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Award Number: W81XWH-05-1-0032

TITLE: Development of STEAP-Based Vaccines for the Treatment of Prostate Cancer

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REPORT DATE: November 2005

TYPE OF REPORT: Annual Summary

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-11-2005	Annual Summary	1 Nov 2004 - 31 Oct 2005

4. TITLE AND SUBTITLE
Development of STEAP-Based Vaccines for the Treatment of Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0032

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The University of Southern California
Los Angeles, CA 90089-1174

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Six-Transmembrane Epithelial Antigen of the Prostate (STEAP) has been considered an attractive target, because it is over-expressed in human prostate cancer tissues. Recently two hSTEAP peptides have been selected for their capacity to bind and stabilize HLA-A*0201 molecules. In addition peptide-specific human CTL cell lines recognized hSTEAP-expressing cells, lysing them. These findings suggest that STEAP is a promising antigen for tumor immunotherapy. In this study we determined the effectiveness of STEAP-based vaccination in prophylactic and therapeutic mice models by using two delivery systems and three vaccination schemes. In the first vaccination scheme, mice were primed and boosted with an expression vector that contains mSTEAP cDNA by gene gun. In the second one, mice were primed with mSTEAP cDNA by gene gun and boosted with Venezuelan Encephalitis Virus Replicons (VRP) encoding mSTEAP and in the last vaccination scheme mice where primed and boosted with mSTEAP-VRP. Our results show that mSTEAP-based vaccination, no matter the scheme of treatment, significantly delays tumor growth. The prime-boost vaccination scheme was able to induce a specific CD8 T cell response responsible for tumor eradication in complete absence of autoimmunity development but was unable to control pre-establish.

15. SUBJECT TERMS
Prostate Cancer, Immunotherapy, Androgen Ablation, Mouse model, Clinical trial

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
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Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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INTRODUCTION

Six-transmembrane epithelial antigen of the prostate (STEAP) was identified in xenografts of advanced human prostate cancer[1], derived from bone and lymph node metastases and propagated in SCID mouse models. Under physiological conditions, low levels of STEAP have been detected in plasma membranes of normal prostate tissues. In contrast, it is highly over-expressed in human prostate cancer tissue. Suggesting it could be a potential candidate for immunotherapy. STEAP expression has also been detected in several colon, bladder, ovarian, and pancreatic cancer cell lines, reinforcing the idea that this gene may be generally up regulated in cancer. We recently identified its murine counterpart, expressed in a prostate tumor cell line (TRAMP-C1) derived from the prostate of mice of the transgenic adenocarcinoma mouse prostate model (TRAMP)[2]. The analysis of the nucleotide and amino acid sequence showed that mouse STEAP has 80% homology with hSTEAP and it also contains six potential membrane-spanning regions. In TRAMP mice, STEAP is over-expressed in prostate cancer tissues whereas low levels are detected in kidney and testis. Recently, we detected STEAP in thymus by immunofluorescence (data not published) and maybe it could participate in mechanisms of central tolerance mediated by medullar epithelial cells. This tumor-associated antigen does not have substantial structural changes when it is compared to the self-Ag. For this reason, it could be difficult to induce a good immune response without disturbing the mechanisms of central and peripheral tolerance potentially associated to this antigen. In a recent report, two human STEAP peptides were identified as excellent inducers of antigen-specific CTLs, which were able to recognize and kill STEAP-expressing tumor cells[3, 4]. This data suggests that STEAP can be considered as a promising candidate for immunotherapy of prostate cancer.

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Task 1. To determine whether mouse STEAP can be used as tumor rejection antigen in C57BL/6 and TRAMP mice.

***Task 1a*. To characterize protein expression levels of mouse STEAP in organs of TRAMP mice.**

We first tested the effectiveness of different mSTEAP-based vaccination in a transplantable tumor model using male C57BL/6 mice, because it was easy and we can get results in a shorter time than in the transgenic adenocarcinoma mouse prostate model (TRAMP). This report includes experiments realized in C57BL/6 mice and therefore we decided to show mSTEAP expression on tissue samples from C57BL/6.

Cryostat sections from different organs such as brain, hearth, kidney, stomach, colon, intestine, bladder, muscle, spleen, adrenal-, salival and seminal gland as well as testis and prostate were incubated with a rabbit anti-mouse STEAP antibody that we developed in our laboratory and then incubated with biotin-conjugated goat anti-rabbit. Finally, bound antibodies were visualized with streptavidin-conjugated to Alexa Fluor-594. A representative image from tissues with high and negative mSTEAP expression are shown in figure 1. Low protein level was detected in bladder and brain. We also detected mSTEAP expression in spleen and we are currently characterizing what cells are producing this protein.

