Pulmonary Macrophage Transplantation Therapy

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Outline

- Background
  - Hereditary pulmonary alveolar proteinosis (hPAP)
  - Validated model – $\text{Csf2rb}^{-/-}$ mice (KO)
- Pulmonary macrophage transplantation (PMT) therapy
  - Overview of approach
  - Therapeutic efficacy
  - Cell localization, engraftment, differentiation
  - Safety
  - WT versus gene-corrected macrophages
  - Nonclinical data status
  - Clinical trial plan
Hereditary pulmonary alveolar proteinosis (hPAP)

Hereditary PAP is a rare disease characterized by accumulation of surfactant in alveolar macrophages and pulmonary alveoli causing restrictive lung impairment, and progressive hypoxemic respiratory failure.

Disease is caused by mutations in the genes encoding the GM-CSF receptor α or β chains (CSF2RA, CSF2RB).

GM-CSF is required for alveolar surfactant clearance.

GM-CSF regulates cholesterol clearance by macrophages constitutively and in reversible fashion.

Surfactant catabolism is not impaired PAP, but clearance is reduced secondary to cholesterol congestion.
CSF2RA/B mutations known to cause hPAP

α Chain (CSF2RA)
- A17G + G196R
- G196R
- 920dupGC
- R217X
- Q170X
- ΔEx7
- ΔEx7-8
- XpΔ0.4
- XpΔ1.6, i(Xq)^Houston

β–Chain (CSF2RB)
- S271L
- 631delC

Mouse GM-CSF-Rβ^KO (ΔEx7)

Adapted from: Suzuki… AJRCCM, 2010
hPAP Pathology Reproduced in iPS Cell-Derived Macrophages

Blood
- hPAP
- Normal

iPS cells

CSF2RA lentivirus

Gene – corrected MΦs

Surfactant Exposure

Oil-red-O stain (neutral lipid)

Surfactant exposure

Normal | hPAP | hPAP + gene Rx

Before

0 hr after

24 hr after

Suzuki... *AJRCCM*. 189: 183-193; 2014
Serum GM-CSF Autoantibody ELISA Test

Optimal cutoff by ROC curve analysis

Sensitivity & specificity = 100% based on ROC analysis

Adapted from Uchida et al. J, Immunologic Methods. 402: 57-70; 2014
Routine ELISA performed using serum
Used to screen for GM-CSF receptor dysfunction

GM-CSF Signaling pSTAT5-Max Identifies Hereditary PAP
Current Therapy: Whole lung lavage

Before

After (5 days)
PMT therapy of hereditary PAP

Defective macrophage due to CSF2RA mutations
Gene-corrected macrophage

CSF2RB
GM-CSF

Pulmonary alveolus
Blood
HSPC
Csf2rb−/- (KO) mice recapitulate human hPAP

Preclinical evaluation of PMT therapy of hPAP

Expansion

Macrophage differentiation

Endotracheal administration of macrophages into lungs

HPSC’s
- WT
- KO; gene-corrected

Csf2rb−/− (KO) mouse

Lung histology 1 year after PMT

WT    KO    KO + PMT

PAS

SP-B

PMT using WT or gene-corrected KO macrophages have similar therapeutic efficacy

PMT corrects secondary polycythemia

PMT improves survival

Suzuki... *Nature*, 2014.
Effect of cell dose on therapeutic efficacy of PMT

Efficacy results summary (Pre-clinical)

• Effector cell - AMs, mature BMDMs, and progenitors have Rx efficacy
• Cell doses $\geq 5 \times 10^5$ => similar efficacy
• $10^5$ cells – Rx effective at two months
• Cell dose/time to Rx effect = constant
• Strong cell survival advantage drives Rx
• Preclinical studies with clinical vector in patient cells completed/successful

Trapnell, Pre-Pre-IND information package
Transplanted macrophages remain in the lungs

**a**

<table>
<thead>
<tr>
<th>MWM</th>
<th>WT Lung</th>
<th>Lys-M^{GFP+} Lung</th>
<th>Vector copy number</th>
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</thead>
<tbody>
<tr>
<td>KO + PMT (1 month)</td>
<td>Lung</td>
<td>Blood</td>
<td>BM</td>
</tr>
<tr>
<td>KO + PMT (1 year)</td>
<td>Lung</td>
<td>Blood</td>
<td>BM</td>
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</tbody>
</table>

