

VEEV replicon-based vaccines used in heterologous prime boost strategies induce lifelong protection against prostate cancer and therapy of cervical cancer in mice and robust cell-mediated immunity in Rhesus macaque

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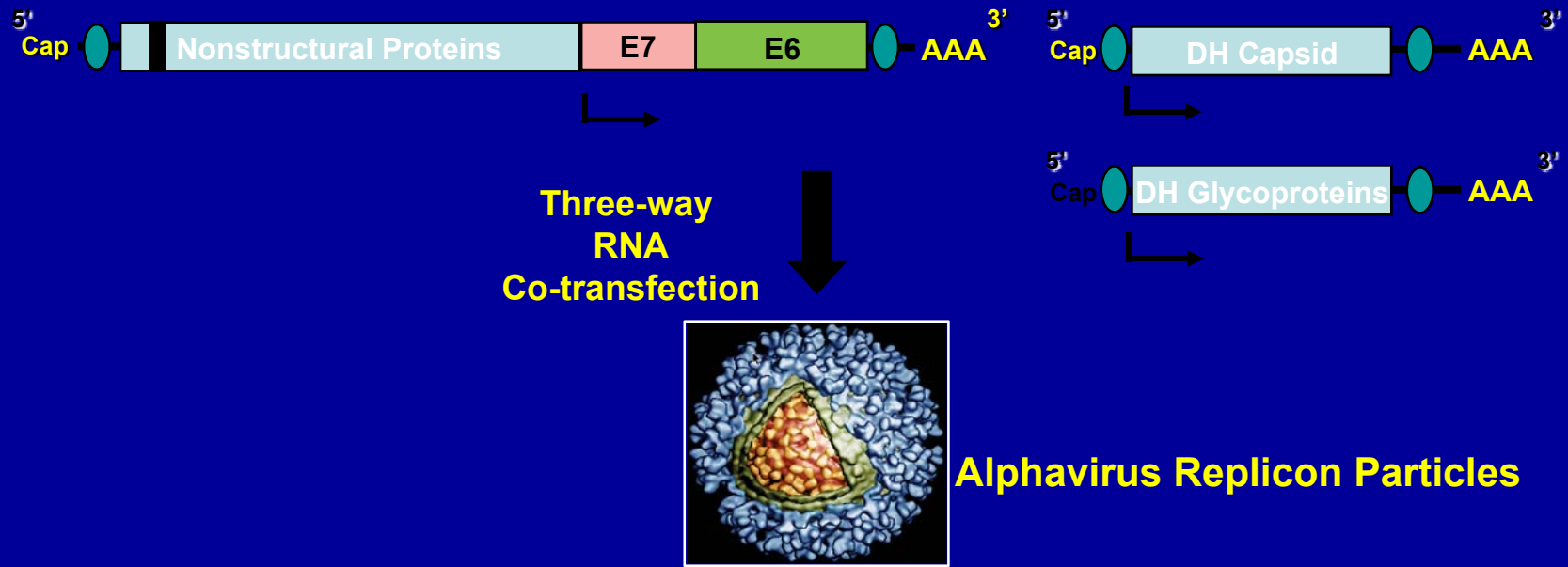
Introduction

- **Persistent Human Papilloma Virus infection is a necessary cause of cervical cancer, the second leading cause of cancer death in women worldwide with 450,000 new cases each year**
- **Two very effective preventive vaccines are now available.**
- **Still as 100's of million women are already infected or will become infected in the future, as the vaccine is expensive and not yet widely applicable and as the preventive vaccines' effects will take decades to materialize in lower rates of cervical cancer there is a medical need for therapeutic vaccines that work after HPV infection or lesion development**
- **HPV can also be a showcase for the demonstration of the effects of therapeutic vaccines**

Therapeutic HPV vaccines in development

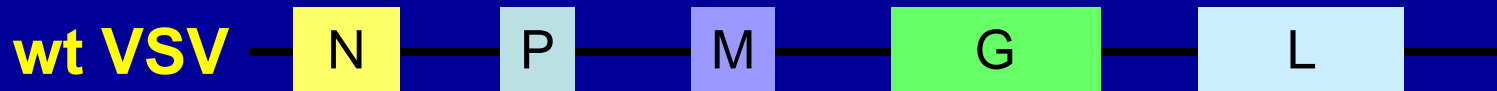
- There are about a dozen biotech companies involved in therapeutic HPV vaccines based on peptide, protein or DNA technology and administered through a variety of delivery platforms ranging from liposomes, adjuvants, in vivo electroporation, gene guns through viral and bacterial vectors.
- Replicons based on Venezuelan Equine Encephalitis Virus
- Recombinant RNA vectors based on Vesicular Stomatitis Virus

VEE-Replicon Particles (VRPs)



- RNA format- no risk of integration
- Apoptosis induction
- Replication incompetent vector (no spreading)
- No widespread pre-existing immunity in humans

Delivery platform: recombinant Vesicular Stomatitis Virus (VSV)



rVSV- N4CT9-(HPVE7E6TM)1



- Single linear negative sense ssRNA genome with cytoplasmic replication
- Attenuated with mutations to limit nucleocapsid formation (N4 Shuffle) reduce particle assembly and pathogenicity (G-CT9 truncation)

