Cancer Vaccines and Cytokines

Elizabeth A. Mittendorf, MD, PhD
Assistant Professor
Department of Surgical Oncology
Disclosures

• I serve as the PI on a phase III trial sponsored by Galena BioPharma investigating NeuVax

• I serve as the PI on a phase II trial sponsored in part by Antigen Express investigating the AE37 vaccine
Goals

- Discuss considerations in vaccine construction/development
- Review specific vaccines currently being evaluated in later stage clinical trials
A vaccine is used for induction of humoral and/or cellular immune responses against an antigen or set of antigens.
Considerations in Vaccine Development

- Target (tumor antigen)
- Effective adjuvant
- Delivery platform
- Clinical setting in which vaccine will be effective
- Patients likely to benefit from vaccination
- Feasibility of large-scale vaccine production
  - Cost
  - Preparation time
Tumor Antigen

- Allows tumor cells to be distinguished from normal
- Overexpressed or abnormally expressed in tumors
- Critical for the survival of the tumor
Tumor Antigen

- Cancer testis
  - MAGE, NY-ESO-1
- Oncogenes
  - HER2, WT1, p53
- Differentiation antigens
  - gp 100, MART-1, tyrosinase
- Glycoproteins
  - MUC-1
- Oncofetal antigens
  - AFP, CEA
Immunoadjuvant

- Nonspecific substance acting to enhance the immune response to an antigen with which it is administered

- Examples
  - Incomplete Freund’s adjuvant (IFA)
  - GM-CSF
  - Monophosphoryl lipid A
  - CpG oligonucleotides
IFA

- Evaluated immune responses to gp100 + IFA
- Peptide/IFA primed tumor-specific CD8+ T cells
- Primed T cells remained at the vaccination site; not tumors

Platforms

- Dendritic cell vaccines
- Peptide vaccines
- Protein vaccines
- Whole tumor cell vaccines
- DNA vaccines
- Recombinant viral vectors
Platforms

• Dendritic cell vaccines
  • Peptide vaccines
  • Protein vaccines
  • Whole tumor cell vaccines
  • DNA vaccines
  • Recombinant viral vectors
Dendritic Cell Vaccines

• Sipuleucel-T (Provenge)
  • Approved by FDA in 2010 for metastatic castration resistant prostate CA
  • Improves OS by 4.1 months

IMPACT Study
HR for death in vaccine group =0.78 (95% CI 0.61-0.98; p=0.03)

Kantoff PW et al. NEJM 2010;363(5):411-422
Dendritic Cell Vaccines

$93,000/patient
Dendritic Cell Vaccines

• Pros
  • Ex vivo DC maturation step
  • ↑ immune activation of infused product over time

• Cons
  • Complex manufacturing process
  • Expensive
  • Inconsistent results
GSK MAGE-A3

- Recombinant protein
- MAGE-A3
  - Tumor specific
  - Expressed in testis and placenta where spermatogonia and trophoblasts lack MHC molecules
- AS15 = GSK proprietary immunologic Adjuvant System
GSK MAGE-A3

- Phase II (n=182)
- Resected stage IB or II NSCLC
- Randomized to post-op vaccine or placebo
- Median f/u = 44 months
- Trend towards improved DFS and OS in vaccine group
- Identified possible gene signature that correlated with clinical activity
MAGRIT - NSCLC

Eligible
- Resected IB-IIIA
- Lobectomy
- MAGE-A3 expression
- Chemotherapy optional
- Patients stratified by +/- chemo

Randomize

MAGE-A3 vaccine x 13 injections over 27 months

Placebo x 13 injections over 27 months

Primary endpoint:
Disease-free survival

Secondary endpoint:
Validation of predictive gene signature
DERMA - Melanoma

Eligible
Stage IIIb or IIIc rendered disease free by surgery
MAGE-A3 expression

Randomize

MAGE-A3 vaccine x 13 injections over 27 months

Placebo x 13 injections over 27 months

Primary endpoint:
Disease-free survival

Secondary endpoint:
Validation of predictive gene signature
Idiotype Vaccine

- Idiotype
  - Molecular determinant on the variable regions of surface Ig on a B-cell
  - Unique to each Ig
  - Can be recognized as antigens
Idiotype Vaccine

• Double-blind, RCT
• Follicular lymphoma
• Bulky stage II, III or IV disease with LN > 2cm accessible for biopsy
• Chemo naïve
• Patients achieving a complete response after chemotherapy were randomized

Vaccine: tumor isotype-matched Id protein manufactured by hybridoma technology.

