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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 Vatuximab^{TM} 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 pathophysiolology.

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 Vatuximab^{TM} was formerly called Bavituximab.

Body

D Statement of Work for Year 1

Phase 1 Evaluate efficacy of Bavituximab to control diverse syngeneic rat prostate tumors: assess physiological parameters (*e.g.*, pO_2) 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. (6 tumors of each of 4 sublines with 3 treatment sizes (0.5 cm, 1 cm, 1.5 cm diameter; respectively 0.06 cm^3, 0.5 cm^3, 1.7 cm^3) = 144 experimental tumors: Tasks 2, 3, 4 and 5 are based on these rats))

Task 2 Months 2-15

Measure baseline pO_2 (*FREDOM*), perfusion characteristics (DCE MRI), and ADC of tumors with respect to Bavituximab infusion to assess acute response over two hours.

Task 3 Months 3-15

Response to multiple doses of Bavituximab. Use MRI to measure pO_2, 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).

Task 4 Months 3-18

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

Task 5 Month 12

Prepare annual report and manuscript.

Key Research Accomplishments

Task 1

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

Measure baseline pO_2 (*FREDOM*), perfusion characteristics (DCE MRI), and ADC of tumors 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, Vatuximab^{TM}).

Apparent diffusion coefficient (ADC) maps are shown for thin slices from representative Dunning prostate tumors of each subline in Figures 1 and 2. Each tumor shows some heterogeneity. In Figure 2 color representations are provided for a representative AT1 tumor, with 3 selected slices before and two hours after administration of Bavituximab. Table 1 provides mean values and Table 2 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.

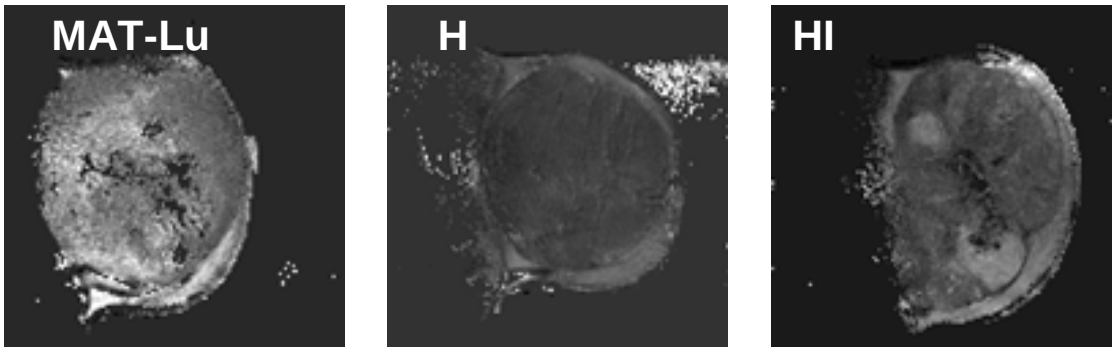


Figure 1 Apparent diffusion maps obtained by proton MRI at 4.7 T of Dunning prostate R3327 tumors growing in rats. Each image represents a slice of a tumor observed *in vivo* presenting diffusion maps obtained with 4 b-value diffusion gradients (MR parameters, FOV = 30 mm, TR = 2,300 ms, TE= 50 ms, in plane resolution 230 um, slice thickness 2 mm with a total acquisition time of 20 mins)

