**Supplementary Figure 1: Expression of MAM-A and HLA-A2 in breast cancer cell lines.** Expression of MAM-A and HLA-A2 was evaluated in breast cancer cell lines by RT-PCR.

**Supplementary Figure 2: MAM-A DNA vaccination increases breast cancer-induced TNF-α production by MAM-A-specific CD8 T cells**. Purified CD8 T cells from subjects vaccinated with the MAM-A DNA vaccine were stimulated with UACC-812 breast cancer cells at a 50:1 T cell to breast cancer cell ratio (S = stimulated, U = unstimulated). Intracellular expression of TNF-α was measured by immunoblot. Actin expression represents a protein loading control.

**Supplementary Figure 3: MAM-A DNA vaccination increases NKG2D expression in MAM-A-specific CD8 T cells.** Purified CD8 T cells from subjects vaccinated with the MAM-A DNA vaccine were stimulated with UACC-812 breast cancer cells at a 50:1 T cell to breast cancer cell ratio. NKG2D expression was determined by immunoblot. UACC-812 stimulation was performed in the presence of isotype or NKG2D antibody; control or NKG2D siRNA, as well as antibodies to IFN-γ and TNF-α. Actin expression represents a protein loading control.

**Supplementary Figure 4: MAM-A DNA vaccination increases DAP10 adapter protein expression in MAM-A-specific CD8 T cells.** Purified CD8 T cells from subjects vaccinated with the MAM-A DNA vaccine were stimulated with UACC-812 breast cancer cells at a 50:1 T cell to breast cancer cell ratio. UACC-812 stimulation was performed in the presence of isotype or NKG2D antibody; control, DAP10 or NKG2D siRNA, as well as antibodies to IFN-γ and TNF-α. (A) DAP10 expression was determined by immunoblot. Actin expression represents a protein loading control. (B) DAP10 expression was determined by qRT-PCR. Actin expression represents an endogenous control to normalize sample RNA.

**Supplementary Figure 5: MAM-A DNA vaccination increases perforin expression in MAM-A-specific CD8 T cells.** Purified CD8 T cells from subjects vaccinated with the MAM-A DNA vaccine were stimulated with UACC-812 breast cancer cells at a 50:1 T cell to breast cancer cell ratio. UACC-812 stimulation was performed in the presence of isotype or NKG2D antibody; control or perforin siRNA, as well as antibodies to IFN-γ and TNF-α. (A) Perforin expression was determined by immunoblot. Actin expression represents a protein loading control. (B) Perforin expression was determined by qRT-PCR. Actin expression represents an endogenous control to normalize sample RNA.

**Supplementary Figure 6: Therapy received by the patients (screen failed and vaccinated).**

# SUPPLEMENTARY FIGURE 1



# SUPPLEMENTARY FIGURE 2



# SUPPLEMENTARY FIGURE 3



# SUPPLEMENTARY FIGURE 4



# SUPPLEMENTARY FIGURE 5



# SUPPLEMENTARY FIGURE 6

|  |  |
| --- | --- |
| Screen Fail ID | Therapy received after study consent form signed |
| SF1 | Intrathecal methotrexate |
| SF2 | Everolimus, exemestane, letrozole |
| SF3 | Fulvestrant, everolimus, exemestane, paclitaxel |
| SF13 | Trastuzumab, anastrozole (continued) |
| SF15 | Gemcitabine, carboplatin, capecitabine |
| SF16 | Estradiol (continued), paclitaxel, Tamoxifen, bevacizumab, ixabepilone, gemcitabine, cisplatin |
| SF21 | Fulvestrant (continued) |
| SF24 | Letrozole (continued) |
| SF25 | Continued fulvestrant and anastrozole, capecitabine, exemestane, everolimus |
| SF26 | Exemestane (continued) |
| SF28 | Anastrozole (continued) |
| SF29 | Tamoxifen, leuprolide, letrozole, capecitabine |
| SF32 | Continued letrozole and trastuzumab, estradiol |
| SF33 | Paclitaxel, doxorubicin |
| SF36 | No additional chemotherapy |
| SF37 | Estradiol (continued), capecitabine, exemestane |
| SF39 | No additional therapy given |
| Vaccinated patient ID | Therapy received after study consent form signed |
| V003 | letrozole (continued); enrolled in CALGB trial (fulvestrant w/ lapatinib or placebo), capecitabine |
| V005 | goserelin and tamoxifen (continued), letrozole |
| V006 | letrozole (continued), estradiol |
| V008 | letrozole (continued),tamoxifen  |
| V013 | letrozole (continued), anastrozole |
| V023 | goserelin, trastuzumab, and anastrozole (continued) |
| V025 | exemestane (continued), CALGB study (ixabepilone w/ bevacizumab), fulvestrant, carboplatin, gemcitabine eribulin |
| V026 | exemestane and trastuzumab (continued) |
| V030 | anastrozole (continued), letrozole, fulvestrant |
| V032 | faslodex (continued) |
| V043 | anastrazole (continued) |
| V048 | exemestane, progressed and subsequently enrolled in another clinical trial at an OSH comparing afinitor vs. aromasin and Herceptin. Assigned to aromasin and herceptin arm |
| V053 | Letrozole (continued) |
| V054 | Letrozole (continued), exemestane |