Exploratory immune assays distinguish healthy volunteer from CF patient cohorts and were validated in a dose escalation study of eluforsen (QR-010) in subjects with cystic fibrosis homozygous for the F508del CFTR mutation

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Methods

Serum ligand-binding assays (LBAs) for chitinase-3-like protein 1 (CHI3L1/YKL-40) and human epididymis protein 4 (HE-4), as well as sputum LBAs for TIMP-1 (all R&D system) and SPLUNC1 (MyBioSource) were successfully set up and optimized with respect to intra- and inter-assay variation, recovery, linearity and sensitivity (LOD); the assay characteristics are summarized in the table on the right. Optimization included a 0.08% DTT pre-treatment incubation at 37°C for 1h for the SPLUNC1 assay. All assays were qualified in a pilot study with samples from CF and non-CF individuals, and subsequently validated in a multi-centre, randomized, double-blind, placebo-controlled dose escalating study of inhaled eluforsen (PQ-010-001). A panel of sputum inflammatory markers were measured using qualified high-sensitivity multiplex ELISA. Results from immune measurements were correlated with patient baseline characteristics, and PQ-010-001 outcome measures.

Assay Development

Assay characteristics - Optimization and qualification using UCL pilot cohort

<table>
<thead>
<tr>
<th>Intra-assay variability</th>
<th>YKL-40 serum</th>
<th>HE-4 serum</th>
<th>SPLUNC1 sputum</th>
<th>TIMP-1 sputum</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2%</td>
<td>2.3%</td>
<td>4.3%</td>
<td>3.1%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inter-assay variability</th>
<th>YKL-40 serum: 7.8%</th>
<th>HE-4 serum: 13.1%</th>
<th>SPLUNC1 sputum: 12.1%</th>
<th>TIMP-1 sputum: 10.9%</th>
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<tr>
<th>Recovery</th>
<th>YKL-40: 96 - 102%</th>
<th>HE-4: 98 - 104%</th>
<th>SPLUNC1: 94.3 - 116.4%</th>
<th>TIMP-1: 93 - 105%</th>
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<tr>
<th>Linearity</th>
<th>YKL-40: 125 - 4 000 pg/mL</th>
<th>HE-4: 71.8 - 5000 pg/mL</th>
<th>SPLUNC1: 3.12 - 100 ng/mL</th>
<th>TIMP-1: 0.313 - 10 ng/mL</th>
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<tr>
<th>Sensitivity</th>
<th>YKL-40: 8.15 pg/mL</th>
<th>HE-4: 32.2 pg/mL</th>
<th>SPLUNC1: 1 ng/mL</th>
<th>TIMP-1: &lt;0.08 pg/mL</th>
</tr>
</thead>
</table>

Sputum biomarkers - Selected immune markers

- NE
- MMP-9
- IL1β
- IL-5
- IL-6
- IL-8
- IL-10
- IL-12p70
- TNFα
- IFNg
- VEGF
- MCP-1
- G-CSF
- MIP-1a
- MIP-1b
- RANTES

For more information on these sputum biomarkers, please turn to poster.
Take home message:

Pilot study UCL: assays distinguished non-CF (HC) from CF cohorts.

Clinical study PQ-010-001: baseline (screening and check in) of the 4 analytes across subjects were not significantly different from the pilot CF cohort:

- YKL-40, HE-4 and TIMP-1 were significantly increased, and
- SPLUNC1 was significantly decreased compared to healthy controls.
**Conclusions**

- Assays to measure YKL-40 and HE-4 in serum as well as SPLUNC1 and TIMP-1 in sputum were successfully optimized, qualified in a pilot study and validated in a clinical study of eluforsen.
- YKL-40 and HE-4 biomarkers are significantly elevated in CF patients compared to non-CF donors; HE-4 distinguished exceptionally well between non-CF and CF populations.
- Overall, sputum biomarker SPLUNC1 was decreased and TIMP-1 was increased in CF patients compared to non-CF donors; however, sputum sample availability in the PQ-010-001 study was low. Induced sputum collection may help to obtain more consistent collection.
- Other known sputum immune inflammatory markers were successfully measured and showed high response variability at baseline (data on poster).
- A trend towards decreased serum analyte concentrations was observed in some patients receiving QR-010, but overall, response variability was high. Further longitudinal studies to establish the marker development over time will help to dissect true biomarker signals from ‘noise’.

**Acknowledgements**

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