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Based on Dynamic MR Tumor Oximetry

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14. ABSTRACT: Targeting the vasculature of tumors promises a new effective therapy for prostate cancer. We propose a new approach targeting the blood vessels in the tumor. Specifically, a novel antibody 3G4, which targets phosphatidylserine (PS) expressed on tumor vasculature was developed by Thorpe et al. and is being developed by Peregrine Pharmaceuticals for clinical trials. Normally, PS exclusively resides on the cytosolic leaflet of the plasma membrane. However, in tumors PS becomes externalized and provides a viable target. The agent not only targets various tumors, but also induces vascular damage and tumor regression with minimal accompanying toxicity. In developing a new therapy, critical issues include scheduling, optimal combination with other interventions to achieve synergy and early assessment of efficacy. Magnetic resonance imaging allows us to follow the induction and development of tumor vascular damage providing new insight into spatial and temporal activity and facilitating effective combination with the hypoxic cell selective cytotoxin tirapazamine. Importantly, this therapy may be effective at any stage of tumor development, and could be most effective for advanced disease. Success will confirm the potential of this new therapeutic approach to prostate cancer in man and lay the foundation for future clinical trials.					
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Introduction

Targeting tumor vasculature promises new effective therapy for prostate cancer (1, 2). It avoids issues of drug delivery and is potentiated by massive downstream effects where one blood vessel may supply the nutrients for thousands of tumor cells. Thus, disrupting the vascular supply should generate magnified tumor cell kill. This research combines the expertise of three laboratories (Pharmacology, Urology, and Radiology) to investigate and optimize a novel therapeutic approach to prostate cancer. Thorpe *et al.* pioneered the concept of targeting tumor vasculature for therapeutic gain using antibodies (3). Recently, they generated a novel antibody 3G4, which targets phosphatidylserine (PS) expressed on tumor vasculature. 3G4 is a naked antibody, which recruits host defense cells to attack tumor vasculature (4-6). In collaboration with Peregrine Pharmaceuticals, this agent has been chimerized and is now being developed for clinical trials as Bavituximab (It should be noted that until last year the name VatuximabTM had been proposed) (7). Normally, PS exclusively resides on the cytosolic leaflet of the plasma membrane. However, in tumors PS becomes externalized and provides a viable target. The agent not only targets various tumors, but also induces vascular damage and tumor regression with minimal accompanying toxicity. In developing any new therapy, critical issues include scheduling, optimal combination with other interventions to achieve synergy and early assessment of efficacy. Magnetic resonance imaging will allow us to follow the induction and development of tumor vascular damage *in vivo* providing new insight into spatial and temporal activity and facilitating effective combination with the hypoxic cell selective cytotoxin tirapazamine.

This research program will evaluate the ability of the agent Bavituximab to generate damage in tumor vasculature and induce prostate tumor growth delay. MRI will be used to assess the onset and distribution of tumor vascular damage in a series of Dunning prostate rat tumors (R3327- AT1, MAT-Lu, HI, and H) (8, 9) (10-14). This will provide an indication of the efficacy with respect to tumors exhibiting diverse histologies (anaplastic to well differentiated), a range of volume doubling times (1.5 to 20 days). Importantly, all these tumors are subclones of the original R3327-H tumor, and hence, together they represent a strong analogy for the clinical situation of advanced multi focal multi clonal prostate cancer. We will assess tumor response at different sizes and the value of repeated doses. Ultimately, we will investigate the synergistic application of Bavituximab with the hypoxia selective cell cytotoxin, tirapazamine (15-17). The experience in diverse subcutaneous models will be translated to human tumor xenografts in intraosseous models of advanced metastatic prostate cancer (18). Here, PSA levels and bioluminescence will provide primary indications of tumor growth and MRI will be applied to examine the tumor pathophysiology.

Successful completion of this project will confirm the potential of this new therapeutic approach to prostate cancer in man. It will lay the foundation for future clinical trials and promises a highly effective novel therapy obviating the need for radical prostatectomy, with its inherent costs, risks, and complications. Ultimately, this approach could lead not only to increased survival time with quality of life, but also cure of the prostate cancer patient.

It should be noted that the antibody VatuximabTM was formerly called Bavituximab.

Body and Progress

Phase 1 Evaluate efficacy of Bavituximab to control diverse syngeneic rat prostate tumors: assess physiological parameters (e.g., pO₂) as surrogate markers of prostate tumor control and mechanisms of response.

Task 1 Months 1-3

Implant tumors of the four Dunning prostate sublines R3327- MAT-Lu, AT1, HI, and H in Copenhagen rats. Tumors of all four sublines have been implanted and are growing routinely in the laboratory. We have also preserved additional tissues to secure the lines and ensure consistency throughout the three year study. Tumors

continue to be implanted to provide tumors for investigation in an orderly manner commensurate with imaging tests and therapy.

Task 2 Months 2-15

Measure baseline pO₂ (*FREDOM*), perfusion characteristics (DCE MRI), and ADC (Apparent diffusion coefficient) of tumors and changes with respect to Bavituximab infusion to assess acute response over two hours.

All the pertinent pulse sequences and MRI hardware are now functional and investigators are now familiar with acquiring the data. We have achieved baseline measurements for tumors of each subline and examined acute changes in each parameter following infusion of the drug bavituximab (formerly, VatuximabTM).

Tumor oximetry

The *FREDOM* (Fluorocarbon Relaxometry using Echo Planar imaging for Dynamic Oxygen Mapping) (19) was successfully applied to measure tumor pO₂ and dynamic response to interventions. Under baseline air breathing conditions all tumors show quite similar oxygenation patterns typically ranging from regions of hypoxia to others with pO₂ ~ 40 torr (Figure 1). Comparison of pO₂ values using Analysis of Variance with Fishers post hoc test showed that the H tumors had significantly lower pO₂ than the AT1 or HI tumors (Tables 1&2). Following bavituximab administration MAT-Lu, AT1 and H tumors showed no particular change. However, several HI tumors showed hypoxiation over about 1 h. One week later both HI and H tumors showed elevated pO₂.