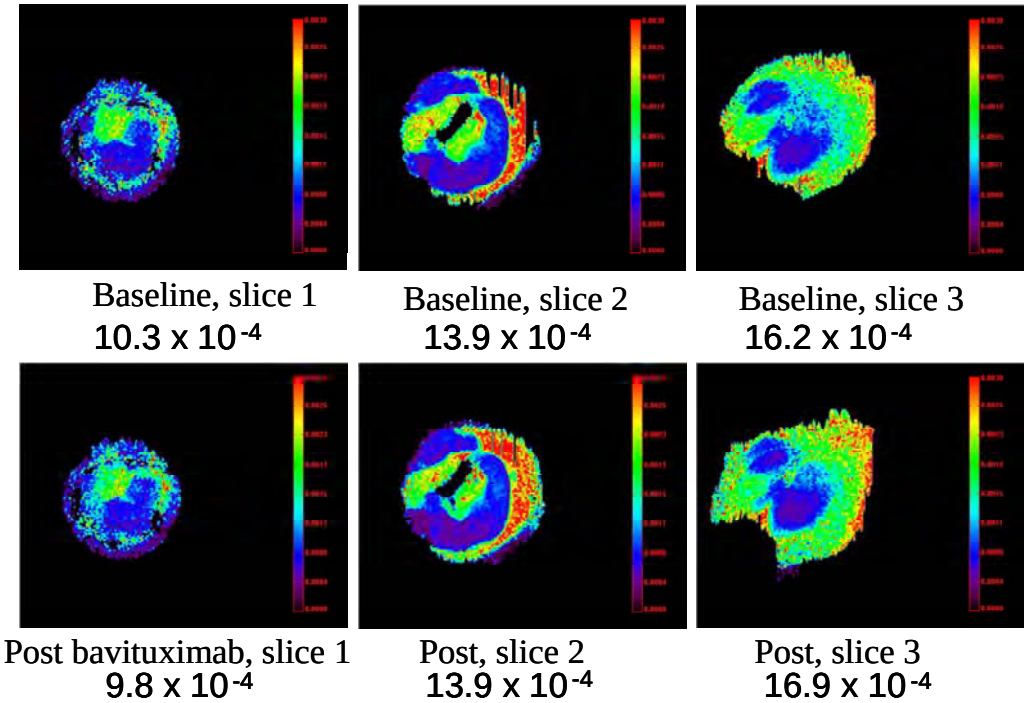


Figure 2 Apparent diffusion maps obtained by proton MRI at 4.7 T of Dunning prostate R3327-AT1 tumor. Data as for Figure 1, but hosing three consecutive image slices in representative AT1 tumor. Distinct baseline heterogeneity is apparent with mean ADC ranging from 10.2×10^{-4} to 16.2×10^{-4} mm^2/s. 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	S
AT1, HI	-3.839	3.153	.0196	S
AT1, MAT-Lu	1.079	3.349	.5083	
H, HI	-14.130	3.387	<.0001	S
H, MAT-Lu	-9.213	3.570	<.0001	S
HI, MAT-Lu	4.918	2.987	.0027	S

Table 1 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

Dynamic contrast enhanced MRI

There was distinct heterogeneity between center and periphery of each tumor type as shown in the following

