according to our defined experimental protocol since May 2003. Thus, accomplishment of the second year goals has been delayed significantly although progress has continued on experimental technique improvements, additional data analysis avenues have been explored and several presentations and publications have been prepared. The full implementation of image-guided treatment of tumors in rats would appear to be unlikely during the originally programmed time period (calendar year 2004) due to these problems, since the goal of reliable identification of an

imaging signature of response to radiation plus antiangiogenic gene therapy, which is necessary for the image-guided treatment protocol, has not yet been completed.

Task 1. Develop imaging techniques to assess early response of tumor to radiation induced gene therapy (months 1-36)

- a. design and construct immobilization jig for mice (months 1-6)
- b. adapt pathology sample preparation apparatus (months 1-6)
- c. develop combined EPRI / MRI imaging protocols (months 1-3)
- d. investigate vasculature-sensitive MRI of TNF-injected mouse tumors (months 1-24)
- e. develop EPRI oxymetry of TNF-injected mouse tumors (months 1-24)
- f. develop and test correlation of EPRI and MRI imaging (months 1-18)
- g. correlate EPRI, MRI and pathology (months 12-36)
- h. statistical analysis of imaging sensitivity for 4 treatment conditions (sham, virus, radiation, radiation + virus) (months 12-36)
- i. implement and deliver adaptive fractionated image-guided therapy in rats (months 18-36)

Major effort has continued to be focused on the imaging and analysis subtasks, (d-h). As described above, MRI scanning has been unavailable for more than half the present year, so we have had to concentrate on EPR imaging and on image analysis of available EPRI and MRI, although the MR images available so far for analysis have not been optimized to our satisfaction.

Continued development of EPR imaging technique has occurred in several areas. Animal management technique continues to improve, and the dual-lumen bladder catheter for preventing accumulation of the EPR spin probe in the mouse bladder has been refined so that it can be routinely utilized. A presentation on the bladder flushing technique was made by Dr. Haney at the 26th International EPR Symposium in Denver, CO in July 2003. A copy of the poster is included in the Appendix. Additionally, a manuscript describing the bladder flushing technique has been prepared for submission to Magnetic Resonance in Medicine.

Image quality has been a continuing problem with the EPR images. Several technical developments under the auspices of Dr. Halpern's EPRI Research Resource have contributed to improved image quality and, importantly, to shortened image acquisition times. This project has not yet realized the full benefit of these improvements, since as noted earlier we have been unable to treat any mice with radiation since mid September 2003 and much of the relevant development has come to fruition since then.

Registration of small animal images from multiple modalities is a challenge, as is widely recognized by investigators who must address this issue in their research(1). We have continued development of several alternative methods for intermodality registration of EPRI and MRI images, and for serial intramodality registration of EPRI and/or MRI images on subsequent days. These two problems present somewhat different challenges

and do not readily admit a single solution. Our initial efforts centered on the use of registration via interactive manual image overlay, and on the use of fiducial markers visible in both EPRI and MRI, as documented in the previous annual report. Manual registration continues to be the most reliable tool at present. Fiducial based registration is compromised by the need to remove the fiducials during EPRI, where their presence causes image artifacts similar to but less severe than those caused by the spin probe accumulating in the bladder. Fiducials (in the form of glass capsules about the size of a grain of rice) are attached to the leg of the sedated mouse with a single suture stitched through the skin. An initial 3D spatial EPR image is made with the fiducials in place, and then they are removed by pulling on sutures tied onto them for this purpose. This allows removal of the fiducials without moving the mouse leg or removing it from the EPR resonator. The full 4D spectroscopic image of the leg is then acquired. The animal is transported still under anesthesia to the MRI lab, a 10 minute trip through the hospital basement. The fiducials are replaced in the sutured loops, as close as possible to their original positions. MRI images are then acquired. There are two problems with this procedure. One is that the replacement of the fiducials is not necessarily perfect, and small mispositioning of a fiducial on a small object like the mouse leg can lead to significant registration errors. The second problem is that the fiducials also affect the quality of the MRI images adversely. We thus nearly always end up using the manual image registration method to make final adjustments.

We are investigating a new strategy for reproducible positioning of the mouse leg in the EPRI and MRI resonators, which was motivated by the techniques used to repeatedly immobilize patients for radiation therapy. This involves the making of a customized insert for the resonator for each mouse leg imaged, that conforms closely to the external contour of the leg and thus enforces a reproducible position when the leg is enclosed in the insert. The insert can be carefully aligned with both the EPRI and MRI resonators, and the mouse leg will be repeatably immobilized by the insert. We believe this will facilitate both accurate intermodality and serial intramodality registration, while none of the other methods can do both. Several materials have been investigated for production of these inserts - urethane foam, Alginate dental impression material and polyvinylsiloxane dental impression material. Practical issues affect the decision as to which material is best - speed of setting, degree of exothermic reaction, cost and availability, possible effects on EPR and MRI signals, longevity of the molded material. An ideal material will have little heat generation during molding, not stick to the animal skin or molding tools, not become brittle over a period of several weeks, not be very expensive, not produce toxic fumes, not degrade the EPR or MRI quality factor Q , and not produce any EPR or MRI signal of its own. Further discussion is included in the Appendix.

An insufficient number of animals in the various treatment conditions has been acquired to do any serious statistical analysis to identify systematic signatures of treatment effect due to the various factors described earlier. However, preliminary analysis of EPR image changes post treatment is encouraging, as reported by Dr. Pelizzari in a presentation at the 26th International EPR Symposium in Denver, CO in July 2003. A printed copy of the slide presentation is included in the Appendix. A consistent change in the appearance

of the EPR images between day 0 (pre treatment) and day 3 (post treatment) is noted, with the characteristic banded structure of the PC3 tumors as seen in the EPR oxygen concentration images pre treatment changing to a more "chaotic" structure and overall lower oxygenation on day 3. Interestingly, on day 16, two weeks post treatment, the EPRI appearance has returned to its pretreatment state. This is consistent with the idea that the single fraction of therapy given initially kills part of the tumor and damages its vasculature, but that the tumor then regrows and reoxygenates since the single fraction is not sufficient to represent a curative dose.

Analysis of the dynamic MRI contrast washout images using a compartment model to shed light on changes in vascular status in treated tumors has also been developed. Dr. Haney was invited to present this work at the Radiological Society of North America Annual Meeting and Scientific Assembly in Chicago, December 2003. A printout of his slide presentation is included in the Appendix. Although the two-compartment model used seems not to be adequate to characterize the contrast curves, as has been observed by others (2), it appears that the vasculature of the tumor is permanently affected by the treatment, in that the day 3 and day 16 parameters are consistently changed relatively to the day 0 parameters. The recovery seen in the EPR images is not supported by this analysis, an observation we will attempt to reproduce and to study further when the MRI and irradiation facilities are back in full operation (March 2004). We have also begun programming analysis codes utilizing more advanced models (3) which will allow more reliable characterization of the vascular performance and of the effects of treatment on vascular status.

As mentioned earlier, no work has been possible on the implementation of image-guided therapy in rats due to the various problems outlined above.

Task 2. Develop image guided injection system (months 1-18):

- a. specify and acquire 3D translation stage (months 1-6)
- b. design and construct stereotactic localizer frame (months 3-9)
- c. integrate positioning and localizer components; interface with imaging (months 6-12)
- d. test accuracy of image guided injection (months 12-18)

Subtasks a-c are essentially complete. Subtask d has not yet been undertaken as we have placed our efforts on the imaging studies since without the imaging signature of therapy, the image guided therapy is not possible.

Task 3. Develop injection planning software (months 1-24):

- a. develop functional specification for final system (months 1-3)
- b. adapt existing brachytherapy planning system as testbed (months 1-6)
- c. test optimization algorithms for injection site placement (months 4-12)
- d. implement final version of nonadaptive software (months 12-18)

- e. incorporate adaptive treatment refinement into planning software (months 18-24)

Subtasks a-c are essentially complete. Subtask d has been delayed due to the concentration on imaging development, but software development for this task is underway. Subtask e has not been begun yet. This entire task is the specific responsibility of Dr. Pelizzari, who will incorporate the adaptive refinement concept of subtask e into the treatment planning software in early 2004.

Task 4. Test adaptive radiation induced gene therapy in rats (months 24-36)

- a. deliver image guided adaptive therapy to test group (months 24-36)
- b. compare EPRI / MRI imaging with histology in rats (months 24-36)
- c. analyze uniform vs adaptive image guided outcomes (months 30-36)

As mentioned above, this task has not been begun since the imaging aims of Task 1 have not yet yielded the required imaging signature. It is anticipated that some image guided treatments may yet be delivered in 2004, although the full clinical trial of uniform vs nonuniform injection strategy may not be possible.

KEY RESEARCH ACCOMPLISHMENTS

- Continued improvement of EPR image quality.
- Identification of consistent image changes on EPR images in treated tumors in mice.
- Identification of contrast dynamics in MRI which is not explained by simple compartment model.
- Development of repeat immobilization for mouse leg based on custom molded cushions.

REPORTABLE OUTCOMES

1. Haney, C.R., K. Ichikawa, A. Parasca, B.B. Williams, E.D. Barth, M. Elas, and H.J. Halpern. "Reduction of Image Artifacts by Bladder Flushing with a Novel Double Lumen Urethral Catheter." in *26th International EPR Symposium*, July 27-31, 2003. Denver, CO.
2. Pelizzari, C.A., C.R. Haney, A. Parasca, K. Ichikawa, E.D. Barth, B.B. Williams, M. Elas, V.S. Subramanian, M.A. Zamora, J.N. River, G.S. Karczmar, H.J. Mauceri, R.R. Weichselbaum, and H.J. Halpern. "Biological Correlates of EPR Oxygen Images: Preliminary Images of Response to Radiation Plus Adenovirus Delivered EGR-TNF Anti-cancer Therapy". in *26th International EPR Symposium*, July 27-31, 2003. Denver, CO.
3. Haney, C.R., C.A. Pelizzari, H.J. Halpern, A. Parasca, H.J. Mauceri, and R.R. Weichselbaum. "Physics Invited Speaker: EPR Oxygen Imaging for Assessment for Radiation Induced Gene Therapy". in *Radiological Society of North America (RSNA)*, December 2, 2003. McCormick Place, Chicago, Illinois. (The abstract will appear in the March 2004 issue of *Radiology*.)
4. C.R. Haney, A. Parasca, K. Ichikawa, M. Elas, M. Zamora, X. Fan, G.S. Karczmar, H.J. Halpern and C.A. Pelizzari, "Assessment Of Radiation Mediated Gene Therapy Via Multi-Modality Imaging," accepted for presentation at 2004 IEEE International Symposium on Biomedical Imaging: From Nano to Macro, April 2004, Arlington, VA.

CONCLUSION

Progress has continued in the second year of this project, though extended unavailability of major experimental facilities has caused serious delay. Identification of a tentative signature of response on EPR images post treatment, consistent with disruption of vasculature immediately following treatment and subsequent return to normal with tumor regrowth, has been made. Several presentations concerning the work have been made, and a paper accepted at the IEEE International Symposium on biomedical Imaging. The use of novel modalities which convey physiologic information to assess the response of disease to therapy has been well received in the scientific community. Dr. Pelizzari's presentation at the International EPR Symposium in Denver in July 2003 was scheduled as the closing talk of the conference, as the organizers felt this was a "keynote" talk showing the promise of the future for EPR in medicine. Comments from reviewers of Dr. Haney's paper for the International Symposium on Biomedical Imaging included the following:

"I think this is an outstanding contribution of using MRI and EPR imaging to assess the outcome of cancer gene therapy. Although the results are preliminary, they serve the purpose of demonstration of proof of principle. The impact of this research can be significant."

"An innovative application of image processing techniques to radiation mediated gene therapy- excellent preliminary data- if successful the technique will have significant impact in molecular imaging."

"Very important topic, an excellent study. The general idea can be adapted to other schemes."

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APPENDICES

- "Custom immobilization for reproducible positioning of tumors in EPRI and MRI"
- Poster presented at 26th International EPR Symposium, July 27-31, 2003, Denver, CO: Haney, C.R., K. Ichikawa, A. Parasca, B.B. Williams, E.D. Barth, M. Elas, and H.J. Halpern. "Reduction of Image Artifacts by Bladder Flushing with a Novel Double Lumen Urethral Catheter."
- Slides from presentation at 26th International EPR Symposium, July 27-31, 2003, Denver, CO: Pelizzari, C.A., C.R. Haney, A. Parasca, K. Ichikawa, E.D. Barth, B.B. Williams, M. Elas, V.S. Subramanian, M.A. Zamora, J.N. River, G.S. Karczmar, H.J. Mauceri, R.R. Weichselbaum, and H.J. Halpern. "Biological Correlates of EPR Oxygen Images: Preliminary Images of Response to Radiation Plus Adenovirus Delivered EGR-TNF Anti-cancer Therapy".
- Slides from presentation at Radiological Society of North America 2003 International Meeting and Scientific Assembly: Haney, C.R., C.A. Pelizzari, H.J. Halpern, A. Parasca, H.J. Mauceri, and R.R. Weichselbaum. "Physics Invited Speaker: EPR Oxygen Imaging for Assessment for Radiation Induced Gene Therapy".
- Manuscript accepted for presentation at 2004 IEEE International Symposium on Biomedical Imaging: From Nano to Macro, April 2004, Arlington, VA: C.R. Haney, A. Parasca, K. Ichikawa, M. Elas, M. Zamora, X. Fan, G.S. Karczmar, H.J. Halpern and C.A. Pelizzari, "Assessment Of Radiation Mediated Gene Therapy Via Multi-Modality Imaging,"

APPENDIX: Custom immobilization for reproducible positioning of tumors in EPRI and MRI

C.R. Haney, Ph.D.