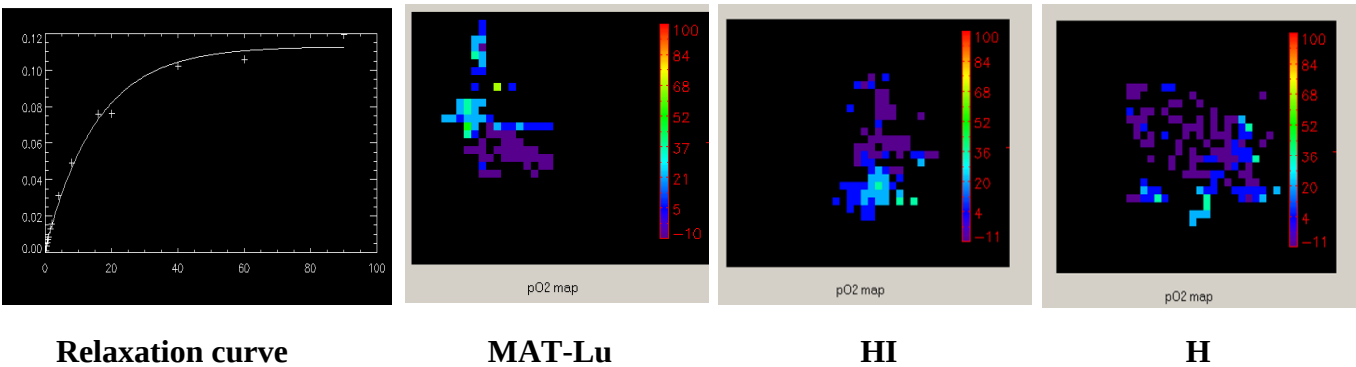


Figure 1 Oximetry in Dunning prostate tumors. Left A typical ¹⁹F NMR T1 relaxation curve for the signal intensity of the reporter molecule hexafluorobenzene from a single voxel within a tumor. The relaxation rate is directly proportional to pO₂. Based on such curves maps were generated for representative MAT-LU, HI and H tumors growing on anesthetized rats breathing air with isoflurane anesthesia. Voxel dimension 1.25 mm in plane with 10 mm thickness.

	Count	Mean	Std. Dev.	Std. Err.		Mean Diff.	Crit. Diff	P-Value	
AT1	15	10.459	9.635	2.488	AT1, H	8.708	6.404	.0085	S
H	12	1.751	2.610	.753	AT1, HI	2.226	5.442	.4170	
HI	24	8.233	9.258	1.890	AT1, MAT-LU	5.176	5.781	.0784	
MAT-LU	18	5.283	8.079	1.904	H, HI	-6.482	5.846	.0303	S
					H, MAT-LU	-3.533	6.162	.2565	
					HI, MAT-LU	2.950	5.156	.2574	

Tables 1 (left) and 2 (right) Baseline oxygenation of four Dunning prostate R3327-tumor lines.

ANOVA showed that H tumors had significantly lower pO₂ than AT1 or HI. This is contrary to our previous observations and we are examining histology and repeating tests to further clarify the pO₂ values observer in the H tumors.