Murine Immunogenicity and Tumor Studies in an HPV16 model

Prime

Boost

Week 0

Week 4

Week 5

↓ **1x10⁷ units
VRP or rVSV
i.m.**

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VRP or rVSV
i.m.**



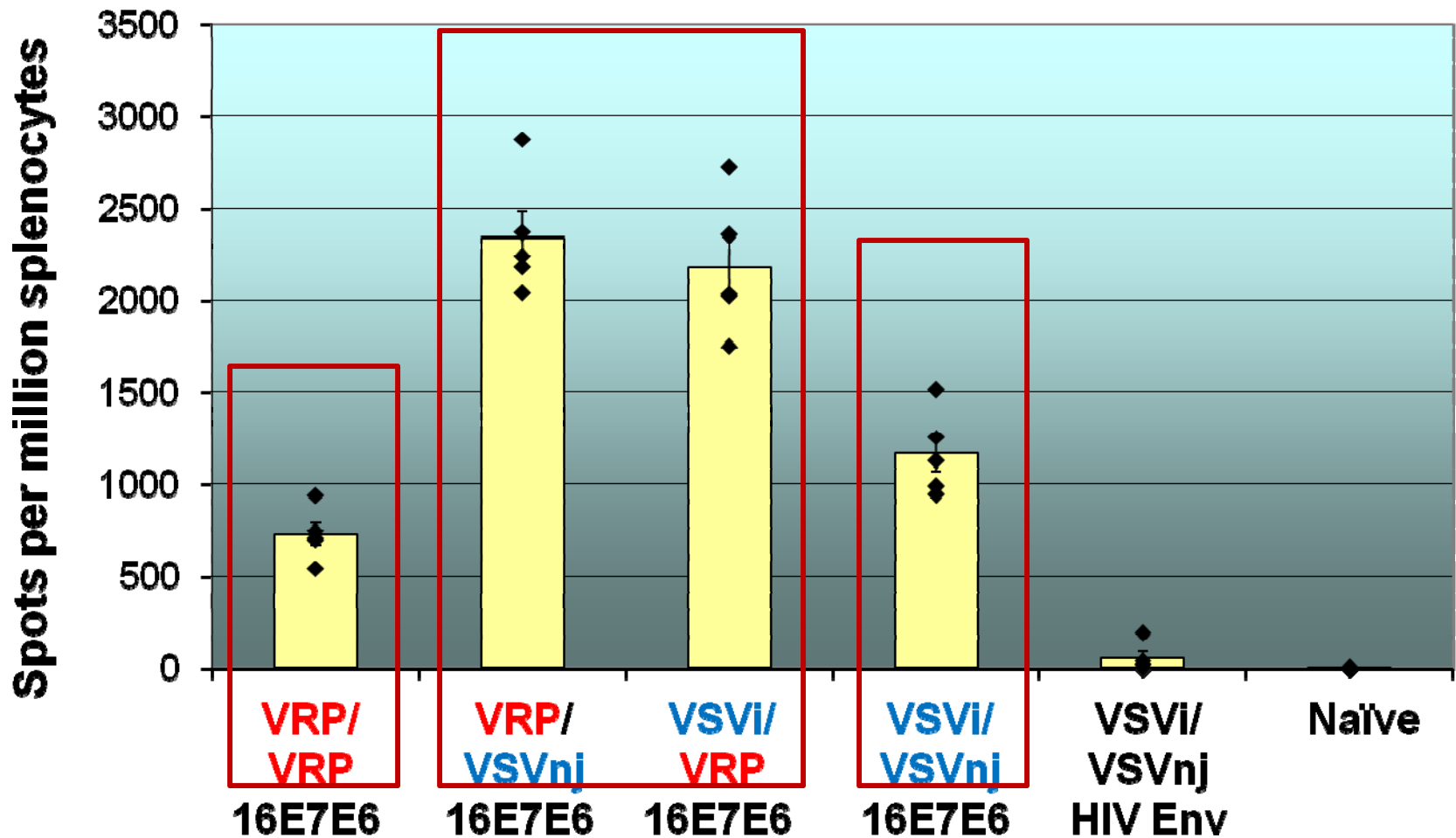
or



**IFN- γ
ELISPOT**

**C3.43
tumor
challenge**

Murine HPV16 E7₍₄₉₋₅₇₎-specific IFN γ ELISPOT responses one week post homologous or heterologous prime/boost