- EGFP
- Lys-M

**b**

Vector copy number

<table>
<thead>
<tr>
<th>Lung</th>
<th>Blood</th>
<th>BM</th>
<th>Spleen</th>
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<tbody>
<tr>
<td>ND</td>
<td>ND</td>
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</tbody>
</table>

Transplanted macrophages localize to alveoli

PMT: Lys-M\textsuperscript{GFP} Knock-in BMDMs

KO - untreated
KO + PMT

Alveolar
Interstial

% PMT - derived cells

Suzuki... *Nature*, 2014.
Transplanted macrophages engraft efficiently

GM-CSF regulates alveolar macrophage population size via a reciprocal feedback mechanism.

Suzuki... *Nature*, 2014.
Importance of pulmonary GM-CSF concentration

GM-CSF Absent
Transplanted macrophages adopt a normal phenotype

Suzuki... *Nature*, 2014.
Alveolar macrophage transcriptome 1 year after PMT

1- Pearson correlation coefficient

-3.0 No change +3.0

Suzuki... *Nature*, 2014.
Results of preclinical safety studies

- PMT using WT or KO gene-corrected macrophages into KO mice:
  - No behavioral changes
  - No cellular inflammation in the lungs
  - No pro-inflammatory cytokine increase in the lungs (TNFα, IL-1β, IL-6)
  - No changes in baseline hematologic parameters
  - Reduction in PAP biomarkers: M-CSF, GM-CSF, MCP-1
  - Doses of 5,000,000 cells/mouse were safe and well-tolerated providing a 10-fold safety margin for cell dose

## Estimating the cell dose for PMT in humans

<table>
<thead>
<tr>
<th>Calculation method</th>
<th>Human cell dose</th>
<th>% AM #</th>
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<tbody>
<tr>
<td>Alveolar number (relative) ¶</td>
<td>0.03 x 10e9</td>
<td>0.5%</td>
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<tr>
<td>Body weight (relative) ¶</td>
<td>1.1 x 10e9</td>
<td>14%</td>
</tr>
<tr>
<td>AM number (relative) ¶</td>
<td>1.1 x 10e9</td>
<td>19%</td>
</tr>
<tr>
<td>Alveolar surface area (relative) ¶</td>
<td>4.4 x 10e9</td>
<td>78%</td>
</tr>
<tr>
<td>AM number (absolute) §</td>
<td>5.6 x 10e9</td>
<td>100%</td>
</tr>
</tbody>
</table>

- Based on values for corresponding mouse and human parameters
  - 500,000 cells/mouse gives good/maximum efficacy at 1 year
  - 100,000 cells/mouse gives detectable therapeutic efficacy at 2 months
  - 4 x 10e6 cells/mouse was well-tolerated and safe without adverse events

¶ Calculated from corresponding mouse and human parameter
§ Calculate from the absolute human parameter
Translating PMT therapy to humans with hPAP

<table>
<thead>
<tr>
<th>Dose No.</th>
<th>Dose (40 kg human)</th>
<th>Safety margin</th>
<th>Delivery site</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>$12 \times 10^6$</td>
<td>293</td>
<td>1 segment</td>
</tr>
<tr>
<td>2</td>
<td>$24 \times 10^6$</td>
<td>146</td>
<td>1 segment</td>
</tr>
<tr>
<td>3</td>
<td>$400 \times 10^6$</td>
<td>8.8</td>
<td>All remaining segments</td>
</tr>
</tbody>
</table>

![Diagram showing observation, treatment, short-term follow-up, and long-term follow-up with visits labeled V1 to V23, and BAL PMT-1, BAL PMT-2, BAL PMT-3, BAL?, and BAL at various time points.](image)
Conclusions

• Pulmonary GM-CSF:
  • Regulates alveolar macrophage population size, surfactant homeostasis, alveolar stability, and lung function
  • GM-CSF is a critical pulmonary hormone

• Pulmonary macrophage transplantation (preclinical):
  • Myeloablation not required
  • Safe and well-tolerated
  • High therapeutic efficacy
  • Durable treatment effect (>1 year)
  • Prolongs lifespan by 20%
  • A strong survival advantage drives efficacy
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