Immunotherapy of Cancer

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Arizona State University
Mayo Clinic Arizona
Anti-Tumor Immunity: A Balancing Act

B cell activation
Antigenic Targets
Antigen Processing
 Trafficking
T cell activation
Innate immunity

Immune evasion
T cell Dysfunction
APC Dysfunction
Cytokine Dysregulation

Regulatory T cells
FoxP3
CTLA-4
B7-H1/PD-L1

B7-H4
IDO
TGF-b
Interleukin-10
FasL/CD95L

B7.1/CD80
B7.2/CD86
CD40
4-1BB
OX40
MICA, MICB
Toll-like receptors
TNF-a
How Specific Does Immunotherapy Need to Be?

**Non-specific**
- Cellular vaccines
- Costimulation
- APC activation
- Cytokines
- Adjuvants

**Specific**
- Peptides
- Antigens
- Adoptive T cells
Irradiated GM-CSF Secreting Tumor Cell Vaccines

• GM-CSF: most potent molecule (of 30 tested) for enhancing tumor immunity following gene transfer into tumor cells

• Mechanism involves improved tumor antigen presentation by mature CD11b\(^+\) dendritic cells

• Tumor rejection requires CD4\(^+\) and CD8\(^+\) T cells, CD1d-restricted NKT cells, and antibodies

Soiffer and Dranoff, J. Clin Oncol. 2003
Hodi and Dranoff, PNAS 2003
Ho and Soiffer, PNAS 2009
Autologous Cellular Vaccines: GVAX

- Recruitment of DCs
- Processing Cancer Antigens
- Activation of T Cells and B cells
- Lysis of Tumor
- Mature DC
Ovarian GVAX with anti-CTLA-4

Anti-CTLA-4

CA-125

VAX

7/9/03
8/3/04
3/6/06

Hodi and Dranoff, PNAS 2008
**Autologous GM-CSF Breast Cancer Vaccine**

- Tumor cells
- Adenovirus-GM-CSF
- Irradiation
- Vaccine

**Dose is dependent on tumor harvest**

- $1 \times 10^5$
- $1 \times 10^6$
- $4 \times 10^6$
- $10 \times 10^6$

<table>
<thead>
<tr>
<th>Week</th>
<th>Action</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Immune monitoring</td>
</tr>
<tr>
<td>2</td>
<td>DTH skin biopsies</td>
</tr>
<tr>
<td>3</td>
<td>VAX# 1</td>
</tr>
<tr>
<td>4</td>
<td>Immune monitoring</td>
</tr>
<tr>
<td>5</td>
<td>DTH skin biopsies</td>
</tr>
<tr>
<td>6</td>
<td>VAX# 2</td>
</tr>
<tr>
<td>7</td>
<td>Immune monitoring</td>
</tr>
<tr>
<td>8</td>
<td>Restaging</td>
</tr>
<tr>
<td></td>
<td>DTH skin biopsies</td>
</tr>
</tbody>
</table>

Anderson and Dranoff, SABCS 2008
Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Result (N=12)</th>
</tr>
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<tbody>
<tr>
<td>Age</td>
<td>34-70 yrs (median 56)</td>
</tr>
<tr>
<td>Duration of Stage IV ca.</td>
<td>0-7 yrs (median 3)</td>
</tr>
<tr>
<td># Prior Chemotherapies</td>
<td>1-7 (median 2.7)</td>
</tr>
<tr>
<td>ER positive</td>
<td>8 (67%)</td>
</tr>
<tr>
<td>Her2 positive (2-3+)</td>
<td>5 (42%)</td>
</tr>
<tr>
<td>Response at 2 months</td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>8</td>
</tr>
<tr>
<td>SD</td>
<td>3 (duration:4,4,14 months)</td>
</tr>
<tr>
<td>NED</td>
<td>1 (duration:18+ months)</td>
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</table>

Toxicity

<table>
<thead>
<tr>
<th>Toxicity (Grade 1/2)</th>
<th>Frequency (N=12)</th>
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<tbody>
<tr>
<td>Injection site reaction</td>
<td>8 (67%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>6 (50%)</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>6 (50%)</td>
</tr>
<tr>
<td>Pruritis</td>
<td>5 (42%)</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>5 (42%)</td>
</tr>
<tr>
<td>Limb Edema</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>2 (17%)</td>
</tr>
</tbody>
</table>
How do you monitor a complex immunotherapy?

