Aerosol delivery, but not intramuscular injection, of adenovirus-vectored tuberculosis vaccine induces respiratory-mucosal immunity in humans

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Conflict of Interest

• License Agreement (#L011-050) of Technology Transfer to CanSino Biotech Inc. of China “Recombinant human type 5 adenovirus-based TB vaccine (AdHuAg85A) and its preparation and use for clinical trials” (Inventors Z. Xing, F Smaill, M. Medina, et al). Filing Date: 2011/07/22
Immunology & Pre-clinical vaccine development

AdHu5Ag85A –
preclinical & clinical development
for respiratory mucosal applications

- murine studies
  - guinea pig (TAMU)
  - murine studies
    - i.m/i.n (Canada)

- guinea pig (TAMU)
  - i.m/i.aerosol (Canada)

- bovine studies (VLA)
  - i.m (Spain)
  - i.d/e.b (UK)

- goat studies (CReSA)
  - i.m (Spain)

- humanized mouse
  - i.m/i.n (Canada)
  - i.m/i.t/aerosol (Canada)

- primate studies (W. Univ)
  - i.m/i.n (Canada)

- human phase 1
  - i.m (Canada)
  - aerosol (Canada)

- human phase 1
  - aerosol (Canada)

AdHu5 backbone

ΔE1  E2  ΔE3  E4

CMV

Ag85A

PolyA
Respiratory mucosal delivery of AdHu5Ag85A by AeroNeb® nebulizer

AeroNeb® Solo
Single Patient Use Vibrating Mesh Nebulizer
Assembled inhaled aerosol delivery system

- Loading volume: 0.5 ml
- Delivery time: 2 min
- Loading vs. emitted dose: 50%
- Droplet size: 85% <5.39 µm
- Viability of aerosolized vaccine: 20%

Aerosol particle size distribution:
- Salbutamol + vaccine vs. salbutamol (red)

Targeting 15-20 generations of small airways
Anti-TB vaccine strategies

**Phase 1b Aerosol Trial Design**

32 BCG+ volunteers (18-55 yrs)

32 BCG+ volunteers (18-55 yrs)

Inhaled aerosol or i.m (10^8 pfu)

AdHu5Ag85A vaccine (1x10^6 or 2x10^6 pfu dose-escalation)

Baseline tests

Ag-specific blood/BAL PBMC
Anti-AdHu5 Ab
Blood hematology/chemistry

Week 16

Week 24

Consent
BCG history
HIV antibody
QFT test*
Chest x-ray
Spirometry
DLCO

Safety evaluation
Spirometry
Blood/PBMC assays

*BronchoAlveolar Lavage

*QFT – Quantiferon TB test used to screen out latent TB
83 ineligible
Lost interest after 1st screening: 58
Not BCG vaccinated: 10
PPD test done within a year: 5
Smoker: 4
Asthmatic: 3
Declined oral contraceptives: 2
QuantiFERON test positive: 1

4 excluded
Withdrew consent: 2
Unable to comply with study visit requirements: 2

32 received intervention

11 assigned to Aerosol 10^6 PFU (LD)
12 assigned to Aerosol 2x10^6 PFU (HD)
09 assigned to IM 10^6 PFU

11 received vaccine
12 received vaccine
09 received vaccine

11 included in intention-to-treat analysis
11 included in intention-to-treat analysis
09 included in intention-to-treat analysis

1 discontinued due to COVID-19 restrictions
## ADVERSE EVENTS

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Low dose (n = 11)</th>
<th>%</th>
<th>High dose (n = 12)</th>
<th>%</th>
<th>i.m. (n = 9)</th>
<th>%</th>
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<tbody>
<tr>
<td>Injection site reaction</td>
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<td>Local pain</td>
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<td>16.6</td>
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<td>Shortness of breath</td>
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<td>Dizziness</td>
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Induction of multifunctional CD4+ T cells in the airways following aerosol (LD and HD) or intramuscular (IM) vaccination

Data in dot plots are expressed as the mean value (horizontal line) with 95% CI. Box plots show mean value (horizontal line) with 95% CI (whiskers), and boxes extend from the 25th to 75th percentiles. Wilcoxon matched pairs signed-rank test was used to compare various time points with baseline values within the same vaccination group.
Frequencies of airway antigen–specific combined total cytokine–producing CD8\(^+\) T cells at various time points in LD aerosol, HD aerosol, and i.m. cohorts.
Frequencies of airway polyfunctional (triple/3+, double/2+, and single/1+ cytokine+) antigen-specific CD4+ T cells at various time points in LD aerosol group.