Therapeutic C3.43 Tumor Model

Tumor injection

Therapeutic Immunization

day 0

day 5

day 12

↓ **1×10^5 tumor cells[s.c.]**

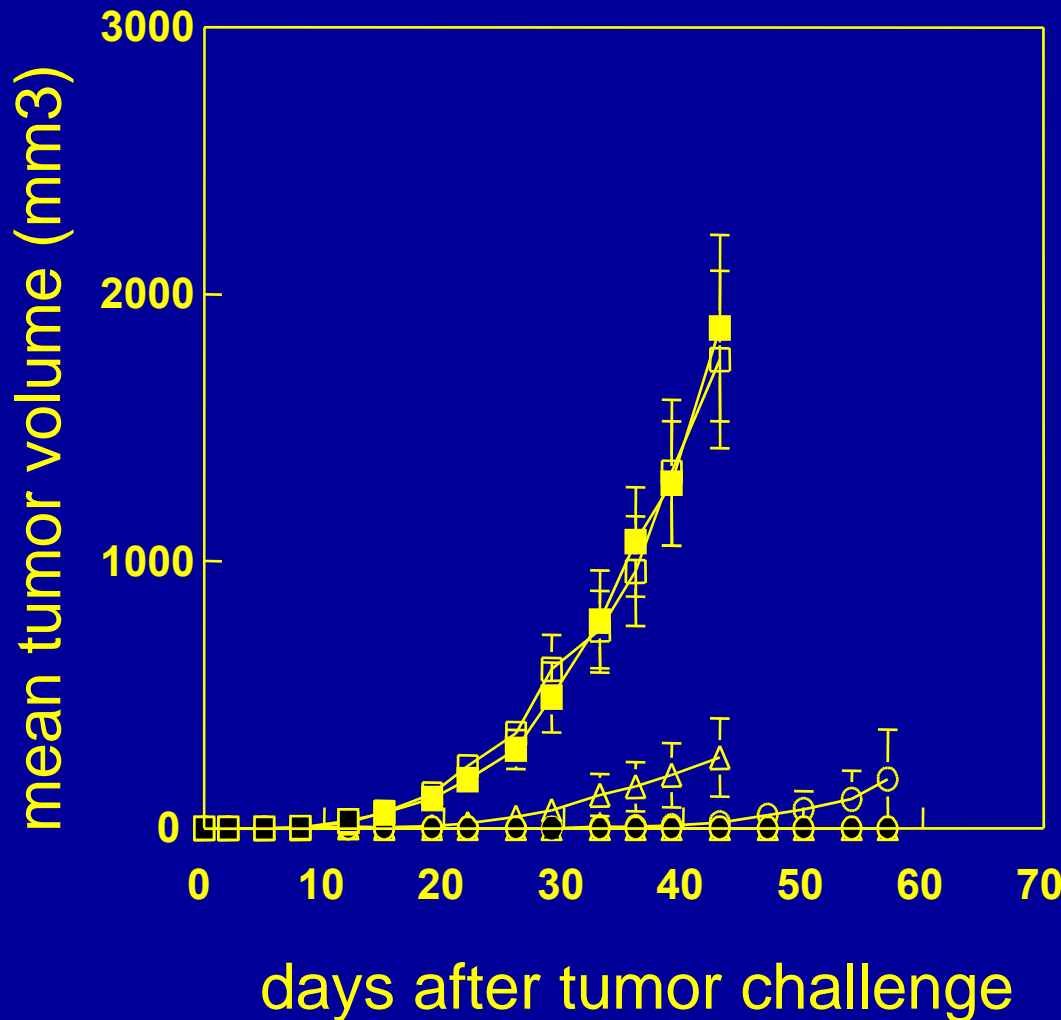
↓ **1×10^7 units VRP or rVSV [i.m.]**

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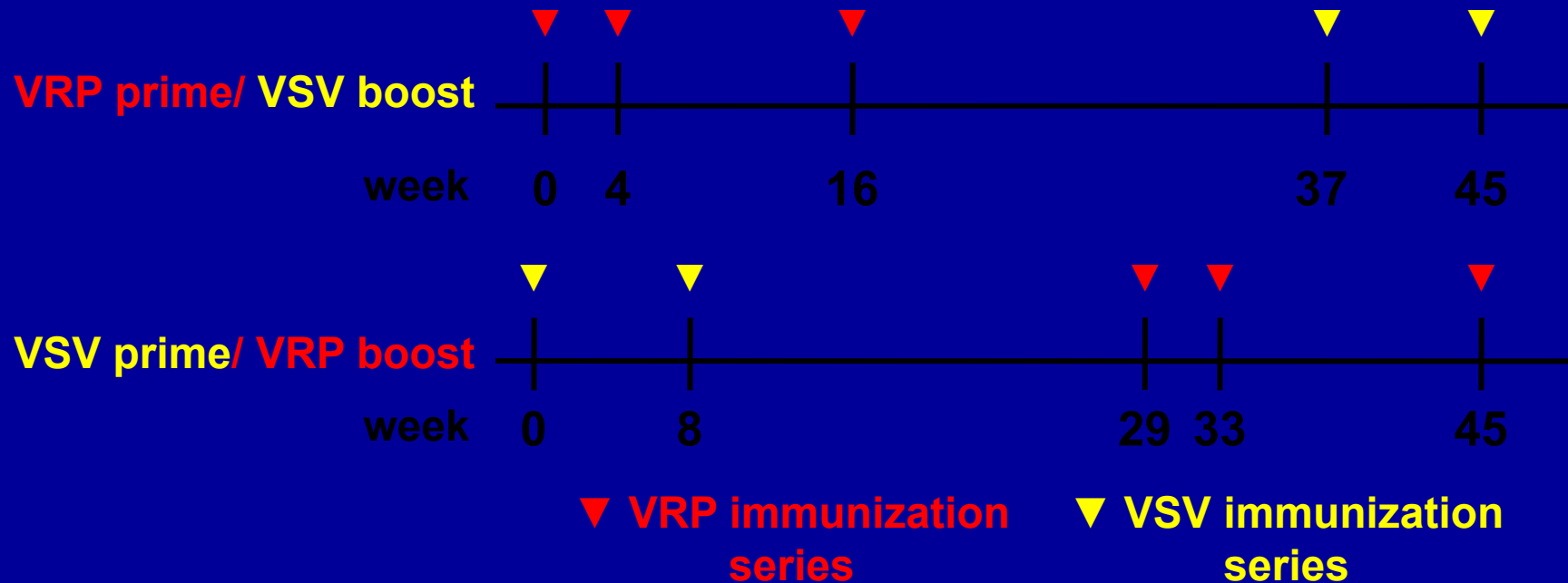
Monitor tumor size 2-3 times/week

Therapy of day 5 established murine C3.43 tumors by homologous or heterologous prime/boost