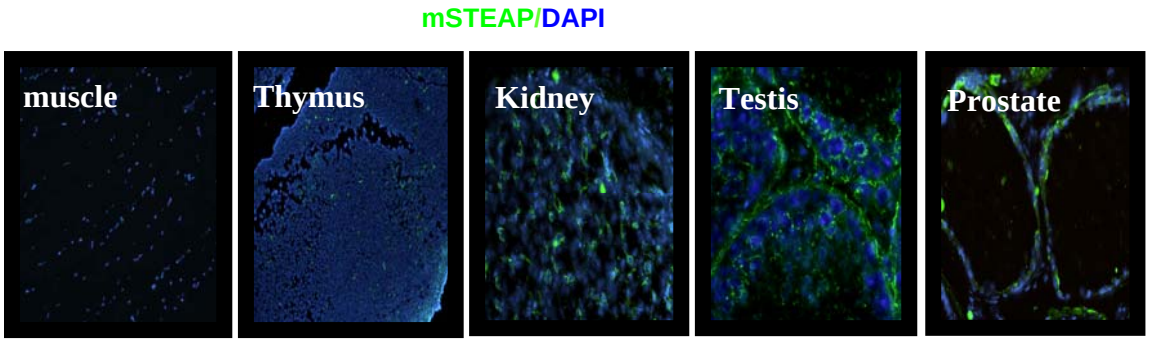

Figure 1 mSTEAP expression in organs from C57BL/6 mice. Different organs including prostate, testis, kidney and muscle were frozen in liquid nitrogen and embedded in OCT. 5 μm frozen sections were incubated with a rabbit anti-mouse STEAP antibody and then with biotin-conjugated goat anti-rabbit. Bound antibodies were visualized with streptavidin-conjugated to Alexa-594. Pictures were taken using a 20X magnification objective.

Body

Task 1b. To obtain a collection of plasmids for expression of mouse STEAP, as well as for production of Venezuelan equine Encephalitis Virus replicon particles (VRP) and Human papillomavirus (HPV) virus like particles (VLPs).

A 1020 DNA fragment encoding mouse STEAP was obtained from the pCR2.1-STEAP plasmid that was previously constructed in our laboratory[7]. For the generation of the STEAP-expressing plasmid (pcDNA3-mSTEAP), cDNA was amplified using specific primers containing sequences recognized by restriction enzymes (Table I) and a high fidelity DNA polymerase.

Table I

	Primer	Restriction site
Expressing vector (pcDNA3)	5'-CCCAAGCTTATGGAGATCAGTGACGAT-3'	Hind III
	5' GGCGAGCTCCTACAACCTGGAGGCCATCT-3'	Xho I
Alphavirus vector (pERK)	5'-TGGAGATCAGTGACGAT-3'	-
	5'-TTAATTAA GGCGAGCTCCTACAACC-3'	Pac I
Baculovirus vector (pFastbac)	5'-CCCGATATCATGGAGATCAGTGACGAT-3'	EcoRV
	5' GGCGAGCTCCTACAACCTGGCTATAGCCC-3	

The specific STEAP product was amplified and cloned into the pcDNA3 expression vector (Fig.2A). Accuracy and correct open reading frame (ORF) of the pcDNA3-mSTEAP construction was confirmed by DNA sequencing. Mouse STEAP expression was tested by transfecting the mSTEAP-pcDNA3 into EL4 cells and protein expression was determined by using western blot from membrane proteins. Fig 2B shows the predicted full-length mSTEAP protein (39kDa) only in transfected EL-4 and TRAMPC2 cells, after probing membranes with an anti-mSTEAP polyclonal antibody generated in our laboratory. mSTEAP expression was detected in lymph nodes obtained from male C57BL/6 mice injected with 1X10 6 IU of mSTEAP-VRP by immunofluorescence (Fig 2D). Also, STEAP open reading frame was amplified and cloned into pERK vector (Fig. 2E, table I), sequenced and sent to the AlphaVax Company, which provided me VRPs that encode either mSTEAP or the green fluorescent protein (GFP), which were used as negative controls. In order to produce the virus like particles containing mSTEAP protein, the ORF

of mSTEAP was amplified and ligated to the L2 gene of HVP-16. Then cloned into pZero, (Fig. 2E) sequenced and subsequently subcloned into pFasbac that contains L1 HPV-16 gene (Fig 2F). However when mSTEAP bacmid DNA was transfected into insect SF9 cells, we were not able to detect the fusion protein (mSTEAP-L2) by western blot in total extracts of transfected cells using a rabbit anti L2 antibody kindly provided by Dr. Schiller (NIH) and only L2 protein was detected in insect SF9 cells transfected with L2-L1-HPV16 bacmid DNA (data no shown) that was used as positive control.