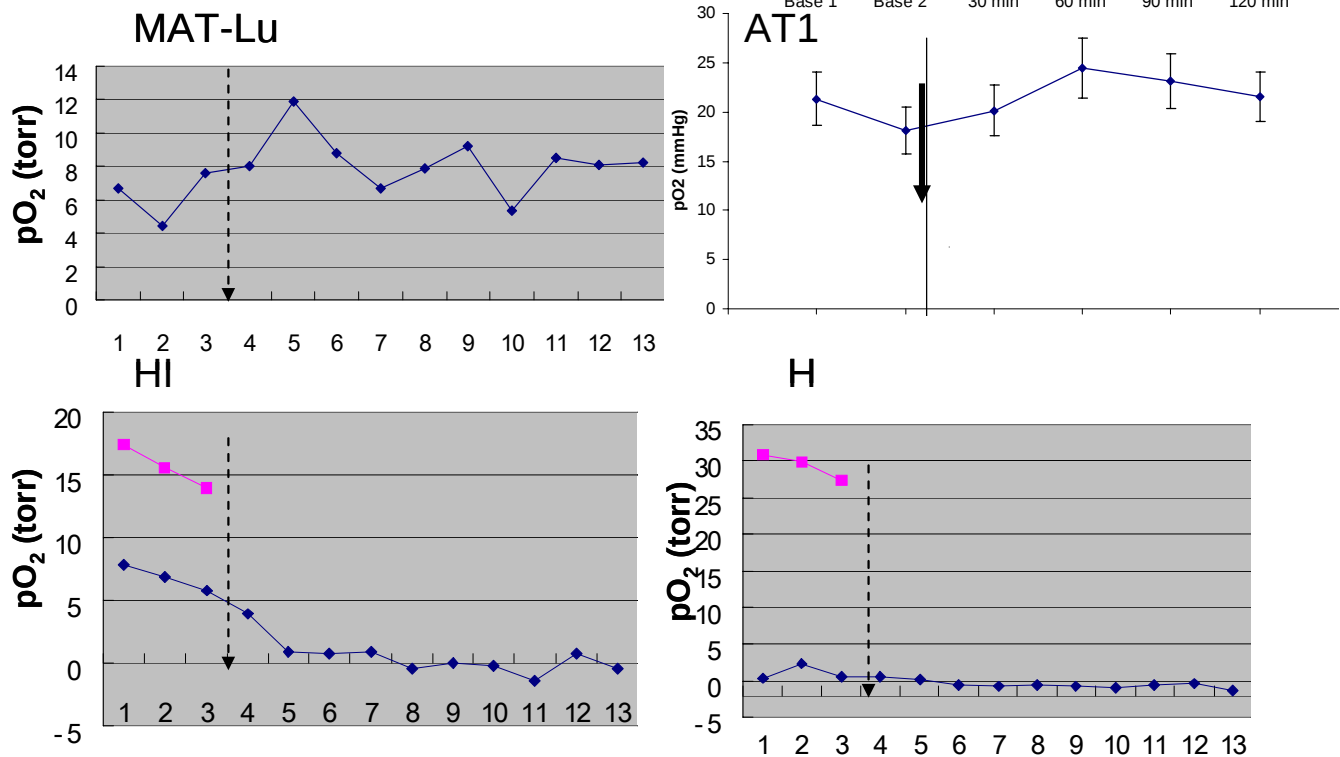


Figure 2 Oxygen dynamic in Dunning prostate R3327 tumors with respect to bavituximab infusion.

Two or three baseline pO₂ maps were generated in individual tumors and then bavituximab was infused IP (arrow). Further pO₂ maps were generated over the following 2 hours. Only HI tumors showed significant change (hypoxiation) following infusion. Pink lines show pO₂ measurements seven days later. For HI tumors the decline in pO₂ was significantly within 30 mins.

Dynamic contrast enhanced MRI

DCE was performed using 1H MRI at 4.7 T with the small paramagnetic contrast agent Omniscan (0.1 $\mu\text{mol/kg}$ (~250 μl) infused IV in tail using catheter vein by hand rapidly (~ 1 s). Data were examined in terms of ΔSI (max change in signal intensity). There was distinct heterogeneity between center and periphery of each tumor type as shown in Figures 3-8. In some cases we have compared regional differences and undertaken measurements of the exchange function k_{ep} .

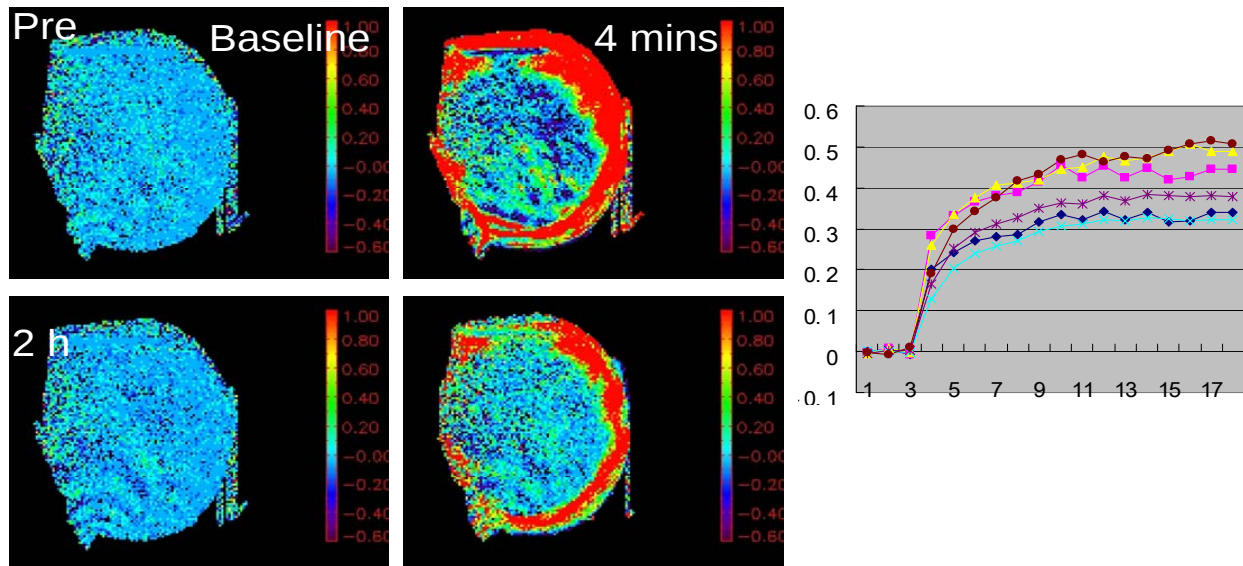


Figure 3 DCE for MAT-Lu tumor. Top left Relative signal intensity map for T1 weighted MRI pre therapy and before contrast agent. Top center: 4 mins after contrast showing strong peripheral enhancement; Bottom left baseline MRI 2 h after administration of bavituximab; Bottom center 4 mins post contrast, 2 h after bavituximab. Right curves show mean signal enhancement for three representative image slices before and 2 h after bavituximab. There were no significant changes. Clearly, further analyses will be required on a regional signal intensity basis.