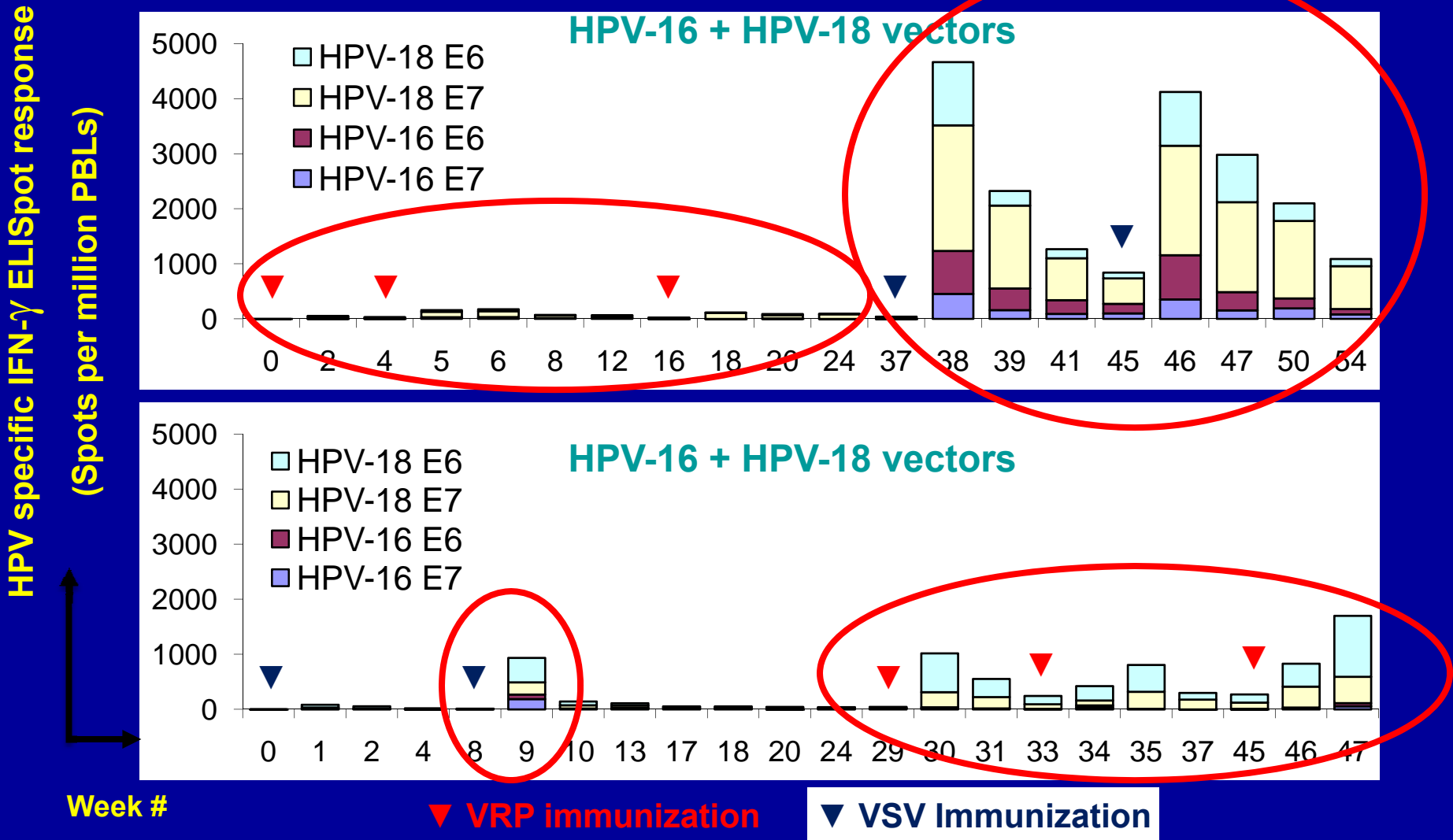


<u>Prime/Boost</u>	<u>Tumor-free mice</u>
—△— VRP E7E6/ VRP E7E6	7/10
—○— VRP E7E6/ VSVnj E7E6	9/10
—▲— VSVi E7E6/ VRP E7E6	10/10
—●— VSVi E7E6/ VSVnj E7E6	9/10
—□— VSVi-ENV/ VSVnj-ENV	0/10
—■— Naive	0/10

Immunogenicity study design for prime/boost vaccination in Rhesus macaques (n=6/group)



Average immunogenicity of combined HPV VRP or VSV vector prime-boost regimen in macaques



Conclusions

- HPV 16 and 18 E6 and E7 antigens are immunogenic
- VRP/VSV heterologous vaccination in non-human primates is dramatically more immunogenic than homologous vaccination with either vector alone.
- Heterologous vaccination in mice with VRP and VSV vectors expressing HPV antigens induces robust T cell immunity that is efficacious in both prophylactic and therapeutic tumor models.

Prostate cancer prevention/therapy through vaccination

- Prostate cancer is the second-leading cause of cancer-related death in males
- Patients who relapse after surgery or radiation therapy may be treated with androgen ablation, but its effects lasts less than 18 months. Most patients develop hormone-refractory prostate cancer and die within 12 months
- Chemotherapy is effective but long-term chemotherapy is not feasible due to its toxicity
- Currently, therapeutic options are very limited for advanced cases
- Therapeutic vaccines might be an option to prevent or treat localized and metastatic disease

Prostate (cancer) associated antigens

- **PSA, prostate specific antigen**
- **PSMA, prostate specific membrane antigen**
- **PSCA, prostate stem cell antigen**
- **PAP, prostatic acid phosphatase**
- **STEAP, six transmembrane epithelial antigen of the prostate**
- **Sperm fibrous sheet proteins**

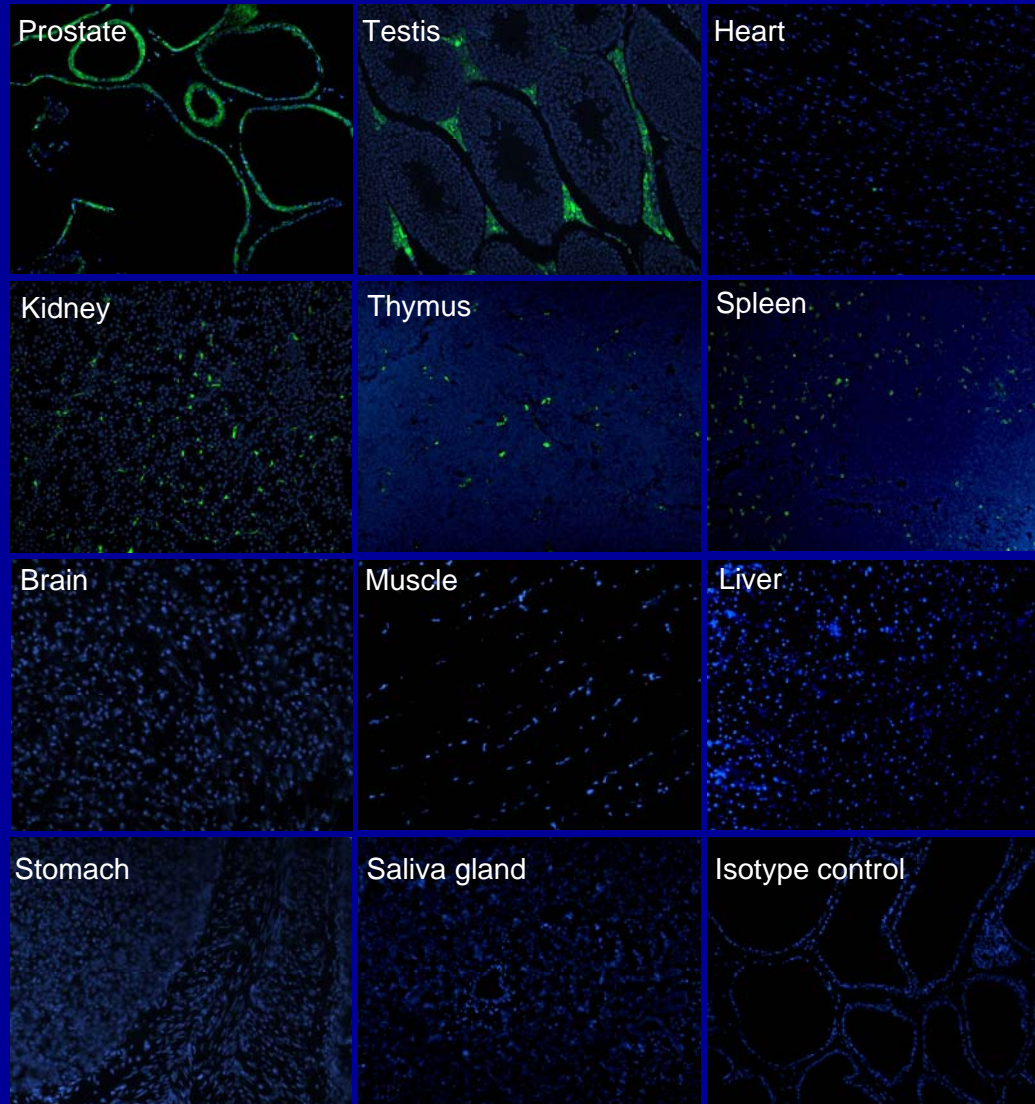
Vaccination strategies chosen

- Prime / boost with heterologous vaccines
- DNA by gene gun followed by VRP (Venezuelan equine encephalitis virus replicons, alpha virus)
- Alpha virus replicons have been shown to break tolerance to self antigens
- VRP have been in the clinic for HIV trials
- Unique access to VRP with STEAP and PSCA through Alphavax