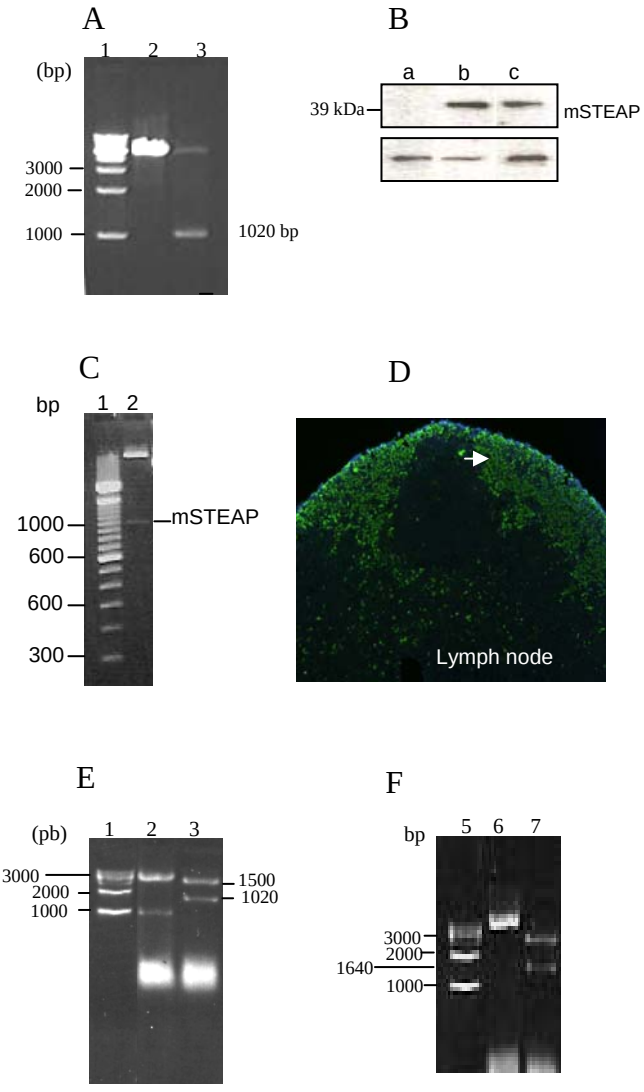

Fig 2. **Cloning of mSTEAP into pcDNA3.** pCDNA3 vector (line 2) and vector that contains the full open reading frame (ORF) of mSTEAP (line 3) were digested with Hind III and Xho I. The restriction product of 1020 pb corresponding to cDNA of mSTEAP was visualized in agarose gels (1%) stained with Ethidium bromide. **B) mSTEAP expression in EL-4 cells.** EL-4 cells were transfected by electroporation and after 48 h membrane proteins were collected. Western blot was performed using membrane proteins from non-transfected cells (a), transfected cells (b) and TRAMPC2 cells (c) as a positive control. **C) Cloning of mSTEAP into pERK vector.** pERK vector or vector that contains full frame of mSTEAP were digested with Sph1.The restriction product (989 bp) was visualized in Ethidium bromide-stained agarose gels (1.5%). **D) mSTEAP expression in lymph nodes.** mSTEAP was detected in lymph nodes from C57BL/6 mice injected with $1X10^6$ IU of mSTEAP-VRP at day 30 post inoculation. Inguinal lymph nodes were collected and embedded in tissue teck and 5 μm cryosections were first incubated with rabbit polyclonal anti-mSTEAP. After that, sections were incubated with biotin-conjugated goat anti-rabbit and bound antibodies were visualized with streptavidin-conjugated to Alexa-594. E) **Cloning of mSTEAP into pZero and pFastbac vectors.** pZero contained the mSTEAP cDNA in ORF (line 2). A clone in wrong orientation (line 3) was also digested with ECORV. The restriction products were visualized in Ethidium bromide-stained agarose gels (1%) and the clone in right ORF (lane 2) was selected for sequencing. F) **Subcloning of mSTEAP-L2 into pFasbac.** mSTEAP-L2 pZero was digested with SNi I and the fusion fragment was subcloned into pFasbac. Empty pFasbac and mSTEAP-L2 pFasbac were treated with SphI and the clone in correct ORF (lane 7) was sequenced

Given that we were not able to produce recombinant virus using the full cDNA of mSTEAP, we will alternatively use the most antigenic region of this protein. Previously, in our laboratory we designed a mini gene containing the most antigenic regions of different prostate antigens included mSTEAP, we will use the same pair of primers to

amplify part of mSTEAP cDNA and it will be ligated to L2 and subcloned into pFasbac containing L1 gene of HVP-16.
We clearly demonstrated in figures 2B and 2D, that mSTEAP-pcDNA3 and mSTEAP-VRP were able to express mSTEAP protein. It was detected in the membrane fraction of transfected EL-4 cells by western blot. Additionally, mSTEAP protein was detected by immunofluorescence in lymph nodes from C57BL/6 injected with mSTEAP-VRP. Therefore, we decided to test whether mSTEAP-based vaccination can induce a specific and protective immune response in C57BL/6 mice by using gene-gun and VRP as delivery systems as was suggested in tasks 1c and 1d.

Task 1c) Vaccination of male C57BL/6 mice with mouse STEAP by gene gun and/or VRP as delivery systems. ***Task 1d)*** To determine whether male C57BL/6 mice can develop a specific immune response against mouse STEAP

In order to characterize whether mSTEAP-based vaccination is able to induce specific immunity against this tumor antigen, two groups of male C57BL/6 mice were vaccinated at day 0 with a vector that contains mSTEAP cDNA (mSTEAP- pcDNA3) by gene gun. Fifteen days later, a group of mice was boosted with mSTEAP-pcDNA3 and the other one was subcutaneously injected with 1.0 $x10^6$ infectious units of mSTEAP-VRP. Another experimental group was vaccinated (sc route) at day 0 and fifteen with 1.0 $x10^6$ infectious units of mSTEAP-VRP. Additional groups were vaccinated with empty vector and/or GFP-VRP as controls for this experiment. 7 days after the second vaccination, splenocytes from vaccinated mice were evaluated for class I-restricted CD8 T-cell responses to the H-$2D^b$ restricted mSTEAP peptide by ELISPOT and chromium-release assays. mSTEAP peptide was predicted by the Parker automated program[5], probed and selected by us in MHC binding assays using RMA-S cells. The induction of a specific T cell response was measured by ELISPOT, detecting antigen-specific IFN-γ producers. As shown in Fig. 3A, the frequency of D^b restricted IFN-γ mSTEAP specific T cell producers, was slightly higher in the group that was vaccinated once with mSTEAP by gene gun and boosted with mSTEAP-VRP than the group immunized two times by gene gun (*p=0.000327, one tail*). On the other hand, there was not significant difference with the group vaccinated two times with mSTEAP-VRP (*p=0.1816*). In contrast, peptide-specific IFN-γ production was absent in control groups of mice vaccinated two times with empty vector and mice vaccinated with empty vector and/or GFP-VRP.
In order to determine whether mSTEAP vaccination is able to induce functional CD8 T cells, their cytotoxic T cell activity was evaluated by a CTL assay. The chromium-release assay measured the antigen specific CTL-mediated killing of EL-4 cells loaded with the mSTEAP $_{326\text{-}336}$ peptide or E7 $_{49\text{-}57}$ (irrelevant peptide). EL-4 cells incubated with medium were used as a control for spontaneous chromium release. The peptide-specific CTL response generated against $mSTEAP_{326\text{-}336}$ was lower in the group of mice vaccinated by gene gun and boosted with VRPs compared with control group. In contrast, mice vaccinated two times by gene gun or VRPs had a complete absence or poor CTL activity, respectively (Fig. 3B). These results suggested that the best vaccination scheme to induce specific CD8 T cells with killing capacity was based on priming with STEAP-DNA and boosting with mSTEAP-VRP. Although the cytotoxic activity between mSTEAP vaccinated and control group in prime-boost scheme was only 12 % and the

