Cancer Vaccines

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Disclosures

I hold equity in BioNTech, and receive research funding from Pharmacyclics
Hallmarks of T lymphocytes

- Immunotherapy
  - CARs
  - TCRs

- Killing ability
- Specificity
- Expansion
- Mobility
- Helper ability
- Memory

- TILs
- Vaccines
- Checkpoint Blockers
- Cancer
- Infections
- Suicide Genes

- Immune-gene therapy
Principles underlying immunotherapeutics

• **Taking advantage of existing spontaneous T cell responses**
  • Adoptive transfer of TILs
  • Checkpoint inhibitors
  • Oncolytic viruses
  • Cancer vaccines

• **Engineering new responses**
  • Antibody therapy
  • CAR-Ts
  • Oncolytic viruses
  • Cancer vaccines
JENNER 1798
Cowpox immunization prevents smallpox

PASTEUR 1870s
Inactivated pathogen
A patient with round cell sarcoma of the jaw and abdominal metastases seen by Coley in 1899. a | Photograph after 63 injections with Coley’s toxins; tumour had diminished to about half its original size. b | Photograph after further treatment with Coley’s toxins. In his 1910 lecture at the Royal Society of Medicine Coley reported that the patient was still alive and well. Images reproduced, with permission, from Ref. 17 © (1910) Royal Society of Medicine.
What is the challenge?
1. The evolutionary capacity of cancer: fuel for therapeutic resistance

Lazarian, Guieze & Wu 2015
2. How do we address tumor-immune co-evolution?
3. What about tumors without pre-existing immunity?

Pre-existing Immunity (20-30%)  Non-functional immune response  Excluded infiltrate  Immune desert

Current immunotherapies work best for patients with a pre-existing anti-tumor immune response

Challenge: driving T cells/immune cells into tumors

- Insufficient priming
- Absence of antigens or dysfunctional antigen presentation leading to immunologic ignorance
-Suppressive soluble factors or inhibitory immune cell populations leading to immune tolerance
- Vascular factors, chemokines or ECM conditions posing barriers to migration of T cells into tumor
What can vaccines do?
Vaccines: an opportunity to expand tumor-directed T cell responses

Pathogens | Prophylactic/Preventive vaccines
---|---
Cancer | Therapeutic vaccines

Hu Ott Wu Nature Reviews Immunology 2018
Vaccines: an opportunity to expand tumor-directed T cell responses

Hu Ott Wu Nature Reviews Immunology 2018
What are the critical components of a vaccine?
Vaccines: a bridge to cure....?
## Diverse choices

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Adjuvant</th>
<th>Formulation and delivery</th>
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<tbody>
<tr>
<td><strong>Tumor-associated</strong></td>
<td><strong>Cytokines</strong></td>
<td><strong>Peptide/protein</strong></td>
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<tr>
<td>Cancer-testis</td>
<td>GM-CSF</td>
<td>DNA</td>
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<tr>
<td>Oncofetal</td>
<td>IL-2</td>
<td>mRNA</td>
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<td>Tissue differentiation</td>
<td>Poly ICLC</td>
<td><strong>Nucleic acid-based</strong></td>
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<tr>
<td>Overexpressed</td>
<td>MPL</td>
<td>DNA</td>
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<tr>
<td>Oncogenic viral</td>
<td>CpG ODN</td>
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<td><strong>Tumor-specific</strong></td>
<td><strong>TLR agonists</strong></td>
<td><strong>Cell-based</strong></td>
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<tr>
<td>Neoantigens</td>
<td>Poly ICLC</td>
<td>Whole tumor cell</td>
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<tr>
<td></td>
<td>MPL</td>
<td>Ag-loaded DC</td>
</tr>
<tr>
<td></td>
<td>CpG ODN</td>
<td>DC-targeting antibody</td>
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<td></td>
<td>DC205</td>
<td>Peptide/protein</td>
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<td></td>
<td>Agonistic αCD40</td>
<td>Nucleic acid-based</td>
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<td><strong>Tetanus/diphtheria toxoid</strong></td>
<td>DNA</td>
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**Emulsions**
- Montanide ISA 51, 720

**Saponin-based**
- ISCOMATRIX
- QS-21

**Liposomes**

**Virosomes**

**Nanoparticles**
So many choices
So little time!
Classes of tumor antigens

Overexpressed Ag, shared

- Tissue-specific expression
  - Melanomas

- Overexpression
  - Some tumours

Melanocytes

Other normal cells

b Antigens: low tumour specificity

No protein
Classes of tumor antigens

Neoantigens
- **Antigens: high tumor specificity**
  - Mutation
  - Most tumors

CT antigens
- **Antigens: low tumor specificity**
  - Tumour-specific expression
  - Many tumors
  - Tissue-specific expression
  - Melanomas

Overexpressed Ag, shared
- Overexpression
- Some tumors

Differentiation between Neoantigens and CT antigens:
- Neoantigens are typically associated with mutations, high tumor specificity, and expression in most tumors.
- CT antigens have low tumor specificity, expressed in many tumors, and specific to tissue types like melanomas.
- Overexpressed Ags are shared by some tumors and can be overexpressed in normal cells.

