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Award Number: DAMD17-02-1-0026

TITLE: Mechanisms and Refinements of PSCA Directed Antibody Therapy

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REPORT DATE: January 2005

TYPE OF REPORT: Final

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

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20051101 114

**REPORT DOCUMENTATION PAGE**Form 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

<b>1. AGENCY USE ONLY</b>		<b>2. REPORT DATE</b> January 2005	<b>3. REPORT TYPE AND DATES COVERED</b> Final (17 Dec 2001 - 17 Dec 2004)	
<b>4. TITLE AND SUBTITLE</b> Mechanisms and Refinements of PSCA Directed Antibody Therapy			<b>5. FUNDING NUMBERS</b> DAMD17-02-1-0026	
<b>6. AUTHOR(S)</b>  Robert E. Reiter, M.D.				
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<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b> U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			<b>10. SPONSORING / MONITORING AGENCY REPORT NUMBER</b>	
<b>11. SUPPLEMENTARY NOTES</b>				
<b>12a. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for Public Release; Distribution Unlimited			<b>12b. DISTRIBUTION CODE</b>	
<b>13. ABSTRACT (Maximum 200 Words)</b>  Prostate stem cell antigen is a cell surface protein expressed by a majority of localized and metastatic prostate cancers. Our laboratory previously showed that a monoclonal antibody against PSCA, mAb 1G8, could inhibit the growth and metastasis of prostate cancers in immunodeficient mice. The overall goal of this study was to determine the mechanism of action of this antibody, primarily whether it inhibited growth by recruiting the immune system or by direct crosslinking and signaling via the antigen on the cell surface. In addition, we proposed to test rational combination therapies to determine if we could improve upon the activity of the antibody itself. The major results reported here are that (1) the antibody 1G8 inhibits tumor growth predominantly through an Fc independent mechanism of action, and (2) that combination therapies are context dependent. In some instances, castration synergized with antibody to prevent androgen independent tumor progression, while in others antibody promoted androgen independent progression. These results are directly relevant to the clinic as a humanized version of PSCA mAb 1G8 generated by our laboratory nears application in the clinic.				
<b>14. SUBJECT TERMS</b>  Prostate Cancer			<b>15. NUMBER OF PAGES</b> 9	
			<b>16. PRICE CODE</b>	
<b>17. SECURITY CLASSIFICATION OF REPORT</b> Unclassified	<b>18. SECURITY CLASSIFICATION OF THIS PAGE</b> Unclassified	<b>19. SECURITY CLASSIFICATION OF ABSTRACT</b> Unclassified	<b>20. LIMITATION OF ABSTRACT</b> Unlimited	

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## SECTION 1: INTRODUCTION

PSCA is a cell surface antigen expressed by a majority of prostate cancers. We have previously shown that PSCA is expressed at particularly high levels in prostate cancer bone metastases, which led us to hypothesize that it might be a suitable target for antibody therapy. We then went on to show that, indeed, antibodies against PSCA have significant anti-tumor activity, inhibiting prostate cancer growth and blocking prostate cancer metastasis in numerous in vivo models. The biological role of PSCA and the mechanism of therapeutic activity of PSCA antibodies are not known. Therefore, the goal of this proposal was to determine the mechanism of anti-tumor activity and try to use this information to improve therapy. In particular, the goal of this grant was to determine if PSCA antibodies work via stimulation of the immune system or by direct binding of the antibody to PSCA on the cell surface. The major tasks of this proposal have been completed. Significant additional experiments have been performed over the past year to further the completed tasks.

## SECTION 2. SUMMARY OF COMPLETED WORK

### Specific Aim 1. To understand the therapeutic activity of monoclonal antibodies directed against PSCA in preclinical models.

