Cancer Vaccines: Current Challenges & Evolving Concepts

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Presentation Highlights

- Understand the key functions and elements of the immune system.
- Review different types of cancer vaccine platforms and other immune-based therapies under investigation.

Current Challenges

- The WHO, WHAT, HOW, WHERE and WHEN of cancer vaccine administration.
- Understand how clinical trials of cancer vaccines differ from clinical trials of molecularly targeted or other chemotherapy drugs.
- Need for imaging criteria that reflect the time course and distinct characteristics of vaccine-induced anti-tumor responses.
- Lack of correlation between vaccine-induced immune responses and clinical outcomes.
Evolving Concepts

- Novel approaches to identify earlier signals of activity:
  - Conduct cancer vaccine studies in earlier stage disease
  - Analysis of growth / regression rate constants as surrogates of early clinical activity.
- More relevant preclinical animal models and a higher threshold for clinical translation.
- Cancer vaccines plus...chemotherapy, radiation therapy, hormone therapy and agents targeting negative regulation.

I will use the NCI Vaccine Branch TARP and AdHER2 DC cancer vaccine trials to highlight discussion of these objectives.
A very complex, redundant network of highly adaptive, specialized tissues and organs, cells, and protein messengers (cytokines and chemokines) that are able to rapidly respond to and eliminate foreign (non-self) pathogens, and control tissue injury and inflammation.

The Bottom Line:
Our immune system protects us from disease.
The Immune System

A very complex, redundant network of highly adaptive, specialized tissues and organs…
The Immune System

...specialized cells...
The Immune System

...and specialized protein messengers (cytokines and chemokines)
The Immune System

...that are able to rapidly respond to and eliminate foreign (non-self) pathogens...
The Immune System - A Practical Analogy

Organs...

Cells...

...and their protein messengers
The Immune System - A Practical Analogy
The Immune System - A Practical Analogy

The Data Closet **AFTER SEQUESTRATION!!**
The Immune System: It’s really a big black box with a LOT of interconnected components!

- **Antigen (Immunogen)**: Given to stimulate the immune system.
- **Immune Response**
- **Clinical Response**
  - Prevention of Disease (**Prophylactic** Vaccines)
  - Treatment of Disease (**Therapeutic** Vaccines)
The *key function* of the immune system is to *distinguish “self” from “non-self”*. 
The Immune System

The main problems we see with immunity are:

- **Too little** immunity - i.e. the immune system is compromised as is seen with malnutrition, cancer, HIV infection and other immune deficiency diseases.

- **Too much** immunity i.e. the immune system is overactive resulting in autoimmune and chronic inflammatory disease where “self” gets attacked as is seen in systemic lupus, rheumatoid arthritis and thyroiditis.
The Immune System

How does the immune system accomplish its key function?

- Whatever the immune system is responding to is always presented in the context of “self”.
- “Self” is described by the human leukocyte antigen (HLA) system - the name of the major histocompatibility complex (MHC) in humans.
- HLA tissue antigens are essential elements to immune function and they constitute our tissue type. They are the major cause of organ transplant rejection and bone marrow transplant failure.
The Immune System

The hand print analogy of what the immune system “sees”:

“Self”- Your HLA Tissue Type Unique to **YOU**

“Self” plus “Non-Self” Non-self is **FOREIGN** to you.
Why Cancer Vaccines?

Because we know that the immune system is inherently capable of controlling and eliminating cancer.
So What Makes Developing Cancer Vaccines So Challenging?

*Cancer* is “self” gone bad but it *is still “self”*, so the immune system is limited in its ability to attack cancer cells.
So What Makes Developing Cancer Vaccines So Challenging?

Once cancer cells take hold and grow into a tumor mass,

the tumor itself disrupts the immune system and even secrets protein messengers that suppress the immune system’s attempts to control and eliminate it.

A MAJOR challenge.
Despite challenges, we have had some success in developing:

**Cancer Vaccines**

**Prevention of Disease** *(Prophylactic Vaccines)*

*HPV VLP* (Virus-Like Particle) *Vaccines:*

- **GARDASIL**, **Cervarix**

**Prevent** cervical, vaginal, vulvar and anal cancer caused by HPV (human papilloma virus)

**Treatment of Disease** *(Therapeutic Vaccines)*

- **PROVENGE**, (sipuleucel-T)

The only FDA approved cancer vaccine.

Used in the treatment of minimally symptomatic, metastatic, castrate resistant prostate cancer (mCRPC).
Specific Cancer Vaccine and Immune-Based Therapy Platforms
Cancer Vaccine and Immune-Based Therapy Platforms

Prophylactic Cancer Vaccines

- Currently only available through the use of prophylactic vaccines targeting chronic infections that are associated with the development of cancer:

  HPV Virus-Like Particle (VLP) Vaccines:
  - GARDASIL® (HPV 6, 11, 16, 18) or CERVARIX® (HPV 16, 18)
  - Prevent persistent HPV infection associated with the development of cervical, vulvovaginal, penile and anal cancer.

  Hepatitis B Vaccines:
  - Energix-B, Recombivax HB
  - Prevent persistent Hepatitis B infection associated with the development of cirrhosis and liver cancer.

- Potential also exists for a therapeutic vaccine for a chronic infectious disease to be a prophylactic vaccine for cancer e.g.
  - Hepatitis C ----> Hepatocellular carcinoma
  - *H. pylori* ----> Gastric carcinoma
Advantages and Disadvantages

Autologous whole tumor cell and tumor lysate vaccines.

- **Advantages:** target the patient’s tumor associated antigens (TAAs) and present a broad selection of antigens to the immune system.

- **Disadvantages:** antigens could be diluted by other cellular components; lack of standardized measures for vaccine potency; difficulty in evaluating antigen-specific immune responses post-vaccination since most of the tumor antigens remain unknown.