**Overall survival**

- Survival Probability
- Months

**Time Interval**

- # events/# at risk
Breast GVAX Induces Local Reactions

Infiltration of lymphocytes, granulocytes, and macrophages
Breast GVAX Induces Local Reactions

One patient developed inflammation at multiple tumor sites post-vaccine.
Infiltration of CD8+ TILs into tumor sites

Pre-GVAX  Post-GVAX

Red: anti-CD8

↑ CD8+ cells

↓ FoxP3+ cells

Green: anti-CD8  Yellow: CD8+CD45RO+ memory
Red: anti-CD45RO  Blue: anti-FoxP3
Conclusions

- Generation of autologous GM-CSF breast cancer vaccine is feasible
- 12 patients with stage IV breast cancer vaccinated; 3 patients had SD and 1 has remained NED
- Toxicities were Grade I/II
- Immune infiltration observed at injection sites and at tumor sites
- Completed trial in the high-risk adjuvant setting

**Hypothesis:** Immunogenic antigens detected in long-term survivors after GVAX will be excellent vaccine targets

- Vaccine limited by ability to prepare primary tumor

![Venn Diagram](image)
GVAX induces T cell immunity to multiple tumor antigens

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Sequence</th>
<th>Position</th>
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<tbody>
<tr>
<td>CEA CAP-1</td>
<td>YLSGANLNL</td>
<td>605-613</td>
</tr>
<tr>
<td>Cyp1 239</td>
<td>SLVDVMPWL</td>
<td>239-247</td>
</tr>
<tr>
<td>HER-2/neu 369</td>
<td>KIFGSLAFL</td>
<td>369-377</td>
</tr>
<tr>
<td>HER-2/neu 435</td>
<td>HILHNGAYSL</td>
<td>435-443</td>
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<tr>
<td>HER-2/neu 689</td>
<td>RLLQETELV</td>
<td>689-697</td>
</tr>
<tr>
<td>MUC-1.2</td>
<td>STAPPVHNV</td>
<td>950-958</td>
</tr>
<tr>
<td>NY-ESO-1</td>
<td>SLLMWITQV</td>
<td>157-165</td>
</tr>
<tr>
<td>Survivin-1</td>
<td>ELTLGEFLKL</td>
<td>95-104</td>
</tr>
<tr>
<td>Survivin-2</td>
<td>LMLGEFLKL</td>
<td>96-104</td>
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<tr>
<td>TPD52_70</td>
<td>KLGINSLQEL</td>
<td>70-79</td>
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<tr>
<td>TPD52_82</td>
<td>NIAKGWQDV</td>
<td>82-90</td>
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<tr>
<td>TPD52_38</td>
<td>ELRRELAKV</td>
<td>38-46</td>
</tr>
<tr>
<td>S100A7_50</td>
<td>KGTVNYLADV</td>
<td>50-58</td>
</tr>
</tbody>
</table>

![Image showing the effectiveness of GVAX therapy over time](image-url)
Nucleic Acid Programmable Protein Microarrays

Replicate microscopic arrays of numerous proteins

Probe with sera

Cell free expression of target protein

GVAX induces B cell immunity

**Z-score Calculations:**

Spot signal - mean st. dev.

Select: $Z>3$ before or after vaccination

Not present in 53 healthy controls
GVAX induces B cell immunity
Conclusions

- Generation of autologous GM-CSF-secreting breast cancer vaccines is feasible both for solid tumor and malignant effusions
- T cell immunity to 5/8 antigens detected at 2 months
- B cell immunity to multiple antigens detected and are distinct from T cell antigens
Primary Tumor Culture: The Next Generation

Mince and digest:
Fresh tissue
Frozen tissue
Needle biopsies

Irradiated feeder cells
+ ROCK inhibitor

Prostate
Feeders + ROCK inhibitor
Feeders
PrEGM + Rock inhibitor
PrEGM

Mammary
Feeders + ROCK inhibitor
Feeders
MEGM + Rock inhibitor
MEGM

Liu and Schlegel, Am J Pathol 2012
Primary Tumor Culture: The Next Generation

<table>
<thead>
<tr>
<th>Day 3</th>
<th>Day 8</th>
<th>Week 8</th>
<th>Tumors/sites</th>
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<tbody>
<tr>
<td>normal</td>
<td><img src="image1" alt="Image of normal culture day 8" /></td>
<td><img src="image2" alt="Image of normal culture week 8" /></td>
<td>0/10</td>
</tr>
<tr>
<td>carcinoma</td>
<td><img src="image3" alt="Image of carcinoma culture day 8" /></td>
<td><img src="image4" alt="Image of carcinoma culture week 8" /></td>
<td>7/10</td>
</tr>
</tbody>
</table>