Task 1. Whole antibody vs. F(ab')<sub>2</sub>. The goal of this task was to compare in vivo efficacy of whole PSCA antibody 1G8 to its F(ab')<sub>2</sub> fragment, thereby testing whether the Fc portion of the antibody was required for antitumor activity. The first and most difficult part of this task was to generate F(ab')<sub>2</sub> fragment and demonstrate its purity and its continued ability to recognize PSCA. This was successfully done and we showed both that there was less than 0.5% contamination and that the F(ab')<sub>2</sub> fragment still recognized PSCA on the cell surface by FACS. We then proceeded to test the fragment and whole antibody in the LNCaP-PSCA and the LAPC 9 models of prostate cancer. Two types of experiments were performed. In the first, antibody and tumor were given concurrently in order to test the ability of antibody to inhibit tumor formation. In the second, antibody was given to animals with established tumors in order to determine the effect of antibody on tumor growth. Molar equivalents of antibody were given. The results for LAPC 9, which expresses endogenous PSCA, are shown below in Figure 1. The results show that F(ab')<sub>2</sub> fragments inhibit tumor growth as well as whole antibody, demonstrating convincingly that the Fc portion of the antibody is not required for tumor inhibition and that the antibody works by crosslinking of antigen, suggesting a direct mechanism of action.

**SUMMARY:** PSCA antibody 1G8 blocks tumor growth and metastasis by an Fc independent mechanism, as evidenced the equivalent effects of F(ab')<sub>2</sub> and whole antibody on tumor growth in vivo. This suggests that PSCA plays an important role in tumor growth and that stimulation or blocking of its function with antibody can inhibit tumor progression. This result has important implications for PSCA as a promising target for anticancer therapy.

Task 2: Comparison of antibody in FcR deficient mice.

A parallel way to approach the question of Fc dependence or independence is to administer whole antibody in mice lacking FcR. These mice were obtained from Jeff Ravetch at Rockefeller University. Although these experiments have been difficult secondary to the very slow breeding of these mice and the fact that we can only use male mice, we have managed to evaluate antibody 1G8 in a small cohort of FcR deficient mice and found that the results were in agreement with those of Task 1, namely that the antibody was still able to inhibit tumor growth in the absence of FcR (data not shown). These experiments bolster the conclusion that PSCA antibody works through a direct mechanism of action.

**SUMMARY:** This task supports the results of Task 1 using a genetic defined model to demonstrate that PSCA antibody 1G8 can inhibit tumor growth in the absence of functional Fc receptor.