A new cast system is under development to remove the need for imaging a tumor bearing leg with fiducials attached. By using a cast, the reference frame (of the resonator) can be taken to another imaging modality for registration, at any time. A side benefit is the creation of a history of the tumors, in the form of positive reliefs. Although the fiducial development is adequate, it requires two images to be taken for EPRI, one spatial with the fiducials, and the other spectral-spatial with the fiducials removed. For the NMR imaging, the fiducials can make shimming the spectrometer difficult, because the fiducials are brighter than the tumor.

We are in the process of developing a casting system that will maintain resonator/tumor registration without imaging the animal twice for EPRI or confounding the shimming process for NMR. A three step process, done the day before imaging, will provide a cast of the tumor bearing leg such that image registration is done without the mouse. Triphasix" Alginate (Parkell, Inc., Farmingdale, NY), will be used to make a negative impression of the tumor bearing leg while the mouse is conscious the day before imaging. Its microfine particles capture detail as small as 50 microns. After returning the mouse to its cage, a positive impression will be made with a threaded rod in the center, back in the lab. The first attempt used polyurethane expanding foam (Foam-iT 5 Eager Pastics, Inc, Chicago, IL). There are two problems with using the polyurethane foam. First, the reaction of the two resins is highly exothermic and would be uncomfortable for the mouse. Secondly, it expands too fast and therefore is hard to control. Voids were present in many of the test casts. The polyurethane is by far harder to clean up compared to the water based alginate. Recent investigation has identified a source of polyvinylsiloxane dental impression material, which has the favorable casting properties of alginate but does not become brittle with age and drying as does alginate. Initial attempts to obtain this material from 3M were frustrated by the vendor's insistence that only dentists could order the material, but a local source of a comparable material has been found where small quantities of material for non-patient related use can be ordered.

After the images are done and the mouse has been taken care of, the casts can be imaged with fiducials or possibly filled with a substance to be imaged. Since EPRI does not give good anatomical information using $8 \times 8 \times 16$, imaging (16^3) the cast filled with contrast agent, e.g., reusable LiPc slurry, can be done overnight to provide anatomical references. This would be helpful if MRI goes down. For image registration, the MRI and EPRI cast will leverage the fact that they both have a nearly perfect impression of the tumor bearing leg. A jig (something like a toothpick with 3 fiducials tied to it) will be used to make a pseudo leg with three fiducials in it. The fiducials could be filled with a nitroxide while the rest of the cast is filled with gelatin. The nitroxide might not overwhelm the MRI fiducial image as trityl might. It has not been determined yet, what is the best combination of materials for the fiducial and filler for registration images.

APPENDIX: Poster presented at 26th International EPR Symposium, July 27-31, 2003.
Denver, CO: Haney, C.R., K. Ichikawa, A. Parasca, B.B. Williams, E.D. Barth, M. Elas,
and H.J. Halpern. "Reduction of Image Artifacts by Bladder Flushing with a Novel
Double Lumen Urethral Catheter."

Reduction of Image Artifacts by Bladder Flushing with a Novel Double Lumen Urethral Catheter.

Chad R. Haney, Kazuhiro Ichikawa, Adrian Parasca, Benjamin B. Williams, Eugene D. Barth, Martyna Elas, and Howard J. Halpern
The University of Chicago, Department of Radiation and Cellular Oncology, MC 1105, 5841 S. Maryland Ave., Chicago, IL 60637-1463

The triarylmethyl spin probe (OX063) developed by Nycomed Innovations (Malmo, Sweden) is useful as a narrow spin probe for *in vivo* imaging. However, the accumulation of spin probe in the bladder creates a tremendous source of signal, often greater than that of the tumor due to self broadening, not the broadening by oxygen (the desired measurement).

Objective:

Due to the paucity of methods available for a non-invasive, MRI/EPRI friendly procedure for flushing a mouse bladder, a novel double lumen urethral catheter was developed. The method also has to accommodate both MRI and EPRI on multiple days, using the same mouse.

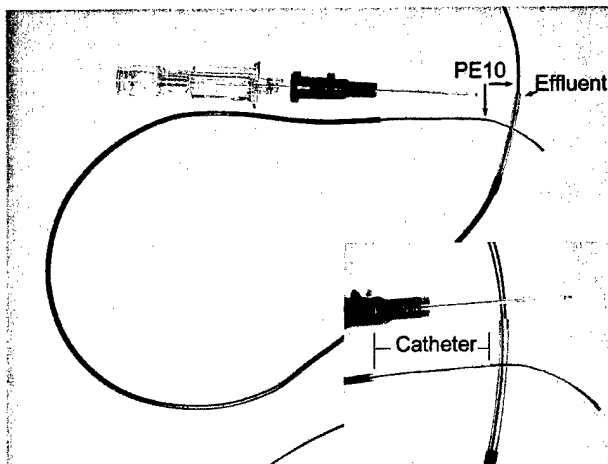


Figure 1

Methods:

A standard 20 gauge IV catheter (1.1 mm ID, Introcan Safety IV Catheter, B. Braun Medical Inc., Bethlehem, PA) is inserted into the urethra of an athymic nude mouse, with a leg bearing a PC3 tumor. After which, the luer-lock connection is cut off and a rubber tube extension is connected with PE10 tubing (0.28 mm ID, 0.61 mm OD, Clay Adams INTRAMEDIC Polyethylene, BD Franklin Lakes, NJ) threaded into the IV catheter. The PE10 was stretched such that it is able to curl within the bladder, minimizing the chance of being impinged against the bladder wall. Using a Harvard 22 syringe pump, (Harvard Apparatus, Inc., Holliston, MA), water at 10-19 mL/hr was infused into the bladder via the PE10 tubing. The effluent from the bladder exits the tubing as shown in figure 1.

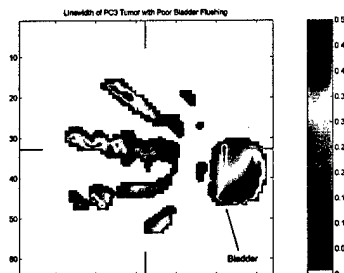


Figure 2

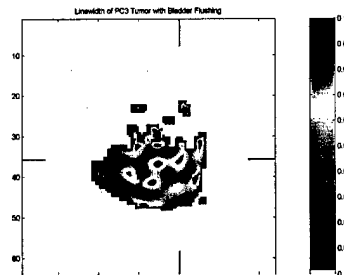


Figure 3

Results:

In figure 2 the bladder is shown with artifacts projecting from it. The self broadening of the trityl makes the bladder appear larger as well. The tumor signal is overwhelmed and cannot be easily viewed. However, in figure 3, when bladder flushing is used properly, there is no hint of bladder signal. Furthermore, the heterogeneity of the tumor is clearly demonstrated, without obstruction of artifacts from the bladder signal.

Discussion:

Further refinement maybe necessary, e.g. the placement of the catheter such that it does not get impinged against the wall of the bladder is critical. However, the double lumen urethral catheter provides a substantial reduction in artifacts when compared to images taken without bladder flushing or poor flushing. Artifacts from the bladder signal have been successfully attenuated using a method which is compatible with our survival, multiple imaging project. Line width images converted to pO_2 are consistent with Oxylite (Oxford Optronix, Oxford, UK) oxygen electrode measurements, when bladder flushing is used. This work was supported by grants P41RR12257 (NIH) and DAMD17-02-1-0034 (DOD).

APPENDIX: Slides from presentation at 26th International EPR Symposium, July 27-31, 2003, Denver, CO: Pelizzari, C.A., C.R. Haney, A. Parasca, K. Ichikawa, E.D. Barth, B.B. Williams, M. Elas, V.S. Subramanian, M.A. Zamora, J.N. River, G.S. Karczmar, H.J. Mauceri, R.R. Weichselbaum, and H.J. Halpern. "Biological Correlates of EPR Oxygen Images: Preliminary Images of Response to Radiation Plus Adenovirus Delivered EGR-TNF Anti-cancer Therapy".

Biological correlates of EPR oxygen images: Preliminary images of response to radiation plus adenovirus delivered EGR-TNF α anti-cancer therapy¹

Charles A. Pelizzari, Chad R. Haney, Adrian Parasca, Kazuhiro Ichikawa, Eugene D. Barth, Benjamin B. Williams, Martyna Elas*, V.S. Subramanian, Marta A. Zamora, Jonathan N. River, Gregory S. Karczmar, Helena J. Mauceri, Ralph R. Weichselbaum, Howard J. Halpern

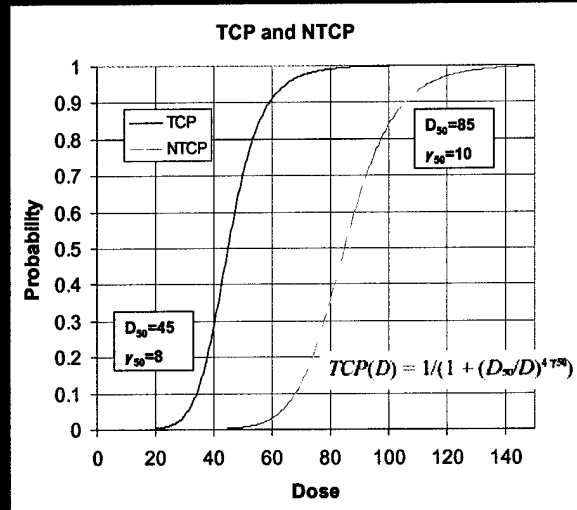
University of Chicago and *Jagiellonian University, Cracow, Poland

¹Supported by US Army Prostate Cancer Research Program (DAMD17-02-1-0034) and NIBIB Center for EPR Imaging for In-vivo Physiology (1 P41 RR12257)

Overview

- Limitation of single modality cancer therapy
- Radiation mediated gene therapy targeting tumor vasculature – a multimodal therapy
- Adaptive image guided therapy concept
- EPR imaging for evaluation of therapy effect
- Image registration and fusion
- Current results (work in progress)

Name of the game: increase dose to improve tumor control,
but do not exceed acceptable normal tissue toxicity



How to improve the odds? Increase toxicity to tumor (moves TCP
curve to left); improve targeting or reduce toxicity to normal tissues
(moves NTCP curve to right)

Gene therapy and radiation

Potential of radiation through
gene therapy: radiation-induced
expression of genes for cytotoxic /
antivascular agents

Enhancement and control of gene
therapy with radiation: IR
modulation of viral replication

Gene therapy for cancer is different from gene replacement therapy

Intention is to achieve cytotoxicity acutely and locally

Prolonged effect is not required

COMBINATION GENE AND RADIATION THERAPY

Objective of Gene Therapy Targeted by Radiation is to Combine Gene Therapy and Radiotherapy and to achieve Spatial and Temporal Control of Gene Therapy

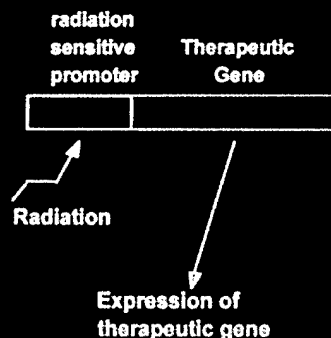
Concept

To utilize a promoter that is sensitive to radiation controlling a gene that is therapeutic

Advantage

The therapeutic gene is controlled (e.g. turned on only when irradiated)

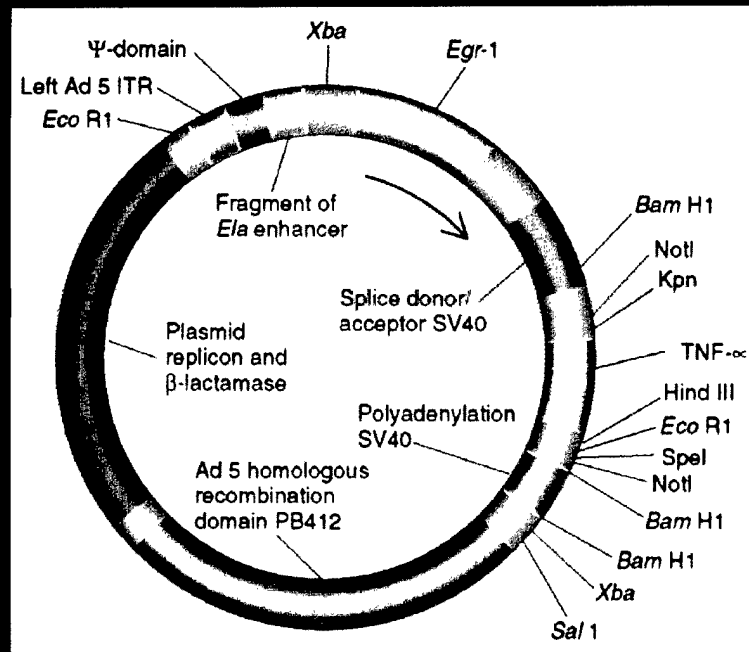
The tumor is exposed to two therapeutic modalities, gene therapy and radiation therapy



