Potent Immunity Achieved by Targeted, Sequential Administration of Recombinant DNA Vectors and Anchor-Modified Epitope Peptides

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- **Diabetes**
  - Late stage development

- **Oncology**
  - Early stage development
Plasmid Vectors: a Typical Case of Yin & Yang

Features
- Co-expression of Tc and Th epitopes
- CpG motifs

Response
- T1 immune profile
- Limited magnitude
Targeted Intra-Lymph Node Delivery

Imaging the inguinal lymph node

Insertion of a needle into a superficial LN

Imaging of draining lymph nodes subsequent to administration of radio-labeled tracer

70 MBq 99mTc-HIG bds 4h p.i.

Intralymphatic Injection
70mBq 99mTc Labelled Human Immunoglobulin

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Immune Reactivity to a Tumor Associated Antigen Correlates with the Clinical Outlook

0.5 – 1.5 mg of pSEM plasmid / infusion

Time to progression versus immune reactivity analysis
(baseline and/or post-treatment)

Ratio of SD patients / total within group

Time to progression (days)

STRATA:

- group=Non-Responder
- Censored group=Non-Responder
- group=Responder
- Censored group=Responder

Responder: patient whose largest tetramer value is >0.1
Non-Responder: patient whose largest tetramer value is <0.1

P=0.0296
Two Mutually Exclusive Possibilities

- TAA immunity is mechanistically relevant
  - The immunization methodology needs improvement

- TAA immunity is largely an epiphenomenon
Optimization of Active Immunotherapeutic Strategies in Development

Preclinical R&D

Proof of concept, exploratory trials

Efficacy trials
Building on the Immune Response Initiated by Plasmid DNA: Preclinical Data

- DNA only
- Peptide only
- DNA priming, Peptide boost

Melan A tetramer percent

Pre final boost | Post final boost

2w

 naïve Controls  
N=5

Plasmid alone  
N=20

Peptide alone  
N=20

Plasmid / Peptide  
N=20
Robust Expansion of T Cell Immunity against Self or Non-Self Epitopes by Targeted, Lymph Node Delivery of Peptide Analogueadapteres

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Tetramer</th>
</tr>
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<tbody>
<tr>
<td>Melan A 26-35</td>
<td>Naïve 0%</td>
</tr>
<tr>
<td>SSX2 41-49</td>
<td>Naïve 0.2%</td>
</tr>
<tr>
<td>NYESO1 157-165</td>
<td>Naïve 0.1%</td>
</tr>
<tr>
<td>PRAME 425-433</td>
<td>Naïve 0%</td>
</tr>
<tr>
<td>PSMA 288-297</td>
<td>Naïve 0.1%</td>
</tr>
<tr>
<td>Tyrosinase 369-377</td>
<td>Naïve 0.1%</td>
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</tbody>
</table>

CD8
A Novel Immunotherapeutic Approach: Features

- **Multi-component**
  - Plasmids and peptide analogues
  - Peptides are anchor-modified

- **Multivalent**
  - Co-targets
    - Cancer cells
    - Neovasculature

- **Targeted Approach**
  - Lymph node delivery
  - ‘Theranostic’ strategy
Prospective Immunization Protocol

- Expression profiling
  - MHC
  - Antigens

- Diagnosis
- Enrollment

Treatment cycles

- Induction Phase
- Maintenance Phase
A Multivalent Immunotherapeutic Candidate for Melanoma

Plasmids (expressed portion)

- MKC1207-D1
  - "Melan A"
  - "Tyrosinase"

- MKC1207-D2
  - "NYESO-1"
  - "SSX2"

Peptides

- MKC1207-P1
  - 27Nva
  - 377Nva
- MKC1207-P2
  - 165V
- MKC1207-P3
  - 158Nva
- MKC1207-P4
  - 42V

Targets

- Melan A
- Tyrosinase
- NYESO-1
- SSX2
A Multivalent Immunotherapeutic Candidate for Melanoma

HHD1 transgenic mice

2w

Plasmids

Peptides

% Tetramer+ CD8 T cells

Naïve controls

(N=5)

Plasmid alone

(N=10)

Plasmid / Peptide

(N=50)

Post boost

Pre boost

Post boost

Pre boost

Melan A tetramer
Tyrosinase tetramer
SSX2 tetramer
NY-ESO tetramer

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A Multivalent Immunotherapeutic Candidate for Ovarian Carcinoma

Plasmids (expressed portion)

Peptides

Targets

PSMA

PRAME

NYESO-1

SSX2

MKC1106-D1

MKC1106-P1

MKC1106-P2

MKC1106-P3

MKC1106-P4

MKC1106-D2

PRAME

PSMA

NYESO-1

SSX2

297V

433Nle

426Nva

58Nva

165V

42V
A Multivalent Immunotherapeutic Candidate for Ovarian Carcinoma

**PSMA**

<table>
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<tr>
<th>% Cytotoxicity</th>
<th>100:1</th>
<th>30:1</th>
<th>10:1</th>
<th>3:1</th>
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<tbody>
<tr>
<td>624.38</td>
<td>624.28</td>
<td>LNCap</td>
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**NYESO-1**

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<tbody>
<tr>
<td>Control</td>
<td>Peptide boost</td>
<td>L158NvaC165V</td>
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**PRAME**

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<th>E:T 30</th>
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<tr>
<td>624.28</td>
<td>IFN treated</td>
<td>IFN treated</td>
<td>T2+SSX2 41-49</td>
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**SSX2**

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<td>T2</td>
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Directions

- Preclinical development
- Proof of principle in exploratory trials
  - Safety, immunity, relationship with clinical outcome
- Optimization
  - Composition
  - Combinatorial approach
    - Approved therapeutics
    - Novel therapeutic candidates
- Efficacy trials
## Acknowledgements

<table>
<thead>
<tr>
<th>Kent Smith</th>
<th>Zhiyong Qiu</th>
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<td>Brenna Meisenburg</td>
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