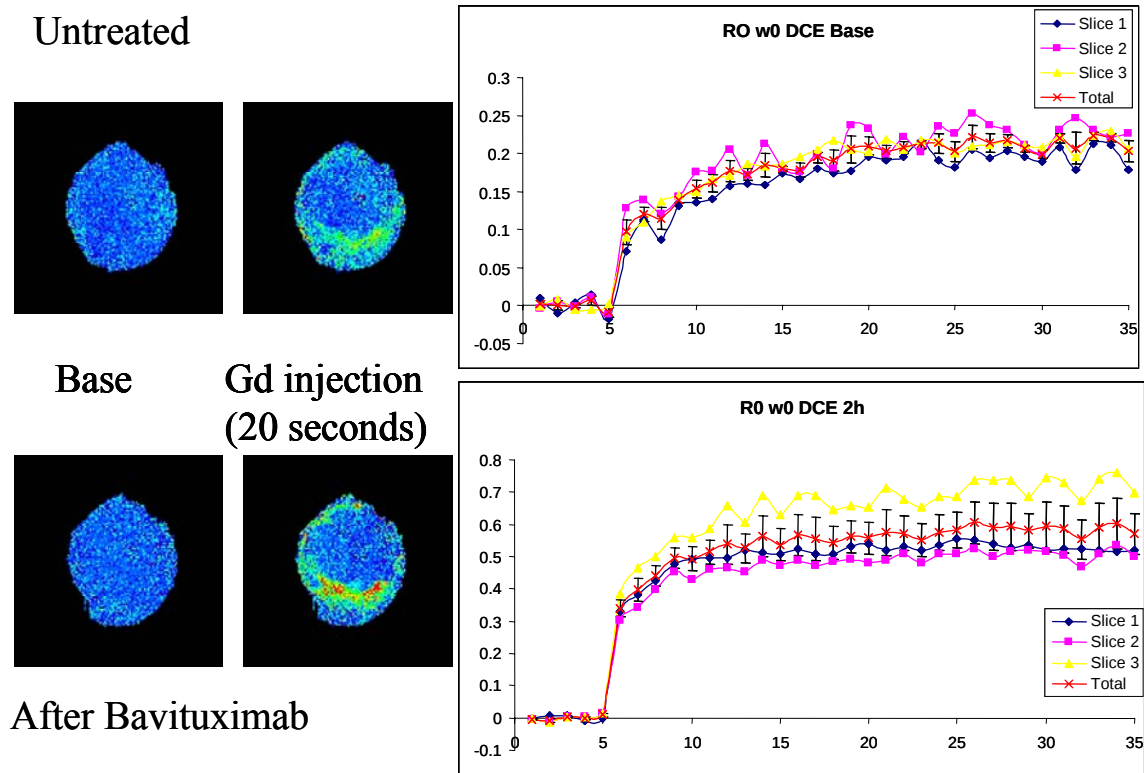


Figure 4 DCE for AT1 tumor. Top left Relative signal intensity map for T1 weighted MRI pre therapy and before contrast agent. Top center: 20 s after contrast showing strong peripheral enhancement; Bottom left baseline MRI 2 h after administration of bavituximab; Bottom center 20 s post contrast, 2 h after bavituximab. Right curves show mean signal enhancement for three representative image slices before (top) and 2 h after (bottom) bavituximab.

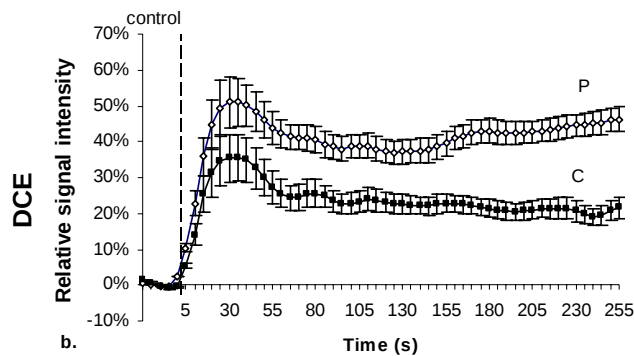


Figure 5 Comparison of signal intensity during DCE experiments for a group of AT1 tumors. A significant difference in signal response was observed between central and peripheral regions of tumor.

	Mean	36±1
(ΔSI)DCE	Periphery	43±1*
%response	Center	24±1
K_{ep} (min ⁻¹)	Mean	3.05±0.37
	Periphery	3.11±0.44
	Center	2.59±0.51

Table 3 Comparison of DCE parameters. For a group of AT1 tumors showing significant difference in signal response between central and peripheral regions of tumor (*). No differences were observed for K_{ep} .

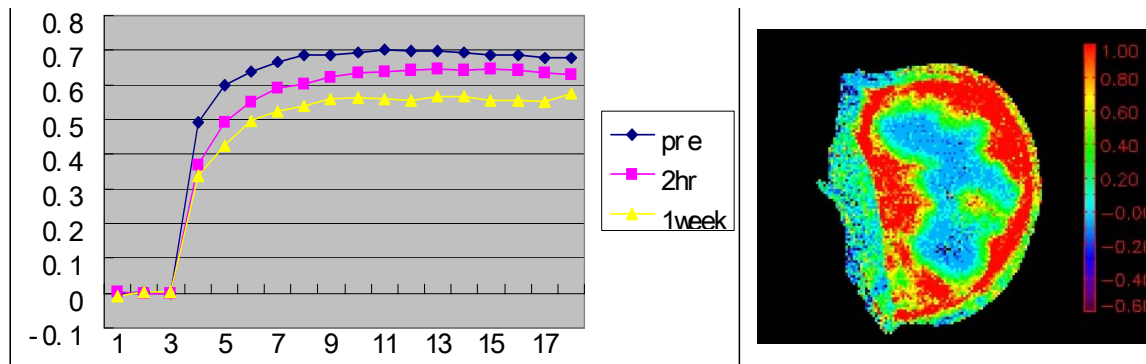


Figure 6 DCE for HI tumor. Left Mean signal intensity kinetics following infusion of contrast agent. Right: Relative signal intensity map for T1 weighted MRI 2 h post therapy (4 minutes after contrast agent) showing heterogeneous perfusion.