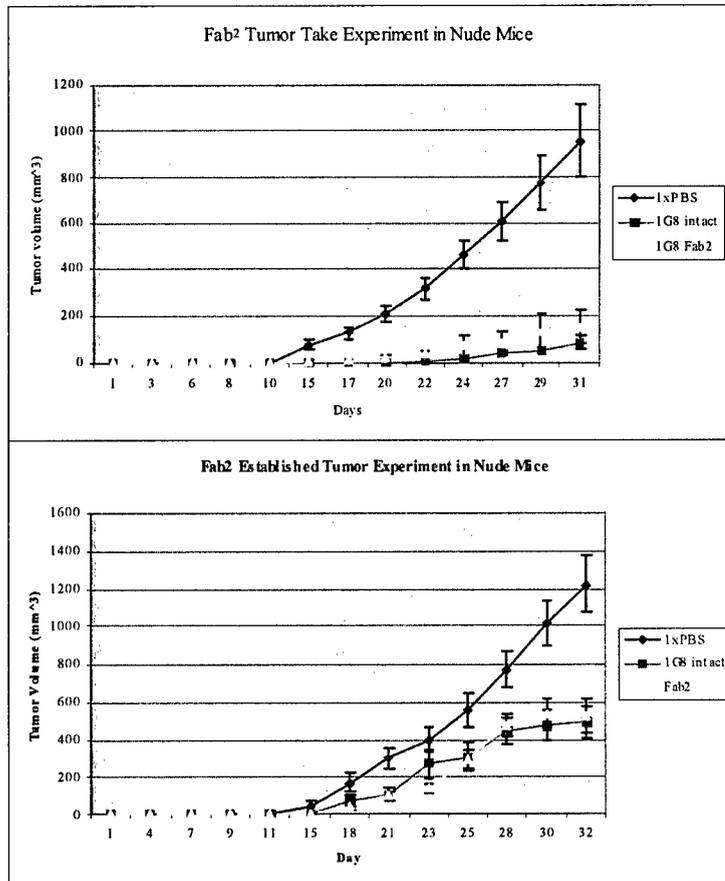


Figure 1. Comparison of whole antibody 1G8 and its F(ab')<sub>2</sub> fragment in nude mice with LAPC 9 prostate tumors. In the top panel, antibody was given twice weekly beginning at the time of tumor inoculation. In the bottom panel, antibody was given when tumors reached ~ 0.5 cm<sup>3</sup> in size. As is seen, F(ab')<sub>2</sub> fragment was equally effective as whole antibody in inhibiting tumor formation and growth.

Task 3: Additional in vivo assays. These experiments were designed as fallbacks if Tasks 1 and 2 did not work, and are not needed at this point. We have, however, examined the mechanism of action of PSCA antibody 1G8 in tissue culture model systems. We found that the antibody can inhibit growth of LNCaP-PSCA cells in vitro, consistent with the direct mechanism of action we have proposed. Preliminary data suggest that antibody works by stimulating cells to undergo apoptosis (Figure 2). We also show that this is a nonclassical apoptotic pathway, independent of caspases. Caspase inhibition did not alter the cell death seen in response to antibody. Also, the response was predominantly one of an increase in PI staining, but no significant increase in annexin. We also showed that crosslinking of antigen is necessary, as single chain F(ab') antibody does not induce cell death (data not shown).

## 1G8 inhibits LNCaP-PSCA growth and induces cell death

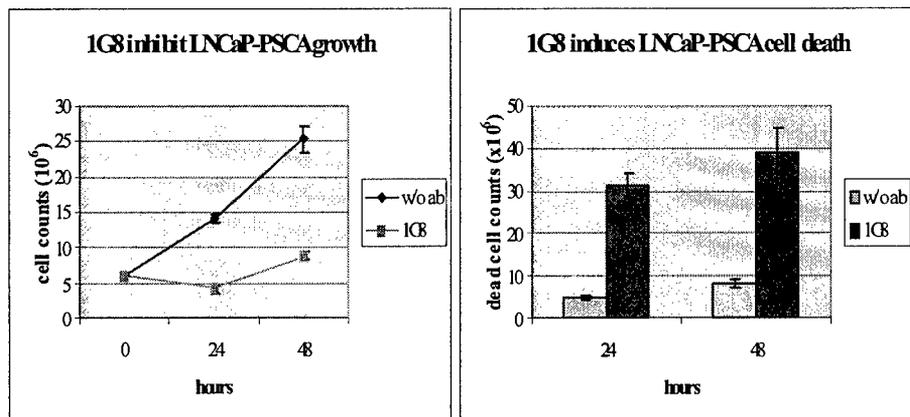


Figure 2. 1G8 can inhibit growth of LNCaP-PSCA cells in vitro (left panel) over 48 hours. On the right, we show that this occurs because of increased cell death after antibody treatment, with 30-40% of cells dying at 24-48 hours. We show in other experiments that this cell death occurs as early as 6 hours after antibody exposure

Over the past year, we have proceeded further to elucidate the mechanism of action.