Allogeneic tumor cell vaccines

- **Advantages:** easier to generate; generally derived from multiple tumor cell lines; able to break tolerance to self-tumor antigens; applicable to a broader number of patients. Example: GVAX platform.

- **Disadvantages:** tumor cells, even when foreign are generally not immunogenic. Must frequently be co-formulated with immunostimulatory proteins e.g. GM-CSF.
Advantages and Disadvantages

- **Dendritic Cell (DC) vaccines**
  - **Advantages:** serve as a bridge between the innate and adaptive immune system; most critical cellular platform to activate both CD4+ and CD8+ T cells and induce a broad immune response. Example: sipuleucel-T/PROVENGE® cellular therapy.
  - **Disadvantages:** requires labor intensive *ex vivo* production --> costly and time consuming; cellular composition and functional potency may vary significantly because each product is patient-specific- challenges with standardization.

- **Synthetic/recombinant peptide-based vaccines:** single epitope, multi-epitope and overlapping.
  - **Advantages:** easy and cost effective to manufacture.
  - **Disadvantages:** hard to definitively establish the best peptide eptiopes for anti-tumor immunity; short peptides (9-11 amino acids in length) lack CD4 help and only generate CD8 cell-mediated responses that are not long lasting; limited number of peptides allows tumor immune escape; must be co-delivered with adjuvants.
Therapeutic Cancer Vaccine Platforms

**Advantages and Disadvantages**

- **Protein-based vaccines**
  - **Advantages:** multiple epitopes presented to the immune system ---\(\rightarrow\) minimizes risk of tumor immune escape; very suitable for induction of CD4 T cell responses.
  - **Disadvantages:** does not induce proper CD8 T cell immunity; mainly induces responses against the *dominant epitopes* - limited reactivity to subdominant epitopes.

- **DNA-based vaccines**
  - **Advantages:** simplicity and low production costs; encodes specific proteins or antigens; excellent priming principle.
  - **Disadvantages:** *poorly immunogenic*; requires large amounts of product co-delivered with plasmids expressing cytokines, TLRs or other immunostimulatory complexes or co-stimulatory molecules; not suitable for induction of robust and sustained T cell responses; administration by electroporation; protein boosting for induction of relevant immune responses.
Other Immune-Based Therapy Platforms

- Cytokines: IL-2, IL-7, IL-15, GM-CSF
- Antibodies to negative immune regulators:
  - Anti-CTLA-4: ipilimumab
  - Anti-TGF-β: GC1008
- Adoptively transferred and genetically engineered lymphocytes.
- Monoclonal antibodies to tumor antigens / epitopes: “the ultimate molecularly targeted therapy”

  Make the cancer more visible to the immune system:
  - Anti-CD20 (found only on B cells): rituximab (Rituxan)
  - Anti-HER2/neu (found on certain types of cancer e.g. breast): trastuzumab (Herceptin), pertuzumab (Perjeta), TDM-1

  Block growth signals:
  - Anti-epidermal growth factor receptor (found on cancer cells): cetuximab (Erbitux).

  Stop new blood vessels from forming:
  - Anti-vascular endothelial growth factor (VEGF) (found on cancer cells): bevacizumab (Avastin)

  Deliver radiation to cancer cells:
  - Combine a radioactive particle with a monoclonal antibody: ibritumomab (Zevalin)
Cancer Vaccines: Current Challenges

- The WHO, WHAT, HOW, WHERE and WHEN of cancer vaccine administration.

- Understanding how clinical trials of cancer vaccines differ from clinical trials of molecularly targeted or other chemotherapy drugs.

- The need for imaging criteria that reflect the time course and distinct characteristics of vaccine-induced anti-tumor responses.

- The lack of correlation between vaccine-induced immune responses and clinical outcomes.
What are the specific challenges we face when designing and developing vaccines to treat cancer?

- **WHO** should we give the vaccine to?
- **WHAT** antigen (or immunogen) should we use?
- **HOW** should we deliver the vaccine antigen, **HOW OFTEN** should we give it, **HOW MUCH** (what dose of antigen) should we give, and **HOW MANY** vaccines should we give to induce immunity?
- **WHERE** should we give the vaccine?
- **WHEN** should we give the vaccine?
**WHO** should we give cancer vaccines to?

**Very Early Disease** ----> **Neoadjuvant Setting**
- Give the vaccine *prior to* surgery ---> allows examination of the tumor when it is removed ---> pathologic complete response (pCR).

**Early Disease** ----> **Adjuvant Setting**
- Give the vaccine *following* surgery ---> allows assessment of *clinical outcomes* such as disease recurrence (may take a while depending on disease natural history).

**Advanced Disease** ----> **Metastatic Tumor**
- Tumor has spread from the tissue of origin to other vital organs e.g. brain, bone, liver, lung etc.
Principle 1:

Giving cancer vaccines earlier in disease while the immune system is still intact is likely to maximize vaccine activity.
WHAT antigen (or immunogen) should we give?

Our goal is to train the immune system to recognize and kill only tumor cells and NOT healthy normal tissue.

Tumor Antigens

- An antigen (usually a protein) that is unique to tumor cells OR over-expressed on tumor cells.
Principle 2:

Cancer vaccines that use tumor antigens direct the immune response to the tumor and minimize the effects on healthy normal tissue.
**HOW** should we deliver the vaccine antigen?

Tumor antigens in cancer vaccines can be delivered in several forms:

- DNA
- RNA
- Protein
- Peptides (smaller pieces of protein)
- Using viruses
- Using bacteria
- Using cells engineered to express tumor antigens
- Using cancer cell lines that are foreign (allogeneic)
- Whole tumor lysate from a person’s own tumor (autologous)
**HOW** often should we give it?

The frequency of cancer vaccination schedules is *HIGHLY VARIABLE*:

- Once a week
- Every 2 or 3 weeks
- Monthly

Sometimes it’s based on pre-clinical animal models, *often it’s not*.

*Generally involves a combination of educated guessing and gut instinct.*
HOW much vaccine i.e. what dose of vaccine should we give?

