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The Medicines Company Ltd
*Tufts University
**LipoScience LLC
<table>
<thead>
<tr>
<th>Disclosure potential conflicts of interest</th>
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</thead>
<tbody>
<tr>
<td><strong>Research contracts:</strong></td>
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<tr>
<td><strong>Consulting:</strong></td>
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<tr>
<td>The Medicines Company (Schweiz) GmbH:</td>
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<td>Herman Kempen</td>
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<td><strong>Employment in industry:</strong></td>
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<td>The Medicines Company:</td>
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<td>David Kallend, Eralp Bellibas, Peter Wijngaard</td>
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<td><strong>Stockholder of a healthcare company:</strong></td>
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<td><strong>Other:</strong></td>
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Phase I study MDCO-216

- Subjects were enrolled and dosed in single dose cohorts as described in the Figure
- Infusion of MDCO-216 or saline for 2 h.
- Blood sampling at baseline, 0.5, 2, 4, 8, 24, 48, 168 and 720 h
- Prior to escalation to the next dose all available data was reviewed by a Safety Review Committee and the decision taken whether to escalate to the next dose.
- Following the completion of the 5 cohorts of healthy volunteers, 4 cohorts of patients with stable CAD were enrolled.
# Pharmacodynamic parameters measured

<table>
<thead>
<tr>
<th>Lipid Profile</th>
<th>Cholesterol efflux capacities</th>
<th>Lipoprotein analysis</th>
<th>HDL subfractions</th>
</tr>
</thead>
<tbody>
<tr>
<td>(AMC-Clin-chem)</td>
<td>(Vascular Strategies)</td>
<td>(Calabresi lab/LipoScience)</td>
<td>(Asztalos Lab/Pacbio)</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td><strong>Basal (J774)</strong></td>
<td>FPLC-TC/FC/TG/PL</td>
<td><strong>2D-PAGGE</strong> reacted</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td><strong>ABCA1 (J774 +cAMP)</strong></td>
<td>FPLC-ApoAI/ApoA-IM/ ApoB</td>
<td>for total apoA-I or apoA-IMilano</td>
</tr>
<tr>
<td>Triglycerides</td>
<td><strong>ABCG1 (BHK)</strong></td>
<td>CER activity</td>
<td></td>
</tr>
<tr>
<td>Phospholipids</td>
<td><strong>SRB1 (Fu5AH)</strong></td>
<td>Lipoprotein particle numbers and sizes</td>
<td>Prebeta-1 HDL ELISA</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td></td>
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<tr>
<td>LDL-cholesterol</td>
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<tr>
<td>Apo A-I (total)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Apo A-II</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Apo B</td>
<td></td>
<td></td>
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<tr>
<td>Apo E</td>
<td></td>
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</tbody>
</table>
Increase in ABCA1 mediated cholesterol efflux capacity after infusion of MDCO-216

Delta ABCA1 in Volunteers

Delta ABCA1 in CAD patients

(Baseline: 4 - 8 %/4h)
Increase in basal cholesterol efflux capacity after infusion of MDCO-216

Delta basal in Volunteers

- 5 mg/kg
- 10 mg/kg
- 20 mg/kg
- 30 mg/kg
- 40 mg/kg

% /4h

0 0,5 2 4 8 24 168

h after start of infusion

Delta basal CAD pats

- 5 mg/kg
- 10 mg/kg
- 20 mg/kg
- 30 mg/kg
- 40 mg/kg

% /4h

0 0,5 2 4 8 24 168

h after start of infusion

(Baseline: ~7 %/4h)
Increase in SRB1 and ABCG1-mediated cholesterol efflux capacity after infusion of MDCO-216.

(Baseline: ~5%/4h)

(Baseline: 6-8%/4h)
Changes in HDL-subfractions after 40 mg/kg MDCO-216 2D-PAGGE of whole plasma; membranes were reacted for total ApoA-I

- \(\alpha-1\)
- \(\alpha-2\)
- \(\alpha-3\)
- \(\alpha-4\)
- prebeta-1
Changes in HDL-subfractions after 40 mg/kg MDCO-216
2D-PAGE of whole plasma; membranes were reacted for
ApoA-I Milano

- α-1
- α-2
- α-3
- α-4
- prebeta-1
Changes in HDL subfractions (2D PAGGE) after infusion of 40 mg/kg MDCO-216 (volunteers)
Change in prebeta-1 HDL (ELISA) after infusion of MDCO-216
(Sandwich ELISA using Dai-ichi Sekisui kit)

(Baseline: 42-84 ug/ml)
Changes in HDL particle concentrations (1H-NMR) after infusion of MDCO-216 in volunteers.
Changes in HDL diameter (1H-NMR) after infusion of MDCO-216 in volunteers.