First, we asked if the GPI anchor of PSCA is necessary for the antibody to be active. We constructed multiple chimeric PSCAs lacking the GPI anchor or replacing the GPI anchor with the transmembrane domain of tissue factor (TM-PSCA). We found that the GPI anchor is not necessary and that the antibody could kill TM-PSCA in vitro and inhibit its growth in vivo. This surprised us, as we had hypothesized that the GPI anchor would be required to localize PSCA to cholesterol rafts on the cell membrane and that such localization was critical for signaling induced by antigen crosslinking. So we looked at membrane localization by fractionating the membrane and staining for PSCA. We found that while TM-PSCA no longer localized to rafts in the absence of antibody, it did localize in part to rafts upon antigen crosslinking. These results suggest that antibody crosslinking can translocate PSCA to rafts, where it can inhibit tumor growth. These results are similar to those reported with other antibodies such as rituxan and suggest that membrane localization is critical for antibody activity. Other ongoing experiments are testing the effect of PSCA loss on tumor growth in NKX3.1 and PTEN knockout mice, since PSCA expression is upregulated in the tumors of these mouse models. We anticipate that these experiments may shed light on PSCA's function in tumorigenesis, and consequently, on the mechanism of action of the antibody. We have also proposed new experiments to look at the effect of PSCA knockdown with shRNA and the effect of PSCA overexpression by making transgenic mice.

**SUMMARY:** In vitro and in vivo models have been developed to elucidate the mechanisms of action of PSCA and PSCA antibody activity and should provide important new information over the coming years.

Task 4 Comparison of antibody activity in tumors expressing high and low levels of PSCA. LNCaP cells expressing high and low levels of PSCA were generated and tested both in vivo and in vitro. LNCaP-PSCA 1 cells express ten-fold higher PSCA than LNCaP-PSCA 2 cells. We found that in vitro the level of PSCA correlated clearly with antibody induced cell death, with LNCaP-PSCA 2 cells responding significantly less. This was also true in vivo, although not as dramatically. LNCaP-1 cells were completely inhibited by 1G8, while LNCaP-2 cells eventually formed tumors (not shown).

**SUMMARY:** The level of PSCA on the cell surface does correlate with response to antibody. However, cells with low-level expression of PSCA were still inhibited by antibody. In other work done by our laboratory, we were able to show that >50% of metastatic prostate cancers express levels of PSCA comparable to or higher than LNCaP-PSCA 1, suggesting that a large percentage of tumors are likely to respond to PSCA antibody.

Importantly, >80% of tumors expressed levels higher than LNCaP-PSCA 2, so even these “low” expressers are potential targets for this therapy.

Specific Aim 2: To enhance the therapeutic activity of monoclonal antibodies directed against PSCA.

Task 1: Hormonal therapy + antibody. These experiments have been performed multiple times, with interesting results. Our hypothesis was that PSCA antibody 1G8 would synergize with castration to inhibit tumor growth and androgen independent progression. When we combined antibody with castration in LNCaP-PSCA 1 cells, this is what we saw. However, when we tested the combination in LAPC 9 cells, which express endogenous PSCA, we found that 1G8 antagonized the effects of castration and actually promoted androgen independent tumor progression (Figure 3). This experiment has been repeated and we also varied the time point at which PSCA antibody treatment was begun, all with similar results.

SUMMARY: The effect of 1G8 on androgen independent growth is context dependent. The cause for the antagonistic effect of 1G8 on castration in LAPC 9 is not known, but suggests that 1G8 (and PSCA) may signal somehow through the androgen receptor, perhaps modulating its activity. This hypothesis is being pursued. It is clear that care needs to be taken in constructing clinical trials using antibodies against PSCA, since one would hypothesize that combination of androgen ablation and PSCA antibody in hormone naïve patients might be harmful.

