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# The use of Her2/neu-specific genetic vaccines for the prevention and treatment of breast cancer

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## Abstract
We are investigating the feasibility of using HER2/neu encoding genetic vaccines to induce potent CD4+ and CD8+ T cell reactivity for the prevention and treatment of breast cancer. Two strategies of genetic vaccination, each with distinct advantages will be assessed. The first strategy entails the use of alphavirus Venezuelan equine encephalitis virus (VEE)-replicons which selectively infect dendritic cells in vivo. Dendritic cells are characterized by a highly potent capacity to activate naïve T cells. Our second strategy is to employ a plasmid DNA (pDNA) vaccine encoding HER2/neu. pDNA vaccination has been shown to be effective in eliciting persistent T cell reactivity in various experimental model systems. VEE-replicon and pDNA vaccines encoding cytokines such as IL-12 and IL-18 will be used to enhance anti-HER2/neu-specific CD4+ Th1 cell and CD8+ CTL activity.

To directly assess the efficacy of our approach to prevent tumor cell growth and/or eradicate established tumors, mice transgenic for a rat HER2/neu gene will be employed. These mice develop mammary tumors and pulmonary metastatic lesions. Furthermore, we will use mice transgenic for HLA-A2.1 to investigate CD8+ CTL responses to HER2/neu-specific peptide epitopes which have been proposed as targets for immunotherapy in a clinical setting.
INTRODUCTION.
The long-term objective of this proposal is to establish a safe, effective, and persistent form of tumor antigen-specific immunotherapy for the prevention and treatment of breast cancer. Specifically, we are investigating the efficacy of tumor antigen encoding genetic vaccines to induce CD4+ Th cell and CD8+ CTL reactivity. We believe that recruitment of both CD4+ and CD8+ T cells is necessary if not essential, to mediate potent and long-term immunity to tumor antigens, which typically are weak immunogens. Our model antigen is HER2/neu, an oncprotein which is overexpressed in 30% of all breast and ovarian cancers. Two strategies of HER2/neu-specific genetic vaccination are being investigated, namely VEE-replicon- and pDNA-based vaccines. Each strategy will be assessed separately, however, we hypothesize that a combination of VEE-replicon and pDNA vaccination will prove to be the most effective form of immunotherapy necessary for prevention and eradication of established tumors. The rationale is to complement the strengths and weaknesses associated with each strategy. For example, administration of a VEE-replicon provides a unique approach to selectively infect dendritic cells in vivo, and in turn initiate potent T cell immunity. However, expression mediated by a VEE-replicon is only transient. On the other hand, the stability and long-term expression of an immunogen encoded by pDNA typically results in persistent T cell and antibody immunity. Yet, it is unlikely that the magnitude of the T cell response elicited via pDNA vaccination will be sufficient to eradicate established tumors. We predict that these two strategies together will elicit a robust and persistent T cell response. In a further attempt to enhance immunotherapeutic efficacy, VEE-replicon and pDNA vaccines encoding cytokines such as IL-12 and IL-18 known to promote CD4+ Th1 and CD8+ CTL reactivity, will be employed. To directly determine the immunotherapeutic efficacy of the treatment regimes, we have established a unique model system incorporating mice transgenic for HER2/neu and HLA-A2.1. In this way, we can: i) critically assess the effectiveness of targeting immunity to a self antigen, ii) directly determine the efficacy of our treatment regimes to prevent tumor progression and eradicate established tumors, and iii) analyze CTL reactivity to HER2/neu-specific epitopes which may have direct application for immunotherapy. In summary, this proposed work should provide the necessary insight to establish a rational, effective, and safe form of HER2/neu-specific genetic vaccination for the long-term prevention and treatment of breast cancer. In addition, this work will generate therapeutic reagents which if effective, can be directly applied to a clinical setting.

BODY.
Specific Aim 1. Determine the optimal conditions for pDNA and VEE-based replicon vaccination to induce HER2/neu-specific CD4+ Th cell and CD8+ CTL reactivity.


As reported previously, we have established pDNA and VEE- replicas encoding rat HER2/neu and shown that the respective recombinants are functional in terms of protein expression. Two different rat HER2/neu encoding pDNA and VEE-replicon constructs have been engineered. Namely, recombinants encoding full length (pDNA-neu, VRP-neu) or the extracellular domain (pDNA-exdneu, VRP-exdneu) of HER2/neu.

