Development of Recombinant Vaccines for the Therapy of Carcinomas
Monotherapy and Combination Therapy

Jeffrey Schlom, Ph.D.
Laboratory of Tumor Immunology and Biology
Center for Cancer Research
National Cancer Institute, NIH
Cancer Vaccine Development:
  – Focus on human carcinoma
  – Focus on development of vaccines that can be widely evaluated

Ultimate Use:
  – Early in disease process/low tumor burden
  – Survival as the endpoint
  – Minimal toxicity

Immunologic Platform:
  – Combination immune therapies
    ➢ immune stimulation strategies
    ➢ reduction of immune inhibitory entities
  – Combination Therapies: **Vaccine plus**:
    ➢ conventional therapies
    ➢ conventional therapies in novel strategies
    ➢ other experimental therapies
Translational Research Programmatic Effort

**PRECLINICAL STUDIES:**

**Laboratory of Tumor Immunology and Biology (LTIB)**
- James Hodge
- Al Tsang
- Claudia Palena
- Jack Greiner
- Connie Rogers
- Benedetto Farsaci
- Sofia Gameiro
- Matteo Vergati
- Mary Litzinger
- Ken Hance

**Laboratory of Molecular Biology**
- Ira Pastan

**Vaccine Branch**
- Jay Berzofsky

**CLINICAL STUDIES:**

**LTIB/Medical Oncology Branch**
- James Gulley
- Philip Arlen
- Ravi Madan
- Mary Pazdur

**Medical Oncology Branch**
- William Dahut
- Tito Fojo
- William Figg

**Radiation Oncology**
- Kevin Camphausen

**Urologic Oncology**
- Marston Linehan
- Peter Pinto

**Biostatistics and Data Management Section**
- Seth Steinberg

**NIH Nuclear Medicine**
- Jorge Carrasquillo
- C.H. Park
Translational Research Programmatic Effort

**CLINICAL STUDIES — EXTRAMURAL:**
- Georgetown – John Marshall
- Dana Farber Cancer Center – Donald Kufe, Paul Eder, Philip Kantoff
- Columbia – Howard Kaufman
- Cancer Institute of New Jersey – Edward Lattime, Robert DiPaola
- Ohio State – William Carson

Eastern Cooperative Oncology Group (ECOG) – Robert DiPaola, Howard Kaufman, Louis Weiner

**CANCER THERAPY EVALUATION PROGRAM (CTEP):**
- Howard Streicher
- Jan Casadei

**PRIVATE SECTOR:**
- GlobeImmune – Alex Franzusoff, David Apelian
- BN ImmunoTherapeutics – Wayne Godfrey, Reiner Laus

**NCI Technology Transfer Center:** Kevin Brand, Karen Maurey
**NIH Office of Technology Transfer:** Mojdeh Bahar
Strategies to Enhance Vaccine Potency

1. Mode of Delivery of the Vaccine
   – place the gene for the tumor antigen into a vector

2. Diversified Vaccine Prime and Boost

3. T-cell Costimulation
   – these molecules are essential for vigorous T-cell activation
   – place costimulatory molecule into vaccine vector

4. Alter the a.a. sequence of the tumor antigen to enhance the immune response “epitope enhancement”

5. Combination therapies
Vaccine Platforms

- Recombinant poxviruses
  - vaccinia; (MVA)
  - fowlpox

- Recombinant *saccharomyces* (yeast)

- Chitosan / nanoparticles
Recombinant Vaccine Vectors

- **Pox vectors**
  - Vaccinia (rV-) elicits a strong immune response
    - host induced immunity limits its continuous use
    - MVA (replication defective)
  - Avipox (fowlpox rF-, ALVAC)
    - derived from avian species
    - safe; does not replicate
    - can be used repeatedly with little if any host neutralizing immunity

- Can insert multiple transgenes
- Do not integrate into host DNA
- Efficiently infect antigen presenting cells including dendritic cells
Costimulatory Molecule Candidates:

- Major Costimulatory Effect must be on the T-cell
- No Overlap of T-cell Ligands
- No Redundancy of Costimulatory Mechanisms
Tumor Antigen Gene
Co-stimulatory molecule genes

- TAA
- B7-1
- LFA-3
- ICAM-1

(TRiad of COstimulatory Molecules)

DNA plasmid
Mammalian cell

Vaccinia(rV) or Fowlpox virus(rF)

Vaccines:
- (rV-TAA-TRICOM)
- (rF-TAA-TRICOM)

Induction of Tumor specific immune responses (T-cells)