Rationale for antivascular therapy + IR

- Tumor viability depends upon vasculature
- One tumor vessel can supply up to 10^6 tumor cells
- Acute vascular destructive effects not generally characteristic of IR alone
- AVT might represent a means of targeting the destructive effect of IR

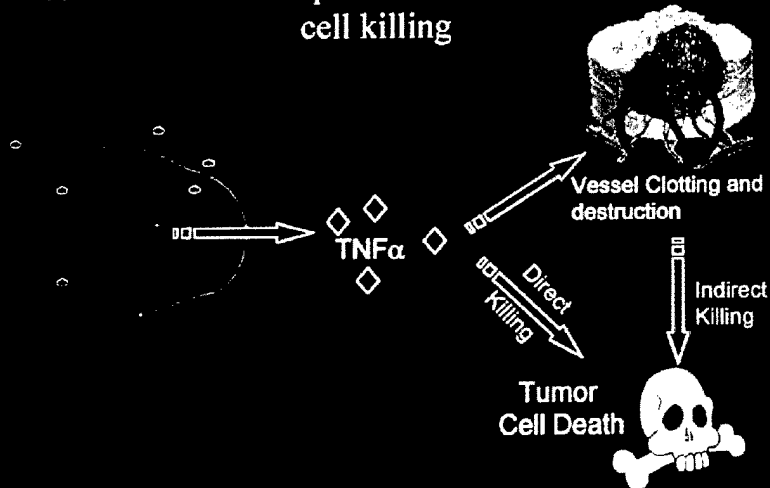
Ad.Egr.
TNF α
virus



Effects of TNF α

- Immune modulation
- Inflammation
- Cell death through Apoptosis
- Vascular Destruction
- Direct cell killing with IR

Induction of TNF α expression enhances IR-mediated cell killing

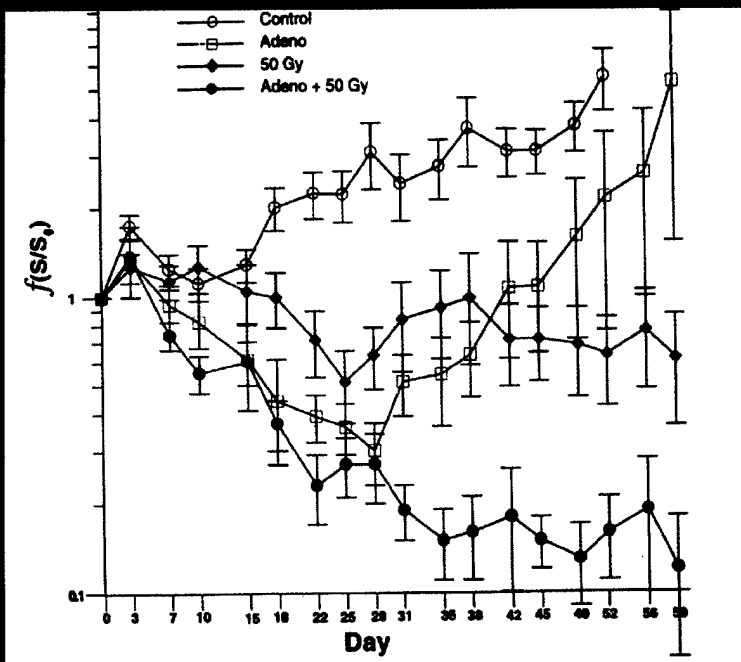


Treatment protocol for SQ-20B xenografts

Inject 1×10^6 SQ-20B cells into right flank on athymic nude mice tumors grown to $>100\text{mm}^3$

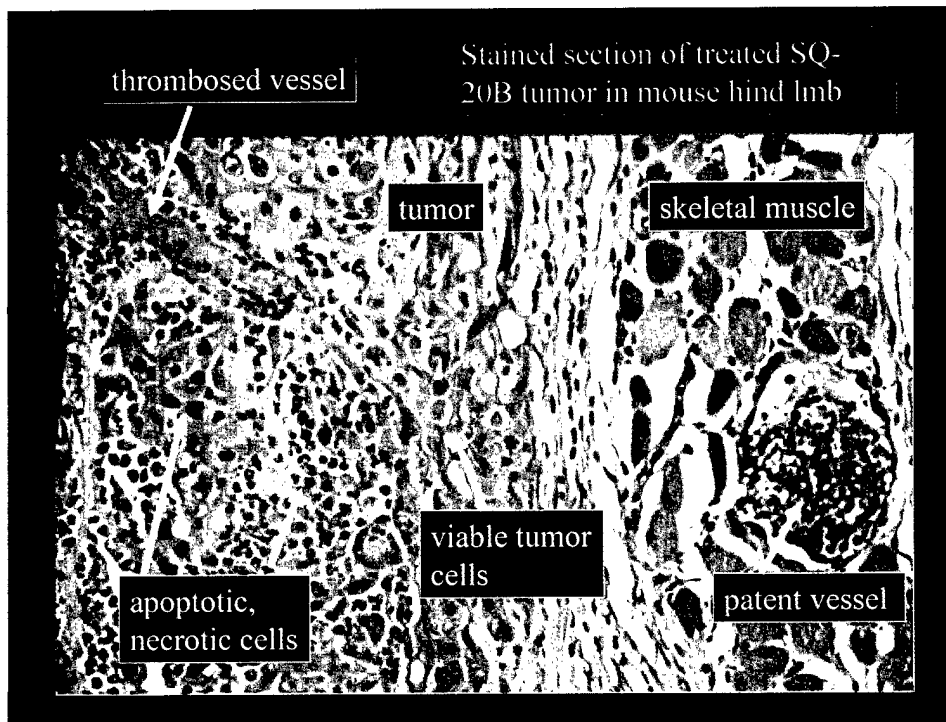
<u>IR:</u>	<u>Ad.EGR.TNFα:</u>	<u>IR+ Ad.EGR.TNFα</u>
50 Gy total	2×10^8 PFU	Virus injected
5Gy fractions	injected in 3 sites	4 hours prior IR
On M, T, Th, Fr	on M, Th, M, Th	on M, Th, M, Th

Tumor volumes ($1 \times w \times h/2$) measured 2 x / week for 60 days
 Fractional tumor volume defined a V/V_0



Intratumoral TNF α (pg/mg protein)

Day	+IR	-IR
7	2486 (20 Gy)	945
14	4300 (40 Gy)	950
21	20000 (50 Gy)	2643



Development of synthetic promoters for radiation-mediated gene therapy

Marples, et. AL, Gene Ther., 7(6); 511-517, 2000

- A synthetic gene promoter was constructed containing four directly repeated CarG elements from the Egr-1 promoter.
- MCF-7 (breast adenocarcinoma) and U87 (glioma) cells were transfected with the plasmid containing the GFP coding sequence under the control of the synthetic promoter
- The synthetic promoter was more radiation-inducible than the wild-type Egr-1 promoter. Maximal GFP expression was observed in both cell lines at the dose of 3 Gy.

Promise of radiation induced gene therapy:

- Gene therapy can be controlled and combined with radiotherapy to increase the therapeutic ratio
- Gene therapy can be controlled in a spatial and temporal manner to limit systemic toxicity
- New promoter elements with enhanced specificity will allow for more toxic gene products to be produced
- But: can be frustrated by poor geometric distribution of viral vector

Hypoxia and radiation inducible promoter

:

EPO-EGR-TNF Construct

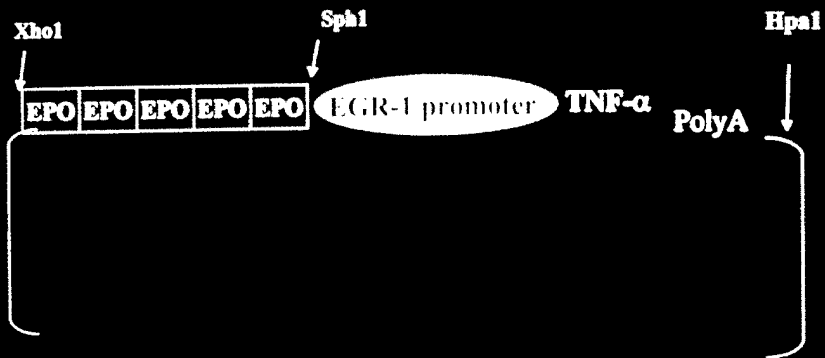


Image Guided Therapy

- Stages of guidance
 - planning
 - setup
 - real time guidance
 - real time feedback
 - adaptation of multiple treatments
 - assessment of effect
 - followup
- Not every application uses all of these

Adaptive Image Guided Therapy

- For fractionated treatments, use imaging to get feedback on spatial distribution of well and not so well treated regions
- “Adapt” subsequent fractions to compensate for any deficiency so identified
- Repeat at each fraction, or until satisfactory distribution is achieved
- Requires recognition of “signature” of well treated regions

Protocol for present work

- Attempt to identify characteristic changes in tissue oxygenation due to treatment
- Nude mice inoculated in hind limb with 10^7 human prostate cancer (PC3) cells
- Tumors grown to $\sim 1\text{cm}^3$
- Day 0: imaging followed by treatment: 2×10^8 PFU Ad.Egr.TNF α + 10 Gy X-rays (250 kVp)
- Day 3, day 16: imaging
- Consecutive EPR and MR images

What might we expect to see?

- Early:
 - destruction of vasculature, necrosis centrally
 - reduced tissue oxygenation?
 - revascularization / reoxygenation peripherally?
- Late:
 - recovery; regrowth
 - return to pretreatment status?

Image registration

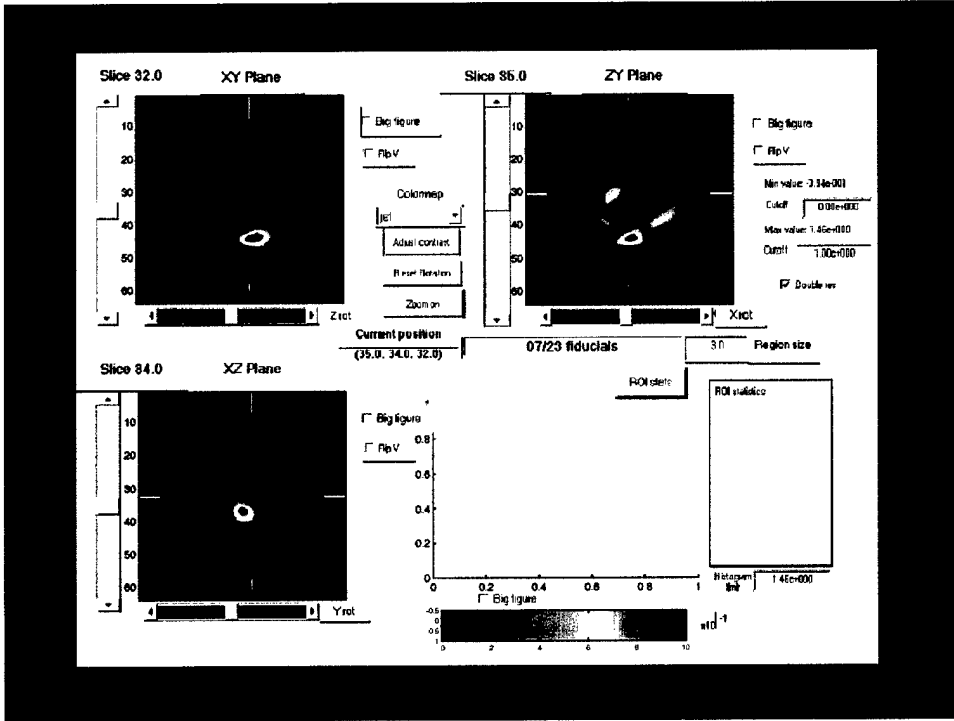
- Note that recognizing image changes due to treatment requires identification of anatomically identical locations in serial images
- Cannot reproduce tumor position with sufficient accuracy (~ 1 mm)
- Utilize registration methods based on matching images