Task 2. Complete construction and testing of pDNA and VEE-replicon recombinants expressing IL-12 and IL-18.

As reported previously, we have established pDNA and VEE- replicas encoding IL-12 and IL-18 and shown that the respective recombinants are functional.
Task 3. Characterize and optimize CD4^+ Th cell and CD8^+ CTL reactivity in FVB/A2.1/K^b mice following treatment with HER2/neu- and cytokine-specific pDNA and VEE-replicon encoding recombinants.

As described in earlier progress reports, we have demonstrated that a HER2/neu-specific response can be induced in FVB/A2.1/K^b and FVB/neu transgenic mice following pDNA-neu immunization. Furthermore, in an initial effort to enhance immunogenicity of pDNA-neu administration, FVB/A2.1/K^b mice were treated with pDNA-neu and pDNA-IL-12/18. However, no significant increase in HER2/neu-specific CD8^+ T cell reactivity was observed relative to animals receiving pDNA-neu alone. In a further attempt to enhance immunogenicity of pDNA-neu, the adjuvant effect of a panel of pDNAs encoding IL-2, IFN_y, GM-CSF and soluble FLT3-ligand was also tested. In view of various studies demonstrating that co-administration of pDNA cytokines enhances T cell immunity (1-4), it was surprising that HER2/neu-specific CD8^+ CTL activity was not increased by co-administration of any of the cytokine encoding pDNAs. Finally, no significant difference in HER2/neu-specific CD8^+ CTL activity was detected in FVB/neu transgenic mice treated with pDNA-neu versus pDNA-exdneu.

As described in the previous report, immunization with VRP-neu resulted in a significant increase in the magnitude of CD8^+ CTL reactivity in either FVB/A2.1/K^b or FVB/neu mice relative to pDNA-neu treated animals. This may reflect the preferential infection of dendritic cells in vivo by VEE-replicons. Also reported earlier, was the finding that co-immunization with VRP-neu and VRP-IL12/IL-18 had no significant enhancing effect on HER2/neu-specific T cell reactivity versus immunization with VRP-neu alone in FVB/A2.1/K^b or FVB/neu mice. However, co-administration of VRP-neu with either VRP-IFN_y or VRP-GMCSF increased HER2/neu-specific CD8^+ T cell reactivity in FVB/neu mice compared to VRP-neu alone (Figure 1). In addition, HER2/neu-specific CD8^+ T cell reactivity was significantly increased in FVB/neu mice receiving VRP encoding the extracellular domain of HER2/neu versus the full length antigen (Figure 2). Since the extracellular domain is approximately half the size of full length HER2/neu relative to mRNA and protein, we believe the increased immunogenicity of VRP-exdneu is in part due to elevated in vivo expression of the recombinant. For example, the maximum sized cDNA accommodated by VRP is approximately 4.0 kilobases, which in turn is the size of the full length HER2/neu cDNA.

Specific Aim 2. Determine the efficacy of HER2/neu-specific pDNA and VEE-replicon vaccination to prevent and eradicate breast tumor cell growth in HER2/neu transgenic mice.

Task 1. Determine efficacy of VEE-replicon and pDNA administration to prevent tumor progression FVB/neu x A2.1/K^b mice.

As previously reported, no significant difference in mammary tumor onset was detected in FVB/neu x A2.1/K^b female mice immunized with pDNA-neu or VRP-neu plus/minus the IL-12/IL-18 encoding recombinants. We next treated groups of 10 FVB/neu x A2.1/K^b female mice 4 weeks of age with 2 injections of 5x10^7 IU of VRP-exdneu or a control VRP encoding influenza hemagglutinin (VRP-HA) or mice were left untreated. The groups of animals have now been followed for tumor progression for approximately 7 months. The majority of untreated (7/10) or VRP-HA treated (8/10) mice developed tumors. In contrast, only 1/10 of the VRP-exdneu immunized animals developed a mammary tumor. We are continuing to monitor these animals. The results suggest that administration of VRP-exdneu can at a minimum delay the onset of tumor appearance. This is in marked contrast to animals receiving pDNA-exdneu in which the onset of tumor appearance was unaltered in FVB/neu x A2.1/K^b female mice.

Task 2. Determine efficacy of VEE-replicon and pDNA administration to eradicate established tumor foci in the mammary glands and pulmonary metastatic lesions in FVB/neu x A2.1/K^b mice.