(Baseline: 9.0 nm)
Conclusions 1

1. Rapid and very pronounced increase in ABCA1-mediated chol efflux
   • peak at 2-4 h, back to baseline at 24 h except after high dose
2. Smaller increase in basal, SRB1-mediated and ABCG1-mediated effluxes
   • Peak at 4-8 h, nearly back to baseline at 24 h
3. Rapid and pronounced increase in prebeta-1 HDL, containing only „wild-type“ apoA-I, no apoA-IMilano
   • peak at 2 h, back to baseline at 24 h at all doses
4. Rapid loss of smaller (α-3 and α-4) HDL and rise of apoA-IMilano in α-1 and α-2 HDL
   • Peak/trough at 4-8 h, back to baseline at 24 h-48 h
5. Rapid increase in HDL-size (shift from small to medium-sized HDL)
   • Peak at 2-4 h, back to or below baseline at 24 h
6. No increase in total HDL particle concentration
**Hypothetical initial events:**
MDCO-216 fuses with small HDL, generating novel alpha-1 and alpha-2 HDL containing apoA-IMilano and displacing endogenous apoA-I to become prebeta-1 HDL (this happens also in vitro upon incubation with plasma: Kempen et al, J Lipids 2014)

- **α-1** with both A-I WT and A-IMilano. Long half-life
- **α-2** with both A-I WT and A-IMilano. Long half life
- Rapid decrease of α-3
- Rapid decrease of endogenous α-4
- Rapid increase in free apoA-I (prebeta-1 HDL). Short half-life.
Question: which changes in HDL subfractions „explain“ the increase in basal and ABCA1 mediated cholesterol efflux capacities?

![Table 2: Univariate and multivariate regression analyses of ABCA1-mediated cholesterol efflux from J774 macrophages on apoA-I-containing HDL subpopulations](image)

- **Univariate Analysis**
  - **Subpopulation**: Preβ-1, Preβ-2, α-1, α-2, α-3, Preα-1, Preα-2, Preα-3
  - **Variables**:
    - **B**: Regression coefficient
    - **se(B)**: Standard error of the regression coefficient
    - **t Value**: Student's t-value
    - **P Value**: Level of significance

  **Results**:
  - **Preβ-1**: B = 0.0617, se(B) = 0.0174, t Value = 3.54, P Value = 0.0006
  - **Preβ-2**: B = 0.1039, se(B) = 0.0987, t Value = 1.05, P Value = 0.2953
  - **α-1**: B = 0.0157, se(B) = 0.0115, t Value = 1.36, P Value = 0.1758
  - **α-2**: B = 0.0359, se(B) = 0.0125, t Value = 2.88, P Value = 0.0048
  - **α-3**: B = 0.0068, se(B) = 0.0101, t Value = 0.67, P Value = 0.5050
  - **Preα-1**: B = 0.0185, se(B) = 0.0300, t Value = 0.62, P Value = 0.5384
  - **Preα-2**: B = 0.0044, se(B) = 0.0427, t Value = 0.10, P Value = 0.9190
  - **Preα-3**: B = -0.0620, se(B) = 0.0777, t Value = -0.80, P Value = 0.4265

- **Multivariate Analysis**
  - **Variables**:
    - **B**: Regression coefficient
    - **se(B)**: Standard error of the regression coefficient
    - **t Value**: Student's t-value
    - **P Value**: Level of significance

  **Results**:
  - **Preβ-1**: B = 0.0686, se(B) = 0.0218, t Value = 3.15, P Value = 0.0022
  - **Preβ-2**: B = 0.0229, se(B) = 0.1145, t Value = 0.20, P Value = 0.8422
  - **α-1**: B = -0.0131, se(B) = 0.0274, t Value = -0.48, P Value = 0.6341
  - **α-2**: B = 0.0402, se(B) = 0.0200, t Value = 2.01, P Value = 0.0477
  - **α-3**: B = -0.0188, se(B) = 0.0173, t Value = -1.08, P Value = 0.2810
  - **Preα-1**: B = 0.0703, se(B) = 0.0736, t Value = 0.95, P Value = 0.3420
  - **Preα-2**: B = -0.0818, se(B) = 0.0931, t Value = -0.88, P Value = 0.3815
  - **Preα-3**: B = 0.0484, se(B) = 0.0947, t Value = 0.51, P Value = 0.6103

B, regression coefficient; se(B), standard error (B); t, t-test [B/se(B)].
„Response“: Area Under the Effect Curve for 24 h after start of infusion, calculated for each subject and each parameter

Example: increase in ABCA1-mediated efflux above baseline after 30 mg/kg MDCO-216 in subject 153

AUEC0-24 = Sum (areas A-E)
Dose-response curves for basal and ABCA1-mediated efflux