other two vaccination schemes did not work as we hoped for, we decided to evaluate whether

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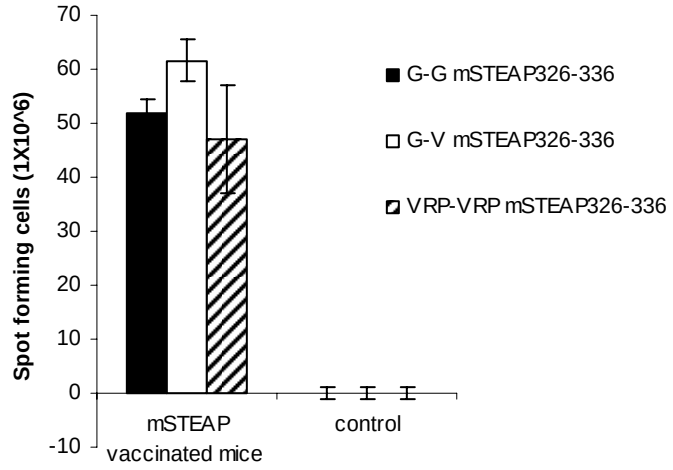

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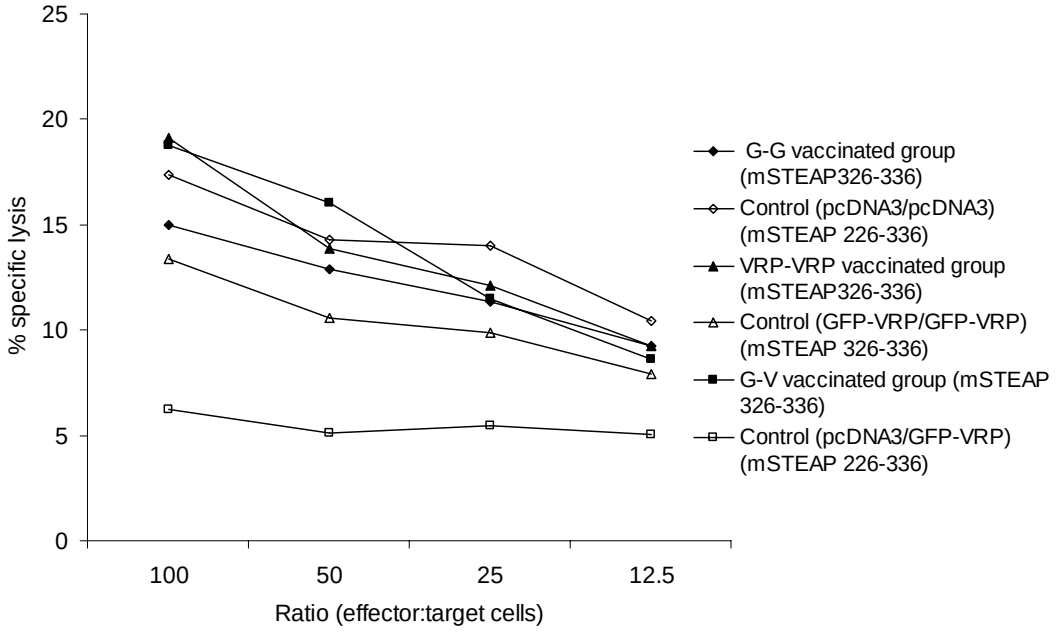


Figure 3. **Characterization of the cellular response induced in secondary lymphoid organs of mSTEAP vaccinated mice after using gene gun and VRPs as delivery systems.** A) ELISPOT assay**.** Male C57BL/6 mice were vaccinated at day 0 with 2μg of pcDNA-mSTEAP by gene gun. Fifteen days later, one group was boosted with 2 μg of pcDNA-mSTEAP (■) and other was injected subcutaneously with $1X10^6$ IU of mSTEAP-VRP (□). In other experimental group, mice were vaccinated at day 0 with $1X10^6$ IU of mSTEAP-VRPs. Mice were boosted with $1X10^6$ IU of mSTEAP-VRP fifteen days later. As controls, two groups of mice were vaccinated and boosted with 2μg of empty vector or $1X10^6$ IU GFP-VRP. Another group was vaccinated at day 0 with 2 μg of empty vector and fifteen days after vaccination,