Idiotype Vaccine

Peptide Vaccines

- Use antigenic peptides derived from tumor associated antigens (TAA)
- Stimulate peptide-specific immune regulators
gp100

- Randomized phase 3
- N=185
- Stage IV or locally advanced stage III melanoma
- HLA-A2+
- Patients randomized to:
  - IL-2 alone
  - Gp100 + IFA followed by IL-2
- Primary endpoint: clinical response

gp100

HER2/neu

Extracellular Domain (aa 1-652)

Trans Membrane Domain (aa 653-675)

Intracellular Domain (aa 676-1255)

E75-peptide vaccine (aa 369-377)
MHC Class I: HLA-A2 & HLA-A3
Stimulate CD8 T cells

I I S A V V G I L
GP2-peptide vaccine (aa 654-662)
MHC Class I: HLA-A2
Stimulate CD8 T cells

K I F G S L A F L
E75-peptide vaccine (aa 369-377)
MHC Class I: HLA-A2 & HLA-A3
Stimulate CD8 T cells

G V G S P Y V S R L L G I C L
AE37-peptide vaccine (aa 776-790)
MHC Class II: multi-allele
Stimulate CD4 T cells
HER2-Derived Peptide Vaccine

- **E75**
  - 9 aa peptide from extracellular domain
  - Immunodominant epitope of HER2/neu
  - MHC class I peptide $\rightarrow$ stimulated CD8$^+$ T cells
  - High affinity for HLA-A2 /A3

HER2/neu $\rightarrow$ E75 + GM-CSF $\rightarrow$
Trial Design

Booster Program

<table>
<thead>
<tr>
<th>2000</th>
<th>'01</th>
<th>'02</th>
<th>'03</th>
<th>'04</th>
<th>'05</th>
<th>'06</th>
<th>'07</th>
<th>'08</th>
<th>'09</th>
<th>'10</th>
<th>'11</th>
<th>'12</th>
<th>'13</th>
</tr>
</thead>
<tbody>
<tr>
<td>IND approved</td>
<td>Enrollment closed</td>
<td>24 mo f/u complete</td>
<td>60 mo f/u complete</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mittendorf E et al. *Cancer* 2012;118(10):2594-2602
Inclusion Criteria

• Histologically confirmed breast cancer
• Node positive or high-risk node negative
• Completed SOC surgery, chemotherapy and radiation
• Immunocompetent
• Any level of HER2 (IHC 1+, 2+, 3+)
## E75 Phase I/II Trial

<table>
<thead>
<tr>
<th></th>
<th>Vaccine</th>
<th>Control</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=</td>
<td>108</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Age (median)</td>
<td>57</td>
<td>53</td>
<td>0.26</td>
</tr>
<tr>
<td>Node Positive</td>
<td>49.1%</td>
<td>55.7%</td>
<td>0.38</td>
</tr>
<tr>
<td>Tumor Size (T2-T4)</td>
<td>34.3%</td>
<td>46.2%</td>
<td>0.13</td>
</tr>
<tr>
<td>Histologic Grade 3</td>
<td>40.0%</td>
<td>39.5%</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>ER/PR negative</strong></td>
<td><strong>31.1%</strong></td>
<td><strong>17.7%</strong></td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td>HER2/neu overexpression</td>
<td>31.7%</td>
<td>26.8%</td>
<td>0.50</td>
</tr>
<tr>
<td>Hormonal Therapy</td>
<td>66.7%</td>
<td>76.9%</td>
<td>0.14</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>75.0%</td>
<td>72.2%</td>
<td>0.74</td>
</tr>
<tr>
<td>XRT</td>
<td>72.2%</td>
<td>81.0%</td>
<td>0.17</td>
</tr>
<tr>
<td>Trastuzumab Therapy</td>
<td>11.1%</td>
<td>3.8%</td>
<td>0.10</td>
</tr>
<tr>
<td>Optimal dose</td>
<td>34.3%</td>
<td>0.0%</td>
<td>n/a</td>
</tr>
</tbody>
</table>
Local Toxicity
In Vivo Immune Response