The determination of optimal vaccine dose is often explored in a phase I dose escalation trial.

An example is a dendritic cell cancer vaccine:
- Dose Level 1: 5 million cells/vaccine
- Dose Level 2: 10 million cells/vaccine
- Dose Level 3: 20 million cells/vaccine

There can be very small doses- several hundred thousand cells or an unlimited dose- give to the patient everything you were able to make.

Still there is usually a minimum dose. An example is PROVENCE (sipuleucel-T): minimum of 50 million autologous CD54+ cells activated with PAP-GM-CSF (the tumor antigen).
**How** many vaccines should we give?

*Priming*: Giving enough doses to generate an initial immune response.

- Generally 1-5 vaccine doses, on average 3.

*Boosting*: Giving additional doses of vaccine to augment or “boost” and maintain the immune response.

- Very dependent on vaccine potency and whether there are strong memory responses following vaccination.
WHERE should we give the vaccine?

Where we deliver the cancer vaccine is often determined by the antigen type and the cancer vaccine platform.

**Routes**
- IM: intramuscularly
- SC: subcutaneously
- ID: intradermally
- IV: intravenously
- IT: intratumoral- directly into tumor
- IN- intranodally- directly into a lymph node near the tumor.
- PO- orally by mouth
- Intranasally

**Examples**
- Most common route; must for DNA
- Proteins, peptides, cell vaccines
- Dendritic cell vaccines
- Cellular therapies- PROVENGE®
- Bacterial and viral vectors
- Bacterial and viral vectors, proteins
- Usually for preventive vaccines
- Usually for preventive vaccines
**WHEN** should we give the vaccine?

**Before Chemotherapy**
- Train the immune system to recognize the tumor followed by direct killing of tumor with chemotherapy. Ensures the immune system is intact (relatively) at the time vaccine is given.

**With Chemotherapy**
- Recent approaches have included low dose chemotherapy to get rid of and diminish immune suppressing cells, followed by administration of the cancer vaccine.

**After Chemotherapy**
- Tumor has regressed and the immune system has recovered (two *major* hurdles). Goal is to deliver vaccine to try and prevent disease recurrence.
Cancer Vaccines: Current Challenges

- The WHO, WHAT, HOW, WHERE and WHEN of cancer vaccine administration.

- Understanding how clinical trials of cancer vaccines differ from clinical trials of molecularly targeted or other chemotherapy drugs.

- The need for imaging criteria that reflect the time course and distinct characteristics of vaccine-induced anti-tumor responses.

- The lack of correlation between vaccine-induced immune responses and clinical outcomes.
How Do Cancer Vaccines Differ From Traditional Chemotherapy?
Hallmarks of Cancer Vaccines and (some) Immune-Based Therapies

- **Clinical responses** to cancer vaccines and immune-based therapies take time.

- Improvement in overall survival (OS) is often seen with little or no improvement in progression free survival (PFS). Examples: Sipuleucel-T (PROVENGE®), PSA-TRICOM (PROSTVAC®).

- Patients sometimes demonstrate “disease progression” prior to regression.

- Are generally more effective in patients with minimal tumor burden / earlier in disease.

- Reliable, validated surrogate markers for clinical responses are often lacking.
How Do Cancer Vaccines Differ From Traditional Chemotherapy?

The impact of cancer vaccines on clinical outcomes and parameters are by their very nature indirect:

A cancer vaccine may or may not induce an immune response, which can include increasing immune cell numbers, stimulating the production of protein messengers i.e. cytokines and chemokines, decreasing or overcoming tumor suppressor mechanisms.

In addition, the immune response induced by the cancer may or may not have an anti-tumor effect.
Hence the possible outcomes of vaccination with therapeutic cancer vaccines are:

Rx Cancer Vaccine ----> **NO** Immune Response ----> **NO** Clinical Response
**ANALOGY:** Immune system is not stimulated = > soldiers have guns but they’re shooting blanks.

Rx Cancer Vaccine ----> + Immune Response ----> **NO** Clinical Response
**ANALOGY:** Immune system is stimulated => soldiers have guns with bullets but they miss their target.

Rx Cancer Vaccine ----> + Immune Response ----> + Clinical Response ----> **IDEAL**
**ANALOGY:** Immune system is stimulated and there is an anti-tumor effect => soldiers have guns with bullets and they’re able to hit the target- Immune Special OPS Forces!

The challenge then becomes **what immune responses correlate with the anti-tumor clinical response?**
Provenge® (Sipuleucel-T) As An Example

- Custom made, patient specific, *personalized cellular immune therapy*.
- **Vaccine antigen** is a *fusion protein PA2024 (PAP-GM-CSF)*: PAP (prostatic acid phosphatase) is a tumor antigen that is over-expressed in prostate cancer. GM-CSF is a protein that recruits cells of the immune system and stimulates them to mature.
- Given at 0, 2 and 4 weeks for a total of 3 doses.
- Approved by the FDA based on a *4.1 month improvement in overall survival (OS)*.
- Upregulation of CD54 (a cell surface marker) on the cellular product was documented to be a measure of potency and immune activation.

**Immune correlates of OS:**
- Cumulative CD54 upregulation dose
- **Antibody titers ≥ 400 at any time against PA2024** (the PAP-GMCSF fusion protein immunizing antigen) or PAP (prostatic acid phosphatase).

- Strong T-cell proliferative responses to PA2024 and PAP was not predictive of improvements in overall survival.
Sipuleucel-T = autologous PBMC cultured with human PAP fused to GM-CSF

HR = 0.759 (95% CI: 0.606, 0.951)
P = 0.017 (Cox model)
Median Survival Benefit = 4.1 months

Median Survival:
- Sipuleucel-T (n = 341): 25.8 mo.
- Control (n = 171): 21.7 mo.