mice were boosted with $1X10^6$ IU GFP-VR. Spleen cells from mSTEAP vaccinated mice were activated with 1μg of mSTEAP $_{326-336}$ or E7 $_{49-57}$ peptide and 5U of IL-2 during 24h. As a control, splenocytes from mice vaccinated with empty vector or GFP-VRP were incubated under the same conditions. Results were calculated by subtracting the background spots produced after stimulation with an irrelevant E7 $_{49-57}$ peptide and presented as spot-forming cells per $1X10^6$ splenocytes. Data represent the mean of four mice in one of three separate experiments. B) Chromium-release assay. Spleens cells from mSTEAP vaccinated mice or control group were stimulated during five days with 20 IU of IL-2 and 10 μg of mSTEAP $_{326-336}$ peptide. Then, they were cultured with ^{51}Cr-labeled EL4 target cells pulsed with mSTEAP $_{326-336}$ (◆, ■, ▲) or E7 $_{49-57}$ (data no shown) peptide at the indicated effector-to-target ratios. Specific CTL activity represents the mean of three pools of three mice in each point ± SE

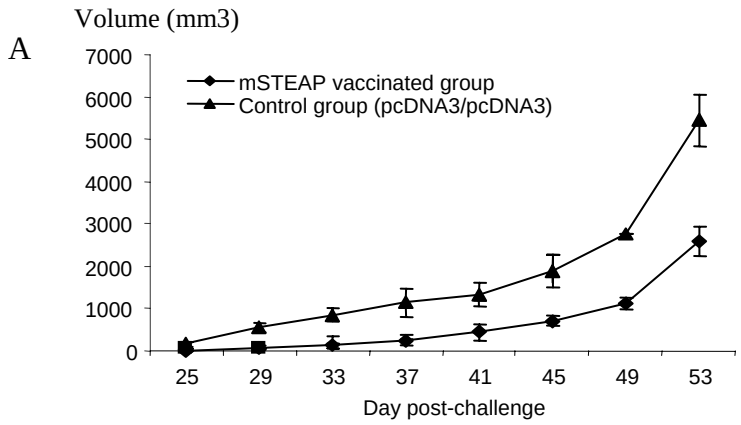
STEAP-based vaccination is able to induce tumor immunity.

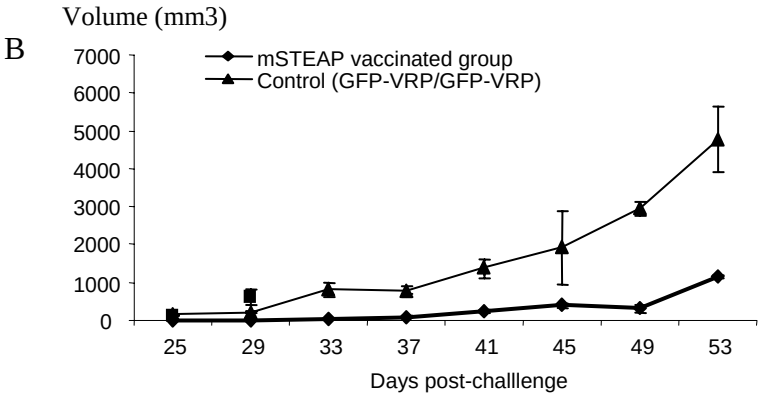
Task 1 d1) **To determine whether mSTEAP vaccination schemes protect C57BL/6 mice against tumor growth promoted by mSTEAP expressing cells (TRAMPC2)**.

We used three experimental groups vaccinated at day 0 and fifteen days later, following the same vaccination schemes as mentioned above. Nine days after the second vaccination, mSTEAP vaccinated and control mice were subcutaneously challenged with $5X10^6$ TRAMP-C2 cells and tumor volume was monitored twice a week. Results show a significantly delayed outgrowth of TRAMP-C2 tumors in mSTEAP vaccinated mice (Fig. 4). Whereas mice vaccinated with the empty vector showed a considerable tumor growth ($p<0.0001$, *one tail*). Despite all vaccination schemes delayed tumor growth ($r = 0.935$), tumor volume in mSTEAP vaccinated mice, using the prime-boost scheme, was smaller than mice vaccinated mice with gene gun or VRPs gene and it was able to induce a specific CD8 T cell response. For this reason, we decided to use this vaccination scheme for future experiments. In an effort to understand the immune mechanisms involved in tumor growth delay and to improve the prime-boost vaccination schemes, we are currently analyzing the populations of infiltrating cells and cytokine levels at the tumor site.

Task 1 d.2**) To determine the therapeutic effect of mSTEAP vaccination in tumor bearing C57BL/6 mice by using a prime-boost vaccination scheme.**

Male C57BL/6 mice with an establishc tumor were immunized with mSTEAP-pcDNA3 at day 24, followed by a sc injection of mSTEAP-VRP at day 39. Results were not statistically significant delay in tumor growth ($p=0.16$) (Fig. 5). It seems that mSTEAP treatment is not enough to control growth of preexisting tumor and additional treatments are necessary. It has been proposed that androgen ablation induces a robust and restricted T cell mediated response in human prostate [6, 7]. Thus, we are planning to evaluate whether combined schemes, involving androgen ablation and mSTEAP therapy, could induce a synergistic effect that will be translated in tumor rejection or at least a delay in tumor growth.