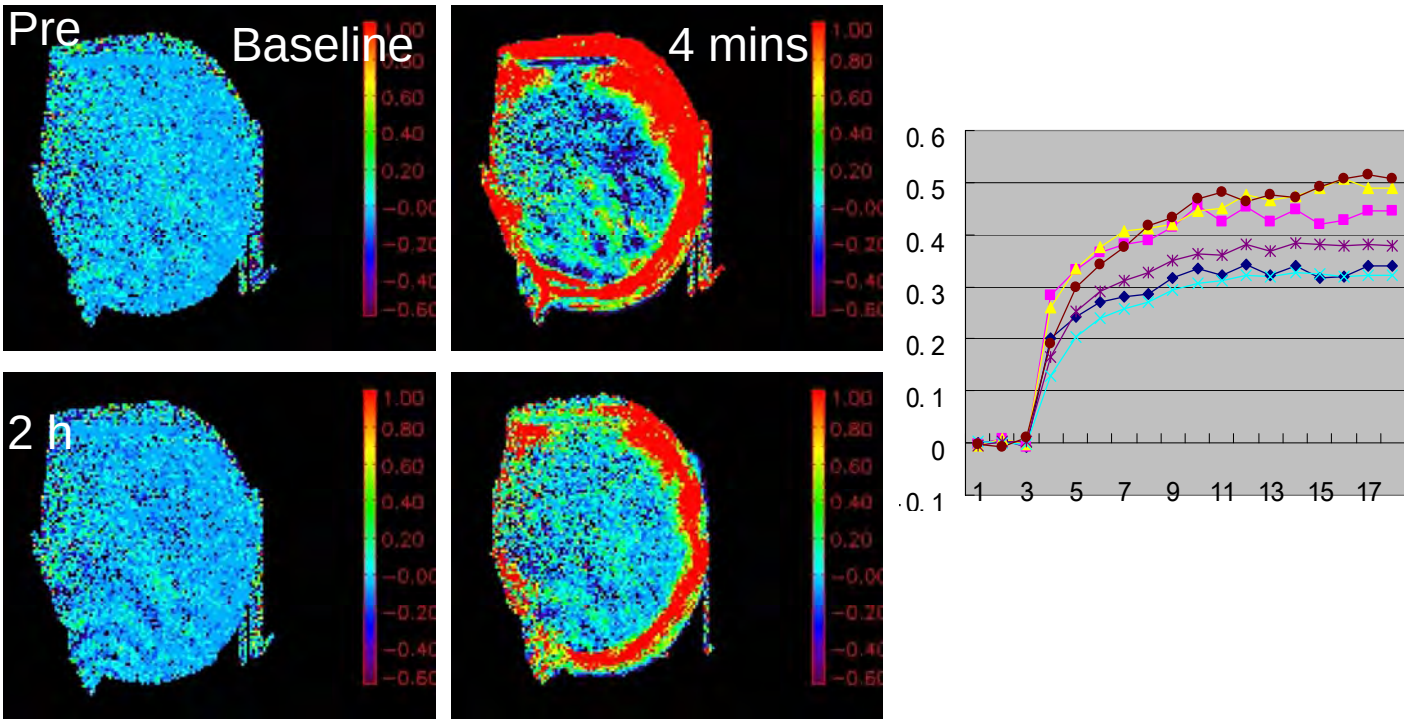


Figure 3 DCE for MAT-Lu tumor. Top left Relative signal intensity map for T1 weighted MRI pre therapy and before contrast agent. Top center: 4 mins after contrast showing strong peripheral enhancement; Bottom left baseline MRI 2 h after administration of bavituximab; Bottom center 4 mins post contrast, 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.

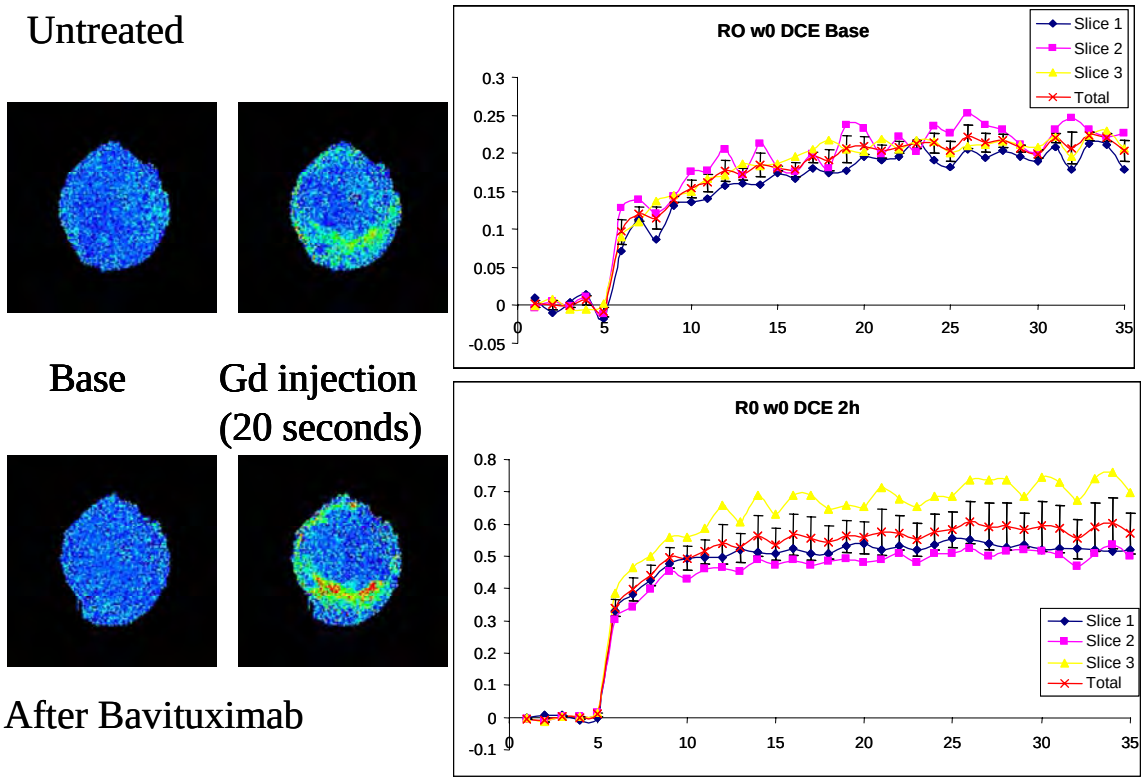


Figure 4 DCE for AT1 tumor. Top left Relative signal intensity map for T1 weighted MRI pre therapy and before contrast agent. Top center: 20 s after contrast showing strong peripheral enhancement; Bottom left baseline MRI 2 h after administration of bavituximab; Bottom center 20 s post contrast, 2 h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

	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 2 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 Kep.