Cellular level distinctions:
- Normal cells lack HLA.
- Neoantigens involve demethylation leading to the expression of proteins specific to spermatocytes, spermatogonia, and trophoblasts.
- CT antigens also involve demethylation, but these proteins are not expressed in normal cells.
- Overexpressed Ags may be shared with melanocytes and other normal cells.
Somatic mutations have the potential to generate neoantigens.
Somatic mutations have the potential to generate neoantigens.
Classes of mutations that can generate potential tumor neoepitopes

**Missense**

LMPKHFIR (parental)
LMPKLFIR (Mutated)

**Splice-site**

Exon A  TGA  Exon B

**Frame-shift**

Deletion or insertion

**Read-through**

**Gene fusion**

Potential neoORFs
Support for neoantigens as effective tumor rejection antigens

- Neoantigen load associated with better clinical outcome
  - TCGA
  - Colorectal cancer
  - Following CPB therapy

- Neoantigen-specific T cells are expanded in settings of effective antitumor immunity
  - After HSCT
  - After TILs
  - After CPB

- Neoantigen-specific CTLs kill tumor cells in vitro and in vivo
  - In mice
  - In humans

• Enhance the magnitude, breadth and durability of the immune response
  • Alum (1920s) → incorporated in hep B, diptheria, tetanus pertussis and HPV vaccines
  • Since 1990s 4 others with licensure

• Modulation of the innate immune system to activate adaptive immunity
  • Through pattern recognition receptors (PRRs)
    • 1990s: TLRs, via pathogen-associated molecular patterns (PAMPs) → activation of DCs
    • Other innate PRRs RIG-I/other RNA sensors, DNA sensors (i.e. STING), C-type lectins, NOD-like receptors (NLRs) and cytosolic receptors (NLRP3-activates the inflammasome)

  • DCs can be also activated through stress signals: pathways of tissue damage, different forms of cell death, and metabolic and nutrient sensors → Release of damage-associated molecular patterns (DAMPs)

• Induction of effective CD8+ T cell responses in humans: requires the optimal adjuvant signaling & sustained presence of antigen
Poly ICLC is a highly effective vaccine adjuvant

- Nucleic acid ligands of TLR/RLRs are effective adjuvants
  - CpG DNA is difficult to obtain for trials
  - dsRNA stimulates several key pathogen sensors

- Stabilization of pIC in a complex with carboxymethylcellulose, poly-lysine and pIC
What studies of cancer vaccines in patients are there?
Cancer immunotherapy: moving beyond current vaccines

Steven A Rosenberg, James C Yang & Nicholas P Restifo

Great progress has been made in the field of tumor immunology in the past decade, but optimism about the clinical application of currently available cancer vaccine approaches is based more on surrogate endpoints than on clinical tumor regression. In our cancer vaccine trials of 440 patients, the objective response rate was low (2.6%), and comparable to the results obtained by others. We consider here results in cancer vaccine trials and highlight alternate strategies that mediate cancer regression in preclinical and clinical models.

We now know the molecular identities of many tumor-associated antigens, and this knowledge has provided a major stimulus for the development of new immunotherapies for the treatment of patients with solid cancers. In the field of cancer immunotherapy, most enthusiasm has been directed at the use of cancer vaccines—active immunizations designed to treat growing tumors. A recent review of dendritic cell vaccines mentioned 98 published studies involving over 12,000 patients. A tabulation in 2003 listed 216 ongoing vaccine clinical trials in cancer patients. These studies were conducted, and others were underway, despite the absence of convincing animal data that cancer patients who achieved clinical responses, many cancer vaccine trials have been optimistically reported because surrogate or subjective endpoints were achieved. Sensitive techniques such as tetramer or ELISPOT assays have been used to demonstrate the generation in vivo of antitumor T cells in vaccinated patients, but the scarcity of clinical responses in these patients has made it difficult to validate any of these assays as a useful surrogate of clinical response.

