

human nasal brushings and human and sheep lung slices. In the mouse nose and lung, the preferred configuration directed up to x500-fold higher transgene expression than the non-viral platform, for the lifetime of the animal. Transgene expression was observed in 14.1% of lung epithelial cells ($p < 0.0001$ compared to controls). Repeated monthly administration (3X) was possible without loss of expression or significant histological inflammatory reactivity. Reassuringly, insertion site profiling from transduced cell lines and mouse nose/lung samples reveals a pattern of integration comparable to those reported for other lentiviral vectors in clinical development, with no evidence for enrichment of insertion at undesirable loci. The stability of rSIV. F/HN vectors was evaluated in two bronchoscope catheters and two aerosol generation devices. Encouragingly for clinical translation, no significant loss of transduction activity was noted with any of these clinically relevant delivery devices ($p = 0.64$). Delivery of rSIV. F/HN expressing CFTR to sheep lung resulted in CFTR mRNA at ~30% the levels of endogenous ovine CFTR ($p < 0.0001$ compared to non-treated lobes), exceeding presumed therapeutic levels. With the majority of translational hurdles addressed, we are now entering toxicology studies and the final stages of pharmaceutical development prior to entering clinical trials.

P204 IMMUNE RESPONSES TO SINGLE AND REPEATED ADMINISTRATION OF PGM169/GL67A: THE UK CF GENE THERAPY CONSORTIUM CLINICAL TRIALS

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10.1136/thoraxjnl-2014-206260.333

Although most CF patients express CFTR protein (albeit mutant) and should therefore not recognise the wild-type CFTR protein as foreign, there is an inherent risk of activation of T-cells against the recombinant wild-type protein after gene therapy. In addition, we have previously shown that approximately 10% of CF and non-CF subjects carry self-reactive CFTR-specific T-cells (Calcedo *et al*, Hum Gene Ther Clin Dev 2013). The reason for this is unknown and it is also unclear whether being positive for self-reactive T-cells affects disease severity or increases the risk of further T-cell activation after gene therapy.

As part of the UKCFGTC Phase I/IIa Pilot study [in which patients received a single dose (5, 10 or 20 mls) of the non-viral formulation pGM169/GL67A] peripheral blood mononuclear cells (PBMC) were collected prior to dosing and approximately 4 weeks after nebulisation of 5 ml ($n = 2$), 10 ml ($n = 6$) or 20 ml ($n = 17$) of pGM169/GL67A. IFN- γ ELISPOT to detect CFTR-specific T-cells in PBMC was performed. CFTR-specific T-cells were detectable in one patient pre- and post-dosing. In the remaining 18 patients we did not detect CFTR-specific T-cells. In addition we quantified anti-DNA antibodies (antinuclear and anti-cytoplasmic) in blood samples taken pre- and approximately 4 weeks post-dosing ($n = 7$ (5 ml), $n = 10$ (10 ml) and $n = 17$ (20 ml)). We did not observe any evidence for induction of anti-DNA antibodies after a single dose of pGM169/GL67A.

The UKCFGTC has now completed a Phase IIB multi-dose clinical trial in May 2014 (ClinicalTrials.gov identification

number – NCT01621867). CF patients received 12 monthly doses of pGM169/GL67A (115 completed nine or more doses), or placebo by aerosol. PBMC were collected on two occasions prior to dose 1 to establish baseline levels for CFTR-specific T-cells, approximately 4 weeks after Dose 4 or 5, and 2 to 4 weeks after Dose 12 and the ELISPOT was performed. In addition anti-DNA antibodies were quantified. The Phase IIB trial will be unblinded in Summer 2014 to allow data analysis and all data will be presented at the conference.

Funded by the NIHR/EME Programme and the Cystic Fibrosis Trust.

