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TITLE: The Use of Venezuelan Equine Encephalitis Encoding the Her-2/neu Tumor Associated Antigen for the Prevention and Treatment of Breast Cancer

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Overview:

Our project has focused developing a vaccination protocol using Venezuelan Equine Encephalitis (VEE) replicons (VRP) encoding the Her-2/*neu* tumor associated antigen to mediate anti tumor immunity in Her-2/*neu* transgenic (Tg) mice. Initial work demonstrated that vaccination with the VRP encoding full-length Her2/*neu* elicited robust Her2/*neu*-specific cytotoxic T lymphocyte (CTL) reactivity in the non-Tg parental strain (FVB/n) of mice (Fig. 1). However, no significant Her2/*neu*-specific CTL reactivity was detected in cultures prepared from the FVB/*neu* Tg mice (Fig. 1). The results demonstrated that an element of self tolerance to Her2/*neu* exists in the transgenic mice. We postulated that due to the size of the cDNA encoding full length Her2/neu (greater than 4 kb), levels of *in vivo* expression were not sufficient to elicit a $CD8^+$ T cell response in FVB/*neu* Tg mice. Accordingly, a VRP encoding the extracellular and transmembrane domains (VRP-EC/TMD) spanning approximately 2 kb was established. More recently, a VRP encoding the intracellular domain (VRP-ICD) has also been generated. Notably, a comparable frequency of IL-2 and IFNγ secreting T cells following stimulation with Her2/*neu* expressing NT2.5 tumor cells[1] was detected in cultures prepared from either FVB/n or FVB/*neu* Tg mice (Fig. 2). These results demonstrated that vaccination with VRP-EC/TMD could elicit significant Her2/*neu*-specific T cell reactivity despite tolerance to the neo-self antigen.

Next, the immunotherapeutic efficacy of VRP-EC/TMD was examined using a tumor challenge model. FVB/n or FVB/neu Tg mice were vaccinated with VRP-EC/TMD or left untreated and then injected s.c. with $2x10^6$ F-H2N1 expressing tumor cells in the hindquarter. This tumor cell line was derived from a spontaneously arising tumor in a FVB/*neu* Tg mouse and tested by FACS for neu expression regularly. As demonstrated in Fig. 3, a significant delay in tumor progression was detected in FVB/*neu* Tg mice vaccinated with VRP-EC/TMD but not in untreated mice. These results demonstrate that administration of VRP-EC/TMD can elicit T cell reactivity sufficient to delay tumor engraftment in FVB/*neu* Tg mice.

We've initiated four separate experiments examining the effect of VRP immunization on spontaneous tumor formation. The first pilot experiment was started over a year ago and has been completed (Figure 4A). These mice were immunized at 6 and 7 weeks of age and monitored for tumor development on a weekly basis. Normally, tumors first begin to appear around 7 months of age (200 days). Here we noticed a delay in the onset of tumor formation in the immunized FVB/*neu* Tg mice ($p = 0.04$, log rank). The second and third experiments were started concurrently, but with two different age groups of mice. The mice in experiment 2 (figure 4B) started treatment at 5 months of age to test the efficacy of late immunization, closer to the onset of tumor formation. While there is a visually obvious shift in the treatment group curve, it is not statistically significant. The third group of mice has been immunized 3 times at two-month intervals beginning at 8 weeks of age. The experimental group of 16 mice has been treated with EC-TMD VRP alone (n=8) or EC-TMD and IL-12 encoding VRPs (n=8). This experiment is still ongoing, however significant delays ($p<0.05$, log rank) have been observed in the appearance of tumors in VRP-EC/TMD immunized mice relative to control FVB/*neu* Tg mice. These preliminary results suggest that VRP-EC/TMD can delay spontaneous mammary adenocarcinoma in FVB/*neu* Tg mice. Further experiments

have been initiated to examine the effect of Full length Her-2/*neu*, VRP-EC/TMD, VRP-ICD and an irrelevant VRP encoding the influenza hemagglutinin protein (HA) on spontaneous tumor formation and are still ongoing.

