Combination Immunotherapies Designed to Target the Tumor Microenvironment

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A special thanks to all of the patients and families who participated in these studies.

Sunrise, Mt. Kilimanjaro Tanzania
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Acknowledgments

- Joy Levi
- James Leatherman
- Xiaobu Ye, MD MS
- Steve Piantadosi, MD PhD
- Silvia Petrik, RN
- Richa Gupta
- Katrina Purtell, RN
- Tianna Dauses, RN
- Maureen Berg, RN
- Maithili Daphtary, PhD
- Marina Laiko, MD
- Irwin Freed
- John Ullman
- Elizabeth Manning, PhD
- Justin Asquith
- Melek Sunay
- Lanquing Huang, PhD
- Nora Disis MD
- Elizabeth Jaffee MD
Conclusions

- Cancer therapeutics can have immune effects even in the absence of active vaccination

- Abrogating the influence of tolerance can release the immune-activating potential of vaccines and distinctly targeted cancer therapeutics
  - Targeted immunotherapy: checkpoint modulators, TLR modulators

- Strongly argues for the scientifically-based integration of tumor vaccines/immunotherapies into standard therapies for early and late stage cancer to maximally harness the therapeutic host immune response

“Hope is not a strategy—you have to follow the science”
Immune-Modulating Chemotherapy Unmasks Synergy Between Anti-VEGF-R2 MAb and Vaccine in *Neu* Mice

Manning et al, 2007, Clin Cancer Res
Anti-VEGF-R2 MAb: FVB vs. Neu Mice

Manning et al, 2007, Clin Cancer Res
Anti-VEGF-R2 MAb Alone Induces T Cell-Dependent Tumor Immunity

Manning et al, 2007, Clin Cancer Res
Tumor Immunity and Monoclonal Antibodies Targeting the Tumor Microenvironment: VEGFR2
Conclusions

• Vaccination sequenced with CY in the setting of standard Trastuzumab therapy is:
  – Safe
  – Well-tolerated
  – Can induce new or augmented HER-2-specific DTH in HER-2+ metastatic breast cancer patients

• The CBR at 6 months was 50%

## Clinical Outcomes

<table>
<thead>
<tr>
<th>Clinical Outcome</th>
<th>Trastuzumab + CY-modulated vaccination</th>
<th>Trastuzumab alone 1(^{st}) line (Vogel 2002)</th>
<th>Trastuzumab alone 2(^{nd}/3^{rd}) line (Cobleigh 1999)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBR at 6 months</td>
<td>50%</td>
<td>48%</td>
<td>56%</td>
</tr>
<tr>
<td>Median survival</td>
<td>39.9 months</td>
<td>24.4 months</td>
<td>13 months</td>
</tr>
</tbody>
</table>

*CBR at 1 year was 35%  
*HER-2-specific DTH developed in 7 out of 20 subjects (35%)  

Systemic Effects of Trastuzumab-Modulated Vaccination

Urticaria

GM-CSF Pharmacokinetics

No Cardiac Toxicity

Treatment Schema

A. Overall Trial Schema

Vaccine 1  Vaccine 2  Vaccine 3  Vaccine 4
C  C  C  C
T  T  T  T

0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 WEEKS

Study Termination

B. Schedule for Treatment Cycles (not to scale)

Trastuzumab (T)
Cyclophosphamide
Vaccine 48° GM-CSF 96° GM-CSF

Pre-Rx -1 0 1 2 3 4 5 6 7 8 14 21 27 to 42 days

Day 14 tumor biopsy

24° GM-CSF 0° GM-CSF 72° GM-CSF

Day 0 tumor biopsy Day 3 skin biopsy

168° GM-CSF

Day 7 skin biopsy

DTH
blood for immune monitoring
disease evaluation
Pre-Rx skin biopsy
Pre-Rx tumor biopsy

Therapeutic HER-2 MAb Augments Fc-Dependent Immune Priming

Table 1
Percentage PKH67+CD11c+ DCs on days 1–5 after in vivo administration of vaccine + mAb

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>3T3 neu/GM + intact 7.16.4 mAb</td>
<td>4.78 ± 0.52</td>
<td>3.02 ± 0.80</td>
<td>2.02 ± 0.40</td>
<td>5.08 ± 0.60</td>
<td>5.36 ± 0.76</td>
</tr>
<tr>
<td>3T3 neu/GM + 7.16.4 F(ab')2</td>
<td>2.13 ± 0.3A</td>
<td>1.98 ± 0.23</td>
<td>2.00 ± 0.28</td>
<td>2.61 ± 0.38A</td>
<td>3.06 ± 0.44A</td>
</tr>
<tr>
<td>3T3 neu/GM + mlgG</td>
<td>2.43 ± 0.57A</td>
<td>2.15 ± 0.38</td>
<td>1.9 ± 0.36</td>
<td>2.90 ± 0.54A</td>
<td>3.32 ± 0.23A</td>
</tr>
<tr>
<td>3T3/GM + mlgG</td>
<td>1.53 ± 0.43A</td>
<td>1.66 ± 0.26A</td>
<td>2.25 ± 0.22</td>
<td>2.54 ± 0.36A</td>
<td>2.88 ± 0.62A</td>
</tr>
<tr>
<td>Naive</td>
<td>0.59 ± 0.12A</td>
<td>0.51 ± 0.14A</td>
<td>0.69 ± 0.06A</td>
<td>0.55 ± 0.18A</td>
<td>0.54 ± 0.16A</td>
</tr>
</tbody>
</table>

Data represent mean ± SD of at least triplicate samples. *P < 0.05 versus 3T3 neu/GM + intact 7.16.4 mAb, Mann-Whitney U test.