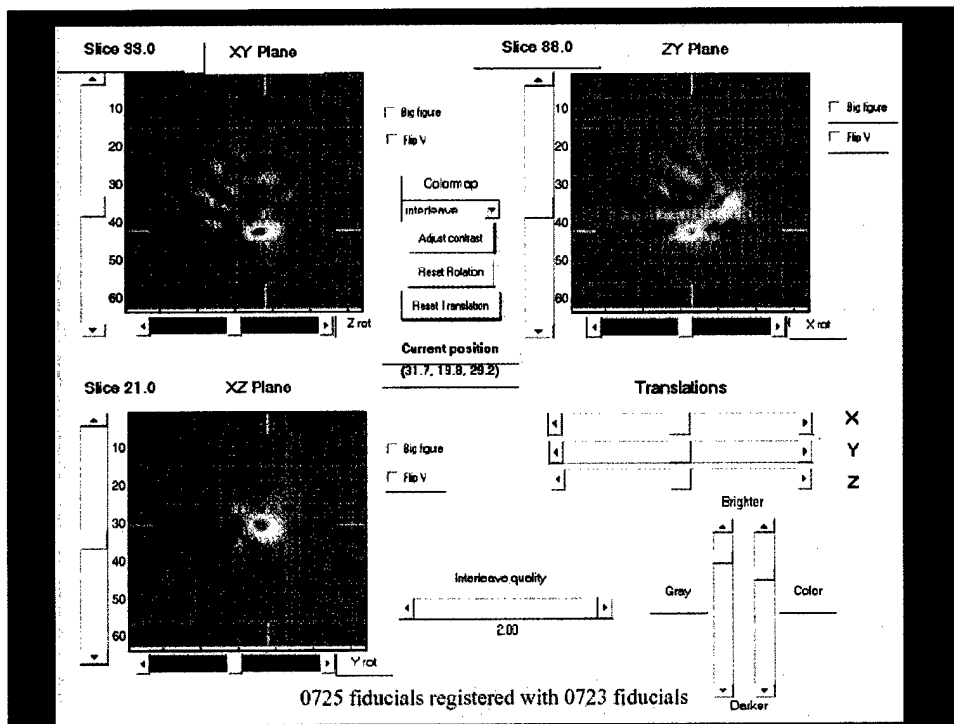
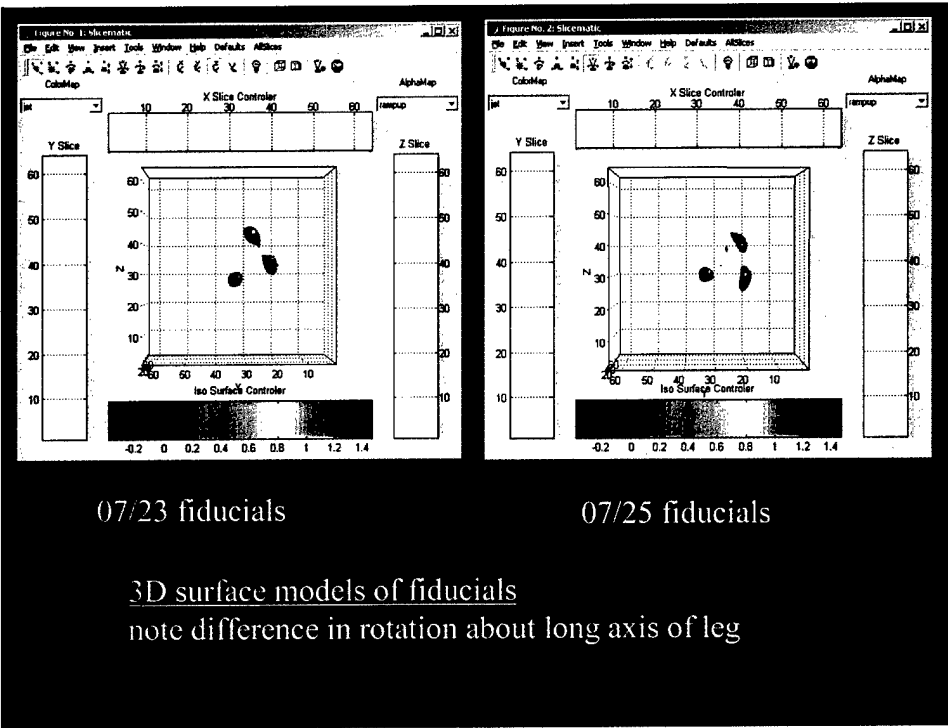
Image registration methods

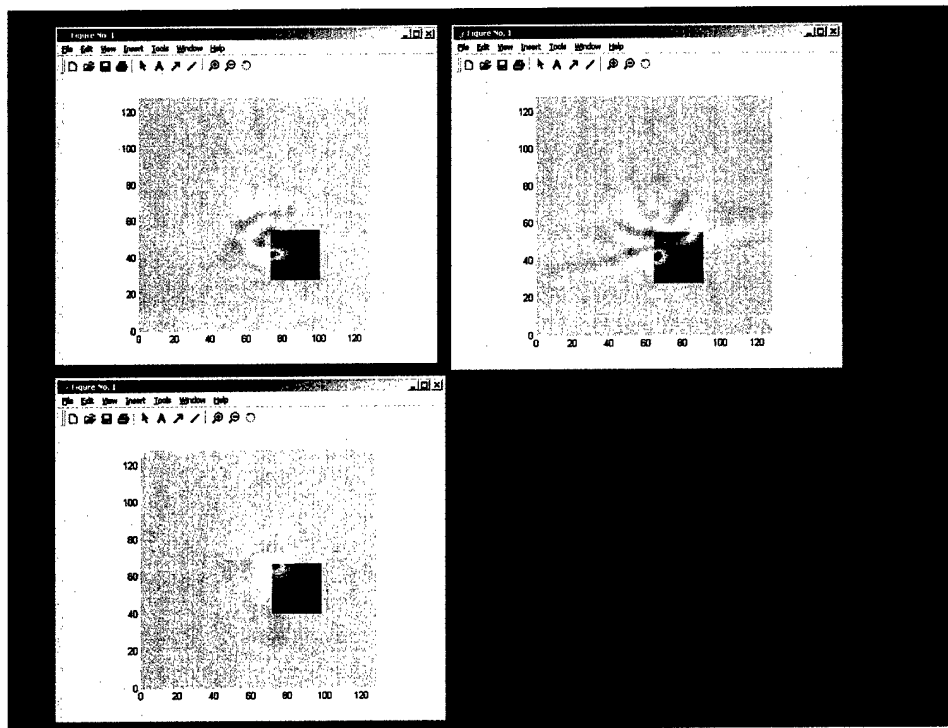
- “Prospective” – add something to the image “scene” that is distinctive and well visualized, and the anatomy is reliably positioned with respect to
 - marker system on animal holder (points, lines)
 - ideal for multiple images on same day if animal doesn’t move
 - fiducial markers on skin or implanted
 - useful for serial imaging as well
 - easy to find transformation
 - requires suitable material for multiple modalities

Image registration methods

- “Retrospective” – determine relative position of anatomy in the image coordinate system by matching the anatomy
 - Manual or automated matching of surface models of 3D objects (e.g. skin, bone)
 - Manual or automated overlay of entire image intensity distribution
 - maximize intensity correlation
 - maximize mutual information

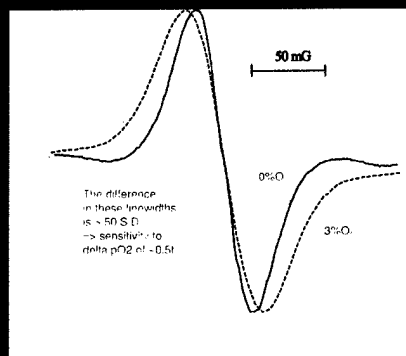




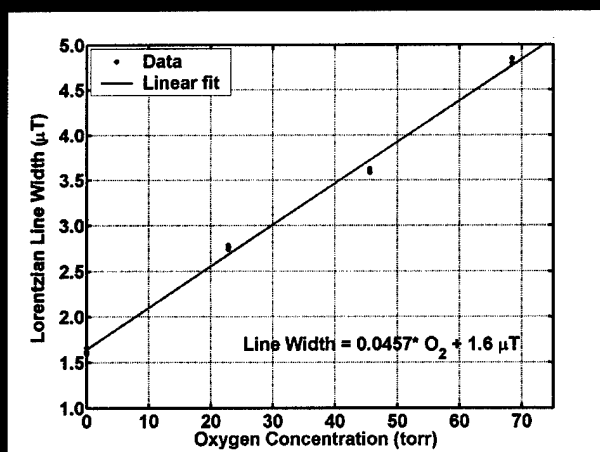


Tissue Property	Spin Probe	Spectral Change
Oxygen	 	
Viscosity		
pH		
RSH (Thiol)		

Response of Symmetric Trityl (deuterated) to Oxygen

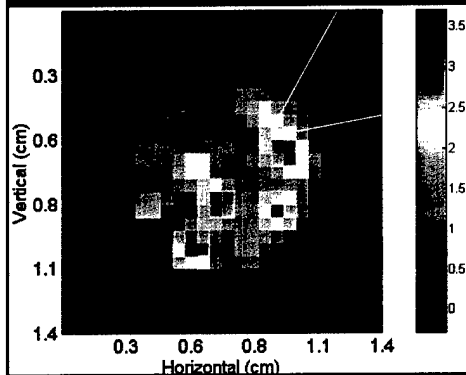
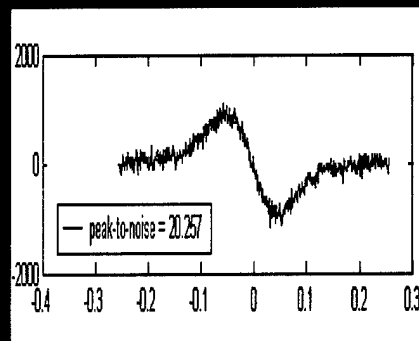


- No temp. dependence: < 0.05 mG/K
- Low viscosity dependence ~ 1 mG/cP
- Minimal self quenching



Oxygen dependence of spin packet width obtained in a series of homogenous solutions of OX31:
 Since minimal viscosity dependence, aqueous=tissue

Spectral spatial imaging:



- CW EPR imager at 250 MHz, with a loop-gap resonator
- OX063 spin probe, 50 mg/mouse





Bladder flushing to prevent accumulation of spin probe is essential to suppress image artifacts

Reduction of Image Artifacts by Bladder Flushing with a Novel Double Lumen Urethral Catheter.

Chad B. Hurre, Karanpal Khanna, Ashraf Parvez, Pragnath B. Williams, Eugene D. Burch, Martin Eric, and Howard J. Halperin
The University of Chicago, Department of Radiology and Urology, MC 1055, 5841 S. Maryland Ave., Chicago, IL 60637, 1997

The intrabulbar spin probe (SP) developed by Accumed Instruments (Moline, IL) is used as a urinary spin probe for void imaging. However, the accumulation of spin probe in the bladder creates a transmission source of signal, often greater than that of the tissue due to void backscattering, not the backscattering from the desired measurement.

OBJECTIVE: Due to the paucity of methods available for a new procedure, MULTIPHASE (University of Chicago) a novel double lumen urethral catheter was developed. The method also has to accommodate both MRI and IFR for multiple views using the same device.

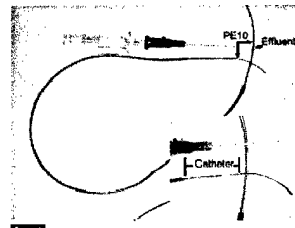


Figure 1

Methods: A standard 20 gauge IV catheter (1.2 mm ID) between urethra IV Catheter B, Becton Dickinson, Franklin Park, IL) is inserted into the urethra of an anesthetized mouse with a 100 mg/kg of 10% urethane. After which the first lumen containing 10 ml of saline and a catheter lumen is connected with PE10 tubing (20 mm ID, 0.34 mm OD) (Accumed Instruments, Moline, IL). Franklin Park, IL) threaded into the IV catheter. The PE10 was threaded until it is able to coil within the bladder maintaining the shape of being trapped against the bladder wall. Using a Harvard 22-Channel pump (Harvard Apparatus, Inc., Holliston, MA) water at 10 ml/hr was infused into the bladder via the PE10 tubing. The effluent from the bladder exit the tubing as shown in Figure 2.