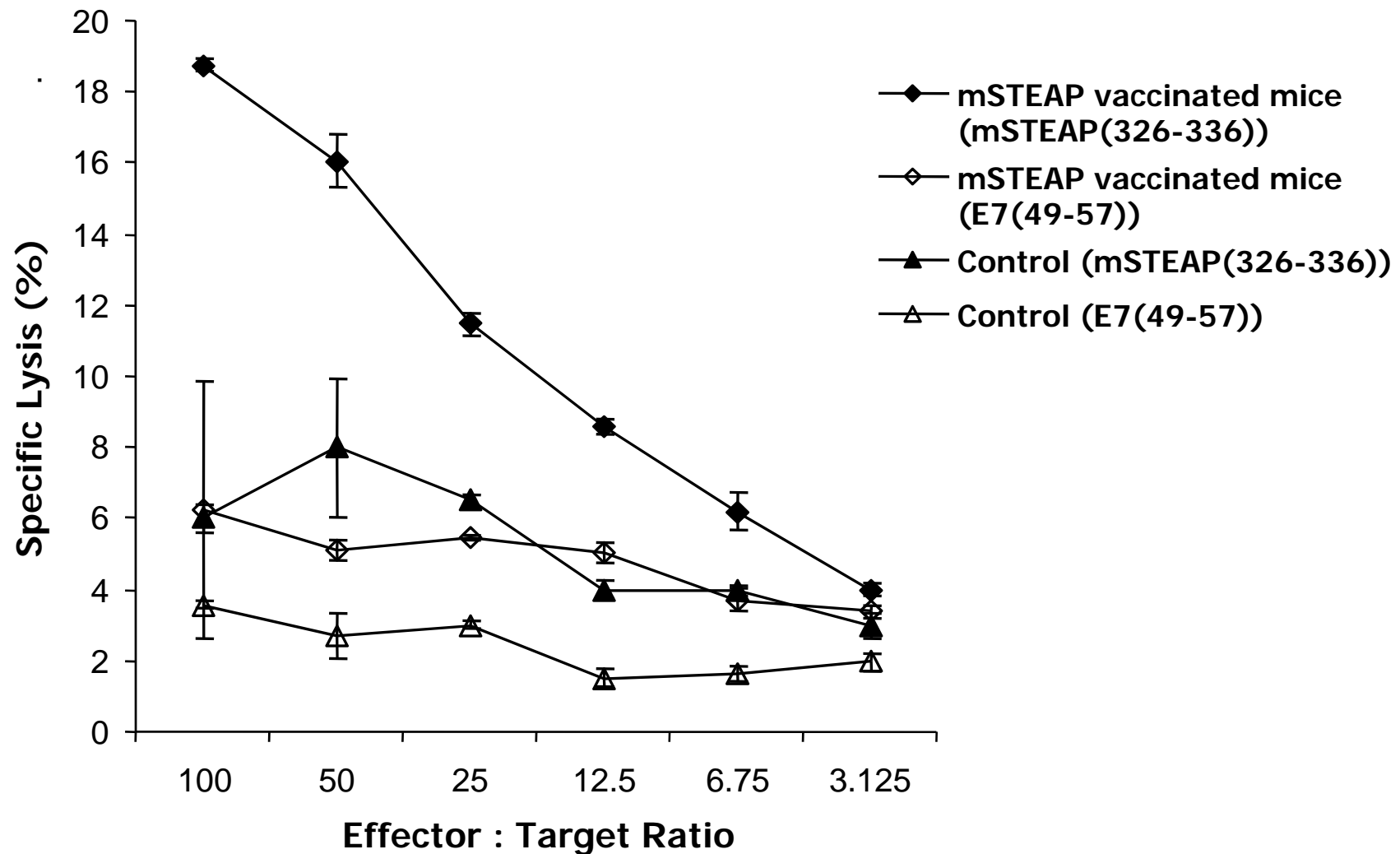
Prostate Cancer Models

- **Male C57 Bl/6 mice challenged with TRAMP-C2 prostate cancer cells**
- **Male TRAMP mice are transgenic for SV40T under control of the probasin promoter and develop neuroendocrine prostate carcinomas**
- **CPP (PTEN KO) mice that develop prostate adenocarcinomas**

