Prostate-Specific Membrane Antigen (PSMA) Targeted Bio-orthogonal Therapy for Metastatic Prostate Cancer

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During the first year of the project we have identified new monoclonal anti-PSMA antibody (mAb), which demonstrated high affinity to the PSMA receptor and excellent targeting of PSMA-expressing prostate cancer cells both in vitro and in vivo. We investigated details of the mAb and therapeutic complexes internalization in these cells and demonstrated rapid perinuclear localization of internalized agents. We have also synthesized and tested click-reactive components for in vivo therapy. First in vivo data for anti-PSMA click-based pretargeting have been obtained with NIR in vivo optical imaging. In addition, all regulatory reviews of the proposed animal procedures have been successfully completed.

Targeted agents, anti-PSMA antibody, image-guided therapy

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1. **Introduction**
During the first year of the project we have identified new monoclonal anti-PSMA antibody (mAb), which demonstrated high affinity to the PSMA receptor and excellent targeting of PSMA-expressing prostate cancer cells both in *vitro* and in *vivo*. We investigated details of the mAb and therapeutic complexes internalization in these cells and demonstrated rapid perinuclear localization of internalized agents. We have also synthesized and tested click-reactive components for *in vivo* therapy. First *in vivo* data for anti-PSMA click-based pretargeting have been obtained with NIR *in vivo* optical imaging. To further understand the tumor delivery patterns contrast enhanced MRI was performed in several animals with PC3-Luc and PC3-PIP tumors. In addition, all regulatory reviews of the proposed animal procedures have been successfully completed.

2. **Keywords**

Anti-PSMA monoclonal antibody, targeted therapy, *in vivo* imaging, antibody internalization, cytotoxic conjugates, two-component therapy, pretargeting

3. **Accomplishments**

**What were the major goals of the project?**

Specific Aims of the proposal are:

**Aim 1.** Synthesize and characterize anti-PSMA J591 mAb-based pretargeting and albumin based nanocarrier components. Optimize the therapeutic efficacy of the delivery strategy *in vitro* in PSMA(+) and PSMA(-) PCa cells.

**Aim 2.** To evaluate the therapeutic system in subcutaneous and intratibial metastatic mouse models using PSMA(+) C4-2 and PC3-PIP and PSMA(-) PC3 cell lines.

Specifically for the first year of the project the approved SOW includes the following activities:

Subtask 1: Synthesize TCO-functionalized anti-PSMA antibody and tetrazine-functionalized HSA-DM1 carriers.
Subtask 2: Characterize stability, solubility, and affinity of specific binding of components in a panel of PSMA-positive cancer cells.
Subtask 3: Study mechanism of internalization of the therapeutic complexes in PSMA-positive cancer cells.
Subtask 4: Regulatory review of the animal procedures proposed.

**What was accomplished under these goals?**

TCO-functionalized anti-PSMA antibodies were synthetized using parent 5D3 mAbs, functionalized TCO moieties for click chemistry reactivity, and fluorophores for microscopy (AF488) and *in vivo* NIR fluorescence imaging (CF680). Examples of *in vitro* and *in vivo* binding of the functionalized antibody in PSMA-expressing (high and low) prostate cancer cells and experimental tumors are shown in Fig. 1.

To synthesize cytotoxic drug carrier components globular albumin molecules were conjugated with PEGylated tetrazine (Tt, reactive group for click chemistry) and microtubule inhibitor mertansine (DM1) therapeutic agent using MCC hetero-bifunctional linker. The high-molecular weight conjugate was purified by ultrafiltration and size-exclusion chromatography.
Mass-spectroscopy of parent albumin, intermediate compounds, and final product are shown in Fig. 2. All compounds were stable for at least 24h in sterile buffers and media at room temperature and at least for 7 days at 4°C. Internalization of components was studied in details in PSMA+ PC3-PIP cells and we demonstrated perinuclear localization of the internalized complexes presumably in the centrosome of the target cell (Fig. 3 - green). The internalization mechanism is different from clathrin-mediated endocytosis, as one can see by comparing signals of internalized dextran and mAbs (Fig. 3 - red).

This new mechanism opens an interesting possibility to deliver therapeutic compounds to the perinuclear location without exposing them (or the carrier) to degradative proteolytic enzymes present in endosomes and lysosomes.

Our most recent NIR imaging data for in vivo distribution of pretargeting and drug carrier components are shown in Fig .4.
Deliverables: during the first year of the project we have synthesized and characterized in vivo and in vitro the following therapeutic components:

(i) 5D3(mAb)-TCO-CF680 for in vivo NIR fluorescent imaging
(ii) 5D3(mAb)-TCO-Alexa488 for confocal microscopy
(iii) Albumin-PEG4-Tt-CF750 for in vivo NIR fluorescence imaging
(iv) Albumin-PEG4-Tt-Rhodamine for confocal microscopy
(v) Albumin-PEG4-Tt-MCC-DM1 cytotoxic drug carrier component

How were the results disseminated to communities of interest?
Scientific presentations were given at laboratory seminars and some results presented at JHU ICMIC seminar series

What do you plan to do during the next reporting period to accomplish the goals?
During the next period of the project (year 2) we will test the synthesized cytotoxic conjugates in cultured prostate cancer cells with different levels of expression of PSMA-receptors. We will optimize the compounds for in vivo studies in orthotopic and metastatic mouse models of human prostate cancer. Intratibial models of metastatic prostate cancer will also be developed.

5. Impact

What was the impact on the development of the principal discipline(s) of the project?
Novel two-component pretargeting system for specific therapy of PSMA-expressing prostate cancer can result in high efficacy and significantly reduced toxicity and side effects. Our synthesized therapeutic components (pretargeting and drug-carrier) are a first generation of such a system and are applicable to experimental therapy in animal models. Once optimized and validated future translation to clinic should be feasible.

What was the impact on other disciplines?
Nothing to report

What was the impact on technology transfer?
Nothing to report

What was the impact on society beyond science and technology?
Nothing to report

6. Changes/Problems

In the course of the study we became aware of a novel monoclonal anti-PSMA antibody, 5D3, produced by Dr. Barinka (Institute of Biotechnology CAS, Vestec, Czech Republic), which have been used in the lab of our consultant, Dr. M. Pomper. These antibodies are significantly less expensive than originally proposed J591 mAb, and have been extensively characterized both in vivo and in vitro and have similar or better affinity to the extracellular domain PSMA target than other anti-PSMA antibodies. The 5D3 antibody is also available in large batches (≥ 10mg). Therefore we have used and planning to use this mAb in all our in vitro and in vivo studies for therapeutic PSMA targeting
7. Products

Research materials (please see Accomplishments)

8. Participants & Other Collaborating Organizations

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<tbody>
<tr>
<td>Name: Dmitri Artemov, Ph.D.</td>
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<tr>
<td>Project Role: PI</td>
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<td>Research Identifier:</td>
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<td>Nearest person month worked:</td>
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Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to report

What other organizations were involved as partners?
Nothing to report

9. Special Reporting Requirements

Nothing to report

10. Appendices

N/A