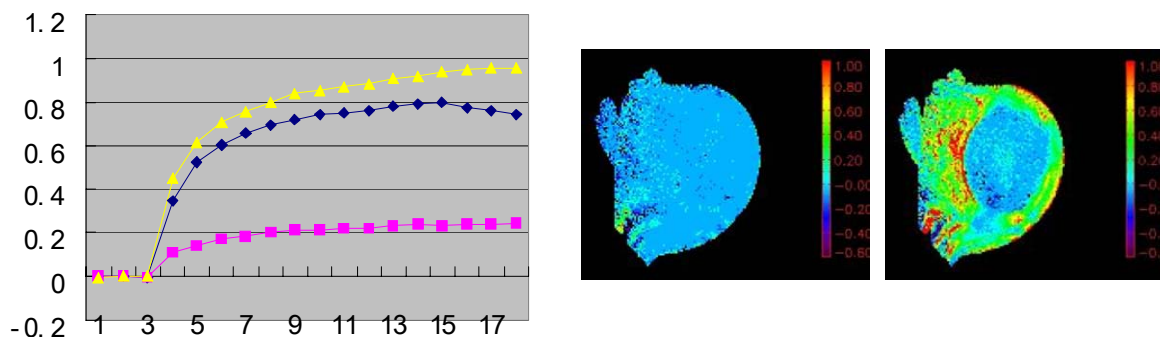


Figure 7 DCE for H tumor. Left Mean signal intensity kinetics following infusion of contrast agent. **Pre (blue), 2h post bavituximab (pink), 7 days post (yellow).** On most occasions DCE indicated considerably lower signal response in the H tumors 2 h after bavituximab. To verify this result we will both use histology following administration of Hoechst perfusion dye and ensure that future studies include normal tissue following assessment of arterial input function. This is cruel to verify that the contrast agent injections are all similarly successful. Right: Relative signal intensity map for T1 weighted MRI pre and 4 minutes after contrast agent showing heterogeneous perfusion pre bavituximab.

$(\Delta SI)_{DCE^+}$ %response	Mean	55±2 [‡]
	Periphery	31±1
	Center	124±6* [‡]
K_{ep} (min ⁻¹)	Mean	3.20±0.39
	Periphery	3.34±0.46
	Center	2.95±0.54

Table 4 Comparison of DCE parameters.

For a group of H tumors there was a significant difference in signal response between central and peripheral regions of tumor. No differences were observed for K_{ep} .

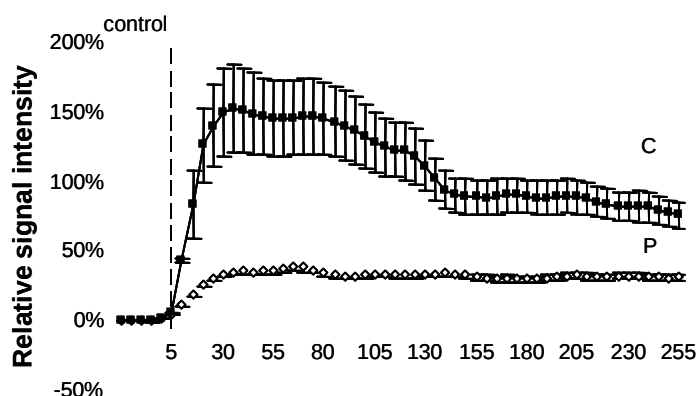


Figure 8 Comparison of signal intensity during DCE experiments for a group of H tumors. A significant difference in signal response was observed between central and peripheral regions of tumor, but here the center showed a larger change, whereas for AT1 tumors in Figure 5, the opposite was observed.

Apparent diffusion coefficient (ADC) maps are shown for thin slices from representative Dunning prostate tumors of each subline in Figures 9 and 10. Each tumor shows some heterogeneity. In Figure 10 color representations are provided for a representative AT1 tumor, with 3 selected slices before and two hours after

administration of Bavituximab. Table 5 provides mean values and compares the statistical significance of difference between the sublines. While the maps showed no significant differences between the AT1 and MAT-Lu tumor types, all the other comparisons revealed significantly differences and the H showed much lower ADC values.

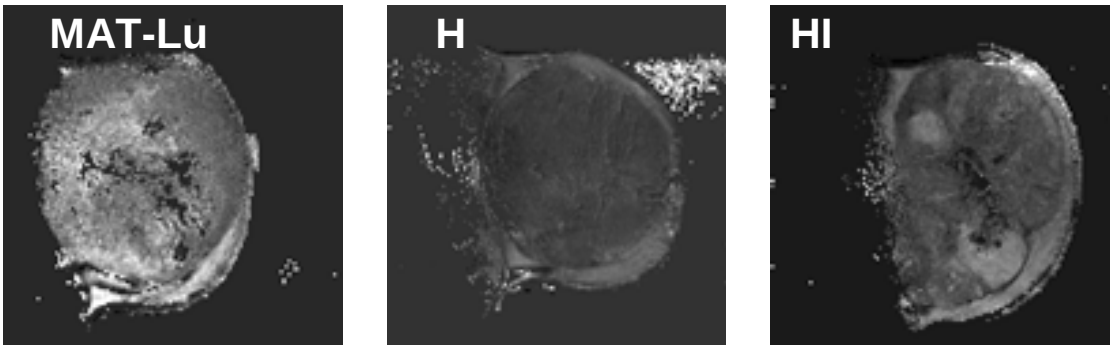


Figure 9 Apparent diffusion maps obtained by proton MRI at 4.7 T of Dunning prostate R3327 tumors growing in rats. Each image represents a slice of a tumor observed *in vivo* presenting diffusion maps obtained with 4 b-value diffusion gradients (MR parameters, FOV = 30 mm, TR = 2,300 ms, TE= 50 ms, in plane resolution 230 um, slice thickness 2 mm with a total acquisition time of 20 mins)