Vaccine
TRICOM
TRIad of COstimulatory Molecules

<table>
<thead>
<tr>
<th>Costimulatory Molecule</th>
<th>Ligand on T cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>B7-1 (CD80)</td>
<td>CD28/CTLA-4</td>
</tr>
<tr>
<td>ICAM-1 (CD54)</td>
<td>LFA-1</td>
</tr>
<tr>
<td>LFA-3 (CD58)</td>
<td>CD2</td>
</tr>
</tbody>
</table>

TRICOM = B7-1/ICAM-1/LFA-3
CEA/TRICOM = CEA/B7-1/ICAM-1/LFA-3
CEA/MUC-1/TRICOM = CEA/MUC-1/B7-1/ICAM-1/LFA-3 (PANVAC)
PSA/TRICOM = PSA/B7-1/ICAM-1/LFA-3 (PROSTVAC)

All vaccines contain: rV- as a prime vaccine
avipox (fowlpox, rF-) as multiple booster vaccines
CEA, MUC-1, and PSA transgenes all contain enhancer agonist epitopes
CEA-specific Lymphoproliferation of T Cells from CEA-Tg Mice Vaccinated with TRICOM Vectors

Therapy of 14-Day Established CEA+ Experimental Metastases in CEA-Tg Mice Using CEA/TRICOM Vectors

VAAA Regimen

- CEA
- CEA/TRICOM

All groups with GM-CSF and low dose IL-2

Aarts WM, Schlom J, Hodge JW. Cancer Res. 62:5770-7, 2002
The Next Frontier: Combinatorial Therapies

The use of cancer vaccines in combination with conventional therapies

- Chemotherapy
- Hormone therapy
- Local radiotherapy of tumor
- Small molecule targeted therapeutics
Vaccine Combination Therapies

1. Vaccines Induce Minimal Toxicity
   – can act independently of concomitant therapy

2. Do NOT confuse
   multiple therapies used prior to vaccine
   vs.
   therapies used with vaccine or following vaccine
3. The vaccine induction of a dynamic host immune response can be boosted by

- concomitant or subsequent therapies

  (a) can alter the phenotype of tumor cells, rendering them more susceptible to T-cell killing

  (b) can lyse populations of tumor cells which, in turn, act as a booster for tumor-specific immune cells

  (c) can kill or inhibit regulatory T cells and thus boost the immune response
Potential Multiple Effects of Local Irradiation of Tumors

Hodge et al, *Oncology* 2008
Combination Therapy: Vaccine + External Beam Radiation

Chakraborty M, Abrams SI, …, Schlom J, Hodge JW. Cancer Res. 15:4328-37, 2004
Radiation-Enhanced Antigen-Specific Lysis of Tumor Cells

CD8+ T-cell

Target Cell

FAS-L  FAS

Cell Death
Persistence of Fas Upregulation on MC38-CEA+ Tumors After External-Beam Irradiation

Fas-PE

CEA-FITC

Histochemistry

Isotype

H&E
QUADRAME is a therapeutic agent consisting of radioactive samarium (\(^{153}\text{Sm}\)) and chelator.

It preferentially binds to osteoblastic metastatic tumor deposits in bone.

\(^{153}\text{Sm}\) is currently FDA approved and clinically utilized for palliation of bone metastasis in multiple tumor histologies.
Low Dose Radiation (25 Gy) of LnCaP Human Prostate Cell Line

Treatment of LnCaP prostate cancer cells with low dose radiation results in the upregulation of MHC and Fas

Gene Expression in LnCaP cells
RT-PCR

<table>
<thead>
<tr>
<th>Tumor Antigen Genes</th>
<th>0 Gy</th>
<th>25 Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA</td>
<td>1</td>
<td>2.79</td>
</tr>
<tr>
<td>PSMA</td>
<td>1</td>
<td>4.14</td>
</tr>
<tr>
<td>PAP</td>
<td>1</td>
<td>29.0</td>
</tr>
<tr>
<td>CEA</td>
<td>1</td>
<td>10.3</td>
</tr>
<tr>
<td>MUC-1</td>
<td>1</td>
<td>3.67</td>
</tr>
</tbody>
</table>
Treatment of LnCaP prostate cancer cells with Palliative doses of $^{153}$Sm results in the upregulation of MHC class I and Fas.

Gene Expression in LNCaP cells after Sm-153 treatment:

<table>
<thead>
<tr>
<th>Accessory Genes</th>
<th>0 Gy</th>
<th>25 Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fas</td>
<td>1</td>
<td>1.96</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>1</td>
<td>29.1</td>
</tr>
</tbody>
</table>

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<th>Tumor Antigen Genes</th>
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<td>3.67</td>
</tr>
</tbody>
</table>

Treatment of LnCaP prostate cancer cells with Palliative doses of $^{153}$Sm results in increased sensitivity to multiple CTLs.