36 mo. survival:
- Sipuleucel-T: 32.1%
- Control: 23.0%

No. at Risk:

<table>
<thead>
<tr>
<th></th>
<th>Sipuleucel-T</th>
<th>Control</th>
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<tbody>
<tr>
<td>At Risk</td>
<td>341</td>
<td>171</td>
</tr>
<tr>
<td>12 mo.</td>
<td>274</td>
<td>123</td>
</tr>
<tr>
<td>24 mo.</td>
<td>142</td>
<td>59</td>
</tr>
<tr>
<td>36 mo.</td>
<td>56</td>
<td>22</td>
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<tr>
<td>48 mo.</td>
<td>18</td>
<td>5</td>
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<tr>
<td>60 mo.</td>
<td>3</td>
<td>2</td>
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And Kantoff et al. NEJM 2010
Challenges in Clinical Development

Documenting Evidence of Cancer Vaccine Clinical Activity Early in Development

Challenge 1: Clinical trial end points for cancer vaccines and immune-based therapies.

- Agent approval is based on demonstration of clinically meaningful superior treatment effect with an acceptable safety profile.
- OS (Overall Survival): platinum standard; unequivocal outcome measure on a continuous time scale.
- DFS (Disease Free Survival), PFS (Progression Free Survival), TTP (Time To Progression): are surrogate end points that are not measured continuously, hence the exact day of recurrence or progression cannot be accurately captured.

- Improvements in OS may not be associated with changes in PFS or TTP.
- Improvements in PFS or TTP may not be associated with changes in OS.
Table 2  
Improvements in Overall Survival Endpoints Associated with Immune-Based, Chemotherapeutic and Hormonal Agents

<table>
<thead>
<tr>
<th>IMMUNE AGENT</th>
<th>Trial Type (N = Enrolled)</th>
<th>Study Population</th>
<th>Improvement in Median OS</th>
<th>Difference in Median OS?* At 6 mo? At 12 mo? At 24 Mo?</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipilimumab</td>
<td>Phase III N = 676</td>
<td>Stage III/IV Melanoma</td>
<td>Alone 3.7 mo (10.1 vs. 6.4 mo)</td>
<td>YES YES YES</td>
<td>^46Hodi FS 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>w/Vaccine 3.6 mo (10.0 vs. 6.4 mo)</td>
<td>No YES YES</td>
<td>Difference in OS @ ~4mo</td>
</tr>
<tr>
<td>Sipuleucel-T vs. Placebo</td>
<td>Phase III N = 512</td>
<td>Minimally or Asymptomatic mCRPC</td>
<td>4.1 mo (25.8 vs. 21.7 mo)</td>
<td>No YES YES</td>
<td>Difference in OS @ ~12mo Earlier studies: Difference OS @ ~8mo</td>
</tr>
<tr>
<td>PSA-TRICOM vs. Control Vector</td>
<td>Phase II N = 125</td>
<td>Minimally or Asymptomatic mCRPC</td>
<td>8.5 mo (25.1 vs. 16.6 mo)</td>
<td>No YES YES</td>
<td>Difference in OS @ ~14mo</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CHEMOTHERAPY / HORMONAL AGENT</th>
<th>Trial Type (N = Enrolled)</th>
<th>Study Population</th>
<th>Improvement in Median OS</th>
<th>Difference in Median OS?* At 6 mo? At 12 mo? At 24 Mo?</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Docetaxel q3 wks plus Prednisone vs. Mitoxantrone plus Prednisone</td>
<td>Phase III N = 1006</td>
<td>Advanced mCRPC</td>
<td>2.4 mo (18.9 vs. 16.4mo)</td>
<td>No YES YES</td>
<td>Difference in OS @ ~8mo</td>
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<tr>
<td>Docetaxel q3 wks plus Estramustine vs. Mitoxantrone plus Prednisone</td>
<td>Phase III N = 770</td>
<td>Advanced mCRPC</td>
<td>1.9 mo (17.5 vs. 15.6 mo)</td>
<td>YES YES YES</td>
<td>Difference in OS @ ~6mo</td>
</tr>
<tr>
<td>Abiraterone Acetate vs. Placebo</td>
<td>Phase III N = 770</td>
<td>Advanced mCRPC, prior docetaxel</td>
<td>3.9mo^ (14.8 vs. 10.9 mo)</td>
<td>YES YES YES</td>
<td>Difference in OS @ ~3mo</td>
</tr>
</tbody>
</table>

^Separation in Kaplan-Meier curve estimates of the probability of overall survival in groups based on visual review of curves in references cited.

^Initial analysis when 552 events had occurred.

Challenges in Clinical Development

Documenting Evidence of Cancer Vaccine Clinical Activity Early in Development

Conclusions regarding clinical trial end point challenges:

- Although improvement in OS has been established as sentinel standard for regulatory evaluation with the approval of sipuleucel-T (PROVENGE), *OS is not a clinically feasible primary end point for first-in-human phase I/II trials.*

- *End points based on objective tumor assessments are unlikely to reliably serve as harbingers of early clinical activity* i.e. no early observable impact on PFS, DFS or TTP.
Cancer Vaccines: Current Challenges

- The WHO, WHAT, HOW, WHERE and WHEN of cancer vaccine administration.

- Understanding how clinical trials of cancer vaccines differ from clinical trials of molecularly targeted or other chemotherapy drugs.

- The need for imaging criteria that reflect the time course and distinct characteristics of vaccine-induced anti-tumor responses.

- The lack of correlation between vaccine-induced immune responses and clinical outcomes.
Evaluation of Response to Immune-Based Therapies: Immune-Related Response Criteria (irRC) vs. Standard RECIST Criteria
Investigators have traditionally relied on Response Evaluation Criteria in Solid Tumors (RECIST) or modified WHO criteria to evaluate antitumor responses to chemotherapeutic agents.

However, the responses that are seen with immunotherapeutic agents may extend beyond those of cytotoxic agents and could include responses after disease progression that are not captured by RECIST or WHO criteria.