Lung function testing: new approaches

P205 MULTIPLE BREATH WASHOUTS IN CHILDREN CAN BE SIGNIFICANTLY SHORTENED WITHOUT COMPROMISING MEASUREMENT QUALITY

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10.1136/thoraxjnl-2014-206260.334

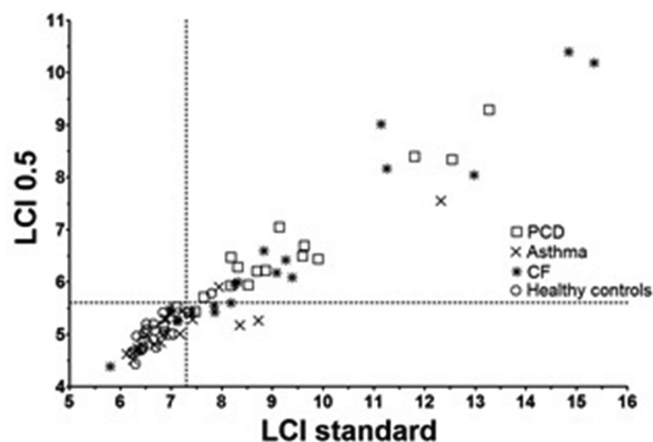
Background Multiple-breath washout (MBW) is used to calculate a measure of ventilation heterogeneity, the lung clearance index (LCI), and requires tidal breathing until a previously inspired tracer gas concentration falls below 1/40th of the initial value, an arbitrary threshold. LCI is usually performed in triplicate, each taking 4–8 min to complete which may be taxing, particularly in young children and those with marked airflow obstruction. Shortened LCI is of interest since a reduction in the test time may increase feasibility and improve the clinical applicability of the measurement.

We hypothesised that LCI measurements could be reliably shortened. We also investigated whether shortened MBW was responsive to an intervention.

Patients and methods We calculated LCI from a fixed time point, and from a fixed number of breaths, as well as LCI and 25% (LCI0.25), 50% (LCI0.5) and 75% (LCI0.75) of 1/40th of the initial concentration of tracer gas (LCIstd) and the time saved, in children aged 6–16 years with asthma ($n = 21$), cystic fibrosis (CF, $n = 20$) and primary ciliary dyskinesia (PCD, $n = 19$), and healthy controls ($n = 17$), aged 3–18 years. Shortened LCI was also calculated in 29 asthmatic children pre and one month post one intra-muscular triamcinolone injection, part of our clinical severe asthma protocol.

Results Calculating shortened LCI from a fixed washout time or breath number was not reliable. However, all shortened LCI measurements from initial gas concentration correlated significantly with LCIstd in each disease group. LCI0.5 presented a balance between correlation with LCIstd (see figure) and time-saving. Mean proportion of time saved per washout, using LCI0.5, was 27% (asthma), 28% (CF) and 31% (PCD). Furthermore, LCI0.5 was significantly reduced after triamcinolone in children with severe asthma (mean LCI0.5 pre, 5.5 and 5.1 post triamcinolone, $p = 0.02$), and the change was similar to that demonstrated using LCIstd (mean LCIstd pre, 7.8 and post 7.0, $p = 0.001$).

Conclusion We show for the first time that LCI measurements can be shortened without loss of information in school-children with asthma, CF and PCD. LCI0.5 was the optimal surrogate measure for LCIstd when proportion of time saved, correlation



Abstract P205 Figure 1 Correlation between LCI0.5 and LCIstd. Shortened MBW, LCI0.5, correlated significantly with LCIstd with r values of 0.84, 0.96 and 0.92 in asthma, CF and PCD groups respectively. The dotted lines indicate the upper limits of normal: LCI0.5 is 5.6 and LCIstd is 7.3

with LCIstd and change following an intervention were considered.

P206 CHANGES IN INDICES DERIVED FROM MULTIBREATH WASHOUT (MBW) FOLLOWING TREATMENT WITH IVACAFTOR IN PATIENTS WITH CYSTIC FIBROSIS

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10.1136/thoraxjnl-2014-206260.335

Background Lung clearance index (LCI) is a measure of gas mixing inhomogeneity derived from multi-breath wash (MBW) out techniques, which has been shown to be more sensitive than conventional spirometry/ FEV₁. The potentiator, Ivacaftor, led to improvement in LCI in patients with mild cystic fibrosis (CF) lung disease however its utility as an outcome measure in more severe disease requires further investigation. Whilst LCI reflects overall ventilation heterogeneity, analysis of the phase III slopes of successive breaths in the MBW; known as Sacin and Scand; are thought to reflect the ventilation heterogeneity generated at branch points in the acinar and conductive lung zones respectively. This study aimed to explore changes in the indices derived from analysis of MBW following a year of treatment with Ivacaftor.