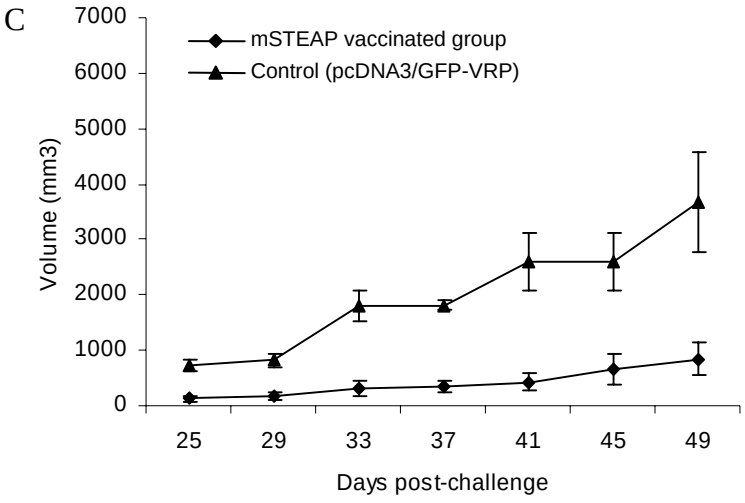


Figure 4 **mSTEAP vaccination delay tumor growth**. Male C57BL/6 mice were vaccinated at day 0 with 2μg of pcDNA-mSTEAP by gene gun. Fifteen days later, one group was boosted with 2 μg of pcDNA-

mSTEAP (A) and other was injected subcutaneously with $1X10^6$ IU of mSTEAP-VRP (B). In other experimental group, mice were vaccinated at day 0 with $1X10^6$ IU of mSTEAP-VRPs. Mice were boosted with $1X10^6$ IU of mSTEAP-VRP, 15 days after first vaccination (C). As controls, two groups of mice were vaccinated and boosted with 2μg of empty vector or $1X10^6$ IU GFP-VRP. Another group was vaccinated at day 0 with 2 μg of empty vector and fifteen days later, mice were boosted with $1X10^6$ IU GFP-VRP. Ten days after second vaccination, mice were challenged with $5x10^5$ TRAMPC2 cells and tumor growth was monitored twice a week with an engineer caliper. The results represent the mean of nine mice + SE in one of two separate experiments with similar results.

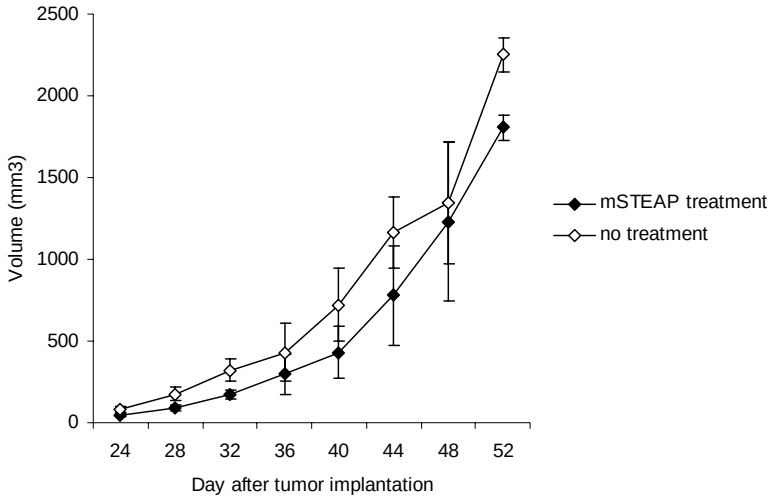


Figure 5**. Therapeutic effect of mSTEAP vaccination using prime-boost vaccination scheme.** Two groups of male C57BL/6 mice were inoculated with 5x105 TRAMPC2 cells at day 0. At day 24, all the mice had a palpable tumor. One group of mice was vaccinated with 2μg of pcDNA-mSTEAP by gene gun. Fifteen days later, mice were immunized with 1X106 IU of mSTEAP-VRPs. The other group did not receive any kind of treatment. Tumor growth was monitored twice a week. Results represent the mean of ten mice ± SE

***Task 1f*. To determine if mSTEAP vaccination induces autoimmunity in male C57BL/6 and TRAMP mice.**

In this section we are showing the measurement of some parameters related to autoimmunity, exclusively in the vaccination scheme mSTEAP-DNA/mSTEAP-VRP. Given that mSTEAP is considered a self-antigen, we decided to analyze development of signs of autoimmunity after the vaccination process. Detection of auto antibodies at systemic level is useful to characterize, in an indirect way, the autoimmune process. As a positive control for rheumatoid factor and anti-ssDNA antibodies (fig 6 A), we used serum from aged MRL/LPR in our study. Even though, rheumatoid factor was only detected at day 50 post vaccination in mSTEAP vaccinated mice, its level was significantly lower compared with aged MRL/LPR mice. In normal conditions, it is likely that auto reactive B cells produce small amounts of auto antibodies without causing any tissue damage. For this reason, we decided to analyze accumulation of Ig M, attraction of inflammatory cells and collagen deposition in kidney where mSTEAP is expressed at low levels in vaccinated mice. It was clear that only MLR/LPR mice developed an active process of auto immunity, associated to accumulation of Ig M and cell infiltrates in the kidney (Fig 6 i, j). Consequently, these mice had a massive collagen deposition, which is a clear sign of tissue damage (fig 6 m). Interestingly, mSTEAP vaccinated mice didn't show any signs of active autoimmunity (Fig 6 b, c and d), Activation of STEAP specific immune cells can be responsible for activation of bystander auto reactive cells that are located in the same environment. To rule out this possibility, we decided to evaluate the production of auto antibodies directed against self proteins produced in organs where STEAP is expressed, in vaccinated mice (kidney, testis and prostate). Briefly, kidney, prostate and testis from RAG mice were incubated with serum from vaccinated- or MLR/LPR mice. Antibodies that recognized self proteins expressed in the above mentioned tissues were detected with a FITC conjugated anti mouse Ig G. Only serum from MLR/LPR mice contained significant levels of auto antibodies that bound self proteins as it was demonstrated by the intense immunofluorescence stain of different cells and structures in tissues of RAG mice (fig 6 g, h and i). These findings suggested that although STEAP vaccination is promoting production of low levels of auto antibodies, it is not causing any autoimmunity that can cause organ pathology. Therefore, STEAP vaccination can be considered as a safe antigen and a key antigen for future vaccination schemes.