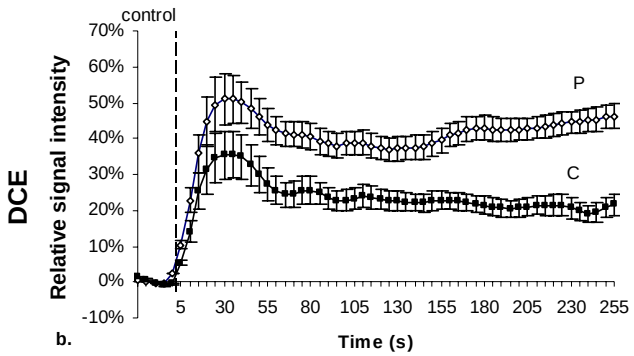


Figure 5 Comparison of signal intensity during DCE experiments for a group of AT1 tumors. A significant difference in signal response was observed between central and peripheral regions of tumor.

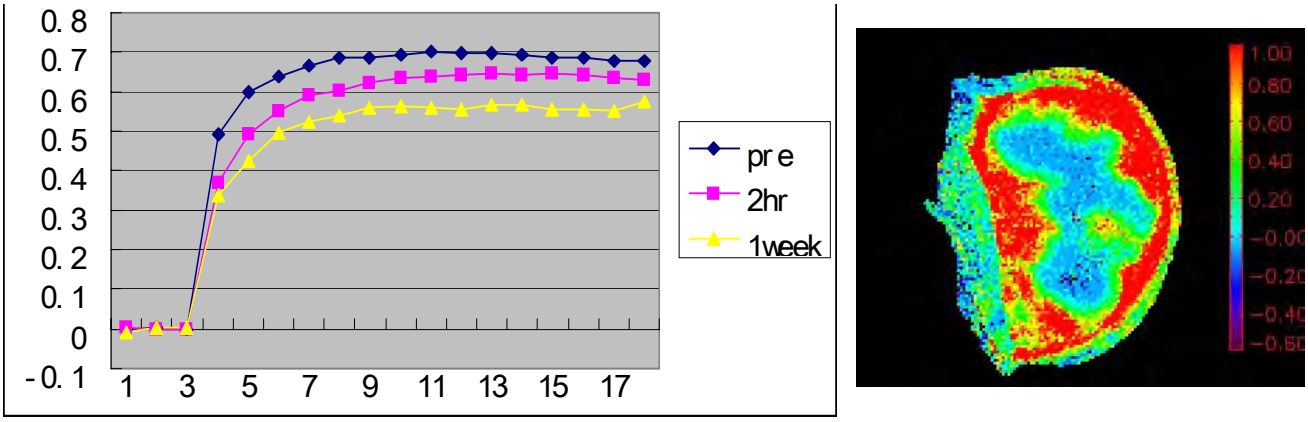


Figure 6 DCE for HI tumor. Left Mean signal intensity kinetics following infusion of contrast agent. Right: Relative signal intensity map for T1 weighted MRI 2 h post therapy and 4 minutes after contrast agent showing heterogeneous perfusion.

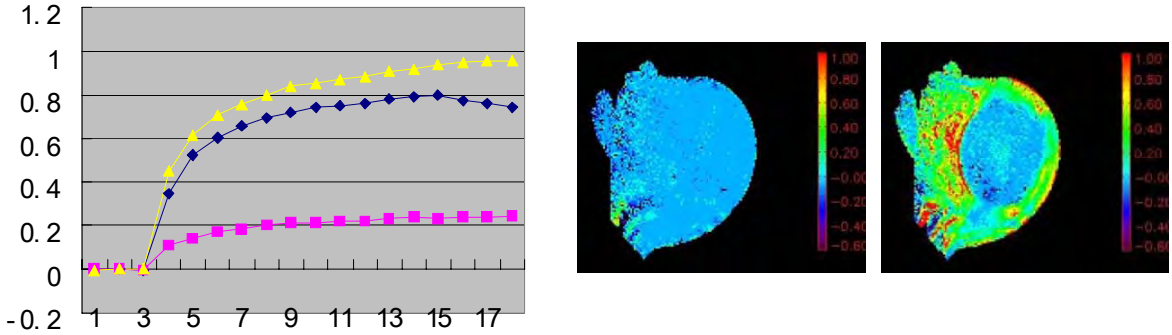


Figure 7 DCE for H tumor. Left Mean signal intensity kinetics following infusion of contrast agent. **Pre (blue), 2h post bavituximab (pink), 7 days post (yellow).** Right: Relative signal intensity map for T1 weighted MRI pre and 4 minutes after contrast agent showing heterogeneous perfusion pre bavituximab.