Method A prospective study was performed between March 2013 and April 2014 on patients with the G551D mutation and eligible for clinically prescribed Ivacaftor. MBW (Innocor SF6 technique) and spirometry were performed immediately prior to commencing Ivacaftor, and at a clinic visit following 9–12 months therapy. FEV₁ is calculated using Stanojevic references and all data are expressed as mean (SD). Paired data were analysed with a Wilcoxon rank sum test and correlations with Spearman's rank correlation. The null hypothesis was rejected at $p < 0.05$.

Results 8 patients were enrolled with ages ranging from 6–27 years. FEV₁ increased from 68.7 (17.2)% before treatment to 80.1 (16.3)% after 9–12 months ($p1$ and LCI did not correlate with each other).

Discussion Patients prescribed Ivacaftor demonstrated improvements in both conventional FEV₁ and the newer measure of

LCI; improvement was not limited to patients with milder disease and was seen throughout the group. In this study, the phase III slope measures did not appear to add further value to the LCI. It is possible that this reflects under powering in this small group; further data will be obtained.

P207 RELIABILITY OF MEASUREMENTS USING INNOCOR BREATH BY BREATH ANALYSER DURING A MAXIMAL EXERCISE TEST IN CYSTIC FIBROSIS PATIENTS

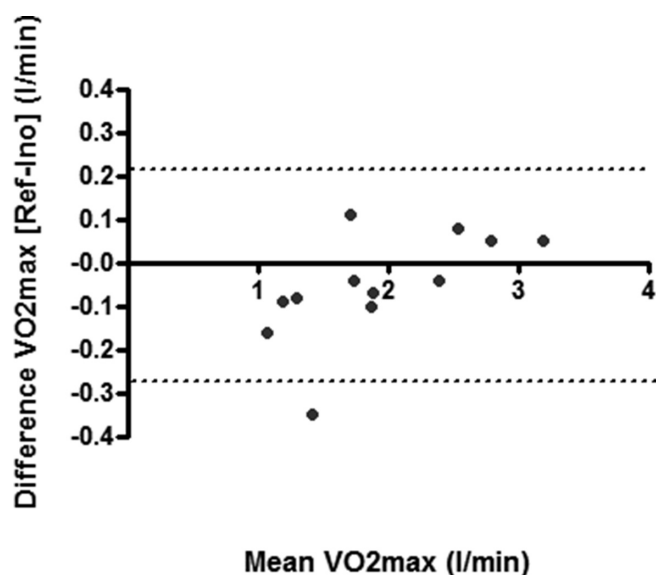
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10.1136/thoraxjnl-2014-206260.336

Introduction Cardiopulmonary exercising testing (CPET) is considered the gold standard to study exercise capacity as an endpoint in clinical trials. Originally the UKCFGTC used the shuttle walk test for exercise capacity measurement but this proved inappropriate for mild, fit cystic fibrosis (CF) patients in our trial cohort (FEV₁ 50–90% predicted). The Innocor device uses photoacoustic gas detection technology and offers metabolic measurement but has not previously been validated for CPET in CF. **Aim** To compare the Innocor with known reliable CPET machines to see if it is suitable to take forward into a multi dose clinical trial of gene therapy.

Methods 12 CF patients (7 Male, 14–47 years) participated in the study recruited from London and Edinburgh sites. They performed two incremental cycle ergometer exercise tests to exhaustion (adapted Godfrey protocol) with breath by breath analysis assessed using a reference system (Jaeger Masterscreen PFT, London; Pulmolink Medisoft, Edinburgh) or the Innocor device. All tests were randomly ordered, completed at least 24 h apart, with no more than two week's separation.

Results VO₂max and V_E max were comparable between the Innocor and reference systems ($p = 0.1790$ and $p = 0.7642$ respectively; paired t tests). For VO₂max, Bland Altman analysis showed the mean difference [Reference equipment-Innocor] was -0.026 l/min and the 95% confidence interval was -0.27 to 0.22 l/min (see Figure). In our experience the Innocor heart rate (HR)



Abstract P207 Figure 1 Bland Altman plot: the difference between VO₂max measured by Innocor vs Reference methods.