Figure 2

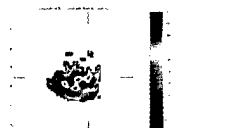
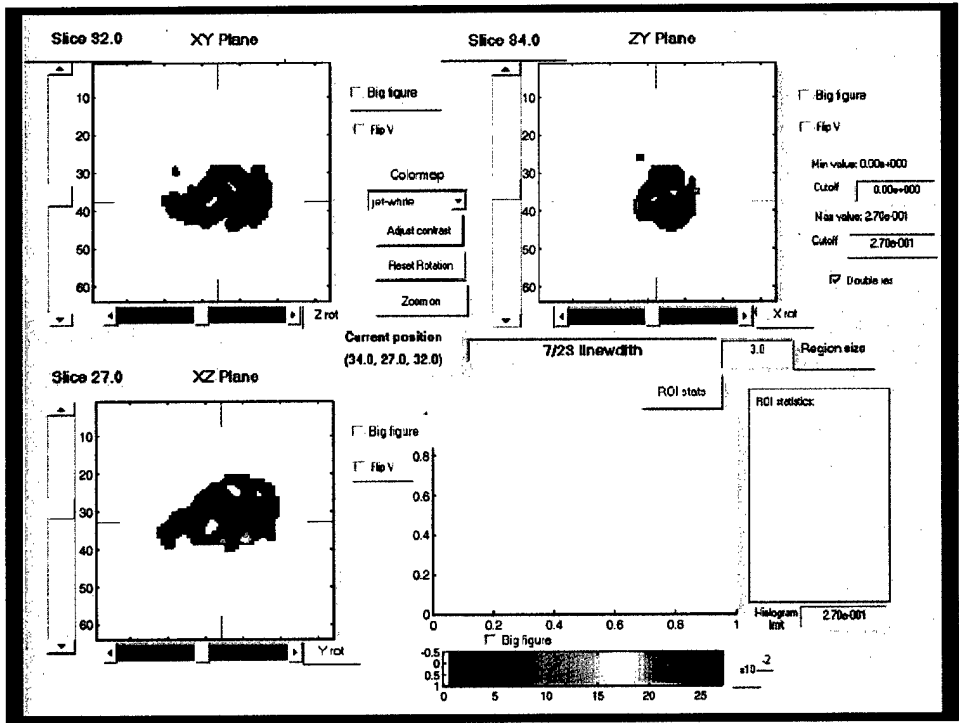
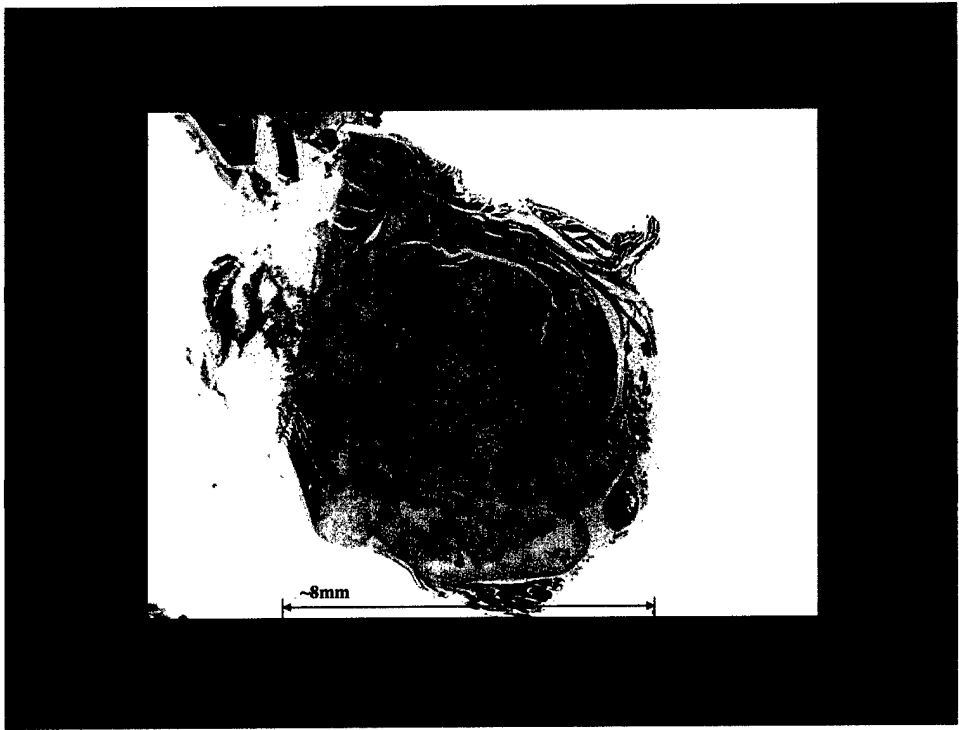
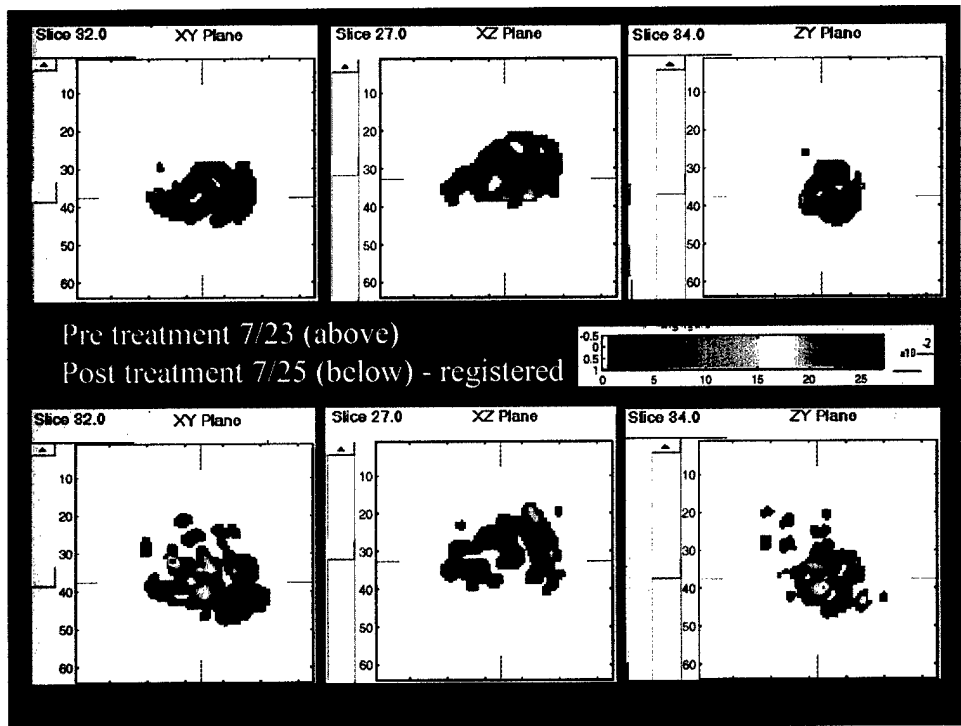
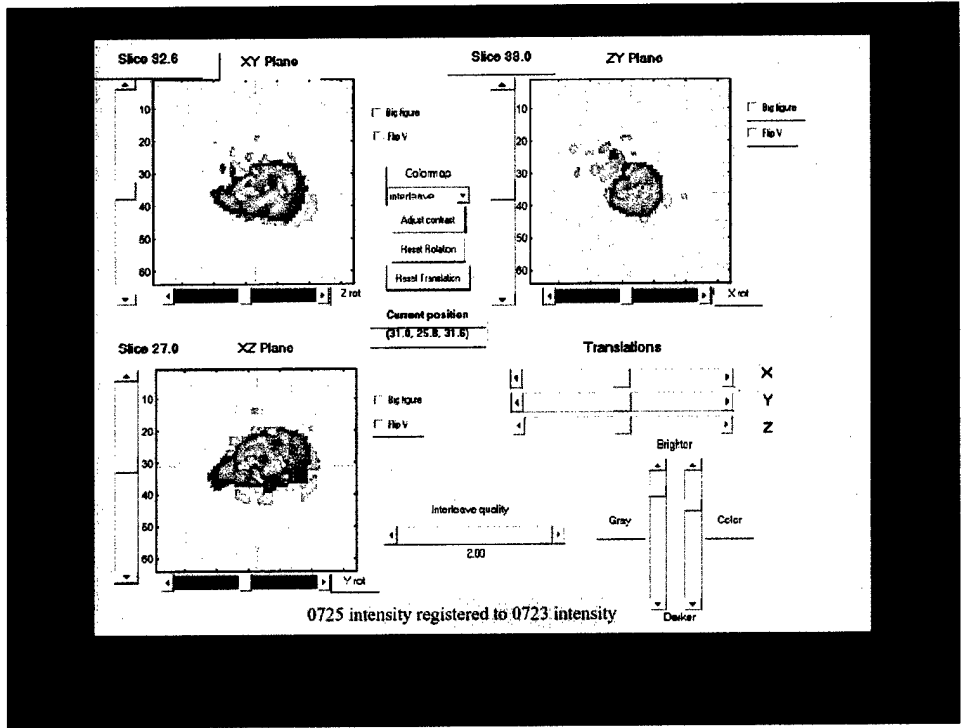


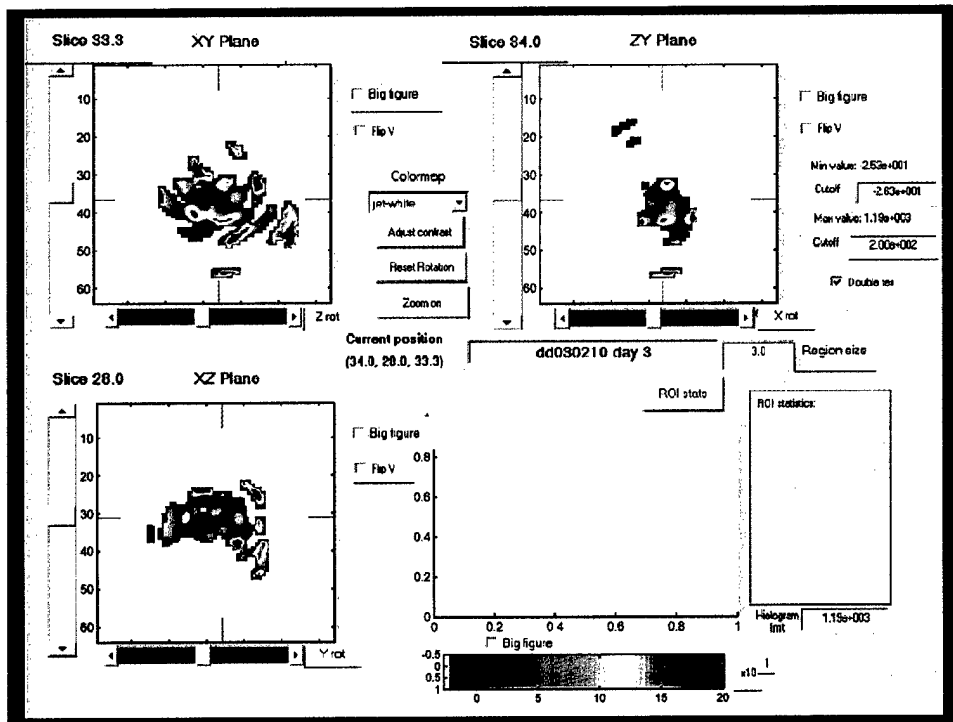
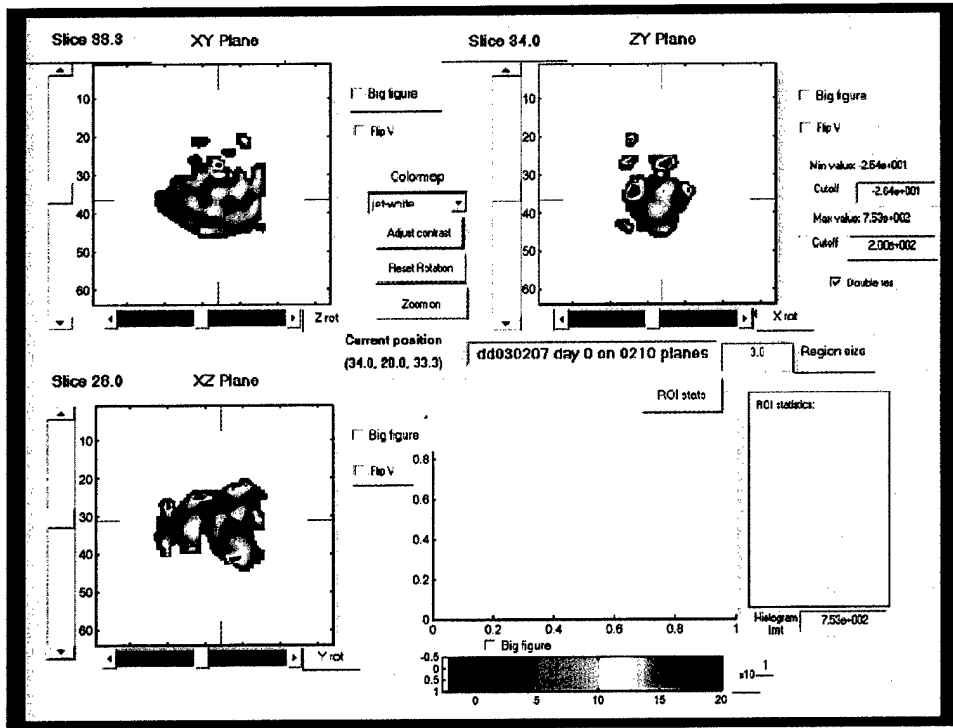
Figure 3

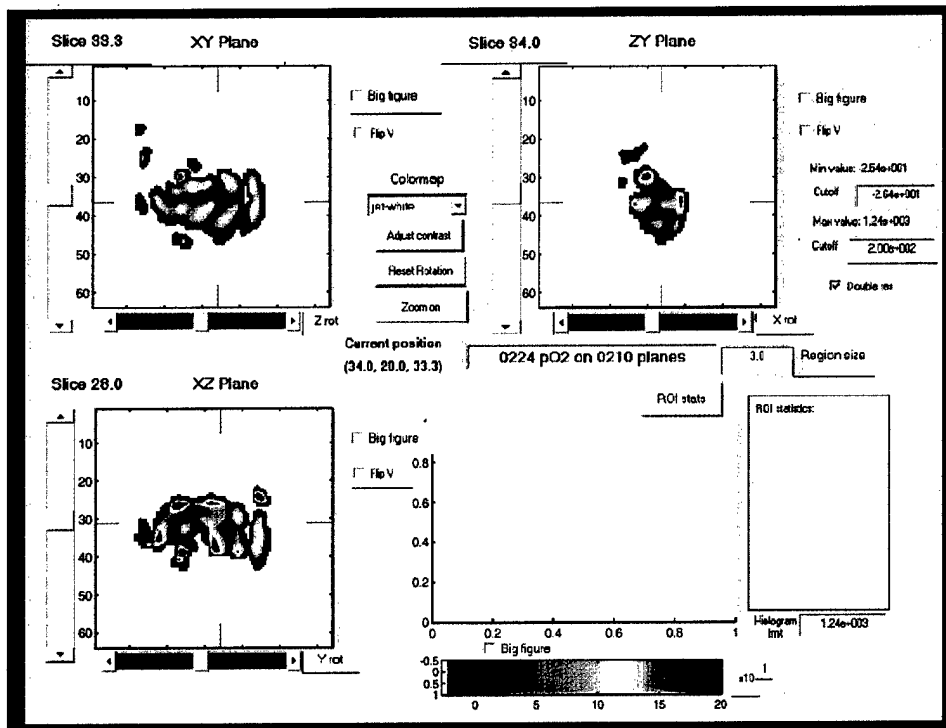
Results: In Figure 2 the bladder is shown with artifact propagating from it. The void backscattering with the SP makes the bladder appear larger as well. The lower signal is overexposed and cannot be seen. However, in Figure 3, when bladder flushing is used properly, there is no loss of bladder signal. Furthermore, the heterogeneity of the tumor is clearly demonstrated without obscuration of artifact from the bladder signal.