(ΔSI)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 3 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 Kep.

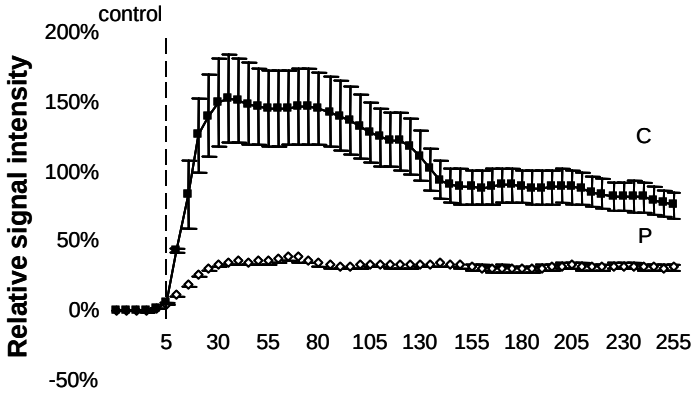
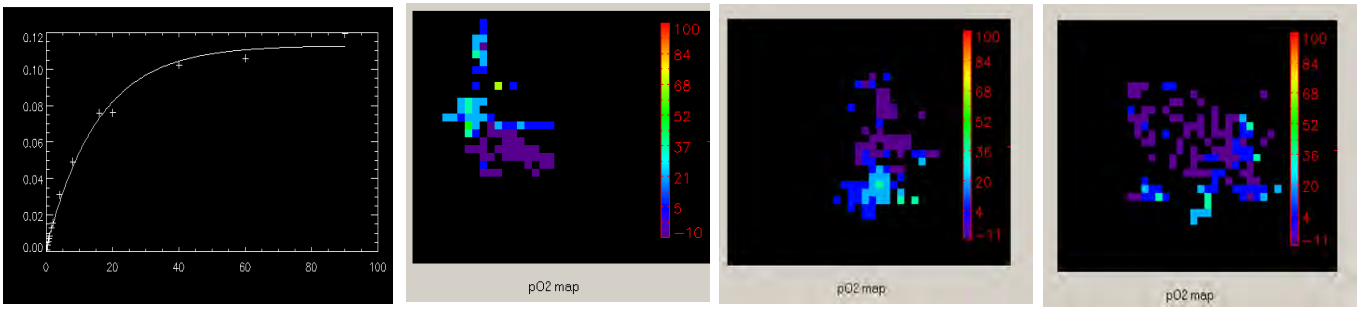


Figure 8 Comparison of signal intensity during DCE experiments for a group of H tumors. A significant difference in signal response was observed between central and peripheral regions of tumor, but here the center showed a larger change, whereas for AT1 tumors in Figure 5, the opposite was observed.

Tumor oximetry

The *FREDOM* (Fluorocarbon Relaxometry using Echo Planar imaging for Dynamic Oxygen Mapping) (19) was successfully applied to measure tumor pO_2 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 pO_2 ~ 40 torr. 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 pO_2.



Relaxation curve **MAT-Lu** **HI** **H**

Figure 9 Oximetry in Dunning prostate tumors. Left A typical ^{19}F 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 pO_2. Based on such curves maps were generated for representative tumors shown for MAT-LU, HI and H. Voxel dimension 1.25 mm in plane with 10 mm thickness.

