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**13. Abstract** ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

PSCA is a cell surface antigen expressed by a majority of prostate, bladder and pancreatic cancers. An antibody against PSCA has preclinical activity against prostate cancer xenografts. The mechanism of this activity is not known. The goals of this project include 1) determining whether anti-tumor activity is Fc-region dependent or independent (or both) and 2) testing combination therapies to identify potential synergies or antagonisms that may be relevant to the clinic. We have also proposed to develop humanized antibodies for potential translation to the clinic.

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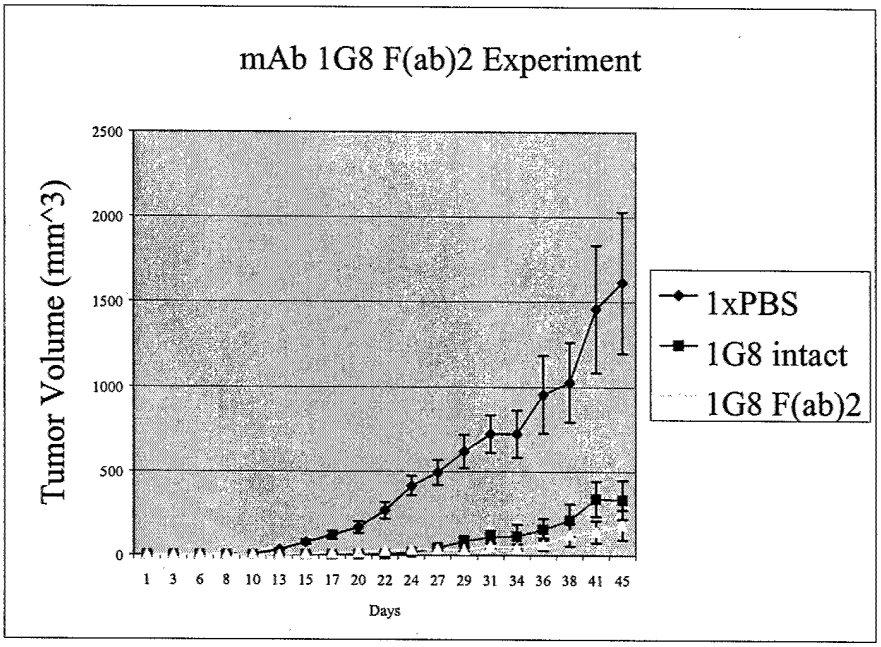
**Introduction:** Prostate cancer is the second leading cause of cancer-related death in American men. Despite advances in local therapy, a significant percentage of men relapse and progress to develop metastatic disease, for which there is currently no cure. Recent advances in molecular target identification, patient selection and antibody engineering have led to the introduction of the first clinically active monoclonal antibodies (i.e. Rituxan and Herceptin) into the marketplace. Prostate stem cell antigen (PSCA), a cell surface homologue of stem cell antigen 2 (SCA-2), is expressed by >80% of prostate cancers and is overexpressed in ~30-40% of local and ~60-100% of bone metastatic tumors. Monoclonal antibodies specific for human PSCA were developed by our laboratory and have been shown to inhibit tumorigenesis, slow tumor growth, prolong survival and block metastasis of androgen dependent and independent prostate cancers in preclinical models. These results suggest that PSCA-directed antibody therapy may have therapeutic activity against human prostate cancer. The Aims of this project are (1) to understand the mechanism of therapeutic activity of monoclonal antibodies directed against PSCA in preclinical models and (2) to enhance the therapeutic activity of monoclonal antibodies directed against PSCA in preclinical models.

**Specific Aim 1. To understand the therapeutic activity of monoclonal antibodies directed against PSCA in preclinical models (months 1-24)**

- Whole antibody vs. F(ab')2
  Production of mAb 1G8 F(ab')2
  In vivo experiments

F(ab')2 fragments were successfully generated and tested to confirm that they recognize PSCA. We also confirmed that the preparation was pure, with less than 0.5% contamination. Molar equivalents of F(ab')2 and whole 1G8 antibody were then tested in our preclincal models. We tested both the ability of antibody to block tumor formation and to inhibit growth of established tumors. As shown below, F(ab')2 was able to inhibit tumor growth as well as whole antbody. These experiments have been repeated and total more than 25 animal per group, clearly establishing that PSCA mAb 1G8 blocks tumor growth at least partly through an Fc-independent mechanism. We have also shown that F(ab')2 internalizes more rapidly than whole antibody, whicn may be relevant.




F(ab')2 and whole 1G8 inhibit LAPC 9 tumor formation in nude mice. Equimolar amounts of PBS, whole antibody and F(ab')2 were injected into mice inoculated with LAPC 9 tumor and then thrice weekly for four weeks. Results show relatively equivalent antitumor activity of 1G8 F(ab')2 and whole 1G8.

- Comparison of antibody in FcR deficient mice (months 1-12)
- Additional in vivo assays (dependent on results of Aims 1.1-1.2, i.e. NK-depleted mice, complement depleted mice etc.) (months 12-36) The above two experiments were proposed in case we found that

F(ab')2 had no anti-tumor activity, since we speculated that this could occur because of poor pharmacokinetic properties of F(ab"2. We are proceeding with these experiemnts anyways, since it is possoble that whole antibody does require its Fc region for activity,while F(ab')2 is small enough to signal on its own. Preliminary data suggest that this may in fact be the case and that the efficacy of whole antibody may at least in part be due to the Fc region.

- Comparison of antibody activity in tumors expressing high and low levels of PSCA (months 18-24). We have now shown that tumors expressing a log less PSCA are still inhibtied by anti-PSCA antibody, although not to the degree fo the high expressors, suggesting efficacy in many PSCA positive tumors.

We have also done considerable work in vitro, confirming the direct activity of PSCA antibody on tumor cell proliferation. Antibody kills tumor cells through a non-classical apoptotic pathway. Also, we are determinign whether antigen crosslinking is necessary. Preliminary data suggest crosslinking is necessary, since single chain antibody has no effect in vitro.

**Specific Aim 2. To enhance the therapeutic utility of monoclonal antibodies directed against PSCA in preclinical models (months 4-36)**

- Hormonal therapy + antibody (months 4-12). These experiments have been started. Initial results are intriguing and suggest that antibody may actually interfere with the anti-tumor activity of castration. Mice treated with the combgination reapidly developed androgen independent tumors. These unexpected results need to be confirmed because of small numbers in the first experiment.
- Chemotherapy + antibody (months 4-24). These experiments are ongoing.
- Cytokines + antibody (months 12-36) (these experiments will be started once the preliminary results of Aim 1 are complete, since the selection of cytokines are premised on a potential finding of an immunological mechanisms of anti-PSCA antibody activity)

**Key Accomplishments:** We have completed a large part of Aim 1, the results suggesting that PSCA antibody can signal directly to tumor cells by antigen crosslinking. These unexpected results may enable us to define pathways downstream of PSCA and to understand its potential contribution to prostate cancer growth. Our intriguing preliminary results combining homronal therpay with antibody also may provide new insight into PSCA function and prostate cancer. It is yet to be determined whether PSCA antibody will become an effective therapy for prostate cancer.

**Outcomes:** We will write up the results to date over the coming year.

**Conclusion:** We have made significant headway on our proposed Aims. The next year promises interestign new data!