P<0.001

PSA-TRICOM + $^{153}\text{Sm}$

**Patient Population:** Metastatic Androgen Independent Prostate Cancer

**Randomize**

Arm A: PSA-TRICOM + $^{153}\text{Sm}$ (n=34)

Arm B: $^{153}\text{Sm}$ (n=34)

**Vaccine:**
- rV-PSA/TRICOM s.c. d 1
- rF-PSA/TRICOM s.c. d 15, 29, q 4 wks
- All vaccines given with GM-CSF 100μg s.c. x 4 d

**$^{153}\text{Sm}$:**
- 1 mCi/kg d 8, may be repeated
- q 12 wks upon hematologic recovery.

PI Gulley  NCI# 7678
Effect of the *pan* Bcl-2 Inhibitor GX15-070 on the Immune System: Preclinical Studies
Activated mature CD8 T lymphocytes are more resistant to GX15-070 than very early activated CD8 T lymphocytes.
- NON SELF-CEA LUNG TUMOR MODEL (C57BL/6 MICE)
- SELF-CEA LUNG TUMOR MODEL (CEA-TG MICE)

**Experimental setup**

- Mice: C57BL/6 or CEA-Tg, female
- Tumor cells: LL2-CEA 3x10⁵ cells/mouse (i.v.)
- Type of tumor model: Pulmonary tumor nodules
- Vaccine prime: PFU 1x10⁸ rV CEA-TRICOM + PFU 1x10⁷ rF GM-CSF / mouse (s.c.)
- Vaccine boost: PFU 1x10⁸ rF CEA-TRICOM + PFU 1x10⁷ rF GM-CSF / mouse (s.c.)
- Inhibitor: GX15-070 0.5 or 2 mg/Kg/mouse (i.v.)
- Groups (6 mice/group):
  1. No treatment
  2. GX15-070 alone
  3. Vaccine alone
  4. Vaccine + GX15-070

**Assays**

- IFN-γ production from splenocyte bulk cultures
- Pulmonary tumor nodules count

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1/29/2009

Benedetto Farsaci
Pulmonary tumor meta-analysis

Closed symbols: CEA-Tg mice. Open symbols: C57BL/6 mice.

* = Statistical significance from two-tailed Mann-Whitney test, 95% confidence interval.
GX15-070 inhibits Treg function

NP68 µg/mL  CD8:Treg ratio

10^{-4}  1:1

10^{-4}  1:2

10^{-5}  1:1

10^{-5}  1:2

CD8 alone  CD8 + Untreated Tregs  CD8 + GX-treated Tregs
Ability of Docetaxel to Alter Tumor-Cell Phenotype: 
Enhanced Sensitivity to Antigen-Specific T-Cell Lysis

Garnett, Schlom, Hodge, Clin Cancer Res., 2008
Docetaxel +/- PANVAC

**Patient Population:** Metastatic Breast Cancer (Docetaxel Naïve) n=48

- **Arm A:** Weekly Docetaxel + PANVAC (rV, rF-CEA-MUC-1-TRICOM)
- **Arm B:** Docetaxel alone

**Primary endpoint:** TTP

**Preliminary Data:** 14 patients enrolled

<table>
<thead>
<tr>
<th></th>
<th>Arm A</th>
<th>Arm B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median TTP</td>
<td>10.5 months</td>
<td>2 months</td>
</tr>
<tr>
<td># on &gt;6 months</td>
<td>5/6 (1 too early)</td>
<td>1/7</td>
</tr>
</tbody>
</table>
Prostate Cancer Vaccine Program
Prostate Cancer and Vaccine Therapy

• Long interval from primary diagnosis to metastatic disease

• Serum PSA (doubling time/velocity) as a surrogate for therapeutic benefit or disease recurrence

• Nomogram (Halabi) at metastatic disease
  – can predict more indolent vs more aggressive disease
Vaccine/Androgen Receptor Antagonist Therapy

Patient Population: Androgen Independent Prostate Cancer with Rising PSA and No Radiographic Evidence of Disease (D = 0.5)

Arm A: Vaccine* (n=21)
- rV-PSA + rV-B7-1 prime, rF-PSA boosts
- monthly IL-2 low dose x 5 days, recombinant GM-CSF x 4 days

Arm B: Nilutamide* (n=21)
(Androgen Receptor Antagonist)

*If patient progressed by PSA but still NED radiographically, they could add in the therapy of the other arm

### Time to Treatment Failure

<table>
<thead>
<tr>
<th>Regimen</th>
<th>n</th>
<th>Median time to treatment failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine</td>
<td>21</td>
<td>9.9 months</td>
</tr>
<tr>
<td>Nilutamide</td>
<td>21</td>
<td>7.6 months</td>
</tr>
</tbody>
</table>

### Time to Treatment Failure After Cross-Over

<table>
<thead>
<tr>
<th>Regimen</th>
<th>n</th>
<th>Median time to treatment failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine → Vaccine + nilutamide</td>
<td>12</td>
<td>13.9 months (after cross-over)*&lt;br&gt;25.9 months (from initiation of therapy)</td>
</tr>
<tr>
<td>Nilutamide → Nilutamide + vaccine</td>
<td>8</td>
<td>5.2 months (after cross-over)&lt;br&gt;15.5 months (from initiation of therapy)</td>
</tr>
</tbody>
</table>

* Median time to cross over was 12.0 months
At progression, patients continued initial therapy and crossed over to also receive other therapy.