Discussion: Further refinement is necessary i.e., the placement of the catheter such that it does not get trapped against the wall of the bladder. Also, to improve the double lumen urethral catheter provides a substantial reduction in artifacts when compared to simple saline wash of bladder. The large volume flushing. Artifacts from the bladder signal have been successfully attenuated using a method which is compatible with our current multiple imaging project. The catheter images connected to PE10 are connected with Catheter (Catheter Optimal, Catheter, IL) except electronic maintenance when bladder flushing is used. The authors appreciate the support of NIH (R01-DC04101) and the support of the University of Chicago.









Summary

- EPR oxygen images appear promising for identification of treated regions in radiation induced antivascular gene therapy.
- Significant experimental difficulties associated with accurate registration of serial and multimodality images are a current focus of activity.
- Bladder flushing to suppress artifacts is essential for acquisition of useful EPR images.



APPENDIX: Slides from presentation at Radiological Society of North America 2003 International Meeting and Scientific Assembly: Haney, C.R., C.A. Pelizzari, H.J. Halpern, A. Parasca, H.J. Mauceri, and R.R. Weichselbaum. "Physics Invited Speaker: EPR Oxygen Imaging for Assessment for Radiation Induced Gene Therapy".



EPR Oxygen Imaging for Assessment for Radiation Induced Gene Therapy ¹

Chad R. Haney, Charles A. Pelizzari, Howard J. Halpern, Adrian Parasca,
Helena J. Mauceri, and Ralph R. Weichselbaum

Department of Radiation and Cellular Oncology
University of Chicago

¹Supported by US Army Prostate Cancer Research Program
(DAMD17-02-1-0034) and NIBIB Center for EPR Imaging for *In
Vivo* Physiology (1 P41 RR12257)



Department of Defense
CONGRESSIONALLY DIRECTED
MEDICAL RESEARCH PROGRAMS

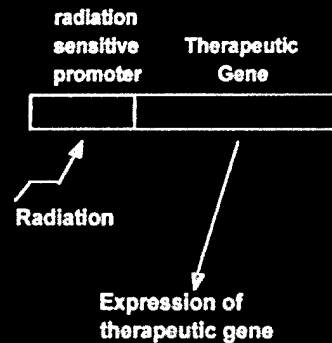


Outline

1. Combined Gene Therapy + Radiotherapy
2. Electron Paramagnetic Resonance (EPR)
Imaging
3. Preliminary Results

Combination Gene and Radiation Therapy

Genetically engineered adenovirus induces expression of gene of interest (TNF α) only in irradiated areas



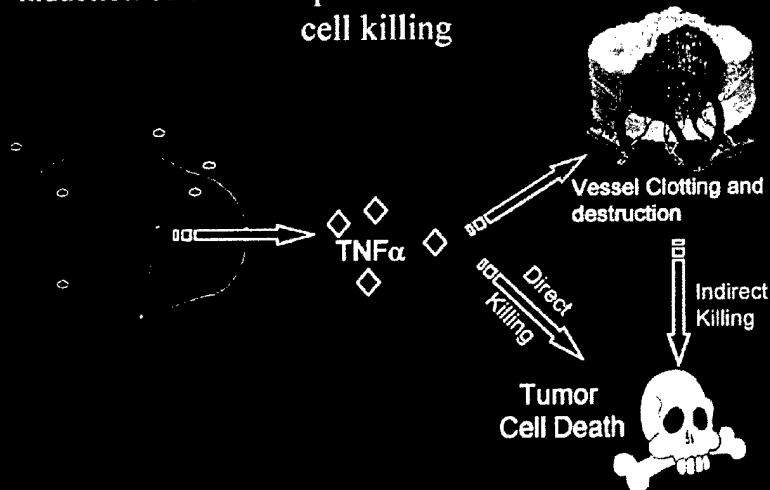
Rationale for anti-vascular therapy + IR

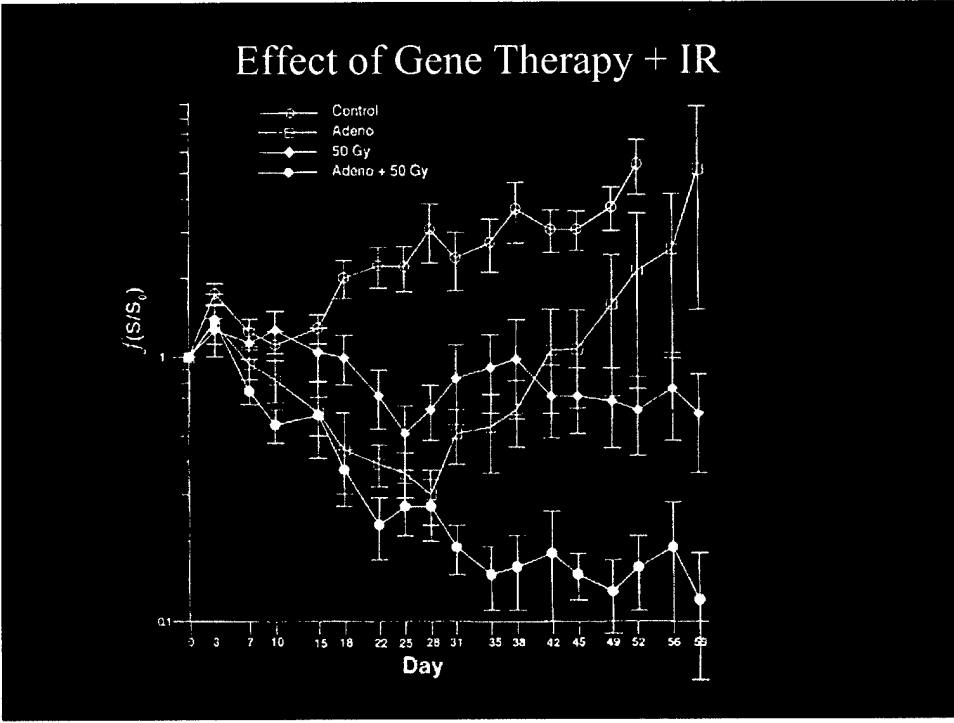
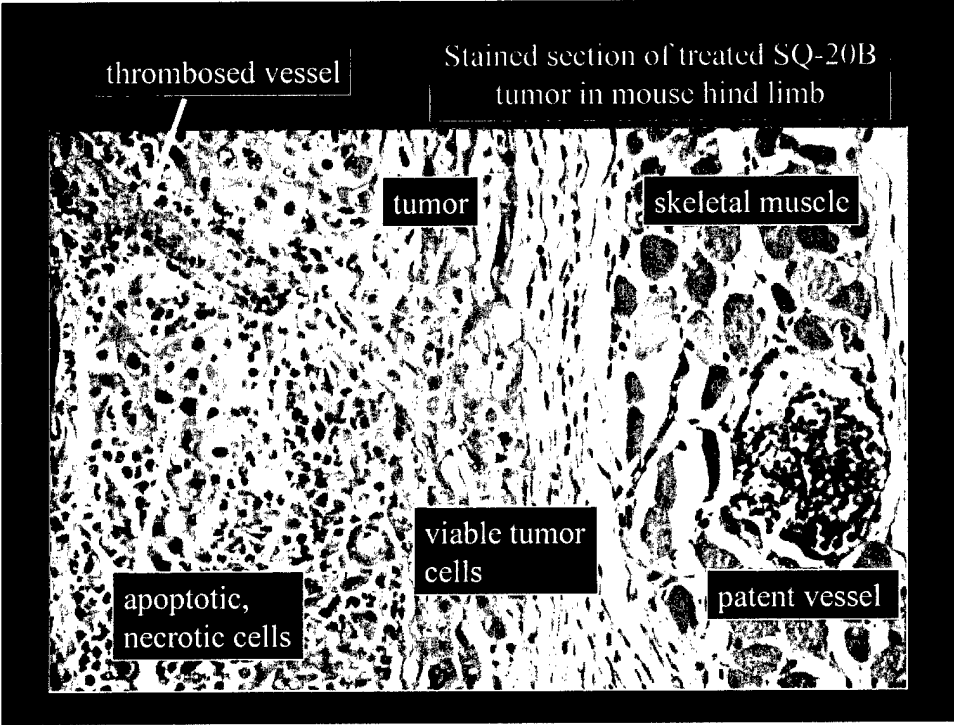
- One blood vessel can supply up to 10^6 tumor cells
- IR alone not specifically associated with acute anti-vascular effects
- Means for targeting the destructive effect of IR
- IR potentiated by anti-vascular therapy

Effects of TNF α

- Cell death through apoptosis
- Immune modulation
- Inflammation
- Vascular destruction
- Direct cell killing with IR

Induction of TNF α expression enhances IR-mediated cell killing





Promise of Radiation Induced Gene Therapy:

- Control of gene therapy:
 - Combination with radiotherapy increases the therapeutic ratio
 - Spatial and temporal control limits systemic toxicity
- Downside: Poor geometric distribution of viral vector can compromise treatment

Adaptive Image Guided Therapy

- Use imaging to get feedback for fractionated treatments
- “Adapt” subsequent fractions
- Repeat at each fraction (unless satisfactory distribution is achieved)
- Requires recognition of “signature” of well treated regions

Protocol for Present Work

- Attempt to identify “signature” of successful treatment (based on oxygenation/perfusion)
- Nude mice inoculated in hind limb with 10^7 human prostate cancer (PC3) cells
- Tumors grown to $\sim 1\text{cm}^3$
- Day 0: Imaging followed by treatment: 2×10^8 PFU Ad.Egr.TNF α + 10 Gy X-rays (250 kVp)
- Day 3, day 16: Imaging
- Consecutive EPR and MR images

What do we expect to see?

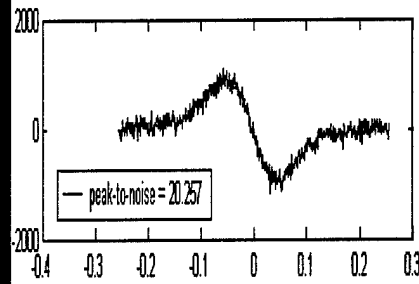
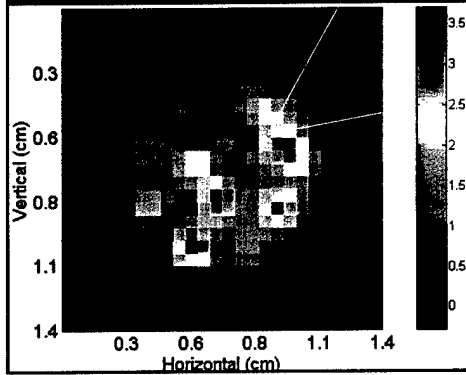
- Early (3 days post treat):
 - destruction of vasculature, necrosis centrally
 - reduced tissue oxygenation?
 - revascularization / reoxygenation peripherally?
- Late (16 days post single fraction):
 - recovery; regrowth
 - return to pretreatment status?

EPR Spectral Spatial 4D Imaging



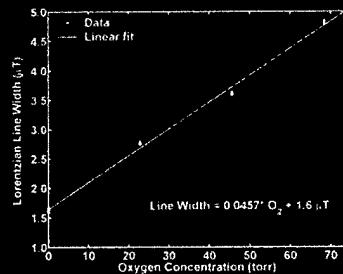
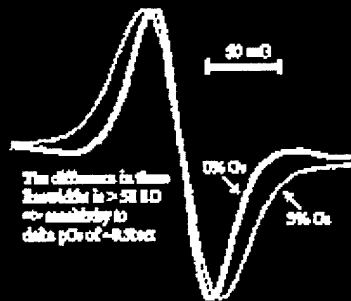
Tissue Property	Spin Probe	Spectral Change
Oxygen		
Viscosity		
pH		
RSH (Thiol)		

Spectral Spatial Imaging:



Each voxel $(0.6 \text{ mm})^3$ contains an EPR spectrum

Response of Trityl (contrast agent) to Oxygen



- No temp. dependence; $< 0.05 \text{ mG/K}$
- Low viscosity dependence $\sim 1 \text{ mG/cP}$
- Minimal self quenching

Oxygen dependence of spin packet width obtained in a series of homogenous solutions of OX031: Since minimal viscosity dependence, aqueous=tissue

Bladder flushing to prevent accumulation of spin probe is essential to suppress image artifacts

Reduction of Image Artifacts by Bladder Flushing with a Novel Double-Lumen Urethral Catheter.