Immune-related response criteria (irRC), are novel criteria that can better capture the response patterns observed with some immunotherapeutic agents (e.g. anti-CTLA-4).

Use of the irRC may allow more comprehensive evaluation of immunotherapeutic agents in clinical trials and potentially, may offer guidance in clinical care. As such, the irRC offer a new tool for clinical investigation of immune therapy in cancer.
# Comparison of RECIST vs. irRC

## RECIST Response Criteria

<table>
<thead>
<tr>
<th>Evaluation of Target Lesions</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CR Complete Response</td>
<td>Disappearance of all target lesions.</td>
</tr>
<tr>
<td>PR Partial Response</td>
<td>( \geq 30% ) decrease in sum of the LD of target lesions, taking as reference the baseline sum LD.</td>
</tr>
<tr>
<td>PD Progressive Disease</td>
<td>( \geq 20% ) increase in sum of LD target lesions, taking as reference the smallest sum LD recorded since treatment start or the appearance of one or more new lesions.</td>
</tr>
<tr>
<td>SD Stable Disease</td>
<td>Neither sufficient shrinkage to qualify as PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since treatment start.</td>
</tr>
</tbody>
</table>

## Immune-Related Response Criteria

<table>
<thead>
<tr>
<th>Evaluation of Target Lesions</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>irCR Complete Response</td>
<td>Complete disappearance of all lesions (whether measurable or not, and no new lesions).</td>
</tr>
<tr>
<td>irPR Partial Response</td>
<td>( \geq 50% ) decrease in tumor burden relative to baseline.</td>
</tr>
<tr>
<td>irPD Progressive Disease</td>
<td>( \geq 25% ) increase in tumor burden relative to nadir (minimum recorded tumor burden).</td>
</tr>
<tr>
<td>irSD Stable Disease</td>
<td>Not meeting criteria for irCR or irPR, in absence of irPD.</td>
</tr>
</tbody>
</table>

### New Measurable Lesions (\( \geq 5\times5\)mm)
*Always represent PD*

### New Non-Measurable Lesions(< 5x5mm)
*Always represent PD*

### Non-Target Lesions
Contribute to defining BOR of CR, PR, SD, and PD.

Incorporated into tumor burden

Do *not* define progression (but preclude irCR)

Contribute to defining irCR (complete disappearance required)
Cancer Vaccines: Current Challenges

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- The lack of correlation between vaccine-induced immune responses and clinical outcomes.
Challenges in Clinical Development

Documenting Evidence of Cancer Vaccine Clinical Activity Early in Development

- **Challenge 2:** *Lack of correlation of immunologic end points* with clinical outcomes.

- Identification of immunologic end points that consistently correlate with clinical outcomes has been sorely absent.

- This may be due to:
  - Lack of standardization in immune monitoring and assays.
  - Unique kinetics of immune responses associated with vaccination.
  - Weak immunogens
  - Usually measured systemically ----> *fail to account for the importance of the local tumor microenvironment.*
Challenges in Clinical Development

Documenting Evidence of Cancer Vaccine Clinical Activity Early in Development

- **Challenge 3:** Lack of assay standardization in obtaining immunologic end points.

- IFN-γ ELISPOT: one of the most common immune assay readouts in cancer vaccine trials but no consensus has been reached on how a positive response is defined.

- Recommendations from a workshop on immune assay standardization*:
  - Utilize accurate, precise, and reproducible immune assay protocols.
  - Use functional assays for primary immunology readouts in clinical trials.
  - Utilize central laboratories for immune monitoring of large multi-institutional trials.
  - Use standardized testing of multiple phenotypic and functional potency assays specific to any cellular product.

Evolving Concepts

- Novel approaches to identify earlier signals of activity:
  - Conduct cancer vaccine studies in earlier stage disease
  - Analysis of growth / regression rate constants as surrogates of early clinical activity.

- More relevant preclinical animal models and a higher threshold for clinical translation.

- Cancer vaccines *plus* chemotherapy, radiation therapy, hormone therapy and agents targeting negative regulation.
Clinical Translation:
TARP Peptide Vaccine
What Is TARP?

**TARP: TCR©Alternative Reading Frame Protein**

- TARP is a novel 58 amino acid protein expressed in ~90-95% of prostate and ~50% of breast cancers that uses a different open reading frame from normal TCR-γ.

- Was discovered by NCI investigator Dr. Ira Pastan and colleagues.

- Additional work by others has shown that TARP is...
  - Highly expressed in primary as well as metastatic prostate cancer
  - Expressed in prostate cancers with a range of Gleason patterns
  - Expressed in both hormone sensitive and castrate resistant prostate cancer.

- ...making TARP an **ideal candidate** for a novel therapeutic vaccine platform in any stage of prostate cancer as well as potentially breast and other cancers.
Proteins: are like a long *sentence*.

Peptides: are like *words* in a sentence.

- Short peptides (small words) are 9-11 amino acids in length
- Long peptides (big words) are 15-20 amino acids in length

Amino Acids: are the *letters* in the words in the sentence.

Epitope Enhancement: *substituting one letter for another in a word*
TARP Epitope Enhancement

**TARP: TCR©Alternative Reading Frame Protein**

In pre-clinical mouse studies, the Berzofsky lab demonstrated that:

- Two HLA-A*0201 overlapping wild type (WT) TARP peptides are associated with killer T-cell responses: TARP27-35 and TARP29-37.

- **TARP** peptide immunogenicity can be improved using *epitope enhancement*: making amino acid substitutions that increase peptide-HLA binding affinity.

- Substitution of Val for Leu at position 9 in TARP29-37 -------> **EETARP29-37-9V**, a peptide with *increased binding affinity* that induces antigen specific T cells able to recognize wild type *and* modified TARP peptides.
Epitope Enhancement

Human CTL Raised Against an Epitope-Enhanced TARP Peptide can Kill Human Tumor Cells Expressing TARP and HLA-A2.