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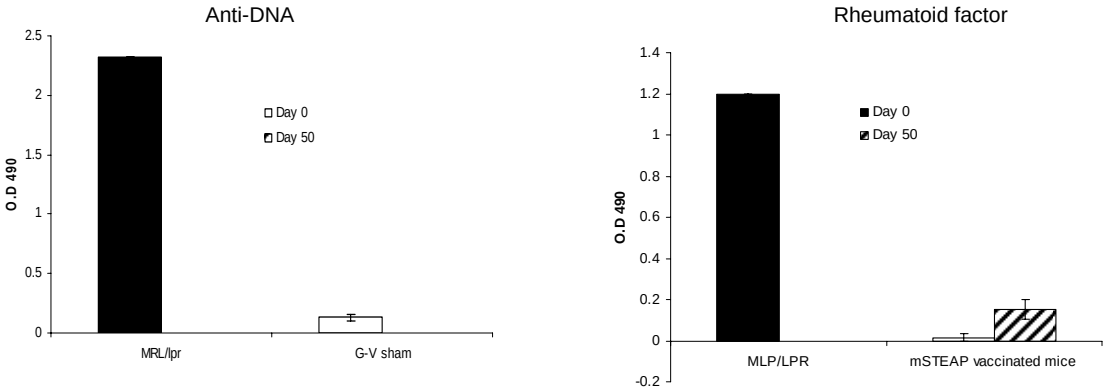
Anti-DNA
O.D 490
2.5
2
1.5
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0.5
0
Day 0
Day 50
MRL/lpr
G-V sham
Rheumatoid factor
O.D 490
1.4
1.2
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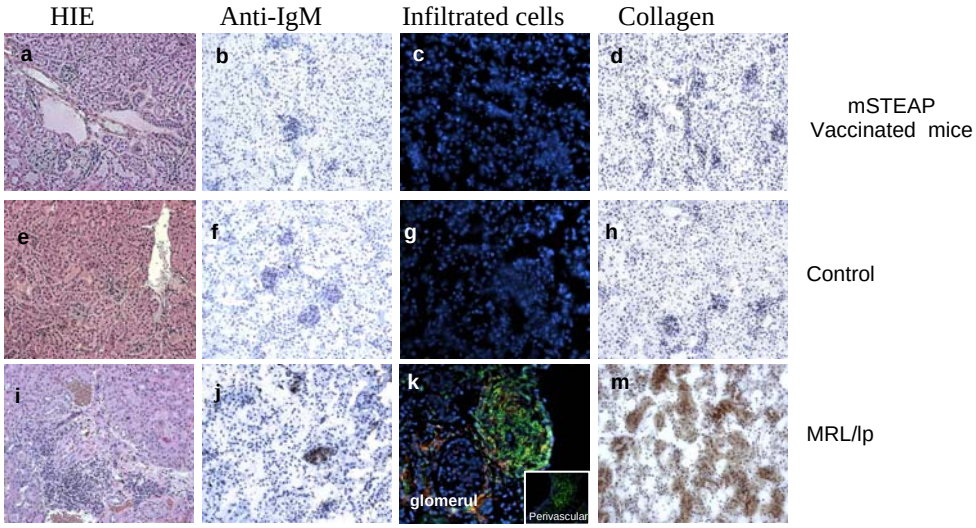
MLP/LPR
mSTEAP vaccinated mice

B


HIE
Anti-IgM
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a
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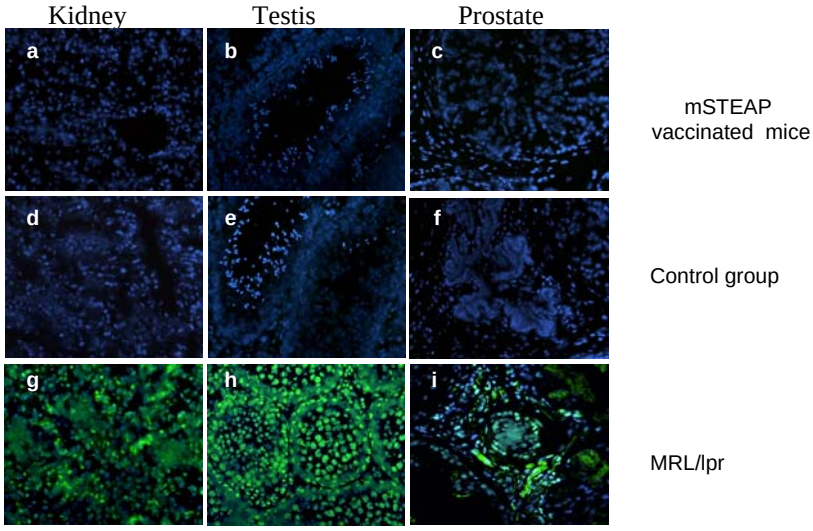
Kidney
Testis
Prostate
a
b
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mSTEAP
vaccinated mice
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Control group
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MRL/lpr