Chad E. Hively, Karolyn K. Williams, John P. Parnis, Gregory B. Williams, Eugene J. Barry, Matthew H. Lee, and Donald H. Meyer

The University of Texas, Department of Radiation Oncology, M.D. Anderson Cancer Center, Houston, TX

The spin probe spin probe (SP) developed by National Instruments (Austin, Texas) is useful as a urinary spin probe for ¹²⁵I spin imaging. However, the accumulation of spin probe in the bladder creates a transient source of signal often greater than that of the tumor during spin imaging, and the resulting by-capture often obscures measurement.

Objective: Due to the paucity of methods available for a non-invasive MRI/SP device, procedures for flushing a novel double-lumen urethral catheter are developed. The method also has to accommodate both MRI and SP, or multiple slices, using the same device.

Methods:

A standard 20-gauge catheter (20 mm ID, Intecum, Irvine, CA) (catheter B, Becton Dickinson, Becton Dickinson) is inserted into the bladder of an animal and secured with a spring-loaded lock. After what the lock mechanism is in place and a radioisotope solution is injected into the bladder, the catheter is connected with PE10 tubing (25 mm ID, 0.4 mm ID, Corning, Irvine, CA) (catheter A). The catheter is then inserted into the bladder, and the PE10 tubing is connected such that it is able to rest within the bladder, minimizing the chance of being impinged against the bladder wall. Using a Harvard 22 syringe pump, 0.5 ml of spin probe (100 μCi) is injected into the bladder via the PE10 tubing. The effluent from the bladder over the tubing is shown in figure 1.

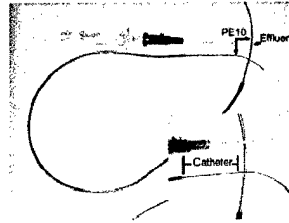


Figure 1

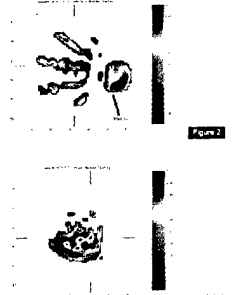


Figure 2

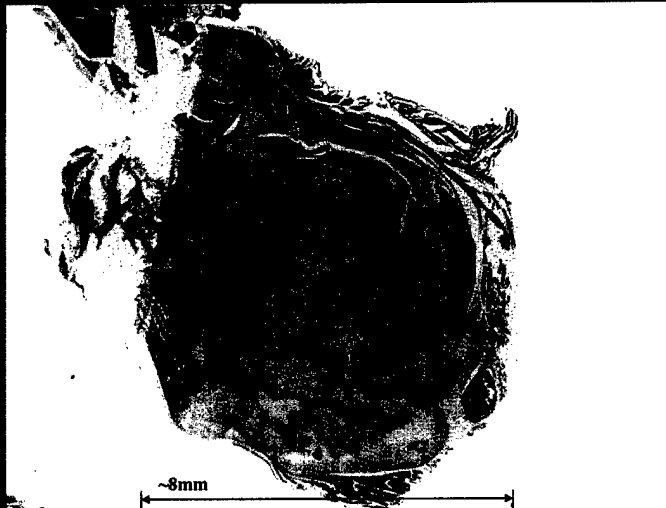
Figure 3

Results: In figure 2 the bladder is shown with spin probe injected from A. The spin probe is visible in the bladder. However, the spin probe is obscured and cannot be easily visualized. However, in figure 3, when the bladder is flushed properly, there is no spin of bladder signal. Furthermore, the spin probe of the tumor is clearly demonstrated, without obscuration of signal from the bladder signal.

Discussion:

Further refinement might involve the placement of the catheter such that it does not get impinged against the wall of the bladder. However, the double-lumen urethral catheter provides a substantial reduction in artifact when compared to groups that do not flush the bladder or use flushing. Artifacts from the bladder signal have been successfully eliminated using a method which is compatible with our current multiple-lumen probe. Use with tumors connected to the bladder is consistent with the use of double-lumen catheters when bladder flushing is used. The use of a double-lumen catheter is a novel approach to the problem of bladder flushing.

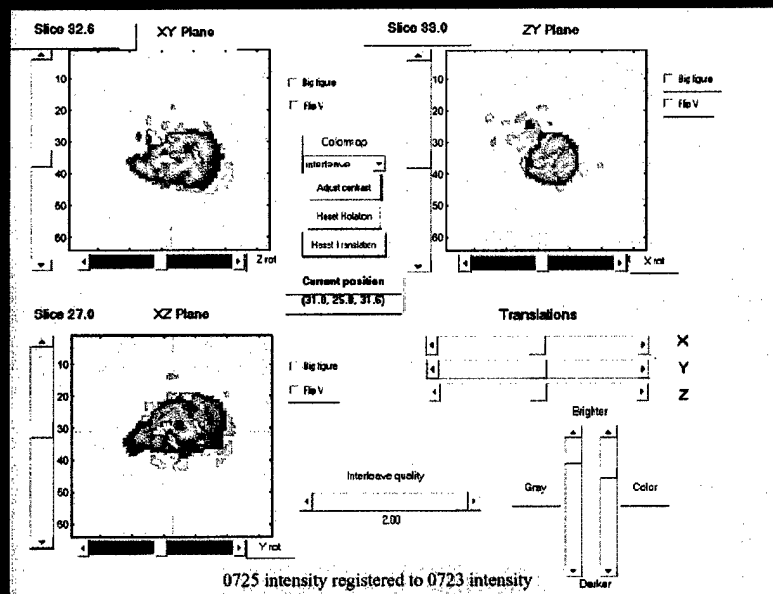
Section of Leg with PC3 Tumor



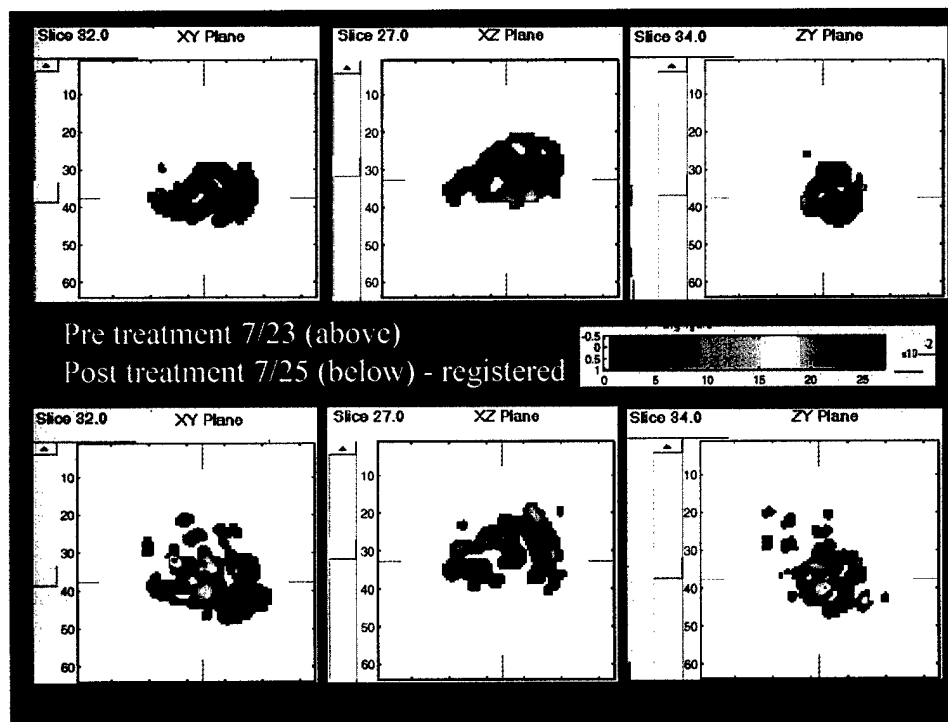
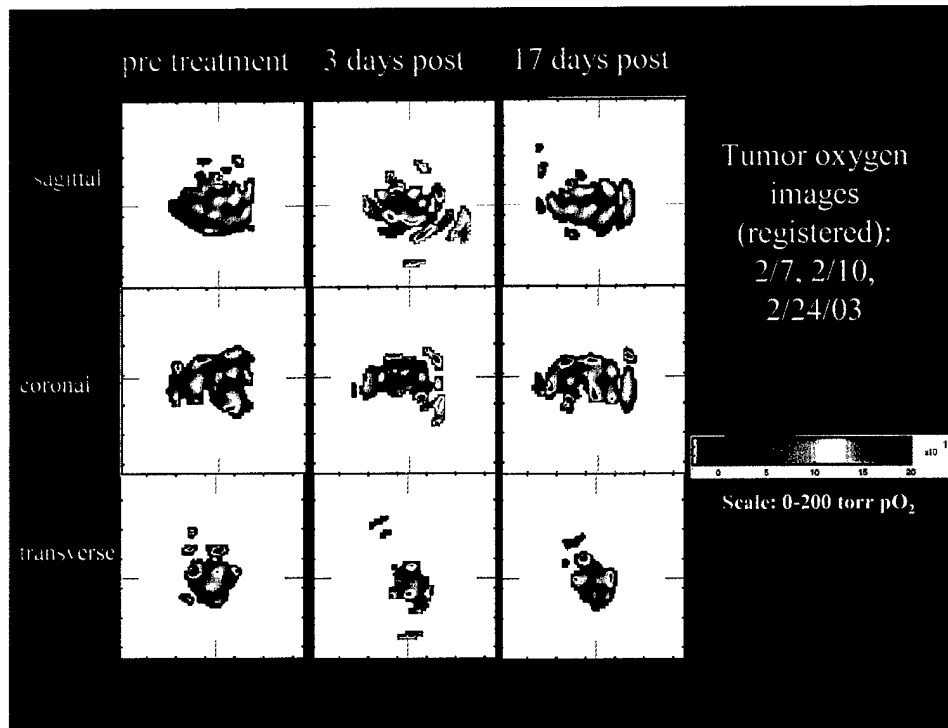
~8mm

Image Registration

- Require identification of anatomically identical locations in serial images
- However, cannot reproduce tumor position with sufficient accuracy ($\sim 1\text{mm}$)
- Utilize registration methods based on matching features in images
- Used to match EPRI with MRI on same day and between days



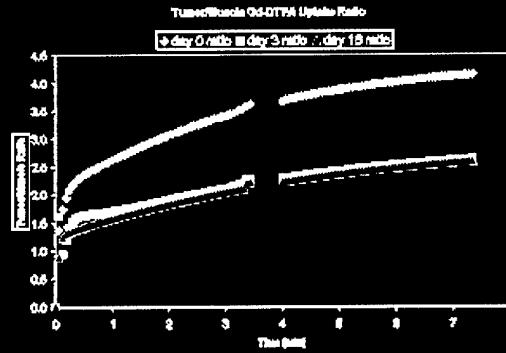
Manual interactive image registration



T₁ Weighted Images



Before and after
TNF α +IR treatment
(2/7/03 & 2/10/03)



Gd-DTPA uptake tumor/muscle
ratio, Day 0, Day 3, and Day 16

Summary

- EPR oxygen images appear promising for identification of treated regions in radiation induced anti-vascular gene therapy.

- Areas of improvement:

- Significant experimental difficulties associated with accurate registration of serial and multimodality images

- Bladder flushing to suppress artifacts is essential for acquisition of useful EPR images.

For more information and pictures see:

<http://rover.bsd.uchicago.edu/lfepr/>

APPENDIX: Manuscript accepted for presentation at 2004 IEEE International Symposium on Biomedical Imaging: From Nano to Macro, April 2004, Arlington, VA:
C.R. Haney, A. Parasca, K. Ichikawa, M. Elas, M. Zamora, X. Fan, G.S. Karczmar, H.J. Halpern and C.A. Pelizzari, "Assessment Of Radiation Mediated Gene Therapy Via Multi-Modality Imaging,"

ASSESSMENT OF RADIATION MEDIATED GENE THERAPY VIA MULTI-MODALITY IMAGING

C.R. Haney*, A. Parasca*, K. Ichikawa*, M. Elas*, M. Zamora#, X. Fan#, G.S. Karczmar#, H.J. Mauceri*, R.R. Weichselbaum*, H.J. Halpern* and C.A. Pelizzari*

The University of Chicago, *Department of Radiation and Cellular Oncology (MC 1105), #Department of Radiology (MC 2026), 5841 S. Maryland Ave, Chicago, IL 60637-1463

1. ABSTRACT

Imaging techniques are under development which, are intended to facilitate early analysis of spatial patterns of tumor response to combined radiation and antivascular gene therapy. We present initial results demonstrating treatment related changes of vascular permeability and blood volume maps using MRI, and tissue oxygenation measurements using electron paramagnetic resonance imaging (EPRI). Using a modified Kety model, the volume fraction accessible to the MRI contrast agent was significantly greater in the tumor (0.81 ± 0.11), compared to muscle (0.20 ± 0.00 , $p < 0.05$). However, the two compartment model is probably not able to account for accumulation of contrast agent in the tumor. A three compartment model may improve fitting.