Oh et al
Cancer Res. 2004

Tumor

<table>
<thead>
<tr>
<th>Tumor</th>
<th>MCF-7</th>
<th>Du145</th>
<th>PC3-TARP</th>
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<tr>
<td>TARP</td>
<td>++</td>
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<tr>
<td>HLA-A2</td>
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</table>
Clinical Translation: TARP Peptide Vaccine

09-C-0139 Pilot Study of Vaccination w/ Epitope-Enhanced TARP Peptide & Peptide-Pulsed DCs in Stage D0 Prostate Cancer

Vaccine Platform: TARP WT27-35 and EE29-37-9V

Study Population: HLA-A*0201 positive men
Stage D0 disease (PSA biochemical recurrence after definitive treatment of primary tumor).
PSADT ≥ 3 months and ≤ 15 months.

Target Accrual: N = 40, 20 patients each arm. (Actual Accrual N = 41)

Objectives: Primary: Safety, toxicity, immunogenicity
Secondary: Change in PSA Doubling Time (PSADT)Slope Log (PSA)
TARP Peptide Vaccine Study Design

**Arm A** TARP peptides plus Montanide ISA51A & GMCSF deep sc

**Arm B** TARP peptide-pulsed dendritic cell vaccine plus KLH administered ID

<table>
<thead>
<tr>
<th>Study Week</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
<th>18</th>
<th>24</th>
<th>36</th>
<th>48</th>
<th>60</th>
<th>72</th>
<th>84</th>
<th>96*</th>
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<tbody>
<tr>
<td>TARP Vaccine</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>(↑)</td>
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<td>Apheresis</td>
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<tr>
<td>CT/Bone Scan Restaging</td>
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</tbody>
</table>

(↑) Conditional vaccine dose if evidence of response by PSADT or immunologic criteria @ study Week 24.

**September 2010**: Study amended and extended for an additional 48 weeks because of observed responses in PSADT.

**June 2011**: Study extended for an additional 48 weeks to 144 weeks total with booster dose of TARP vaccine @ Wk 96.
Clinical Translation: TARP Peptide Vaccine

**TARP Baseline Study Demographics**

<table>
<thead>
<tr>
<th>Arm</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arm A</td>
<td>TARP peptides plus Montanide ISA51VG and GMCSF</td>
</tr>
<tr>
<td>Arm B</td>
<td>Autologous TARP peptide-pulsed dendritic cell vaccine</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>GS</th>
<th>PSA</th>
<th>Vit D</th>
<th>ALC</th>
<th>CD4%</th>
<th>CD4#</th>
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</thead>
<tbody>
<tr>
<td><strong>Arm A (N = 21)</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>64</td>
<td>7</td>
<td>3.44</td>
<td>26</td>
<td>1360</td>
<td>40.4</td>
<td>584</td>
</tr>
<tr>
<td>Range</td>
<td>45 - 74</td>
<td>6 - 9</td>
<td>0.64 - 16.70</td>
<td>6 - 79</td>
<td>610 - 2160</td>
<td>28.5 - 58.4</td>
<td>206 - 915</td>
</tr>
<tr>
<td><strong>Arm B (N = 20)</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Median</td>
<td>60</td>
<td>7</td>
<td>2.74</td>
<td>28.5</td>
<td>1230</td>
<td>44.7</td>
<td>536</td>
</tr>
<tr>
<td>Range</td>
<td>50 - 82</td>
<td>4 - 9</td>
<td>0.48 - 30.70</td>
<td>5 - 70</td>
<td>690 - 3270</td>
<td>26.5 - 62.9</td>
<td>288 - 1283</td>
</tr>
<tr>
<td><strong>All Patients (N = 41)</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Median</td>
<td>62</td>
<td>7</td>
<td>3.44</td>
<td>28</td>
<td>1270</td>
<td>42.5</td>
<td>547</td>
</tr>
<tr>
<td>Range</td>
<td>45 - 82</td>
<td>4 - 9</td>
<td>0.48 - 30.70</td>
<td>5 - 79</td>
<td>610 – 3270</td>
<td>26.5 – 62.9</td>
<td>206 - 1283</td>
</tr>
</tbody>
</table>

Radical Prostatectomy:  N = 30 of 41 (73%)

S/p EBRT for PSA Biochemical Recurrence:  N = 26 of 41 (63%)
A decrease in slope log (PSA) is equivalent to an increase in PSADT.
Clinical Translation: TARP Peptide Vaccine

Maximal Slowing of PSA Occurs During **Active** TARP Vaccination: Arm A Patients

**FRB PSA Values (Pre-NIH through On-Study)**

**E-D PSA Values (Pre-NIH through On-Study)**
Clinical Translation: TARP Peptide Vaccine

Maximal Slowing of PSA Occurs During **Active** TARP Vaccination: Arm B Patients
TARP Vaccination Results in a Decrease in Slope Log PSA at 24 Weeks

Waterfall Plot Week 24 Data (through 07/31/12): TOTAL N = 39*

*Patient 213 not shown due to mixed vaccines; Patient 219 is Off Study.

Patients with Decreased Slope Log PSA: YES = 28 (71.8%)
No = 11 (28.9%)

P = 0.0012 for change in magnitude of slope log PSA
TARP Vaccination Results in a Decrease in Slope Log PSA at 48 Weeks

Difference Slope Log PSA Wk 3-48 vs. Pre-NIH Slope Log PSA

Waterfall Plot Week 48 Data (through 07/31/12): TOTAL N = 31

Patients with Decreased Slope Log PSA: YES = 23 (74.2%)
No = 8 (25.8%)

^Three patients off study by Week 48: 203 (patient request), 213 (DC vaccine issues) and 219 (PSADT)

P = 0.0004 for change in magnitude of slope log PSA
Summary of Key Findings

- No statistically significant difference between Arm A and Arm B when examining differences in slope log PSA between Weeks 3-24 or 3-48 minus the pre-NIH slope, allowing us to pool them.