Pie chart of median proportions of antigen-specific airway CD4+ and CD8+ T cells expressing a specific single or combination of 2 or 3 cytokines at various time points in LD aerosol group.
Frequencies of airway antigen–specific IFN-γ+ CD4+ TRM co-expressing CD69 and CD103 surface markers at various time points in LD aerosol, HD aerosol, and i.m. vaccine cohorts.

Data in dot plots are expressed as the mean value (horizontal line) with 95% CI. Wilcoxon matched pairs signed-rank test was used to compare various time points with baseline values within the same vaccination group.

Frequencies of airway antigen–specific IFN-γ+ CD8+ TRM co-expressing CD69 and CD103 at various time points in LD aerosol, HD aerosol, and i.m. vaccine cohorts.
Transcriptomic analysis of alveolar macrophages following low dose aerosol vaccination

Group 1 – Week 0 (unstimulated)
Group 2 - Week 8 (unstimulated)
Group 3 – Week 0 (stimulated)
Group 4 – Week 8 (stimulated)
Induction of antigen-specific T cell responses in the peripheral blood following aerosol or intramuscular vaccination.

Antigen-specific cytokine production in whole blood culture at various time points after LD aerosol, HD aerosol, and i.m. vaccine groups. The measurements were subtracted from unstimulated control values.
# Anti-Ad5 antibody titres before and after low dose, high dose and intramuscular vaccination

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ad5 humoral immunity</th>
<th>Study time (week)</th>
<th>LD aerosol</th>
<th>HD aerosol</th>
<th>i.m.</th>
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<tr>
<td>Serum</td>
<td>Anti-Ad5 total IgG titre</td>
<td>0</td>
<td>11</td>
<td>9513 (700, 11,5520)</td>
<td>11</td>
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<tr>
<td></td>
<td></td>
<td>4</td>
<td>11</td>
<td>4974 (611, 326,600)</td>
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<td></td>
<td>Ad5 neutralizing antibody titre</td>
<td>0</td>
<td>11</td>
<td>22 (2, 7937)</td>
<td>11</td>
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<td></td>
<td></td>
<td>4</td>
<td>11</td>
<td>32 (3, 7692)</td>
<td>11</td>
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<tr>
<td>BALF</td>
<td>Anti-Ad5 total IgG titre</td>
<td>0</td>
<td>11</td>
<td>469 (222, 2855)</td>
<td>11</td>
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<tr>
<td></td>
<td></td>
<td>8</td>
<td>10</td>
<td>502 (115, 1188)</td>
<td>3</td>
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<tr>
<td></td>
<td>Ad5 neutralizing antibody titre</td>
<td>0</td>
<td>11</td>
<td>2.13 (1, 55)</td>
<td>11</td>
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<tr>
<td></td>
<td></td>
<td>8</td>
<td>10</td>
<td>2.8 (1, 46)</td>
<td>3</td>
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</tbody>
</table>

*P values obtained by comparing 4-week time point values with baseline values within the same vaccination group using Wilcoxon matched-pairs signed-rank test. Units of measure within table are as follows: Anti-Ad5 total IgG titre: arbitrary units/mL. Ad5 neutralizing antibody titre: reciprocal value of the serum dilution corresponding to 50% of inhibition in the frequency of GFP* cells.
Respiratory mucosal immunity

Polyfunctional tissue resident memory T cells (T_{RM}), and trained innate immunity

Systemic immunity

Inhaled aerosol viral-vectored vaccine

Intramuscularly-injected viral-vectored vaccine