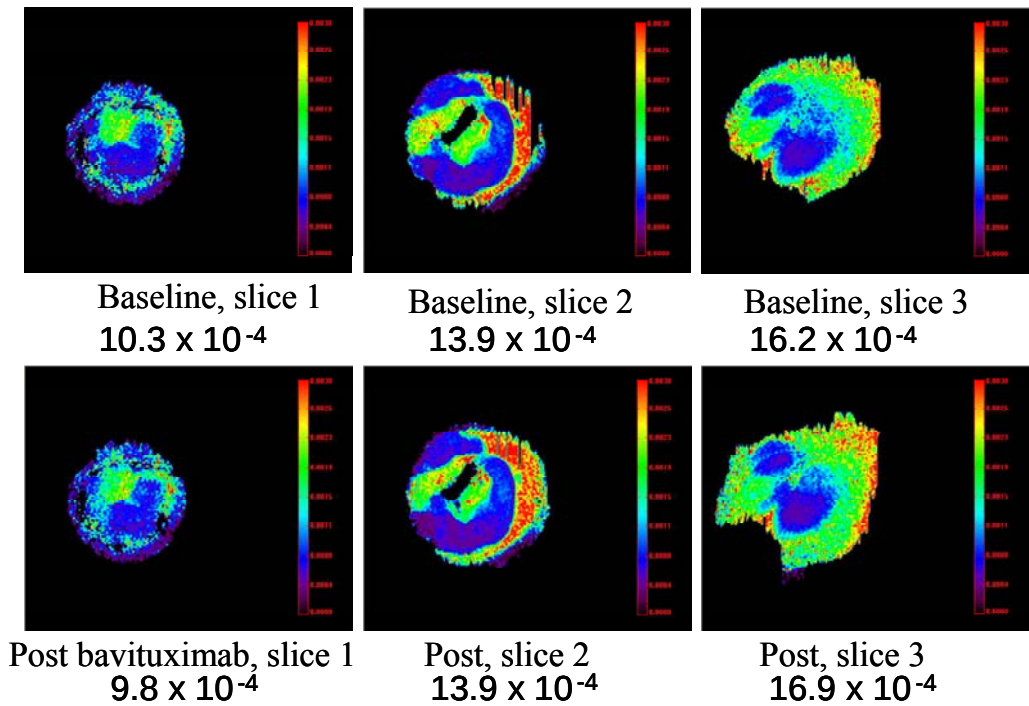


Figure 10 Apparent diffusion maps obtained by proton MRI at 4.7 T of Dunning prostate R3327-AT1 tumor. Data as for Figure 9, but showing three consecutive image slices in representative AT1 tumor. Distinct baseline heterogeneity is apparent with mean ADC ranging from 10.2×10^{-4} to 16.2×10^{-4} mm²/s. The lower image shows the same slices 2 h after administration of 2.5 mg/kg bavituximab. There were no significant acute changes.

	Mean	Std. Dev.
AT1	12.9	.92
H	2.6	3.83
HI	16.7	2.80
MAT-Lu	11.8	2.44

Fisher's PLSD for ADC pre			
Effect: Tumor type			
Significance Level: 5 %			
	Mean Diff.	Crit. Diff	P-Value
AT1, H	10.292	3.710	<.0001
AT1, HI	-3.839	3.153	.0196
AT1, MAT-Lu	1.079	3.349	.5083
H, HI	-14.130	3.387	<.0001
H, MAT-Lu	-9.213	3.570	<.0001
HI, MAT-Lu	4.918	2.987	.0027

Table 5 Left Relative ADC values for groups of Dunning prostate tumors. **Right** Statistical comparison of ADC values for tumor types showing levels of significance for analysis of variance based on Fisher’s test

Task 3 Months 3-15

Response to multiple doses of Bavituximab. Use MRI to measure pO₂, perfusion characteristics and diffusion characteristics of tumors with respect to repeated Bavituximab administration (assess response over a period of weeks/months by MRI and tumor volume).

Administration of bavituximab produced no significant acute changes in ADC over a period of 2 h (e.g. Figs. 1 and 2). In most tumors there appeared to be no changes in perfusion based on DCE. However, several H tumors indicated reduced perfusion at 2 h, which was restored after 1 week. pO₂ values were quite variable among individual tumors. Most showed no significant response to administration of bavituximab. However, several HI tumors showed significant hypoxiation during the 2 h following administration.

H tumors indicated strong therapeutic response (Figure 11). Each tumor showed either reduction in growth or tumor shrinkage. Tumors of the faster growing cell lines appeared to respond less well to therapy. However, they generally develop massive central necrosis with only a thin peripheral rim of viable tumors. In many cases this was revealed as ulceration leaving a donut cavity. Thus, there is extensive tumor control, but volume measurement based on respective dimensions alone does not appropriately reveal the control.

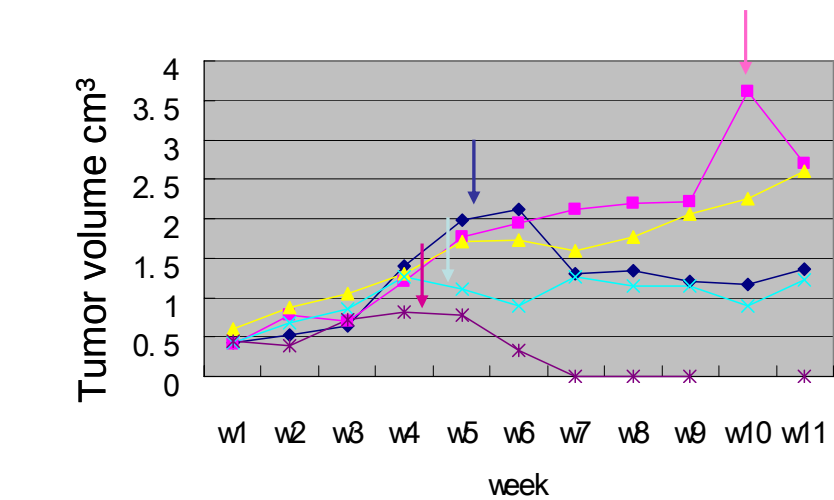


Figure 11 Growth curves for H tumors with respect to bavituximab therapy. Therapy (2.5 mg/kg thrice weekly IP) was initiated at times shown by arrows. In each case tumor growth was controlled and in one case the tumor disappeared. There rats are still alive and growth studies are ongoing.