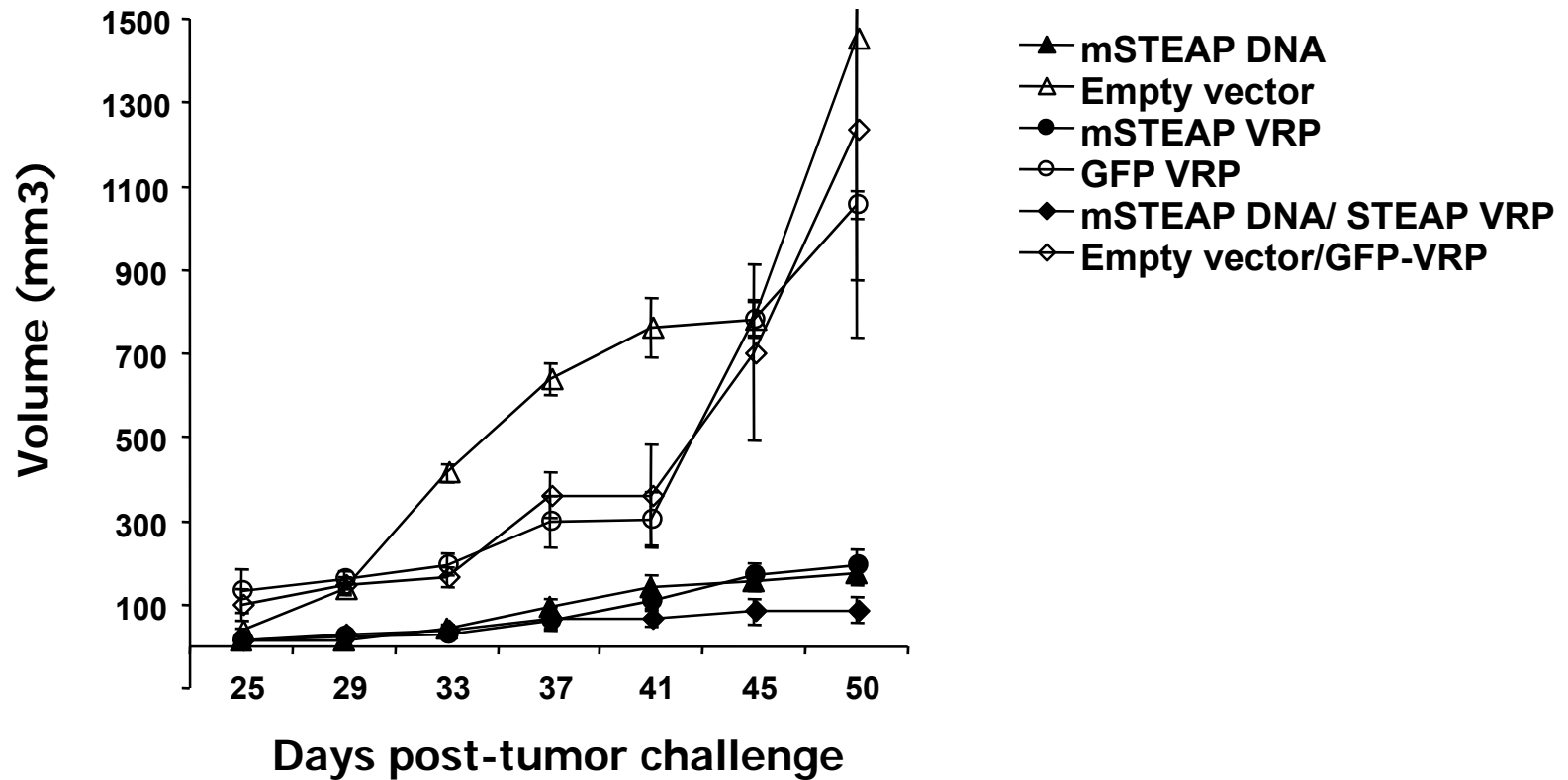
STEAP tissue expression



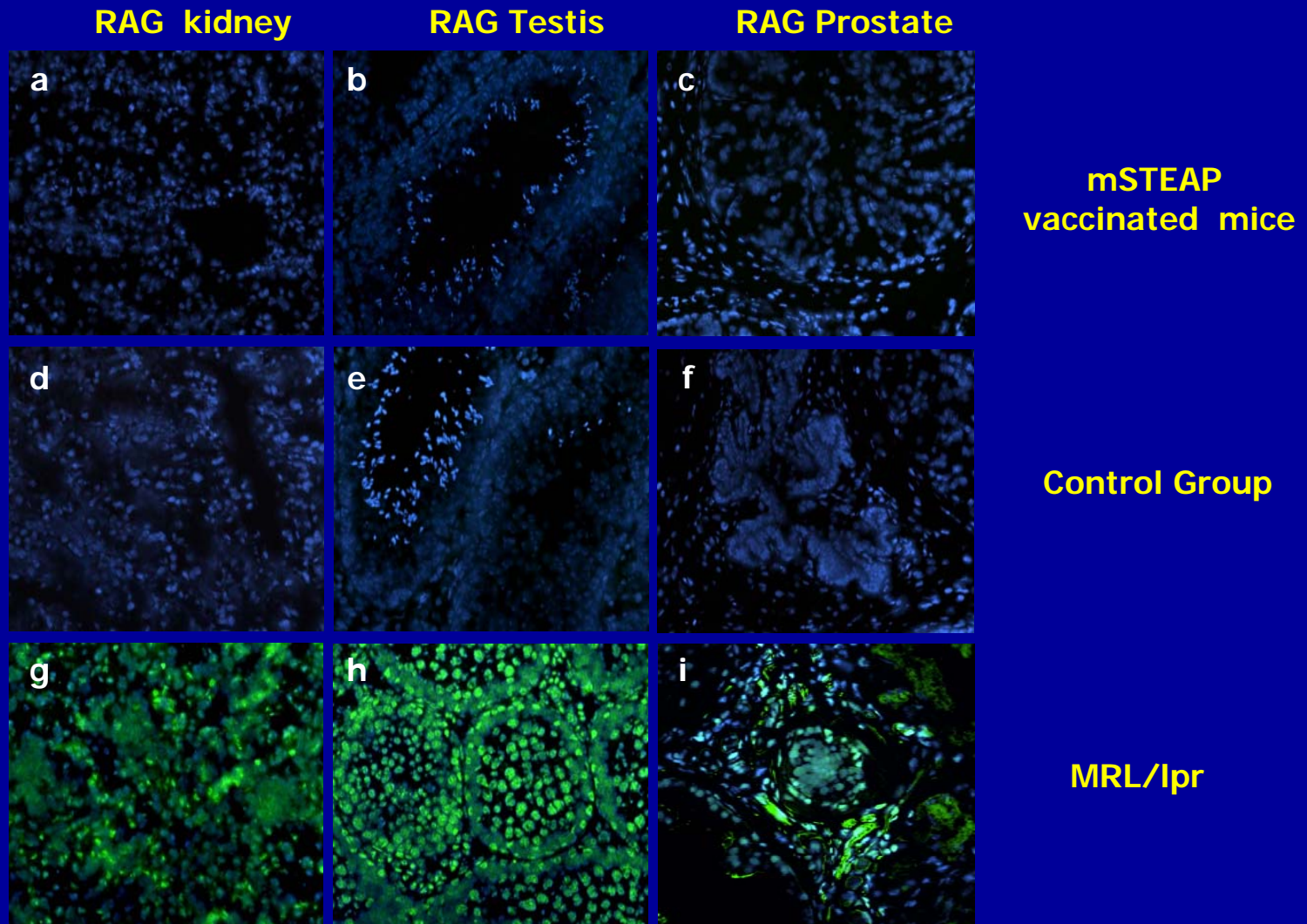
Vaccination leads to mSTEAP specific CTL