Analysis of trials using standard oncologic criteria

Standard oncologic criteria for evaluating and reporting objective clinical responses to treatment are well established in oncology, and adherence to these guidelines is essential in comparing the results of treatment protocols. A set of criteria proposed recently is the Response Evaluation Criteria in Solid Tumors (RECIST): a 30% reduction in the sum of the maximum diameters of lesions to indicate a response, along with the appearance of no new or progressive lesions. The most commonly used definition of objective clinical response, however, is at least a 50% reduction in the sum of the products of the perpendicular diameters of all lesions without the 25% growth of any lesion or the appearance of new lesions. The latter definition has been used in our analysis of our own protocols as well as

Pitfalls of the single antigen-targeting vaccine
Antigen-specific vaccination: the opportunity to focus the response and broaden the T cell repertoire

• Less impressive….
  • Single immunogen vaccine studies

• Glimmers of success!
  • Whole tumor cell vaccines
  • Improved delivery
    • Long peptides (HPV)
    • Dendritic cell-based vaccines (DC fusion, Provenge)
Whole tumor cell vaccines

- Complex vaccines—many antigens available to stimulate B and T cell responses
- Potential to be a ‘personal’ vaccine
- Potentially poorer expression of any one ag
- Low-ish activity
Sipuleucel: “DC-based” vaccine

Final product cell composition reflects the major components of the immune system

Day 0
Apheresis
Buoyant Density Separations

Day 2
Ex Vivo Culture
Wash
Resuspend Cells in Lactated Ringer’s Solution

Final Product

Patient cells are incubated with PA2024, a fusion protein of prostatic acid phosphatase (PAP) and GM-CSF
Survival, %

Time Since Randomization, Months

Sipuleucel-T (n=341)
Control (n=171)

4.1-month survival benefit

Reduction in risk of death:
22.5%

HR=0.775
(95% CI: 0.614, 0.979)
P=.032

21.7 months
25.8 months

Hacohen CIR (2013)
• Safe, feasible
• Highly immunogenic
• Promising combination with CPB

Sahin Nature (2017)
Carreno Science (2015)


Adjuvant setting

Tissue procurement
Antigenic target selection
Preparation of personalized vaccine
Therapeutic immunization

- SLPs
- Poly-ICLC
- Up to 20 targets, separate pools
Table 1. Summary of Neoantigen Vaccines

<table>
<thead>
<tr>
<th></th>
<th>Ott et al. [4]</th>
<th>Sahin et al. [3]</th>
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</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>Vaccine</td>
<td>Synthetic peptide+ poly IC:LC</td>
<td>RNA</td>
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<tr>
<td>Administration route</td>
<td>Subcutaneous</td>
<td>Intranodal</td>
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<tr>
<td>Epitope length</td>
<td>15–30 aa</td>
<td>27 aa</td>
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<tr>
<td>No. of epitopes/patient</td>
<td>13–20</td>
<td>10</td>
</tr>
<tr>
<td>No. of doses</td>
<td>7</td>
<td>8–20</td>
</tr>
<tr>
<td>Immunogenicity (total no. peptides tested)</td>
<td>91 peptides</td>
<td>125 epitopes</td>
</tr>
<tr>
<td>CD8+ T cell response ratea</td>
<td>16%</td>
<td>25%</td>
</tr>
<tr>
<td>CD4+ T cell response rateb</td>
<td>60%</td>
<td>66%</td>
</tr>
</tbody>
</table>

aEx vivo manufactured and pulsed with synthetic peptides.
bImmune response rate to MHC class I or class II epitopes (per vaccine trial).

Linette & Carreno Trends in Molecular Medicine (2017)
Melanoma Neovax: Enduring CRs after Neovax + α-PD-1

Durable and encouraging long-term responses

Generation of memory response

T cell diversification & persistence

Epitope spreading

Hu Leet & Allesoe Nat Med (2021); Ott PA Cell 2020
Tracking of NeoAg T cells to the site of tumor after vax

Keskin et al. *Nature (2019)*

**GBM**

Pt 7: GBM infiltrating lymphocytes

- 37 singles
- 63 singles
- 101 singles

Neoantigen-reactive T cells from peripheral blood

- CD8 (n = 125)
- CD4 (n = 240)

**Met melanoma**

Hu Leet & Allesoe. *Nat Med (2021)*

Pt. 6

- Recurrent tumor, bulk TCR sequencing
- Tetramer + scTCR-seq derived clonotypes

- Mutant
- Wildtype
- OVA

**Graphs**

- ARHGAP35 peptide (pg/ml)
- IL-2 (pg/ml)
# Ongoing Clinical Trials Testing Neoantigen Targeted Vaccines