2. INTRODUCTION

Progress in radiation therapy has been facilitated by the development of methods for quantitative estimation of the distribution of radiation dose, so the dose distribution can be optimized to accommodate the particular geometry of both tumor and normal tissues in an individual patient. For example, in image-guided prostate brachytherapy, dose is estimated both prospectively in computerized treatment planning calculations based on a planned ideal configuration of implanted seeds, and retrospectively using imaging to detect the actual location of seeds and calculate the as-delivered dose to target and normal tissues. Intraoperative image-guided adaptation of the seed distribution is also possible, using ultrasound or X-ray fluoroscopic localization of the seeds as they are implanted. As each succeeding seed is added to the implant, its position can be optimized taking into consideration the actual positions of all preceding seeds, which inevitably deviate somewhat from the ideal planned positions. Extra seeds can be added to treat identified cold spots, and seed spacing can be adjusted to avoid overdosing critical structures such as the rectum and urethra. This

adaptive image guidance strategy is applicable in principle to many other forms of therapy.

Radiation inducible gene therapy is radiation combined with gene therapy, where gene expression is spatially and temporally controlled by the irradiation. In the modality used in the present study, radio-inducible elements from the Early Growth Response-1 (EGR-1) gene promoter are inserted upstream of a cDNA encoding Tumor Necrosis Factor-alpha (TNF- α). Replication-defective adenovirus is used as the vector and the construct is called Ad.EGR-TNF. TNF- α is a potent antitumor and antivascular agent, with a variety of potential undesirable side effects on normal tissues. By limiting its expression only to the areas irradiated, the systemic side effects may be minimized, while the synergistic effect of irradiation and TNF- α are exploited. Preclinical studies have shown that tumors infected with Ad.EGR-TNF respond to radiation with induction of TNF- α expression and substantial increases in antitumor activity. [1, 2]

Ultimately, this form of therapy is likely to be delivered in a fractionated scheme, with daily doses of radiation and semi-weekly or weekly injections of gene vector. It is the premise of the overall project of which the present work is a part, that imaging may provide feedback as to the spatial distribution of vascular damage and cell death due to the combined therapy, and that this information may be used to optimize subsequent fractions (both injection pattern and radiation dose distribution) to accommodate the possible imperfections of the observed spatial distribution of response. This "adaptive image guided therapy" approach clearly depends on the availability of suitable images to provide the necessary feedback. The present report summarizes our experience to date with the development of electron and nuclear magnetic resonance imaging techniques for this purpose.

The use of TNF- α to target tumor vasculature will reduce tumor blood vessels or induce thrombosis and necrosis. This effect should be detectable directly with MRI sensitive to local blood volume or microvessel area-permeability product. Changes in

tumor oxygenation can also be imaged with a novel high resolution, high sensitivity modality, Electron Paramagnetic Resonance Imaging (EPRI). Subsequent reoxygenation during the course of fractionated radiation can also be imaged and used as a marker for response. We present here preliminary results from MRI demonstrating decreased perfusion with treatment. Complementary reduction in oxygenation demonstrated by EPR images will also be discussed.

3. METHODS

Athymic nude mice (average weight 25 g) were injected subcutaneously with PC3 human prostate cancer tumor cells in the right hind limb. In approximately two weeks the tumors reached a mean diameter of 5 - 8 mm. On the initial treatment day ("day 0"), both EPRI and MRI imaging were performed, followed by injection of the Ad.EGR-TNF vector (2×10^8 plaque forming units), and irradiation with 10 Gy of 250 kVp X-ray 2-3 hours post injection. Three and sixteen days later ("day 3" and "day 16"), the mice were again imaged with EPRI and MRI. No additional doses of either viral vector or radiation were given after day 0.

A mixture of ketamine HCl (50 mg/kg)/xylazine (2.1 mg/kg) was used to anesthetize the mice during imaging via an IP line. A standard 24 gauge IV catheter (1.1 mm ID, Introcan Safety IV Catheter, B. Braun Medical Inc., Bethlehem, PA) was inserted into the tail vein for the injection of contrast agent. Unless preventive measures are taken, the EPR contrast agent (OX063 spin probe, Nycomed Innovation AB, Malmö, Sweden) accumulates in the bladder, creating large image artifacts. To address this problem, we developed a novel double lumen catheter which was inserted into the bladder via the urethra, and through which the bladder contents were flushed continuously during EPR imaging with a slow flow of sterile water driven by an infusion pump. All animal procedures were performed under IACUC approved protocols.

The EPR imaging methodology has been described earlier [3, 4]. Briefly, 3 g/kg OX063 was given as an IV bolus. A 4D spectral spatial image was acquired with 8×8 spatial and 16 spectral projections, and reconstructed using filtered back projection to yield a 64 point spectrum at each of $64 \times 64 \times 64$ voxels. The reconstructed voxel spacing was 0.55 mm. The spectrum at each voxel was fitted to a physics-based model [5]. The fitted Lorentzian linewidth, which is broadened linearly with oxygen concentration, was used to map the pO_2 . The linewidth vs. pO_2 characteristic of OX063 was measured utilizing

samples equilibrated with gas mixtures with known oxygen concentration.

Images were acquired at 4.7 Tesla, using a 30 cm diameter bore Omega MRI Scanner (General Electric/Bruker) equipped with self-shielded imaging gradients with a saddle coil around the tumor bearing leg. A gradient echo sequence (TR/TE = 10 msec/3 msec) was used with slice thickness 3 mm, and in plane resolution approximately $400 \times 400 \mu\text{m}$. Images were acquired in planes roughly parallel to the long axis of the leg, approximating a sagittal plane.

Uptake of contrast agent (Gd-DTPA dimeglumine, 0.2 mM/kg IV) in the dynamic T_1 MR images was analyzed to assess vascular structure and function. Plots of contrast agent concentration as a function of time were used to determine blood volume and capillary permeability following methods previously described [6-10]. Briefly, blood volume was estimated from the early increase in image intensity due to the contrast agent. This increase in image intensity was used to calculate tissue concentration of contrast agent. Since the contrast agent is entirely intravascular during the first few minutes after injection, blood volume can be calculated from the early concentration of contrast agent in each pixel, if the blood concentration of contrast agent is known. Blood concentration of the contrast agent can be estimated based on the change in T_1 in muscle, since the blood volume of muscle is assumed to be a biological constant. Vascular function in tumor regions was characterized in terms of $F \times E$ (flow times extraction fraction) and extracellular-extravascular volume (α) by fitting the time dependent concentration curves to a simple two compartment model [8].

$$\frac{dC(t)}{dt} = \frac{F \cdot E}{V_t} \cdot \left(C_p(t) - \frac{1}{\alpha} \cdot C(t) \right)$$

where $C(t)$ is the Gd-DTPA concentration as a function of time, V_t is the volume accessible to water, $C_p(t)$ is the plasma concentration of Gd-DTPA.

Each of the EPR oxygen images was performed within 2 hours before an MRI of the tumor blood volume and vasculature. Thus, we have blood vessel and oxygen information on the same tumors at similar times at all time points. Therefore differences in metabolic state can be ignored. Using a manual image registration method developed by us, we correlated the EPRI-derived oxymetric measurements with the MRI vasculature/blood volume images. ANOVA was used to compare tissue parameters for day 0, 3, and 16 and a *t-test* was used to compare

muscle and tumor on each day ($p < 0.05$, was considered significant).

4. RESULTS

In Figure 1 there are three areas of high oxygenation with one area with essentially zero oxygenation (denoted with the arrow). Comparing with Figure 2, one can see the zero oxygenation region along with one remaining well oxygenated area. Note the day 3 image volume was registered to that of day 0 using manual image registration. The slices shown here are approximately the same anatomical sections. The oxygenation overall, has been reduced after treatment suggesting less vascularization.

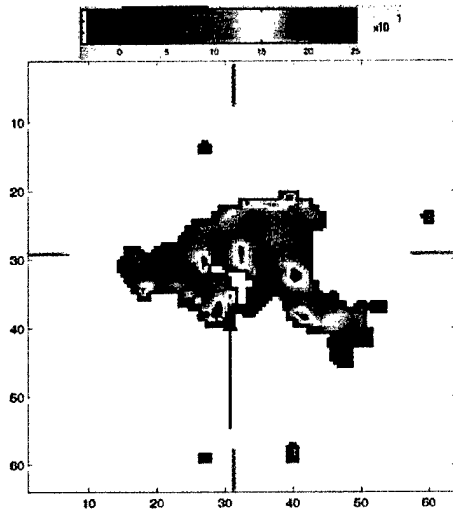


Figure 1 Day 0 pO_2 map, colorbar is in mmHg, axes are in pixels

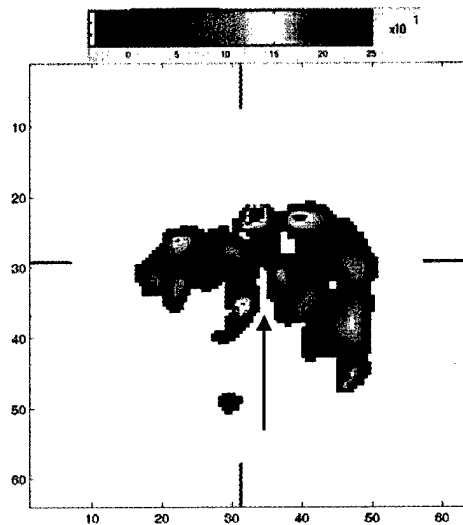


Figure 2 Day 3 pO_2 map, registered to Day 0, colorbar is in mmHg, axes are in pixels

Similarly, looking at the arrows in Figure 3, the area where the tumor was on day zero appears to be disrupted in the image taken three days after treatment.

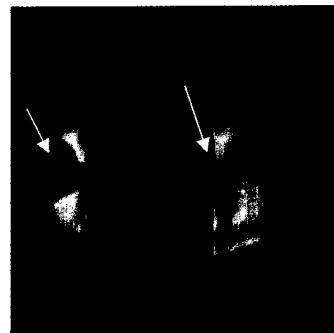


Figure 3 T_1 weighted image before (left) and after (right) Ad.EGR-TNF treatment.

The kinetic parameters of the muscle did not significantly change over the sixteen day experiment as shown by α and FE/V_t in Table 1 and Figure 4. Kinetic parameters for tumor tissue also did not significantly change. However, on day 0, α and FE/V_t , are significantly greater than muscle values.

Table 1 Kinetic parameters

Day	α		FE/Vt	
	Muscle	Tumor	Muscle	Tumor
0	0.20±0.00	0.81±0.11*	0.12±0.01	0.25±0.05*
3	0.20±0.00	0.59±0.20	0.12±0.02	0.21±0.08
16	0.20±0.00	0.49±0.27	0.12±0.00	0.16±0.04

Data are mean ± SE with Day 0 n=6, day 3 n=4, and day 16 n=2, * significantly different from muscle p < 0.05

Figure 4 demonstrates the difference in permeability, i.e., reduced contrast uptake between the muscle and tumor and suggest a reduction after treatment.

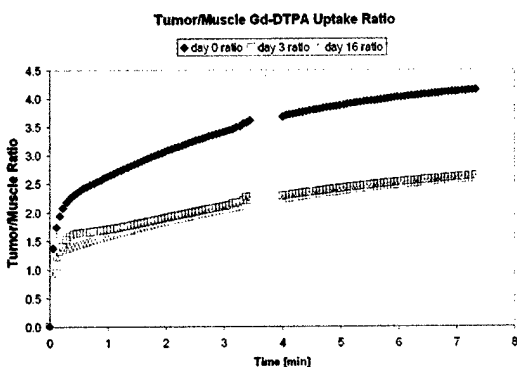


Figure 4 Gd-DTPA tumor/muscle uptake ratio. ♦ Day 0 n=6, □ day 3 n=4, and △ day 16 n=2.

5. CONCLUSION

Clinical trials are ongoing for a treatment similar to the Ad.EGR-TNF used here, differing only in the vector. The present results indicate that MR and EPR images are responsive to antiangiogenic activity of the combined radiation and TNF- α therapy. Imaging a small animal twice on three different days led to many experimental difficulties and the variability in fitted kinetic parameters reflect this. Furthermore, the two compartment model used in the kinetic modeling may not adequately account for the chaotic vasculature of the tumor. With improved experimental technique and more sophisticated analysis, multimodality imaging as described here appears to hold promise for early detection of response to combined radiation and gene therapy

Acknowledgement: this research was supported by US Army Prostate Cancer Research Program (DAMD17-02-1-0034) and NIBIB Center for EPR Imaging for *In Vivo* Physiology (1 P41 RR12257)

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