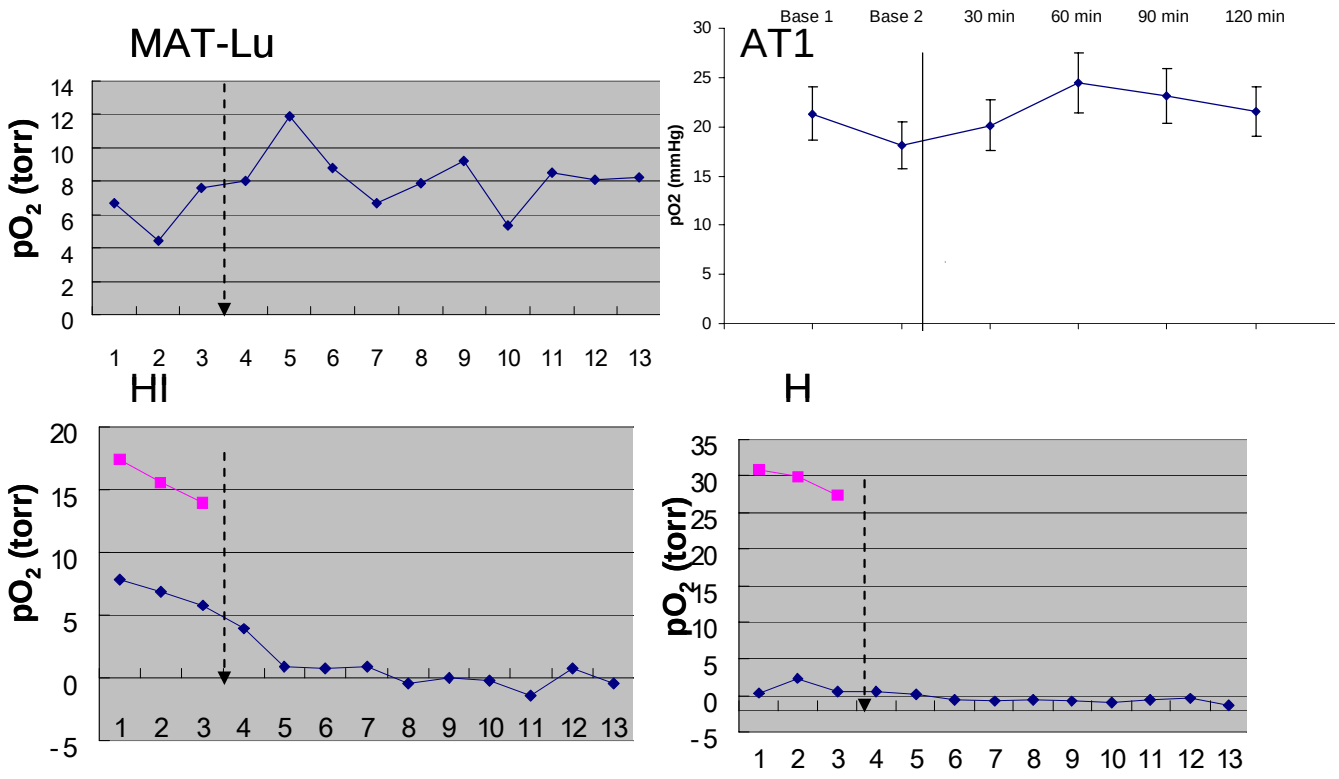


Figure 10 Oxygen dynamic in Dunning prostate R3327 tumors with respect to bavituximab infusion.

Two or three baseline pO_2 maps were generated in individual tumors and then bavituximab was infused IP arrow). Further pO_2 maps were generated over the following 2 hours. Only HI tumors showed significant change (hypoxiation) following infusion. Pink lines show pO_2 measurements seven days later.

Task 3 Months 3-15

Response to multiple doses of Bavituximab.

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. 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 (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.

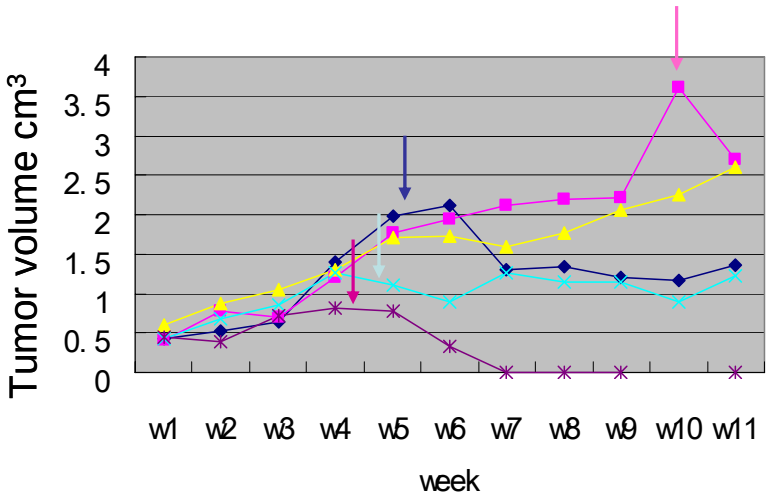


Figure 11 Growth curves for H tumors with respect to bavituximab therapy. Therapy (2.5 mg/kg thrice weekly IP) was initiated at times shown by arrows. In each case tumor growth was controlled and in one case the tumor disappeared. There rats are still alive and growth studies are ongoing.