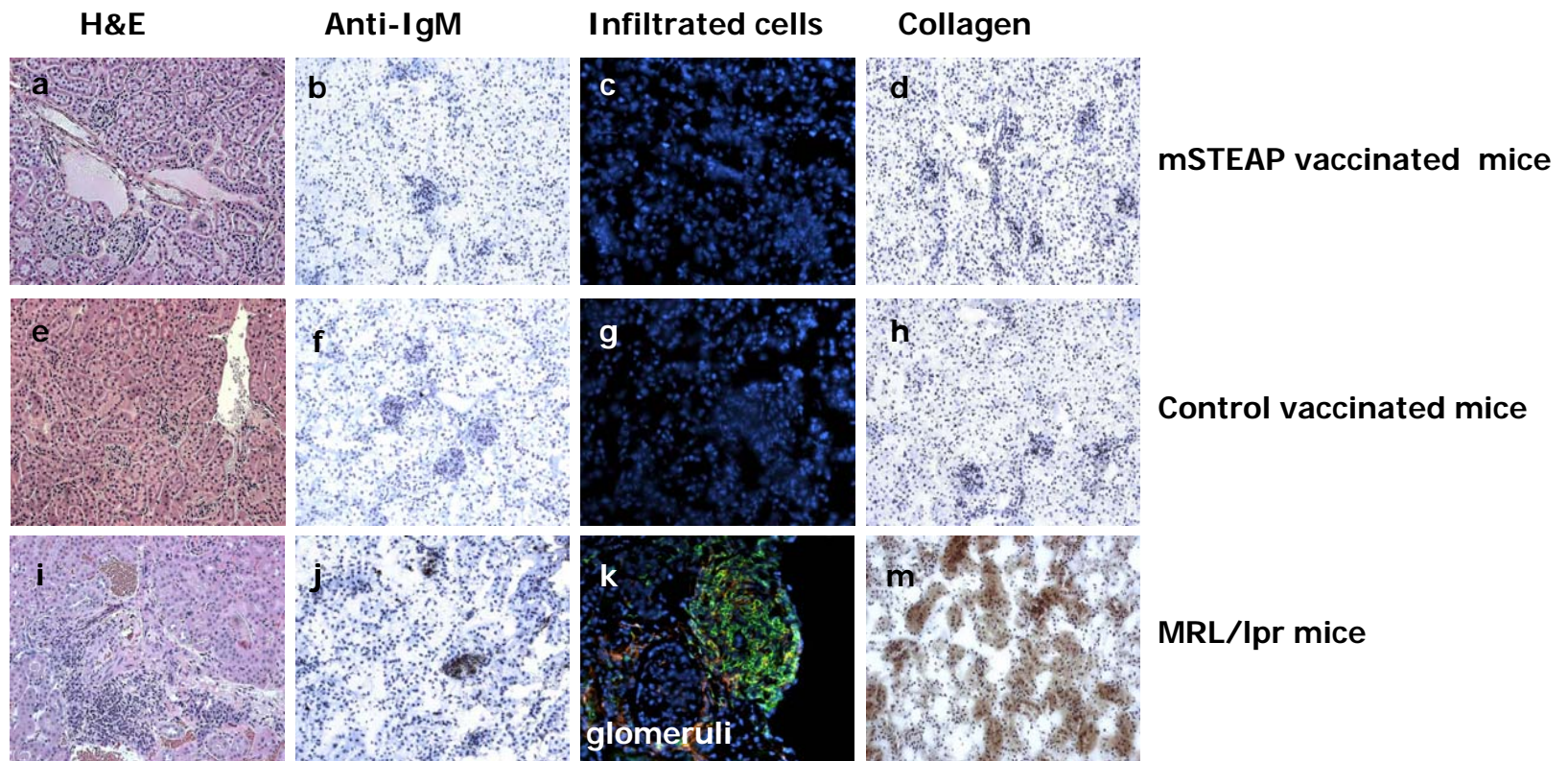
mSteap vaccination protects against TRAMP-C2 tumor challenge



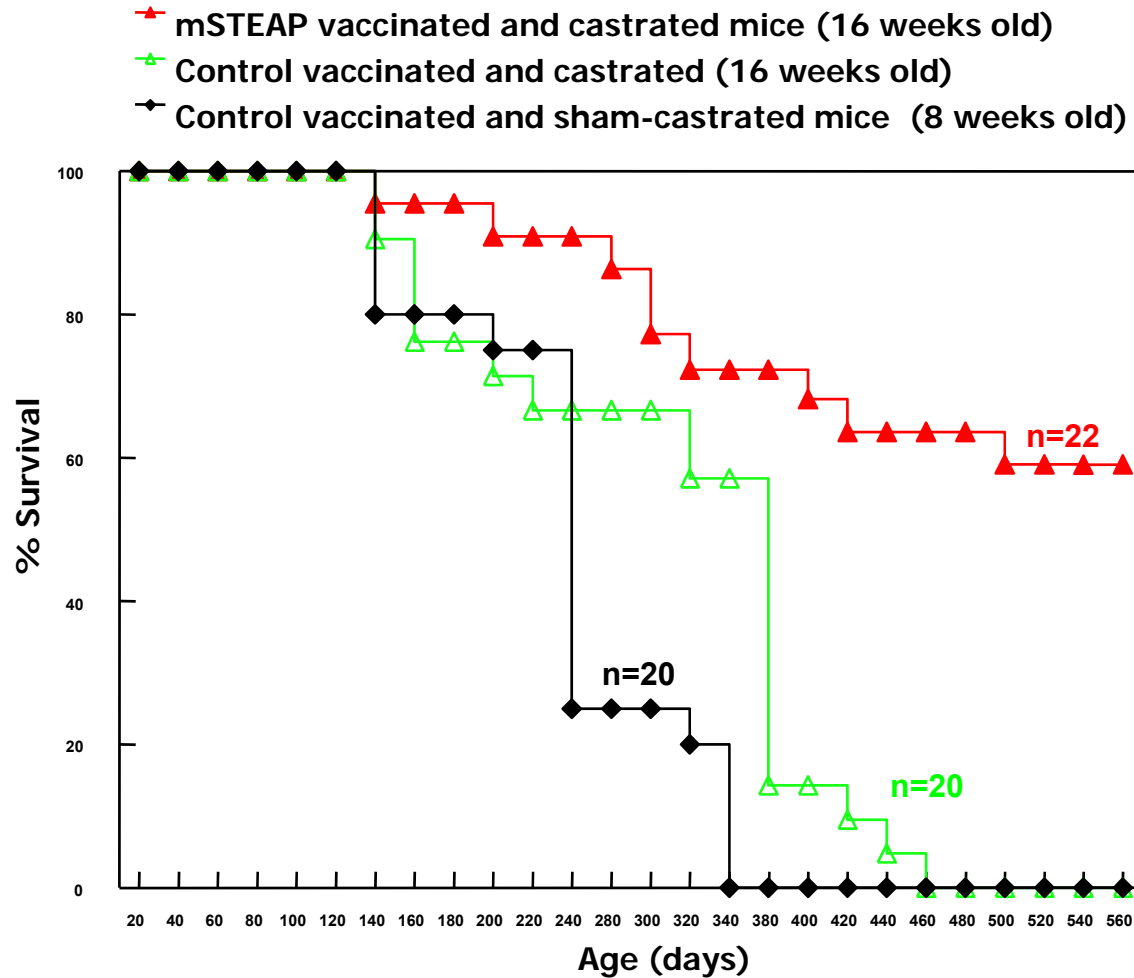
mSTEAP vaccination does not induce auto-antibodies