Our plans are to further establish the efficacy of VRP vaccination using a series of *in vitro* assays to determine the cellular phenotype mediating anti tumor prodtection in the FVB/*neu* Tg mice. The relative contribution of $CD4^+$ Th and $CD8^+$ CTL will be evaluated. We further plan to assess *in vivo* therapeutic efficacy using the *neu* expressing tumor challange model and evaluatet the effect of coimmunization with cytokine encoding VRP (and irrelevant replicon controls). This series of experiments will include vaccination prior to tumor cell engraftment as well as more clinically relevant experiments in which the ability of vaccination to mediate rejection post establishement will be evaluated. Finally, we plan to assess whether VRP immunization can prevent or inhibit the metastatic progression of transferred tumor to the lung. Spontaneous adenocarcinomas arising in FVB/*neu* Tg mice have been shown to metatasize to the lung and assay protocols have been developed in which cultured tumor cells are transferred to recipient mice I.V. and tumor engraftment in the lung is evaluated microscopically following india ink inflation of the lung.

Key Accomplishments:

- Demonstrated $CD8^+$ CTL reactivity in FVB/n mice.
- Splenocytes from VRP immunized FVB/n and Her-2/*neu* Tg mice contain increased number of antigen specific IFNγ producing cells.
- We have completed two tumor challange experiments demonstratic the *in vivo* efficacy of Her-2/*neu* encoding VRP toward inhibiting the engraftment of transferrred neu expressing tumor cells.
- Three experiments assessing the efficiacy of Her-2/*neu* VRP vaccinations toward preventing spontaneous adenocarcinoma in Her-2/*neu* Tg mice have been completed. These results demonstrate VRP encodong Her-2/*neu* can delay spontaneous mammary adenocarcinoma.
- Completed *in vitro* testing and packaging of an additional truncated Her-2/*nue* encoding replicon expressing only the intracellular domain of the protein.

Reportable outcomes:

Results were presented at the Era of Hope Department of Defense Breast Cancer Research conference (oral communication and poster) in September of 2002.

Figures:

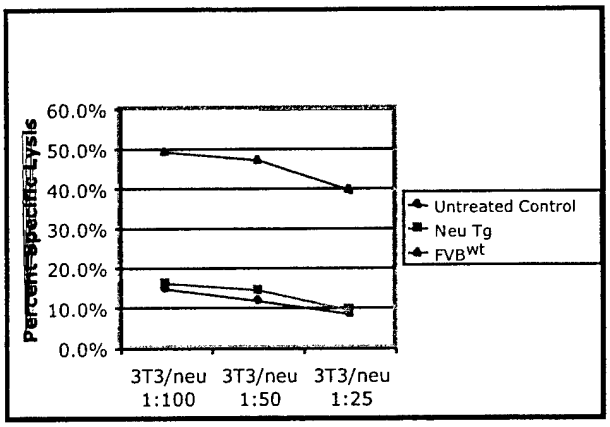


Figure 1. Standard 5 hr chromium release assay for Her-2/*neu* specific lysis. Wild type FVB and FVB/*neu* Tg mice were immunized I.P. 3 times at 7 day intervals with VRP-neu (5.0×10^5 I.U.). Splenocytes were prepared from immunized mice 10 days following the final injection and re-stimulated in vitro for 6 days with psorelen inactivated NIH-3T3 cells permanently transfected with a Her-2/*neu* encoding construct. Stimulated lymphocytes were incubated with ^{51}Cr labeled targets in the indicated ratios for 5 hours.