- **Again, in the pooled analysis, a statistically significant decrease was observed in the slope log PSA compared to the Pre-NIH baseline at both 3-24 and 3-48 weeks.**

- The effect of decreased slope log PSA at Weeks 3-24 doesn’t wane significantly over time and isn’t impacted by an additional vaccine dose at Week 36.

- Only a minority of patients (6/39, 15.4%) experienced an absolute decrease in PSA values at Week 24.

- The effect was persistent: Week 48-72 slope log PSA minus the pre-NIH slope, showed a statistically significant decline, \( p = 0.0035 \), (\( N = 21 \) patients).

- **There were no correlations or associations with any baseline variables** including CD4 percent/absolute count, CD8 percent/absolute count, 25 OH vitamin D levels, Gleason score, PSA, s/p RP or a baseline PSADT < 6 vs. ≥ 6 months.
Effect of TARP Vaccination on Tumor Growth Rate Constant

Fit of PSA Data to Exponential Growth Curves

Analysis by T. Fojo, J. Wilkerson, W. Stein
Effect of TARP Vaccination on Tumor Growth Rate Constant

- Each black dot at a given time point on treatment is the mean of calculated growth rates to that time point for all patients on study at that time point.
- The blue vertical lines with horizontal cross hatches represent 95% confidence intervals for each respective mean.
TARP Immunologic Correlates of Clinical Outcomes
The short answer: There are none identified to date.
7 Day IVS IFN-γ ELISPOT Does **NOT** Correlate with Changes in Slope Log PSA Response at Week 24 (or Week 48: Data Not Shown)

Positive 7 Day IVS ELISPOT Response in 31 of 40 (77.5%) Patients Tested
Ongoing Attempts to Identify Additional Correlates of Response to TARP Vaccination

- **TARP Tetramers:** data uninformative to date.

- **Polyfunctional T cells by ICS** for assessment of TARP-specific cellular reactivity is planned including Granzyme A and perforin.

- **Anti-TARP and Anti-PSA Antibody Responses:** negative.

- **Changes in NKT cells.**

- **Circulating Tumor Cells (CTC)** at Weeks 0, 12, 18 and 24.
TARP Vaccination Conclusions

- TARP vaccination was safe and well tolerated with adverse events limited to local injection site reactions ≤ Grade 2.

- Induction of TARP-specific IFN-γ ELISPOT responses was observed in a majority of patients but did not correlate with changes in PSA slope.

- Evidence of clinical activity was observed in a surprisingly high percentage of patients (74%) reflected in decreased PSA slope at one year compared to pre-treatment baseline.

- Since all patients received treatment in this study, a randomized, placebo-controlled trial is planned to confirm the preliminary evidence of clinical activity.
Multi-Epitope (ME) TARP Vaccine Phase II Study Design Schema

ME TARP Vaccine Platform:
Original two HLA A*0201 restricted peptides plus an additional five 20mer peptides overlapping by 10 mer to cover the entire 58 amino acid TARP protein --> eliminates need for HLA restriction and provides TARP-specific CD4 help --> cellular and humoral immune responses.

Lead-in-Accrual

N = 6

Study # 101-106

Assess ME TARP DC Vaccine Safety and Immunogenicity Through 12 Weeks.

2:1 RANDOMIZATION

ME TARP DC Vaccine
N = 44
Study # 201-244

PBMC Placebo Vaccine
N = 22
Study # 301-322

Study Week
0 3 6 9 12 15 18 24 36 48 60 72 84 96

ME TARP or Placebo Vaccine
Apheresis
CT/Bone Scan Restaging
TARP-Specific Immunity
Evolving Concepts

- Novel approaches to identify earlier signals of activity:
  - Conduct cancer vaccine studies in earlier stage disease
  - Analysis of growth / regression rate constants as surrogates of early clinical activity.
- More relevant preclinical animal models and a higher threshold for clinical translation.
- Cancer vaccines plus...chemotherapy, radiation therapy, hormone therapy and agents targeting negative regulation.
Clinical Translation: AdHER2ECTM Autologous DC Vaccine
Key Background Highlights

- HER2-directed therapy with trastuzumab and lapatinib (and soon to be pertuzumab and TDM-1) have resulted in expansion of therapeutic options and improved clinical outcomes.

- Clinical limitations of trastuzumab therapy:
  - Restricted to patients with HER2 3+ by IHC or Vysis FISH ratio >2.2.
  - Many patients are unresponsive
  - Many patients progress, due to emergence of resistance.
  - Cardiotoxicity associated with administration.

- Vaccines that induce anti-HER2 antibodies in cancer patients are NOT available.

- Therapeutic vaccines against HER-2/neu have been difficult to develop:
  - DNA vaccines: limited immunogenicity, no clinical activity
  - Protein-based vaccines: ineffective to date
  - Peptide-based vaccines: induction of T cell responses observed

- We have documented eradication of large established primary and metastatic cancers in the Balb/c TUBO mouse model using a therapeutic adenoviral HER2/neu vaccine. (Park JP et al Cancer Res 2008 Mar 15;68(6):1979-87)
Key Highlights Pre-Clinical Animal Data

- AdneuECTM vector caused regression and cure of large established tumors, whether delivered alone or as a DC vaccine.

- Vaccine anti-tumor activity is:
  - Antibody mediated.
  - However, unlike trastuzumab, antibody activity is Fc Receptor independent.
  - Does not require CD4+ T cell help (except at the time of vaccine delivery) or CD8+ T cells.
  - Induces polyclonal antibody responses.

- Ad-neuECTM serum:
  - Can prevent tumor growth and inhibit established tumors in transfer experiments.
  - Downmodulates ErbB2 and inhibits its phosphorylation.