Task 4 Months 3-18

Histological analysis- assess distribution of Bavituximab, necrosis, hypoxia, perfusion based on dyes and antibodies.

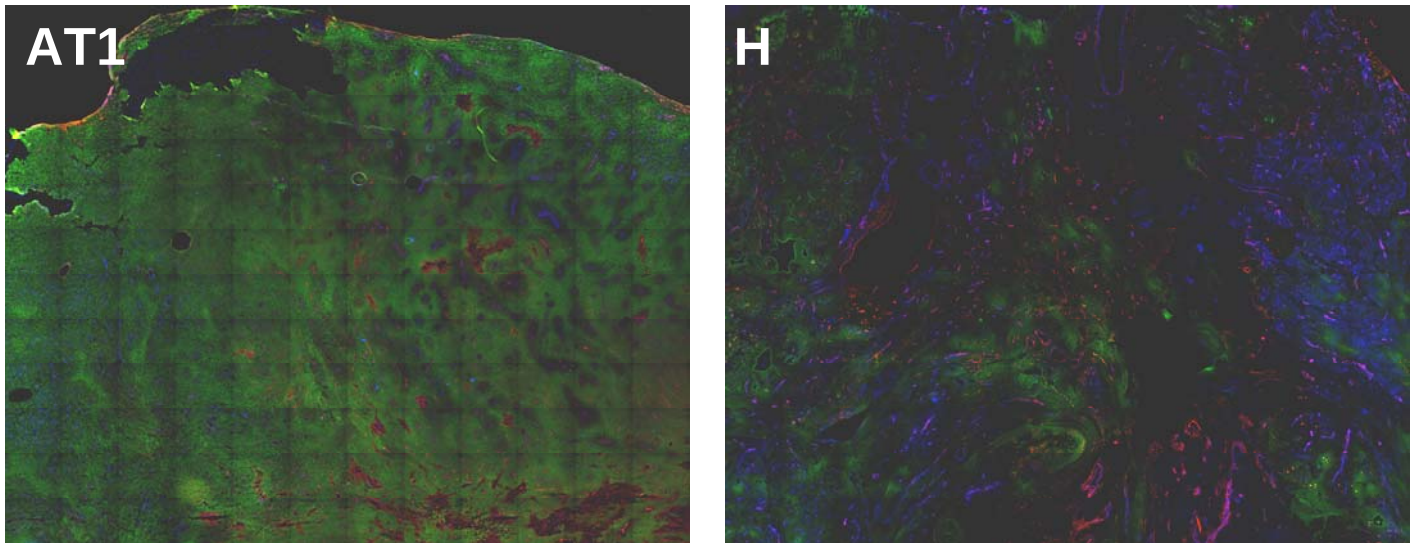


Figure 12 Comparison of microvasculature and hypoxia in control AT1 and H tumors.

Vascular endothelium marked by CD-31 (red), perfused vessels marked by Hoechst dye 33342 (blue) and hypoxia by pimonidazole hydrochloride (green). Images obtained with the assistance of Dr Bert van der Kogel, Univ. Nijmegen.

- a) The AT1 tumor shows extensive hypoxia and many vessels appeared to be non-perfused. Near the tumor periphery, perfusion is more effective as revealed by the purple appearance of vessels (red overlapping blue).
- b) The H tumor shows more extensive vascular endothelium, which is well perfused throughout the tumor. Hypoxia occurs distant to perfused vessels and is less extensive.

Treated tumors have been stored and histology is underway.

Task 5 Month 12

Prepare annual report and manuscript.

KEY RESEARCH ACCOMPLISHMENTS:

- Examined changes in tumor oxygenation in response to bavituximab administration. Only HI tumors showed significant hypoxiation.
- Examined changes in tumor perfusion following bavituximab. Only H tumor showed significant change (reduction)
- No changes in apparent diffusion coefficients were found following batuximab administration.
- Most H tumors showed significant reduction in growth rate (based on tumor volume) and growth delay (or shrinkage) were maintained over many weeks while additional doses of bavituximab were administered.

- The faster growing tumors showed central necrosis and tumor control based on histological examination, but simply measuring whole tumor volume did not readily reveal tumor control due to peripheral rim which continued to grow.

REPORTABLE OUTCOMES: Accepted Abstracts for conference presentations:

1 DOD "Innovative Minds in Prostate Cancer Today - IMPaCT" meeting on September 5-8, 2007, at the Hyatt Regency in Atlanta, Georgia.

“Vatuximab™: Optimizing Therapeutic Strategies For Prostate Cancer Based on Dynamic MR Tumor Oximetry”, Ralph P. Mason; Weina Cui; Dawen Zhao; Albert J. van der Kogel; Johan Bussink; Jesús Pacheco Torres; Jennifer McAnally; Linda Watkins; Peter Peschke; and Philip Thorpe.

2 Second International Conference of European Society for Molecular Imaging, June 14-15, 2007 in Naples, Italy

“Differential physiological response to carbogen of two diverse prostate tumor lines detected by tissue water ¹H MRI”, J. Pacheco-Torres, D. Zhao, J. McAnally, and R. P. Mason

CONCLUSION: As expected based on previous observations all prostate tumors show considerable hypoxia. However, only HI tumors showed significant acute increased hypoxia following administration of bavituximab. Thus, the hypoxia selective cytotoxin tirapazamine is expected to be effective on the prostate tumors, but in terms of combined effects and potential synergy, we now only expect this for the HI tumors, where additional hypoxia is induced by bavituximab.

It appears that the faster growing sublines have a rapidly proliferating edge, which escapes control from bavituximab alone. Thus, we propose to add the standard chemotherapy treatment with docetaxel to some groups of tumor bearing rats to establish whether this can effectively control the tumors. We have obtained IACUC approval for this deviation and will formally propose the additional treatment to the CDMRP.