![Bar chart showing DTH (mm) for E75 Pre-PVS, E75 Post PVS, and Saline Post-PVS. The E75 Post PVS group has a significantly higher DTH compared to the other two groups (P<.001).]
Clinical Benefit to Vaccination

Primary analysis at 18 months median follow-up

<table>
<thead>
<tr>
<th></th>
<th>Vaccinated</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrence Rate</td>
<td>5.6%</td>
<td>14.2%</td>
<td>0.04</td>
</tr>
<tr>
<td>Disease Free Survival</td>
<td>92.5%</td>
<td>77.0%</td>
<td>0.04</td>
</tr>
<tr>
<td>Overall Survival</td>
<td>99.0%</td>
<td>95.1%</td>
<td>0.10</td>
</tr>
</tbody>
</table>

DFS – 60 mo median f/u

Vreeland T, et al. SABCS 2012
E75 Trial Summary

• Largest breast cancer adjuvant vaccine trial
• Safe and effective in raising HER2 immunity
• Appears to have clinical impact
• HER2 low expressing patients with best immunologic response
Data Limitations

- No true control group (HLA-A2/3+ vaccinated, A2/A3- controls)
- No GM-CSF alone group
- Analyzed phase I/II together
  - Not all patients received optimal dose
  - Not all patients received booster
DFS – Optimal Dosing

Vreeland T, et al. SABCS 2012
HLA-E75 Peptide-TCR complex
Induces proliferation of E75-specific T-cell clones

E75 isolated from HLA in human tumors

E75 derived from HER2 endogenous pathway

HLA-A2
T-Cell Receptor

NeuVax: E75 Peptide + GM-CSF

E75 Peptide

APC

Signalizing

Activated, Cancer-Fighting “Killer T-Cells”

Cancer Cells

Dying Cancer Cell

Induces proliferation of E75-specific T-cell clones

Millions of HER2-targeted “CTL” clones seek and destroy HER2 expressing cancer cells

E75 derived from HER2 endogenous pathway

E75 isolated from HLA in human tumors

NeuVax: E75 Peptide + GM-CSF

E75 Peptide

APC

Signalizing

Activated, Cancer-Fighting “Killer T-Cells”

Cancer Cells

Dying Cancer Cell

Induces proliferation of E75-specific T-cell clones

Millions of HER2-targeted “CTL” clones seek and destroy HER2 expressing cancer cells
Phase III Study Schema: PRESENT (Prevention of Recurrence in Early Stage Node-Positive Breast Cancer with Low to Intermediate HER2 Expression with NeuVax Treatment)

**Study Population**

Adjuvant Breast cancer (BC) patients, n=700, randomized 1:1

- Node positive (NP), HLA A2/A3+, low and intermediate HER2 expression
- Achieve CR with standard of care (SOC)
- Stratified by Stage (IIA-IIIA), Type of Surgery, Hormone Receptor and Menopausal status
- Single dose level of GM-CSF +/- NeuVax

**Dosing by Month**

- NeuVax (E75) + GM-CSF

- Placebo + GM-CSF

**Interim analysis by DSMB at n=70 events**

**Endpoint DFS at n=139 events / 36 mos.**

PI: E.A. Mittendorf
Combination Immunotherapy

• Pretreatment of tumor cells with trastuzumab results in increased specific cytotoxicity
Possible Mechanisms

- Increased antigen availability
- Altered MHC class I expression
- Altered APM
- Antibody response
Combination Immunotherapy

Enhanced antigen presentation

Trastuzumab binds to HER2/neu receptors on breast cancer cell. Trastuzumab/HER2 complexes are internalized and processed by proteasomes into short peptides (such as E75 or GP2), which are then presented on MHC class I molecules.

