In situ targeting of antigen presenting cells within secondary lymphoid organs as a means to control immune responses

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MannKind’s Active Immunotherapy Approach

- **Method of immunization**
  - Plasmid-prime, peptide-boost

- **Route of administration**
  - Intralymphatic targeted delivery of actives
    - Independent control of signal 1 and 2
Model Actives: pSEM and Melan A\textsubscript{26-35} (ELAGIGILTV)

- **1**: CMV Enhancer/promoter
- **2**: Melan A minigene
- **3**: BGH polyadenylation region
- **4**: kanamycin resistance gene
- **5**: PMB Origin of Replication
- **6**: ISS
- **7**: Nuclear Import Sequence

**pSEM**

- **3315 bp**

**Melan A 26-35**

**ELAGIGILTV**

**MLLA VLYCL**

<table>
<thead>
<tr>
<th>Elagigiltv</th>
<th>YMDGTMSQV</th>
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<tbody>
<tr>
<td>Melan A 26-35A27L</td>
<td>Tyr 369-377</td>
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Melan A 31-96

**Tyr 1-9**

**Tyr 31GILT……CEPV\textsuperscript{96}**

Tyr 369-377

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Preclinical model: HHD-1 mice (Human HLA-A*0201 transgenic H-2D^b/- β2m/- double knockout mice)

HHD construct designed:
Hβ2μ-Hα1-Hα2-mα3-mTM-mcyt

Construct injected into C57Bl/6 x SJL oocytes

Mice breed on H-2D^b/-X β2μ/- background

Express A*0201 on somatic and BM cells

CTL repertoire selection on A*0201

Mount A*0201-restricted CTL responses

MHC Tetramers for the *ex vivo* quantification of cellular immune response

MHC complex

CD8

PE

19.5% Melan A Tet+

100
101
102
103
104

 ELAGIGILTV

MHC Tetramer

MART-1 (Beckman Coulter)
More effective induction of immune responses by intra-lymphatic immunization

*Targeting the lymph node yielded the most robust immune responses

N=12 per Group
More effective induction of immune responses by alternating plasmid (priming) with peptide (boost)

N=12 per Group

Completion of Immunization

Peptide Boost

Weeks Following Immunization
Refinement of the prime-boost regimen to maximize the expansion of antigen specific T cells

**Graph A**
- Priming: #391: 1.4 +/- 0.7%
- Post peptide boost: #360: 8.0 +/- 2.3%
- Animal Number: #332, #284, #399, #369

**Graph B**
- Priming: #241: 8.0 +/- 2.3%
- Post peptide boost: #370: 21.8 +/- 3.2%
- Animal Number: #331, #399, #389

**Graph C**
- Priming: #392: 21.8 +/- 3.2%
- Post peptide boost: #379: 1.4 +/- 0.7%
- Animal Number: #400, #266, #371

*Immunization schedules: Plasmid DNA (D), Peptide (P)*
Enhanced response seen in lymphoid and non-lymphoid organs correlates with efficacy

Control  DNA/DNA/Peptide

Blood

LN

Spleen

Lung

Total Lymphocyte Melan A Teraemer (%)
Clearance of human tumor cells in animals immunized by DNA priming – peptide boost

In vivo cytotoxicity

Control
- 0% TET=2%

DNA/DNA/DNA
- TET=11%

DNA/Pept/Pept
- TET=31%

DNA/DNA/Pept
- TET=83%

CFSE
Mechanism of Action Studies

Characterization of the phenotypic profile and functional status of the T cell response induced by plasmid-priming and peptide-boost
Phenotypic analysis of Melan A\textsubscript{26-35} specific CD8\textsuperscript{+} T cells before and after \textit{ex vivo} stimulation
Functional specific T cells rapidly acquired the expression of CD107a and IFN-\(\gamma\) following Melan A tetramer stimulation.

A lower stimulating threshold for CD107a expression was observed following limiting dilution of the Melan A tetramer (right panel).
Control of the immune response by targeted lymph node delivery

pSEM plasmid and/or Melan A 26-35 peptide (intra lymph node admin)

Pro-inflammatory cytokines

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<tr>
<th>pg/ml</th>
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<tr>
<td>IFN-γ</td>
</tr>
<tr>
<td>Peptide / DNA</td>
</tr>
<tr>
<td>DNA / DNA</td>
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<td>DNA / Peptide</td>
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Chemokines

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<thead>
<tr>
<th>pg/ml</th>
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<tbody>
<tr>
<td>RANTES</td>
</tr>
<tr>
<td>Peptide / Peptide</td>
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<tr>
<td>DNA / DNA</td>
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<td>DNA / Peptide</td>
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Immune regulatory cytokines

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<tr>
<th>pg/ml</th>
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<tbody>
<tr>
<td>TGF-β</td>
</tr>
<tr>
<td>Peptide / Peptide</td>
</tr>
<tr>
<td>DNA / DNA</td>
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<td>DNA / Peptide</td>
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Dichotomy between T cell profile elicited by DNA priming or peptide priming
Proposed mechanism of action of plasmid versus peptide priming

Priming with DNA and boosting with peptide achieves substantially higher immune responses (Th1), and is associated with an increase in the rate of responders and the magnitude of response.
Summary

- Targeted delivery of plasmid primes a long lasting population of central and peripheral memory cells, with significant expansion capability.
- Effector cells resulting from plasmid prime – peptide boost display a complex functional profile.
- Immune signatures of DNA versus peptide are substantially different.
  - Independent control of signal 1 and 2 in context of lymph node targeting may allow effective manipulation of the magnitude and profile of immune response.
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Liz Lantzy
Enhanced immunity following two therapeutic cycles: Rational for clinical protocol

One Immunization Cycle

Two Immunization Cycles

Naive Controls

DNA/DNA/DNA Group 1

DNA/PT/PT Group 2

DNA/DNA/PT Group 3

N=12 per Group

Melan A specific tetramer percent
Luminex multiplex cytokine analysis of sorted CD62L negative cells from Melan A\textsubscript{26-35} immunized animals

**Presort**

- CD62L
- 55% Melan A tetramer

**Post sort**

- CD62L
- 99.5% Melan A tetramer

**Cytokine production (pg/ml)**

- GM-CSF
- IFN-\(\gamma\)
- IL-12
- IL-2
- IL-4
- IL-6
- MIP-1\(\alpha\)
- RANTES
- TNF-\(\alpha\)
CD62L negative population of Melan A immunized animals identified from CD8+ lymphocytes

Animals immunized with 4 injections of pSEM plasmid (1mg/mL) or 4 injections of Melan A (A27L) peptide analogue (1mg/mL). CD62L negative population analysis performed on CD8+ and Melan A+/CD8+ populations.

A student’s t-test value of 4.74 was determined from the Melan A tetramer+/CD8+ population comparing the pSEM immunized group and the ELA peptide immunized group.
MannKinds Active Immunotherapy Approach

- **Route of administration**
  - Intralymphatic vs. subcutaneous injection

- **Method of immunization**
  - Optimized protocol in transgenic mice

- **Mechanism of Action**
  - Antigen specific effector cells

- **Conclusions**