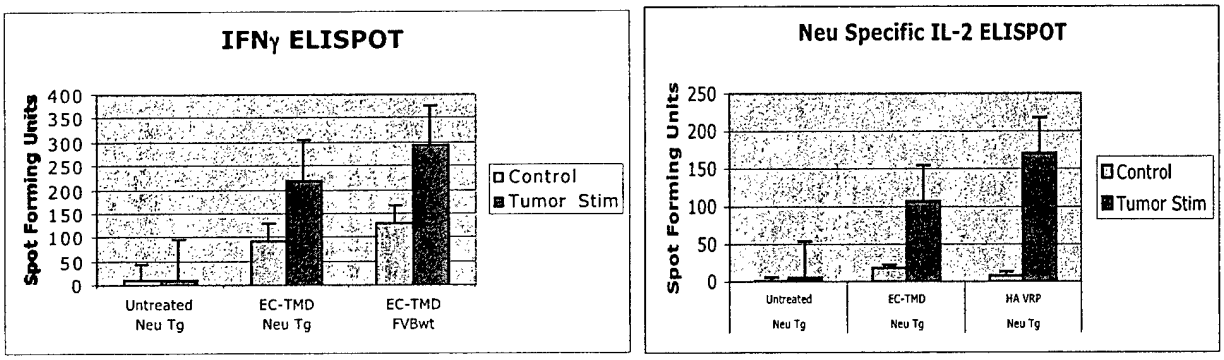


Figure 2. Her2/neu-specific ELISPOT assay. FVB/n and/or FVB/*neu* Tg mice were immunized I.P. twice 2 weeks apart with 5.0×10^5 I.U. VRP-EC/TMD and splenocytes were harvested 7 – 10 days later. 7.5×10^5 (IFNγ) or 1.0×10^6 (IL-2) splenocytes were cultured with 1.0×10^5 mitomycin-C treated NT2.5 tumor cells for 24 hours. Cells were than aspirated and developed for spot forming units. For all treated mice, $p < 0.05$ for tumor stimulated versus control.

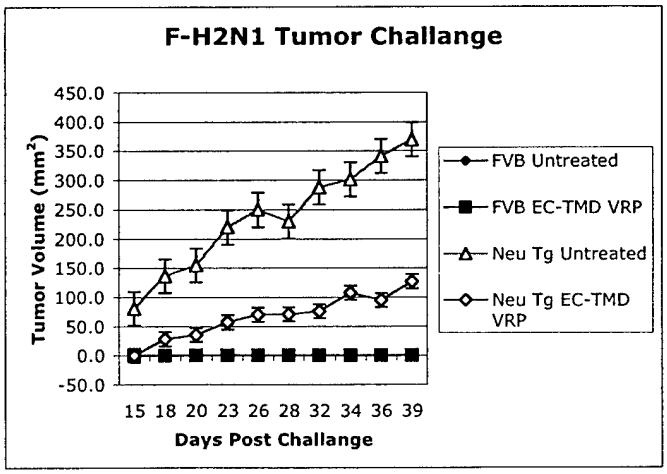
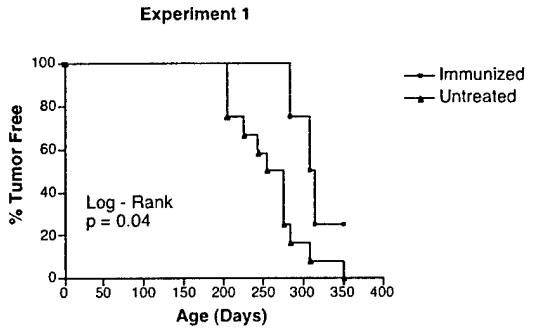


Figure 3. Delayed tumor growth in VRP immunized mice. Wild type FVB and FVB/*neu* Tg mice were immunized I.P. twice at 10-day intervals with 5.0×10^5 I.U. VRP-EC/TMD. One week later, mice received 2.0×10^6 F-H2N1 tumor cells subcutaneously in the left flank. Challenged mice were monitored for tumor growth over a 39-day period and palpable tumors measured using a Vernier type caliper. Data is representative of two different experiments, four mice per group. P=0.037 at day 32 (t-test) Untreated vs. immunized FVB/neu Tg mice.