Correlation between HER2 and MHC-I

Inoue M, et al. *Oncoimmunology* 2012;1(7):1104-1110
Antibody Response

HER2-specific IgG Ab response

Arm A  N=22

Antibody Levels (RI)

Before After

Time point

p=0.4

Arm B  N=14

Antibody Levels (RI)

Before After

Time point

p=0.004

Arm C  N=14

Antibody Levels (RI)

Before After

Time point

p=0.2

Knutson, et al.  ASCO 2013
Phase I Trial

• Combination therapy with vaccine + trastuzumab is:
  – Safe
  – Immunogenic
  – No dose limiting toxicity or cardiac events
Phase II: NeuVax (E75) + Trastuzumab v. Trastuzumab alone in HER2 IHC 1+/2+, early-stage breast cancer

**Study Population**
Adjuvant Breast cancer (BC) patients, n=300, randomized 1:1
- HLA A2/A3+, low and intermediate HER2 (IHC 1+/2+) expression; node positive (HR+/−) or node negative (HR−)
- Stratified by nodal status and HER2 status
- Single dose level of Trastuzumab + NeuVax vs Trastuzumab + GM-CSF alone

**Primary Endpoint**
DFS at 24 mos.

**Trastuzumab**
- Standard Herceptin dosing every 3 weeks for 1 year
- + 1 booster dose every 6 months thereafter
- + Dosing to disease progression or 36 months

**NeuVax (E75)**
- 6 doses of NeuVax given every 3 weeks starting with third dose of Herceptin

**Secondary Endpoint**
DFS at 36 mos.
HER2/neu

Extracellular Domain (aa 1-652)

**ECD**

Trans Membrane Domain (aa 653-675)

**TMD**

Intracellular Domain (aa 676-1255)

**ICD**

**I I S A V V G I L**

GP2-peptide vaccine (aa 654-662)
MHC Class I : HLA-A2
Stimulate CD8 T cells

**K I F G S L A F L**

E75-peptide vaccine (aa 369-377)
MHC Class I : HLA-A2 & HLA-A3
Stimulate CD8 T cells

**G V G S P Y V S R L L G I C L**

AE37-peptide vaccine (aa 776-790)
MHC Class II : multi-allele
Stimulate CD4 T cells
AE37

• Modified HER2/neu class II epitope
  • Naturally occurring AE36
  • Linked to Ii-Key moiety of the invariant chain
    • Facilitates epitope charging of MHC class II molecules
    • ↑ antigen presentation
    • ↑ potency to > 250 times that of unmodified class II epitope in vitro
Specific Aims

1. Recurrence
2. Time to Recurrence
3. Immunologic response correlation

National PI: E.A. Mittendorf
AE37/GP2 Phase II Trial

• What are we learning?
  • Toxicity is due primarily to GM-CSF
  • GM-CSF alone is not responsible for the immune response
  • DTH continues to be a good predictor of clinical response
# Interim Analysis: AE37

**AE37 Disease Free Survival – All Patients**

<table>
<thead>
<tr>
<th></th>
<th>Vaccine (n=115)</th>
<th>Control (n=166)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age</td>
<td>49</td>
<td>51</td>
<td>.13</td>
</tr>
<tr>
<td>Tumor ≥ 2 cm</td>
<td>57%</td>
<td>63%</td>
<td>.42</td>
</tr>
<tr>
<td>Grade 3</td>
<td>50%</td>
<td>54%</td>
<td>.52</td>
</tr>
<tr>
<td>Node positive</td>
<td>73%</td>
<td>66%</td>
<td>.24</td>
</tr>
<tr>
<td>ER/PR neg</td>
<td>40%</td>
<td>38%</td>
<td>.73</td>
</tr>
<tr>
<td>HER2 pos</td>
<td>50%</td>
<td>48%</td>
<td>.75</td>
</tr>
</tbody>
</table>

Log Rank = 0.328

- **Vaccine** n=115
- **Control** n=166

- **Fraction Surviving Disease Free**
  - **90.1%**
  - **83.0%**
  - **RRR 41.8%**