Task 4 Months 3-18

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

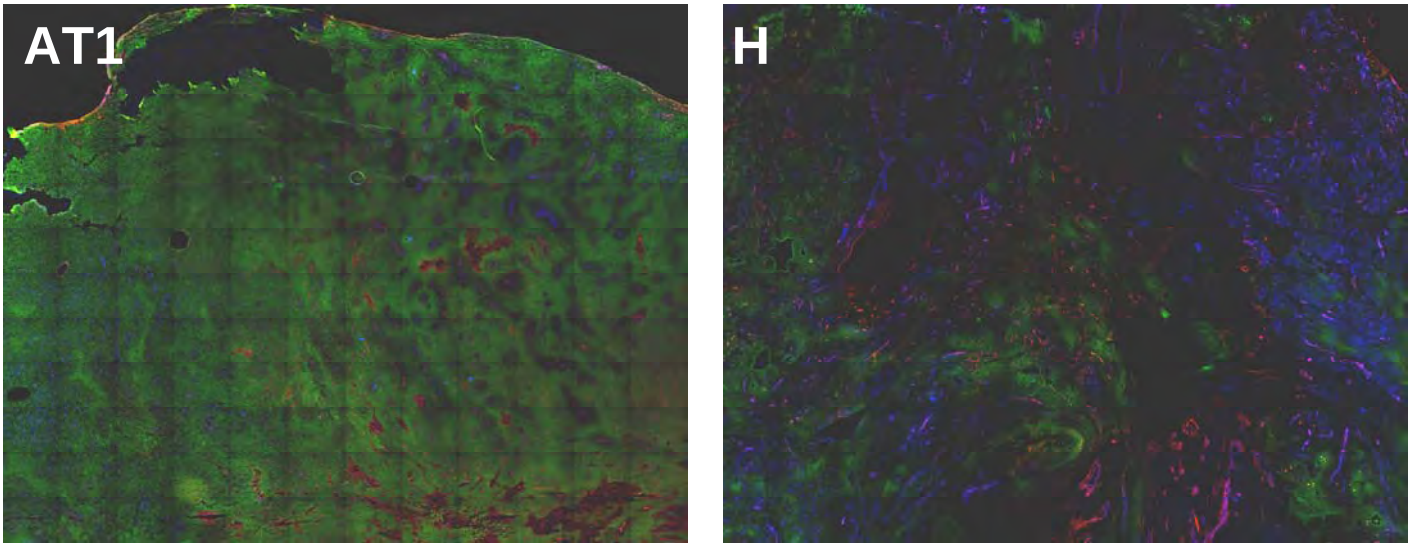


Figure 12 Comparison of microvasculature and hypoxia in control AT1 and H tumors.

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. Nineteen.
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 Prepare annual report and manuscript.

Annual report is provided here and a manuscript is in preparation.

Reportable Outcomes

At this time there are no specific reportable outcomes, but manuscripts are in preparation.

Conclusions

1 Non-invasive evaluation of Bavituximab activity *in vivo*. Assessment of efficacy in syngeneic rat prostate tumor sublines of diverse characteristics at different sizes and with respect to multiple doses is underway and continues. MRI has been used to assess onset of tumor vascular damage and evaluate the earliest and most definitive indications of drug efficacy. Methods will include Dynamic Contrast Enhanced MRI, diffusion, and pO_2 based on *FREDOM* (Fluorocarbon relaxometry using Echo planar Imaging for Dynamic Oxygen mapping). We had expected bavituximab to cause acute hypoxiation and thus act synergistically with hypoxia selective cytotoxins. Only HI tumors show any acute hypoxiation. Thus, for this tumor line we continue to hypothesize that combination of bavituximab and tirapazamine should be effective. We will still undertake the combined therapy trials for the other sublines since the tumors naturally show some hypoxia and thus combined therapy should show some advantage. However it appears that for the faster growing sublines have a rapidly proliferating edge escapes control. 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 are currently seeking IACUC approval for this deviation. We will then formally propose the additional treatment to the CDMRP.

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