A



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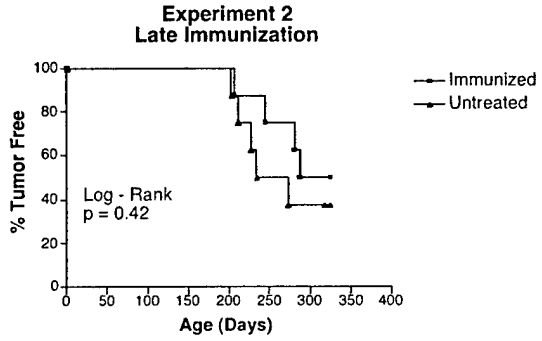

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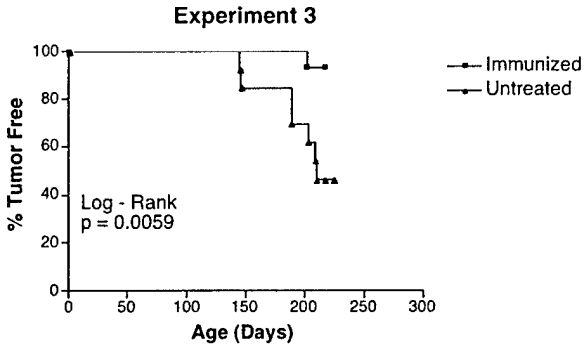

Figure 4. Spontaneous tumor formation. Neu Tg mice were immunized with 5.0 x 10^5 I.U. neu EC-TMD VRP I.P. at 6 and 7 weeks of age (A), at five and 7 months of age (B) or at two, four and six months of age (C). Mice were visually monitored on a weekly basis for development of breast adenocarcinoma.

Summary:

We have completed much of the work described for Specific Aim 1 in which we set out to establish and characterize VRP vaccination to induce Her-2/*neu* specific $CD4^+$ Th1 and $CD8^+$ CTL responses. We have completed all in vitro testing and packaging of replicons including two truncated Her-2/*neu* replicons encoding the extracellular and transmembrane domains, and encoding the intracellular domain of the protein. We have observed IL-2 and IFNγ secreting cells in cultures prepared form vaccinated FVB/*neu* Tg mice indicating we could elicit significant Her-2/*neu* specific T cell reactivity despite

tolerance to the neo-self antigen. A H-2^q restricted immunodominant epitope of Her-2/neu has recently been identified which should aid us in further characterizing the responding $CD8^+$ T cell populion.[2]

For the second specific aim, we wished to determine the efficacy VRP vaccination for the prevention and/or treatment of mammary adenocarcinoma in FVB/*neu* Tg mice. We have made progress in evaluating the therapeutic efficacy of VRP toward preventing the progression of adenocarcinoma in mice challenged with F-H2N1 tumor cells. Specifically, we have demonstrated delayed onset and inhibited growth in challenged mice immunized with the truncated VRP-EC/TMD. We have begun to evaluate the efficacy of Her-2/*neu* encoding VRP in preventing the spontaneous development of mammary adenocarcinoma in FVB/*neu* Tg mice. These results demonstrate vaccinated mice display a delay in the development of spontaneous mammary adenocarcinoma as compared to untreated control mice.

Comparative analyses are ongoing to determine the magnitude of $CD4^+$ and $CD8^+$ T cell reactivity induced in FVB/*neu* Tg mice following vaccination with VRP-EC/TMD plus/minus cytokine encoding VRPs, in addition to VRP-ICD (plus/minus cytokine VRPs). Experiments will examine the efficacy of VRP treatment *after* FVB/*neu* Tg mice are challenged with tumor cells. Depending on their adjuvant effect, VRP encoding the appropriate cytokine will be co-administered with VRP-EC/TMD (and VRP-ICD) and the efficacy of this regime to suppress the progression of established tumors in the challenge model will be investigated. Studies will continue to evaluate the immunotherapeutic efficacy of VRP-EC/TMD (and VRP-ICD) in the prevention of spontaneous tumor formation in FVB/*neu* Tg mice. Finally, we have established adenoviral-associated viral (AAV) recombinants encoding the EC/TMD and ICD of Her2/*neu*. Once packaged, T cell reactivity induced following vaccination with the AAV vectors will be characterized.

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1. **R. Todd Reilly**, Morris B. C. Gottlieb, Anne M. Ercolini, Jean-Pascal H. Machiels, Colin E. Kane, Francesca I. Okoye, William J. Muller, Katherine H. Dixon, and Elizabeth M. Jaffee. **2000**. HER-2/*neu* Is a Tumor Rejection Target in Tolorized HER-2/*neu* Trangenic Mice. Cancer Research 60, 3569-3576
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