Figure 6 **mSTEAP vaccination did not induce any signs of autoimmunity.** A) Autoantibody production in serum samples from male C57BL/6 mice immunized with mSTEAP. Anti-ssDNA antibodies and rheumatoid factor were measured in serum from mSTEAP vaccinated and control group by ELISA and titers were compared with a serum sample from MRL/lpr mice (positive control of active autoimmunity). Results were calculated by subtracting autoantibody titers of C57BL/6 mice vaccinated with empty vector and GFP-VRP to titers found mice vaccinated with mSTEAP-DNA and mSTEAP-VRPs. Results show the mean of 8 mice $\pm$ SE. Data from one of two similar independent experiments is shown. B) Absence of inflammation and cell infiltrates in in kidney from mSTEAP vaccinated C57BL/6 mice. A kidney section taken at day 50 after vaccination (mSTEAP-DNA and mSTEAP-VRP) and stained by haematoxylin and eosin (H&E), shows a complete absence of inflammatory cells (a, c). Control mice didn't show any significant cell infiltration (e, g) compared with the massive inflammatory infiltration seen in MRL/lpr mice (i, k). Inflammatory infiltrates were basically composed of macrophages, T cells and dentritic cells (k). Immunohistochemistry analysis using an HPR-conjugated anti mouse IgM and mouse anti-collagen type IV showed IgM deposited on glomerular basement membrane in MRL/lpr (j), which had a considerable collagen deposition (m). No positive stain was detected in kidney from mSTEAP vaccinated (b, d) or control mice (g, h). Representative images of similar lesions found in an experimental group of 8 mice are shown. Pictures were taken using a 20X magnification. C) mSTEAP vaccination does not induce auto antibodies against self-antigens on tissues were STEAP is expressed. Frozen sections of kidney, testis and prostate from RAG mice were incubated with serum from mSTEAP vaccinated or control group. Serums were diluted 1:40 and bound antibodies were detected with FITC-conjugated an affiniPure Fab fragment goat anti-mouse IgG. Both mSTEAP vaccinated (a, b, c) and control group (d, e, f) did not show any positive reaction against self-antigen present on those tissues. In contrast, tissues from MRL/lpr mice show a strong positive reaction against self-components of STEAP expressing organs (g, h, i).

Key research accomplishments

1) Characterization of STEAP expression in C57BL/6 mice
2) Selection of the best vaccination scheme to protect against prostate cancer using C57BL/6 mice as a model.
3) Successful vaccination with mSTEAP without induction of autoimmunity in STEAP expressing tissues.

Reportable outcomes

Manuscripts, abstracts, presentations:

Poster presentation at AAI meeting 2005, San Diego Convention Center, April 2-6 2005
PSCA vaccination induces a prostate cancer protective immune response in the absence of autoimmunity
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Patents and licenses applied for and/or issued:

None

Degrees obtained that are supported by this award:

None

Development of cell lines, tissue or serum repositories:

None

Informatics such as databases and animal models, etc:

None

Funding applied for based on work supported by this award:

None

Employment or research opportunities applied for and/or received on experiences/training supported by this award:

None

Conclusions.

IFNγ production is a good marker of an adequate Th1 response and it is very important for activation of CD8 T cells. Also, IFNγ can induce chemokines with a potent angiostatic activity such as CXCL19 (MIG), CXCL10 (IP-10), CXL11 (I-TAC), molecules that interfere with successful tumor growth. Therefore, it was relevant to measure IFNγ production by STEAP specific CD8 T cells. mSTEAP vaccinated mice mice had a considerable number of IFN-γ producing cells in the spleen, in all vaccination schemes: gene-gene, gene-VRP and VRP-VRP. It suggested that there was efficient T cell priming in the splenic environment and it could be responsible for the control of tumor growth. However, according to the functional activity of STEAP specific CD8 T cells, measured by a chromium release assay, the gene-VRP scheme had the best induction of cytotoxic CD8 T cells. The number of STEAP specific CD8 T cells was low, something suggesting that it would be necessary to add adjuvants to the vaccination schemes to have a more potent CD8 T cell response. Another goal in this part of the project was focused to determine if therapeutic mSTEAP vaccination can be useful to control tumor growth. We found a slightly delayed tumor growth that was statistically significant when compared to control mice, but it was not as impressing as to consider STEAP vaccination alone as a therapy able to control tumor growth after tumor is implanted and palpable. Additional molecules with adjuvant properties are required for promoting a strong T cell response to kill established prostate tumors. Given that STEAP is a self-antigen, there was a potential risk to induce autoimmunity during the vaccination process, which can cause tissue damage where STEAP is produced at low levels. Although, mice vaccinated with STEAP (gene-VRP) produced low levels of rheumatoid factor and antibodies against ss-DNA, they didn't show any sign of tissue damage in kidney, prostate and testis, tissues where STEAP is expressed. Also, there was a complete absence of Ig M trapped in glomeruli, collagen deposition or recruitment of inflammatory T cells to the kidney. In addition, they didn't produce auto antibodies against proteins produced in the tissues above mentioned. Based on these findings, we can propose that STEAP vaccination can be useful for the induction of CD8 T cells responses without inducing secondary concomitant auto immunity. However, it is required to find the right combination of adjuvant molecules to enhance the production of STEAP specific CD8 T cells with a considerable CTL activity.

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