Kim et al, 2008, J Clin Invest
Chemotherapy-Modulated Vaccination Induces Maximal Tumor Apoptosis When Combined with Therapeutic HER-2 MAb

Tumors were harvested and snap frozen. 5 um sections were cut onto slides and immunohistochemistry was performed using antibodies specific for cleaved caspase 3.

Emens, unpublished data
Therapeutic HER-2 MAb Augments Vaccine-Induced HER-2-specific CD8⁺ T Cells and Tumor-Free Survival

Emens, unpublished data
Rationale for Combining Active Vaccination with Trastuzumab Therapy

- Minimal antibody responses in vaccinated *neu* mice
- Cell cycle arrest (PI3K/Akt pathways)
- Inhibits pro-growth signals/angiogenesis (VEGF)
- Promotes apoptosis
- Trastuzumab+chemotherapy induces T cells
- Inhibits DNA damage repair
- Promotes ADCC
- Augments CD8⁺ CTL activity/antigen processing
- Enhances immune priming, immune memory

HER-2-specific Monoclonal Antibodies and Vaccination
Conclusions

- CY 200 mg/m² augments HER-2-specific humoral immunity
- Doses of CY >200 mg/m² suppress both DTH and antibody responses
- The optimal chemotherapy dose combination: CY 200 mg/m² with DOX 35 mg/m²
Three-Dimensional and Contour Plots of the Predicted Responses Surfaces Using a Second Order Polynomial Regression Model

\[ y_u = \beta_0 + \beta_1 x_{1u} + \beta_2 x_{2u} + \beta_{11} x_{1u}^2 + \beta_{22} x_{2u}^2 + \beta_{12} x_{1u} x_{2u} + e_u \]

Predicted Max Peak Increase is 0.739 μg/ml, at CY= 193 mg/m², DOX=35 mg/m²

Emens et al, 2009, J Clin Oncol
Impact of Increasing Chemotherapy Dose on Vaccine-Induced Immunity—Serum HER-2 Ab

CY

DOX

Emens et al, 2009, J Clin Oncol
GM-CSF Pharmacokinetics

Vaccine Alone

Vaccine + Chemo

Emens et al, 2009, J Clin Oncol
**Treatment Schema**

**B. Overall Trial Schema**

<table>
<thead>
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<th>Vaccine 1</th>
<th>Vaccine 2</th>
<th>Vaccine 3</th>
<th>Vaccine 4</th>
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<tr>
<td>C</td>
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**Study Termination**

**C. Schedule for Treatment Cycles (not to scale)**

- Pre-Rx -1

- 0° GM-CSF

- Pre-Rx biopsy

- 0° GM-CSF

- DTH

- DTH biopsy

- 24° GM-CSF

- Day 3 biopsy

- 96° GM-CSF

- Day 7 biopsy

- 48° GM-CSF

- 168° GM-CSF

- DTH blood for immune monitoring disease evaluation

- Doxorubicin

- 28 days
Study Design Matrix

Emens et al, 2009, J Clin Oncol
A Human GM-CSF-secreting Breast Cancer Vaccine

- Allogeneic breast tumor cells
  - SKBR3: HER-2+, ER-
  - T47D: HER-2-, ER+
- Generalizable
- Allows unbiased antigen delivery
- Secretes human GM-CSF 324 ng/10^6 cells/24 hrs

Davis-Sproul et al, 2003, Cytotherapy
Cyclophosphamide Treatment Temporarily Suppresses Peripheral Regulatory T Cell Numbers

Polychemotherapy Maximizes HER-2/neu-Targeted Vaccination in Neu Mice

NT cells → Cyclophosphamide → vaccine → Doxorubicin

- Day 0
- Day 2
- Day 3
- Day 10

Graph showing tumor free probability over days post tumor injection:

- Controls 3T3/GM (n=10)
- Vaccination 3T3-neu/GM (n=14) p = .3
- 3T3/GM + Cyclophosphamide 100 mg/kg + Doxorubicin 5 mg/kg (n=11) p = .02
- Vaccination 3T3-neu/GM + Cyclophosphamide 100 mg/kg +

Machiels et al, 2001, Cancer Res
Chemotherapy and Vaccination
Immune Tolerance to HER-2/neu in Neu Transgenic Mice

Parental mice
Vaccination day 15

neu transgenic mice
Vaccination day 1

Tumor size mm² (mean ± I. SEM)

Days post tumor injection

* p < .05

Machiels et al, 2001, Cancer Res
Approaches

• Integrate with established breast cancer therapeutics
  – Chemotherapy
  – Tumor-specific monoclonal antibodies (HER-2)
• Target distinct components of the tumor microenvironment
  – VEGFR2
  – Multi-kinase inhibitors
Conflict of Interest Statement

**Biosante:** Under a licensing agreement between Biosante and the Johns Hopkins University, the University is entitled to milestone payments and royalty on sales of the vaccine product described in the presentation. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.

**Genentech/Roche:** Breast Cancer Advisory Board, Research Funding

**Bristol Myers Squibb:** PD-1/PD-L1 Breast Advisory Board