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APPENDICES:

Abstract for the DOD "Innovative Minds in Prostate Cancer Today - IMPaCT" meeting on September 5-8, 2007, at the Hyatt Regency in Atlanta, Georgia.

Ralph P. Mason; Weina Cui; Dawen Zhao; Albert J. van der Kogel (Department of Radiation Oncology, University Medical Center Nijmegen, Nijmegen, Netherlands); Johan Bussink (Department of Radiation Oncology, University Medical Center Nijmegen, Nijmegen, Netherlands); Jesús Pacheco Torres (Instituto de Investigaciones Biomédicas "Alberto Sols" CSIC/UAM, Madrid, Spain); Jennifer McAnally; Linda Watkins; Peter Peschke (German Cancer Center, Heidelberg, Germany); and Philip Thorpe.

Targeting tumor vasculature promises a new effective therapy for prostate cancer, since a blood supply is required for the tumor to grow and develop. We proposed a new approach, using the novel antibody 3G4 (now called baviximab), which targets phosphatidylserine (PS) expressed on tumor vasculature. In collaboration with Peregrine Pharmaceuticals, this agent is being developed for clinical trials. Normally, PS exclusively

resides on the cytosolic leaflet of the plasma membrane. However, in tumors PS becomes externalized and provides a target. The agent not only targets various tumors, but also induces vascular damage and tumor regression with minimal accompanying toxicity. Our goal is to evaluate the dynamic effects of 3G4 on tumor pathophysiology, so as to optimize combination with additional drugs for synergistic therapeutic response.

Magnetic resonance imaging is used to follow the induction and development of tumor vascular damage *in vivo* in diverse syngeneic rat tumors (Dunning R3327-MAT-Lu, AT1, HI and H) known to exhibit differential vascular extent and growth rates. Specifically, we are examining changes in diffusion coefficients (ADCs), perfusion and vascular leakiness based on dynamic contrast enhancement (DCE) and hypoxiation based on NMR oximetry (*FREDOM*-Fluorocarbon Relaxometry using Echo Planar imaging for Dynamic Oxygen Mapping).

Before therapy all tumors exhibit substantial heterogeneity. MAT-Lu and AT1 tumors exhibit similar ADCs, while HI tumors are significantly greater and H tumors substantially lower. Groups of AT1 and H tumors showed significant differences in DCE signal response between central and peripheral regions. No regional differences were observed for the pharmacokinetic parameter *Kep* for either tumor type. Histology confirmed that H tumors are better perfused and exhibit much less hypoxia (based on Hoechst dye distribution, CD31-staining, and pimonidazole uptake). During baseline air breathing all tumors show quite similar oxygenation patterns typically ranging from regions of hypoxia ($pO_2 < 5$ torr) to normoxia ($pO_2 \sim 40$ torr).

Administration of bavituximab produced no significant acute changes in ADC over 2 h. In most tumors there appeared to be no changes in perfusion based on DCE. However, several H tumors indicated reduced perfusion at 2 h, which was restored after 1 week. pO_2 values were quite variable among individual tumors. Most showed no significant response to administration of bavituximab. However, several HI tumors showed significant hypoxiation during the 2 h following administration.

H tumors indicated strong therapeutic response. Each tumor showed reduction in growth or tumor shrinkage. Tumors of the faster growing cell lines appeared to respond less well to therapy. However, many develop massive central necrosis with only a thin peripheral rim of viable tumor, often revealed as ulceration leaving a donut cavity. Thus, there is extensive tumor control, but volume measurement based on respective dimensions alone does not appropriately reveal the extent of control.

Our data are preliminary, but we have demonstrated proof of principle. Importantly, this therapy is associated with little or no toxicity and could be effective at any stage of tumor development. We are initiating combined studies with tirapazamine to exploit the observed hypoxiation. Success will confirm the potential of this new therapeutic approach to prostate cancer in man and lay the foundation for future clinical trials.

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“Differential physiological response to carbogen of two diverse prostate tumor lines detected by tissue water 1H MRI” J. Pacheco-Torres^{1,2}, D. Zhao², J. McAnally², and R. P. Mason²

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Tumor oxygenation play important roles in cancer malignancy. Recently, studies have suggested a possibility of assessing tissue oxygenation based on the shortening of the tissue water T1 due to oxygen. Here, we are investigating differences in T1- and T2*-weighted signal intensity, as well as maps of R1, R2 and R2* in response to carbogen between two Dunning prostate R3327 rat tumor sublines: AT1 (anaplastic and poorly vascularized) and HI (moderately well differentiated and vascularized).

In response to carbogen breathing, significantly increased signal intensity in both T1 and T2*-weighted images was found in both the tumor lines. Much higher enhancement in both T1 and T2*-weighted signal was observed

in HI compared with AT1 tumors (mean maximum $\Delta SI(\%) = 8.6 \pm 2.2$ vs. 5.4 ± 0.7 in T_1 -weighted; 23.9 ± 8 vs. 9.8 ± 1.8 in T_2^* -weighted). R_1 maps revealed that carbogen induced significantly increased R_1 values in both periphery and center of the HI tumors (mean $\Delta R_1 = 0.012$ (periphery) vs. 0.006 s^{-1} (center); $p < 0.01$), while no significant increase was seen in the AT1 tumors. Similarly, reduction in R_2^* values in response to carbogen was found in the HI tumor, but not the AT1 tumors. These results are in line with previous studies in these two tumor lines.

While SI of T_1 -weighted image increased, T_1 values were not shortened in the AT1 tumors with carbogen inhalation. This may be attributed to an increase in blood flow associated with carbogen. Since this approach is totally non-invasive it appears worthy of further investigations for characterizing tumors and response to adjuvant interventions.