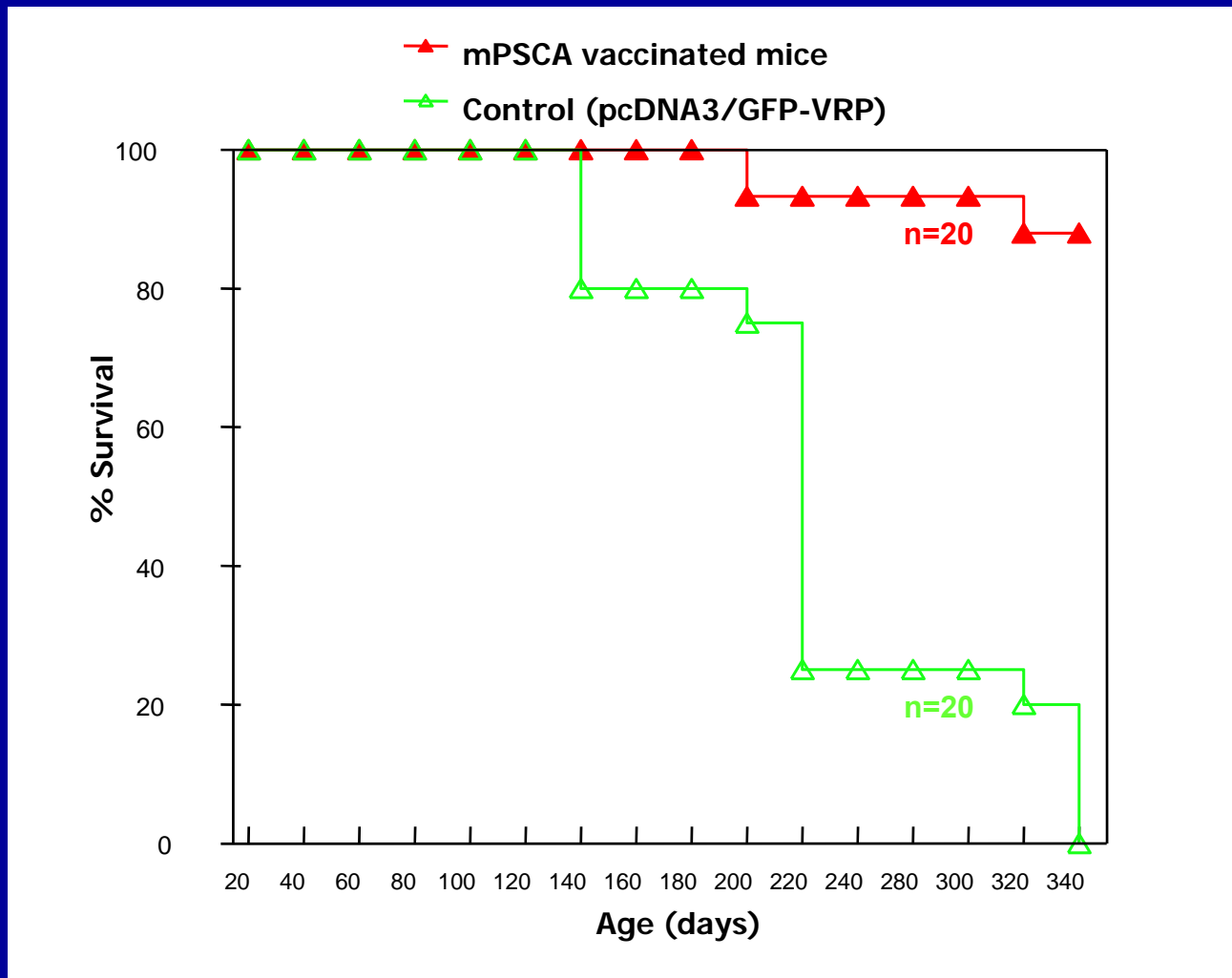
No other signs of autoimmunity after STEAP vaccination



mSTEAP vaccination with androgen ablation improves survival in tumor-bearing TRAMP mice



mPSCA based-vaccination induces long term protection in 8-10 week old TRAMP mice



Conclusions

- **There are several prostate antigens that can induce T cell immunity without measurable autoimmune side effects**
- **Our vaccination data in the TRAMP model show spectacular lifelong survival outcomes. This might induce a paradigm shift in how we would handle prostate cancer in patients by using therapeutic vaccines in the preventive setting rather than aggressive treatments**

The Kast USC Team

- Steven Fausch
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Publications:

Cooper et al, In Preparation

Kast et al Cancer Res 2008

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<http://www.aacr.org/home/about-us/news.aspx?d=957>



“Of Mouse Vaccine and Men”

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