<table>
<thead>
<tr>
<th>Vaccine (format)</th>
<th>Number of neoantigens included</th>
<th>Neoantigen discovery platform</th>
<th>Adjunct and/or delivery system</th>
<th>Study phase</th>
<th>Tumor types</th>
<th>Treatment approach</th>
<th>ClinicalTrials.gov identifier (Ref Id)</th>
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<tbody>
<tr>
<td>NeoVax (GLP)</td>
<td>P=20</td>
<td>Broad Institute/EPCC</td>
<td>Poly-ICCL</td>
<td>Phase I</td>
<td>Melanoma, RCC, NSCLC, BCC, or unclassified carcinomas</td>
<td>NeoVax plus locally administered adjuvant (Modified CTA/4 antibody)</td>
<td>NCT019050216</td>
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<tr>
<td>GEM-001 (GLP)</td>
<td>4=20</td>
<td>ATLAS</td>
<td>Poly-ICCL</td>
<td>Phase I</td>
<td>Melanoma, RCC, NSCLC, BCC, or unclassified carcinomas</td>
<td>GEM-001 alone for patients with no evidence of disease after completion of cycles of treatment with nivolumab or pembrolizumab (anti-PD-1 antibody) for those with unresectable advanced stage tumors</td>
<td>NCT00633830</td>
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<tr>
<td>PENVax (GLP)</td>
<td>Up to 10</td>
<td>Personalized genomics vaccine pipeline</td>
<td>Poly-ICCL</td>
<td>Phase I</td>
<td>Melanoma, RCC, NSCLC, BCC, or unclassified carcinomas</td>
<td>PENVax alone</td>
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<td>AutoSynVax (HSV)</td>
<td>Up to 24</td>
<td>ARN, QS-21 Stimulus</td>
<td>AutoSynVax alone</td>
<td>Phase I</td>
<td>Advanced stage solid tumors</td>
<td>AutoSynVax alone</td>
<td>NCT00900277</td>
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<td>ROTI0457</td>
<td>Up to 20</td>
<td>Not disclosed</td>
<td>NA</td>
<td>Phase I</td>
<td>Advanced stage solid tumors, most commonly NSCLC, TNEC, renal cell, and CRC</td>
<td>ROTI0457 alone or with stereotactic radiosurgery (anti-PD-1 antibody)</td>
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<td>Randomized phase I</td>
<td>cDNA positive, randomized phase III</td>
<td>NSCLC</td>
<td>NCT01267237</td>
<td>Phase I</td>
<td>Advanced stage renal cell carcinoma, after adjuvant or chemotherapeutic</td>
<td>cDNA positive or stereotactic radiosurgery (anti-PD-1 antibody)</td>
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<td>Randomized phase I</td>
<td>Advanced stage melanoma (treatment naïve)</td>
<td>NSCLC</td>
<td>NCT00351558</td>
<td>Phase I</td>
<td>Advanced stage melanoma (treatment naïve)</td>
<td>cDNA positive or stereotactic radiosurgery (anti-PD-1 antibody)</td>
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<tbody>
<tr>
<td>VB10.NEO (plasmid/DNA)</td>
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<td>NeoSELECT</td>
<td>Pharmacologic System</td>
<td>Phase I</td>
<td>Advanced stage RCC, NSCLC, melanoma or NSCLC without a complete response to SGC immune checkpoint therapy</td>
<td>VB10.NEO plus stereotactic radiosurgery (anti-PD-1 antibody)</td>
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<td>ONO-8043 (plasmid/DNA)</td>
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<td>Newly-diagnosed MPMI, gastrointestinal carcinomas</td>
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<tr>
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<td>NA</td>
<td>Phase I</td>
<td>Edge</td>
<td>NA</td>
<td>ONO-8043 alone</td>
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<td>mRNA-4157 (plasmid/DNA)</td>
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<td>Interleukin-2 delivery system (IL-2) or interferon</td>
<td>Randomized phase I</td>
<td>Advanced stage solid tumors</td>
<td>mRNA-4157 alone for patients with unresectable tumors or with pembrolizumab for those with unresectable tumors</td>
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<td>mRNA-4157 (plasmid/DNA)</td>
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Blass, Ott Nature Rev Clin Oncol, 2021
Composite results across studies….

CRs with α-PD-1 post long peptide Vax in Melanoma

Decreased Recurrences post RNA Vax in Melanoma

Epitope Spreading post long-peptide Vax in mel, NSCLC, bladder Ca

Tracking of T cells to site of tumor

Randomized open label phase 2
Ongoing bedside to bench and back again efforts

Disease-specific research

- Tumour biopsy
  - Non-malignant tissue (peripheral blood)
- Somatic mutation identification and HLA typing
- Identification of immunogenic epitopes
  + Confirm expression of mutated genes

Target discovery → Vaccine formulation and administration → Immune assessment

ELISpot

- Number of SFCs
  - Weeks after vaccination

T cells
  - Wild type
  - Mutated epitope
  - Control

scRNA-seq
  - Population 1
  - Population 2
  - Population 3
  - Population 4
  - Population 5

Building new capabilities

Blass, Ott Nature Rev Clin Oncol, 2021