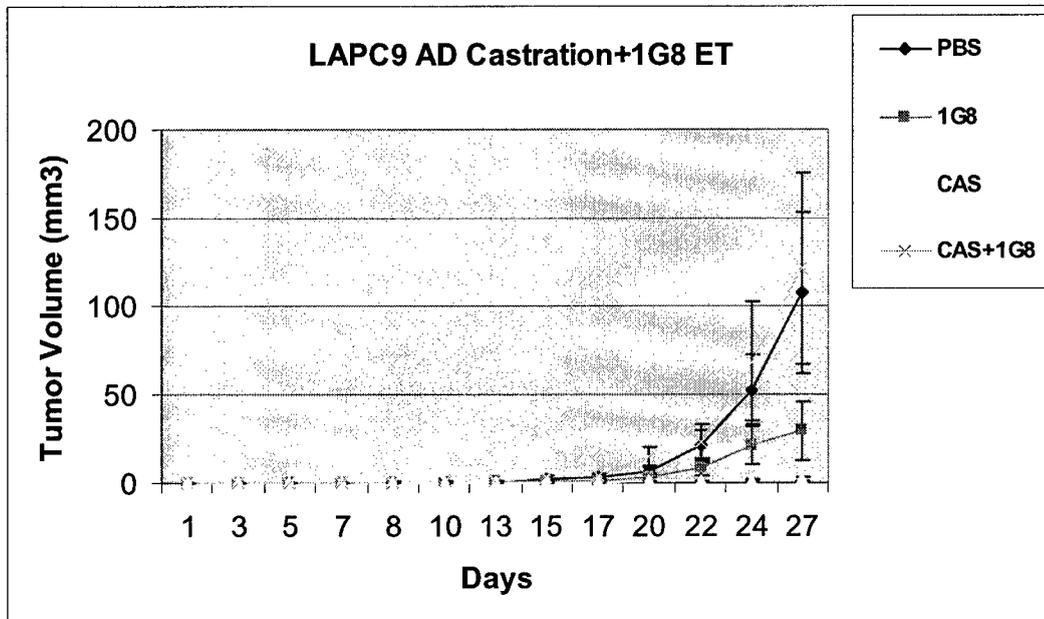


Figure 3: PSCA mAb 1G8 antagonizes castration. Mice were treated with PBS, 1G8, castration, or castration + 1G8 and tumor growth evaluated. Castration led to tumor regression, as predicted (if left longer, these mice eventually developed androgen independent tumors). 1G8 inhibited tumor growth compared to PBS, but not as much as castration. However, 1G8 abrogated any effect of castration and permitted tumor growth similar to noncastrate controls.

Tasks 2-3. We evaluated the combination of antibody with taxol chemotherapy in LAPC 9 cells and saw no synergy, although there was an additive effect. Experiments combining antibody 1G8 with mTOR antagonists are underway, based on the observation that PTEN null prostate cancers express elevated levels of PSCA. We also did a small experiment combining GM-CSF with 1G8 and saw no additive effect, presumably because 1G8 does not appear to work via stimulation of the immune system. This was a small experiment limited by the cost of GM-CSF (~\$1,000/mouse).

#### KEY RESEARCH ACCOMPLISHMENTS:

1. The major outcome of this study was the determination that PSCA mAb 1G8 works by crosslinking of PSCA on the cell surface in an Fc independent manner.
2. Demonstration that PSCA antibody activity may be contextual, in some cases synergizing with, in others antagonizing hormonal therapy.

#### REPORTABLE OUTCOMES

Three manuscripts are in preparation at this time, one describing the Fc independent mode of action, one describing signaling through cholesterol rafts, and a third describing the development of chimeric and humanized version of PSCA mAb 1G8.

## CONCLUSIONS

Our major goal was to determine if PSCA mAb 1G8 inhibits tumor growth by interaction with the immune system or by direct antigen crosslinking. We proposed to answer this question using multiple approaches, all of which showed that crosslinking is the major mode of action. This result is in contrast to work by others with Herceptin and Rituxan, showing that the immune system plays a dominant role in activity. Our results suggest that elucidation of the normal function of PSCA will be critical for understanding the mechanism of action of PSCA antibody. We also suspect that elucidation of the signaling pathway(s) controlled by PSCA will lead to rational therapeutic combinations. Our empiric attempts to identify combination therapies led to the surprise result that PSCA mAb can actually promote androgen independent growth in some contexts, a result that needs additional validation and experimentation.