*2D culture* | *3D culture* | *Xenograft*

Liu and Schlegel, Am J Pathol 2012
Autologous Vaccination Version 2.0

Unique opportunity to identify tumor-specific antigens
Acknowledgements

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- Barb Pockaj

**Our Patients**
- NCI/Early Detection Research Network
- Avon Foundation
- Komen Foundation
What are the Antigen Targets in Breast Cancer?

**Cancer-Testis**
BAGE 1

**Chimeric**
ABL1  AML1  BCR1  CAN  DEK  ETV6  
FUT  NPM1  PML  RARA  SYT

**Differentiation**
CEA  KLK3  Mammaglobin A  
NY-BR-1  OA1  RAB38  TRP-1  TRP-2  Tyrosinase

**Mutation**
a-actinin-4  ARTC1  Beta-catenin  B-RAF  Caspase 8  CDC27  
CDK4  CDKN2A  COA-1  EFTUD2  ELF2  FN1  
GPNMB  HSP70-2  iCE  KIAA0205  K-ras  MUM1  
Myosin class 1Neo-PAP  NFYC  N-ras  OGT  
OS9  PRDX5  RBAF600  SIRT2  SNRPD1  TPI

**Overexpressed**
BING-4  CLCA2  CPSF  Cyclin-D1  Cyp1B1  ep-CAM  
ephA2  ephA3  Her2/neu  mCSF  MDM2  MMP2  
MUC1  p15  p53  PRAME  PSMA  RAGE1  
RNF43  RU1  SART1  SART3  Secernin 1  SOX10  
STEAP1  Survivin  PDEF  hTERT  S100A7  TPD52

**Shared tumor specific**
CSAG2  Gnt-V  LAGE-1  LDLR  MAGE-A1  MAGE-A12  
MAGE-A2  MAGE-A3  MAGE-A6

**Unclassified**
ACPP  Annexin II  CYP1B1  Lbc  LCK  NY-REN-18  
PRAP6  PSCA  RHAMM  RPL10  RPS2  SPA17
Cyp1B1 as a Tumor Antigen

Maecker and Nadler Blood 2003

Anderson et al, Cancer Immunol Immunother. 2011
Cyp239 epitope is naturally processed
### Not All Antigen Presenting Cells are Equal; Not All Antigens Are Equal

<table>
<thead>
<tr>
<th></th>
<th>FluM1 clone</th>
<th>Mart1 clone</th>
<th>Cyp239 clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>No peptide</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
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<td>Specific RNA</td>
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<td><img src="image15.png" alt="Image" /></td>
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<tr>
<td>Specific RNA</td>
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<td><img src="image17.png" alt="Image" /></td>
<td><img src="image18.png" alt="Image" /></td>
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<tr>
<td>Control RNA</td>
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<td><img src="image20.png" alt="Image" /></td>
<td><img src="image21.png" alt="Image" /></td>
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<tr>
<td>Specific RNA</td>
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<td><img src="image23.png" alt="Image" /></td>
<td><img src="image24.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**Diagram:**
- **T cell** → IFN-γ → APC
- **Ag RNA** → **APC**
- **K562/A2**
  - Lactacytin
  - MG-132
APCs Express Different Proteasomes

W. J. Gastroent. 2010

mDC  CD40-B  K562

Proteasome content

Constitutive  Immunoproteasome

β1  LMP2
β2  LMP10
β5  LMP7

LMP10  LMP2  LMP7

Fold change RNA

B

DC  B  K562

C

DC  B  K562 + IFNγ

β1  LMP2
β2  LMP10
β5  LMP7
Conclusions:

- K562 cells, compared with DCs and CD40-B cells, have low immunoproteasome content

- The tumor antigens Mart1 and Cyp1B1 are poorly presented by DCs and CD40B cells compared with K562

- Selection of APCs for antigen delivery may vary between antigens