**Five-Year Overall Survival:**
- 38%: Nilutamide first
- 59%: Vaccine first

Madan, Gulley, Schlom et al.
Randomized Multicenter Placebo-controlled Vaccine Therapy Trial in Castrate-resistant Metastatic Prostate Cancer Patients

**Patients** (n = 125)
- Metastatic prostate cancer (CT or bone scan +)
- Gleason score ≤ 7; no visceral disease
- Chemotherapy naïve

**Vaccine:** rV, rF-PSA-TRICOM (PROSTVAC) + GM-CSF

**Control arm:** empty vector

**Randomization:** 2:1 (double blind)

**P.I.:** P. Kantoff, Dana-Farber Cancer Center

**Analyses:** W. Godfrey, BNIT
- B. Blumenstein, statistician
Survival Full Analysis Set

$P = 0.006$ (stratified logrank)

Hazard Ratio $= 0.801$ (0.396 to 0.912)

Randomized Multicenter Placebo-controlled Vaccine Therapy Trial in Castrate-resistant Metastatic Prostate Cancer Patients

Observations:

A. Time to Progression: no difference in arms

B. Median survival at 4 years
   Placebo: 16.6 months
   Vaccine: 25.1 months (p=0.006)

C. 40% reduction in death rate in vaccine arm

Phase III Trial Planned
Overall survival analysis of a Phase II study of PSA-TRICOM in the treatment of metastatic, castrate-resistant prostate cancer

Ravi A. Madan¹, James L. Gulley¹, William L. Dahut², Kwong Y. Tsang¹, Seth M. Steinberg³, Jeffrey Schlom¹ and Philip M. Arlen¹

¹Laboratory of Tumor Immunology and Biology, ²Medical Oncology Branch, and ³Biostatistics and Data Management Section, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland
Predicted vs. Actual Survival of PSA-TRICOM Patients

Updated with March 10, 2008 data

Predicted Survival of ≥ 18 Months

- Halabi Predicted Survival
- Survival less than Predicted
- Survival greater than Predicted
- Still Alive

Survival less than Predicted

Survival greater than Predicted

Still Alive

Patient Number

n=17

n=15

Halabi Predicted Survival of < 18 Months

Halabi Predicted Survival of ≥ 18 Months

Months From On-Study Date

0 10 20 30 40 50
Actual survival (*) compared with the Halabi predicted survival (o).
## Phase II/III Trials: Metastatic Prostate Cancer

<table>
<thead>
<tr>
<th>Phase III</th>
<th>Median Overall Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitoxantrone</td>
<td>16.4 mo</td>
</tr>
<tr>
<td>Docetaxel (weekly)</td>
<td>17.3 mo Δ 0.9 mo</td>
</tr>
<tr>
<td>Docetaxel (3 weekly)</td>
<td>18.9 mo Δ 2.5 mo</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NCI Phase II</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Docetaxel (HPS 16.5 mo)</td>
<td>15.5 mo (Δ 1.0 mo)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Randomized Phase II</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Vector control</td>
<td>16.3 mo</td>
</tr>
<tr>
<td>PSA-TRICOM (p = 0.006)</td>
<td>24.4 mo Δ 8.1 mo</td>
</tr>
<tr>
<td>(HR = 0.6)</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>NCI Phase II</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA-TRICOM</td>
<td>26.6 mo</td>
</tr>
<tr>
<td>(HPS 17.4 mo)</td>
<td>Δ 9.2 mo</td>
</tr>
<tr>
<td>(ave HPS 12.3 mo)</td>
<td>14.6 mo Δ 2.2 mo</td>
</tr>
<tr>
<td>(ave HPS 20.9 mo)</td>
<td>≥37.3 mo Δ ≥16.4 mo</td>
</tr>
</tbody>
</table>
Vaccine Combination Therapies

The vaccine induction of a dynamic host immune response can be boosted by

– concomitant or subsequent therapies

(a) can alter the phenotype of tumor cells, rendering them more susceptible to T-cell killing

(b) can lyse populations of tumor cells which, in turn, act as a booster for tumor-specific immune cells

(c) can kill or inhibit regulatory T cells and thus boost the immune response