We have continued short-term experiments employing a HER2/neu expressing tumor cell line (F-H2N) established in the laboratory from a mammary tumor excised from FVB/neu mice. As we reported earlier, F-H2N cells are tumorigenic in FVB/neu mice upon adoptive transfer. Furthermore, an earlier experiment demonstrated that F-H2N tumor progression was somewhat delayed and tumor vascularization was reduced in FVB/neu mice immunized with VRP-neu (independent of VRP-IL12/IL18 co-immunization) relative to animals treated with
VRP-HA. We have repeated the experiment employing VRP-exdneu. As demonstrated in Figure 3, a significant
delay in tumor progression was detected in mice immunized with VRP-exdneu relative to VRP-HA treated
animals. Furthermore, tumors failed to develop in 2/8 mice receiving VRP-exdneu. These results indicate that
administration of VRP-exdneu can also influence tumor progression in the F-H2N challenge model. We are
currently assessing the immunotherapeutic efficacy of recombinants encoding extracellular HER2/neu and the
extended panel of cytokines in this adoptive transfer model.

In contrast to findings made with VRP-exdneu, no significant immunotherapeutic effect was observed in
FVB/neu mice immunized with pDNA-exdneu. This result further supports the notion that a more potent T cell
response is elicited via VRP versus pDNA administration.

KEY RESEARCH ACCOMPLISHMENTS.

• Administration of pDNAs encoding either full length or the extracellular domain of HER2/neu fail to effectively
prevent spontaneous mammary tumor development in FVB/neu mice or elicit protection to a challenge of
HER2/neu expressing tumor cells.

• HER2/neu-specific T cell reactivity induced following treatment with VRP-neu can be enhanced by co-
administration with VRPs encoding either IFNγ or GM-CSF.

• Increased immunogenicity is observed for VRP encoding the extracellular domain of HER2/neu versus the full
length recombinant.

• Administration of VRP-exdneu mediates protection from spontaneous tumor development and a tumor cell
challenge.

REPORTABLE OUTCOMES.
Currently not applicable.

CONCLUSIONS.
Our results demonstrate that VEE-replicon versus pDNA administration is a more effective strategy of genetic
vaccination. This is based on data indicating that the magnitude of HER2/neu-specific T cell reactivity and
protection elicited against the development of spontaneous mammary tumors or a tumor cell challenge are
enhanced following VRP immunization relative to treatment with pDNA. Our findings with VRP-exdneu are
promising and efforts are underway to determine whether the observed protection can be further enhanced by co-
administration of VRPs encoding IFNγ and GM-CSF, for example.

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APPENDICES.

Figure 1. The immunogenicity of VRP-neu is enhanced by co-administration of VRPs encoding murine IFNγ or GM-CSF. Groups of three FVB/neu female mice received two intraperitoneal (i.p.) injections of 5x10^5 IU of VRP-neu alone or VRP-neu plus VRP-IFNγ or VRP-GMCSF. Three weeks after the final immunization, splenocytes were prepared from individual mice, and the frequency of IFNγ spot forming T cells (SFC) in response to irradiated HER2/neu expressing NIH3T3 cells (3T3-neu) was measured via ELISPOT. p<0.001: VRP-neu+VRP-IFNγ versus VRP-neu, and VRP-neu+VRP-GMCSF versus VRP-neu.

Figure 2. VRP-exdneu exhibits increased immunogenicity relative to VRP-neu. Groups of three FVB/neu female mice received two i.p. injections of 5x10^5 IU of VRP-neu or VRP-exdneu. Three weeks after the final immunization, splenocytes were prepared from individual mice, and the frequency of IFNγ secreting T cells in response to irradiated HER2/neu expressing NIH3T3 cells was measured via ELISPOT. p<0.001: VRP-exdneu versus VRP-neu.
Figure 3. Administration of VRP-exdneu protects against a challenge with the F-H2N tumor cell line. Groups of eight FVB/neu female mice received two i.p. injections of $5 \times 10^5$ IU of VRP-exdneu, VRP-HA or were left untreated. Seven days after the final injection, mice received $2 \times 10^6$ F-H2N cells subcutaneously in the hind flank. Tumor size was then measured with time. Note that the tumor progression depicted for VRP-exdneu treatment is for only six mice since tumors failed to develop in two of the animals. $p < 0.001$: VRP-exdneu versus untreated or VRP-HA.