Vreeland T, et al. ASCO 2012
DFS: HER2 low-expressors

63% RRR

Vreeland, et al. ASCO 2012
Interim Analysis: GP2

- **GM-CSF Only**: p = 0.25, n = 43, 11.63%
- **GP2 + GM-CSF**: n = 46, 4.65%

Trappey, et al. ASCO 2013
Conclusions

• Cancer vaccines represent a nontoxic therapeutic modality with great specificity
• Multiple ongoing phase III trials to assess efficacy
Conclusions

• Ongoing challenges
  • Identification of appropriate patient populations
  • Integration into current treatment algorithms
  • Determination of immune response that correlate with outcome
  • Elucidation of gene signatures predictive of response
Vaccination in Less Aggressive Disease

Vaccines for Solid Tumors

<table>
<thead>
<tr>
<th>Peptide</th>
<th>HLA restriction</th>
<th>Total patients</th>
<th>NR</th>
<th>PR</th>
<th>CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MART127-35</td>
<td>A2</td>
<td>23</td>
<td>22</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>MART127-35 + IL-12</td>
<td>A2</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MART126-35(27L)</td>
<td>A2</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TRP-2160-166</td>
<td>A2</td>
<td>20</td>
<td>19</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>gp100209-217</td>
<td>A2</td>
<td>9</td>
<td>8</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>gp100209-217(210M)*</td>
<td>A2</td>
<td>32</td>
<td>32</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>gp100209-217(210M) + IL-12</td>
<td>A2</td>
<td>28</td>
<td>28</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>gp100209-217(210M) + GM-CSF</td>
<td>A2</td>
<td>18</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>gp100210-206</td>
<td>A2</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>gp100210-206(2889V)*</td>
<td>A2</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>gp100126-102</td>
<td>A2</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>gp100E1203-217(210I)</td>
<td>A2</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>g209-2M + MART-27L</td>
<td>A2</td>
<td>23</td>
<td>23</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>g209-2M, g280-9V, MART-27L, tyr304</td>
<td>A2</td>
<td>16</td>
<td>14</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>gp100A4-59</td>
<td>DR4</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>gp100A4-55 + g209-2M + MART-27L</td>
<td>A2/DR4</td>
<td>22</td>
<td>21</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Tyrosinase240-251</td>
<td>A1</td>
<td>16</td>
<td>16</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>gp10017-23</td>
<td>A3</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tyrosinase256-214</td>
<td>A2</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TRP-1 ORF1-9</td>
<td>A31</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Combination peptides</td>
<td>Non-A2</td>
<td>15</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MAGE-12170-178</td>
<td>Cw7</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>NYESO-1-157-165(165V)</td>
<td>A2</td>
<td>19</td>
<td>19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NYESO-1-157-165(165V)</td>
<td>DP4</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Her2/neu256-379</td>
<td>A2</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Telomerase540-545</td>
<td>A2</td>
<td>13</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dendritic cells + g209-2M + MART-27L</td>
<td>A2</td>
<td>15</td>
<td>13</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>381</td>
<td>370</td>
<td>9</td>
<td>2</td>
</tr>
</tbody>
</table>

Overall objective response rate = 2.9%. HLA, human leukocyte antigen; CR, patients showing complete response; PR, patients showing partial response; NR, patients showing no response. *g209-2M, *g280-9V, †MART-126-27L, ‡Tyrosinase240-251, §37CD.

Vaccines for Solid Tumors

- 440 patients
  - 422 metastatic melanoma
    - 65% visceral disease
    - 20% lymph node disease ± subcutaneous disease
    - 15% subcutaneous or cutaneous disease only
  - 18 with other metastatic CA

Minimal (Residual) Disease

- Idiotype vaccines
- GSK MAGE-A3
- HER2
Conclusions

• Ongoing challenges
  • Identification of appropriate patient populations
  • Integration into current treatment algorithms
  • Determination of immune response that correlate with outcome
  • Elucidation of gene signatures predictive of response
Conclusions

• Ongoing challenges
  • Identification of appropriate patient populations
  • Integration into current treatment algorithms
  • Determination of immune response that correlates with outcome
  • Elucidation of gene signatures predictive of response
Conclusions

- Ongoing challenges
  - Identification of appropriate patient populations
  - Integration into current treatment algorithms
  - Determination of immune response that correlates with outcome
  - Elucidation of gene signatures predictive of response
Thank You