Response basal efflux

- CAD pts
- HV

Response ABCA1 efflux

- CAD pts
- HV

(dose (mg/kg))

(means +/- SEM)
Dose-response curves for HDL-subfractions (total apoA-I after 2D-PAGGE)

- **AUC0-24 prebeta-1 2D**
  - CAD pts
  - HV

- **AUC0-24 prebeta-1 ELISA**
  - CAD pts
  - HV

- **AUC0-24 alpha-1**
  - CAD pts
  - HV

- **AUC0-24 alpha-2**
  - CAD pts
  - HV

- **AUC0-24 alpha-3**
  - CAD pts
  - HV

- **AUC0-24 alpha-4**
  - CAD pts
  - HV

*Note: AUC0-24* indicates the area under the curve from 0 to 24 hours.
Dose-response curves for HDL-subfraction particle concentrations (1H-NMR)

AUEC0-24 small HDL

AUEC0-24 medium HDL

AUEC0-24 large HDL

- CAD pts  - HV

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Conclusions 2

- Responses for basal and ABCA1-mediated cholesterol efflux keep increasing nearly linearly over the tested dose-range.

- Responses for HDL subfractions reach plateau, except response for prebeta-1 HDL ELISA and α-1 HDL in volunteers which were linear.

- Responses in apoA-I in α-1 and α-2 HDL correspond with response in medium-sized, not large-sized HDL (may explain lack of effect on ABCG1-mediated efflux).
Correlation of responses for basal and ABCA1-mediated cholesterol efflux with responses for HDL-subfractions (n=32)

Yellow: significant at p<0.05

<table>
<thead>
<tr>
<th>Response in:</th>
<th>basal efflux</th>
<th>ABCA1 mediated efflux</th>
</tr>
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<tbody>
<tr>
<td>Preβ-1 Elisa</td>
<td>0.72</td>
<td>0.74</td>
</tr>
<tr>
<td>Preβ-1 2D</td>
<td>NS</td>
<td>0.47</td>
</tr>
<tr>
<td>α-1 2D</td>
<td>0.68</td>
<td>0.76</td>
</tr>
<tr>
<td>α-2 2D</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>α-3 2D</td>
<td>-0.41</td>
<td>NS</td>
</tr>
<tr>
<td>α-4 2D</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>large HDL-P</td>
<td>0.55</td>
<td>NS</td>
</tr>
<tr>
<td>medium HDL-P</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>small HDL-P</td>
<td>-0.53</td>
<td>-0.55</td>
</tr>
</tbody>
</table>
Response of ABCA1-efflux correlates strongly with response of preβ-1 HDL and α-1 HDL.

$r=0.74$

**ABCA1 vs preβ-1**

$r=0.76$

**ABCA1 vs α-1**
## Multiple regressions for basal and ABCA1-mediated efflux responses

### Response in basal efflux

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>SE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>7.57</td>
<td>3.59</td>
<td>0.043</td>
</tr>
<tr>
<td>α-1</td>
<td>0.043</td>
<td>0.01</td>
<td>0.008</td>
</tr>
<tr>
<td>Preβ-1 (E)</td>
<td>0.0075</td>
<td>0.0021</td>
<td>0.001</td>
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### Response in ABCA1 mediated efflux

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>SE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>13.2</td>
<td>15.4</td>
<td>0.39805</td>
</tr>
<tr>
<td>α-1</td>
<td>0.27</td>
<td>0.06</td>
<td>0.0002</td>
</tr>
<tr>
<td>Preβ-1 (E)</td>
<td>0.033</td>
<td>0.009</td>
<td>0.0008</td>
</tr>
</tbody>
</table>
Conclusions 3

• Responses of basal and ABCA1 mediated effluxes correlate positively with responses of preβ-1 HDL and α-1 HDL, and inversely with that of small HDL

• In multiple regression both preβ-1HDL and α-1 HDL responses remain significantly related with basal and ABCA1-mediated efflux responses

• α-1 HDL loaded with apoA-I Milano may drive cholesterol effluxes even when preβ-1 HDL is back to baseline
More on this Phase I study:

- Xchange Session 15 “HDL functionality”: Monday 14:45 - 16:30 (presentation by C Sirtori)
- Clinical Breakthroughs: modifying lipids, recent developments Tuesday 14.59 – 16.29 (presentations by SE Bellibas and D Kallend)

- **Further Q&A**: Poster Session 6.4: “Therapy” Tuesday from 13.45 - 17.00 (posters by Bellibas, Kallend, Kempen)
Acknowledgements

• Joannes Reijers and Matthijs Moerland, Center of Human Drug Research, Leiden, NL
• Heidi Collins, Vascular Strategies LLC, Plymouth Meeting, USA
• Monica Gomaraschi, Univ. Milan, Italy