- Tumor may continue to grow for 1-3 weeks before starting to regress, so patients who exhibit modest increases in tumor size should not be taken off study too soon.
Ad-neuECTM Treatment Causes Regression of Established s.c. TUBO Mammary Carcinomas
Ad-neuECTM Vaccine Induces Regression of Established Lung Tumors from IV Injection of TUBO Breast Cancer Cells

Park et al., *Cancer Research* 2008
The HER2 protein is over-expressed in breast and many other types of cancers. It sends signals to tumor cells to make them grow and prevents them from dying. Tumors that over-express HER2 are associated with more aggressive disease, higher recurrence rates and reduced survival rates.
Trastuzumab (Herceptin®) and pertuzumab (PERJETA™) are monoclonal antibodies that each recognize a distinct, small portion of the HER2 protein.

These monoclonal antibodies have to be given repeatedly to have an effect.

Despite their effectiveness in some cancers, trastuzumab and pertuzumab don’t work for everyone and even when they are effective, they may stop working after some time.
The AdHER2 DC vaccine is being tested in patients with HER2 expressing cancers for the very first time to see if it is safe and identify the best dose of vaccine to give. We are studying whether the vaccine can induce a patient’s own immune system to make multiple, different types of antibodies to HER2, called POLYCLONAL antibodies. We also want to know if these antibodies can help slow the growth of or possibly even shrink tumors.
Clinical Translation: AdenoHER2/neu DC Vaccine

Part I  N = 30 patients

- This part of the study is designed to examine vaccine safety and toxicity in patients whose tumors are not driven by amplification of HER2.

- **Dose Escalation:**
  - Dose Cohort 1: $5 \times 10^6$ cells/vaccine
  - Dose Cohort 2: $10 \times 10^6$ cells/vaccine
  - Dose Cohort 3: $20 \times 10^6$ cells/vaccine

- **Study Population:** vaccine dose escalation in a population with no prior exposure to trastuzumab or other HER2-targeted therapies to determine if there is a significant, adverse safety signal regarding cardiac toxicity, in addition to preliminary assessment of the vaccine’s immunogenicity and clinical activity.

- Adults ≥ 18 with recurrent, metastatic solid tumors characterized by some HER2/neu expression but for whom trastuzumab is not clinically indicated:
  - Patients with ovarian, colon, non-small cell lung, renal cell, bladder and prostate cancer that is known to be HER2 1+, 2+ or 3+ by IHC OR have a Vysis FISH result > 1.8.
  - Patients with breast cancer that is known to be HER2 1+ or 2+ by IHC or with a Vysis FISH result of 1.8 - < 2.2.

- Adults ≥ 18 with HER2+ bladder cancer in the adjuvant setting (adjuvant bladder cancer patients)
  - Naïve to trastuzumab, lapatinib and other HER2-directed therapies
Evolving Concepts

- Novel approaches to identify earlier signals of activity:
  - Conduct cancer vaccine studies in earlier stage disease
  - Analysis of growth / regression rate constants as surrogates of early clinical activity.
- More relevant preclinical animal models and a higher threshold for clinical translation.
- Cancer vaccines plus...chemotherapy, radiation therapy, hormone therapy and agents targeting negative regulation.
Cancer Vaccines Plus…

The Tantalizing Promise of Combination Therapy

Chemotherapy plus vaccines:
- Chemotherapy may induce anti-cancer immune responses as a result of tumor cell lysis, upregulation of MHC class I expression, increasing immunostimulatory cytokines, and decreasing regulatory T cells.

Agents targeting negative regulation plus vaccines:
- Combining agents that address negative checkpoint regulation with therapeutic vaccines have been shown to be synergistic in animal models. Clinical issues: systemic delivery vs. local co-delivery of the agent with the vaccine.

Hormone therapy plus vaccines:
- Androgen deprivation therapy has been shown to potentiate immune responses in pre-clinical animal models and clinical studies.

Radiation therapy plus vaccines:
- Radiation therapy has direct cytotoxic effects as well as indirect, immune-mediated anti-tumor effects. Abscopal effect: reduction in tumor growth outside the field of radiation (mediated by T cells).
Cancer Vaccines Plus...

The Tantalizing Promise of Combination Therapy...

....and Its Challenges

When to give:
- Neoadjuvant, adjuvant, treatment of primary disease, treatment of recurrent disease.

How to give:
- Sequentially vs. concurrently.

How to monitor:
- RECIST (tumor shrinkage associated with chemotherapy and radiation therapy) vs. immune-related Response Criteria (irRC) (slowing of tumor growth and anti-tumor activity associated with immune-based therapies and vaccines).

How to attribute toxicity:
- Many negative regulators e.g. anti-CTLA-4 or anti-PD-1 are associated with *autoimmune* conditions that also correlate positively with outcome. How to differentiate between autoimmunity induced by a vaccine immunogen vs. an agent that targets negative regulation.

*Do NOT assume* that immune-based therapies are always “benign, relatively non-toxic” therapies when compared to chemo or radiation.
Clinical Investigators, Staff, and Collaborators

- **Vaccine Branch, CCR, NCI, NIH**
  - Lauren V. Wood
  - Jay A. Berzofsky
  - Masaki Terabe
  - *Brenda Roberson, RN*
  - *Meghan Hughes*

- **Department of Transfusion Medicine, CC, NIH**
  - Luciano Castiello
  - Susan Leitman
  - Hanh Khuu
  - Marianna Sabatino
  - David Stroncek
  - and the *INCREDBLE STAFF of DTM!*

- **Medical Oncology Branch, CCR, NCI**
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  - Tito Fojo
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  - Julia Wilkerson
  - Phil Arlen
  - Wilfred Stein
  - Ravi Madan
  - Min Jung Lee
  - Jane Trepel
  - Sunmin Lee
  - Ira Pastan
  - Tapan Bera
  - University of Cincinnati
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  - Jason Steele

- **Molecular Imaging Program, CCR, NCI**
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  - BDMS, CCR, NCI
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  - Georgetown: Lou Wiener, Claudine Isaacs
  - Hitachi Chemical: Masato Mitsuhashi