Human blood eosinophils were prepared from healthy donors using hetastarch-sedimentation and EasySep® immunomagnetic beads, and cultured in RPMI + 10% autologous serum for 6–24 h. Apoptosis and effectorcytosis were quantified using standard morphology, AnV/PI staining and myeloperoxidase counter-stain methods. Hypoxic incubation (H) (media PO₂ 2.9±0.1 kPa) caused a marked survival response in eosinophils compared to normoxia (N) (% apoptosis at 24 h: N 16.3±3.0%; H 1.2±0.2%; n=5), which was of similar magnitude to that observed with IL-5 (N 4.2±0.3%). This hypoxic survival effect was mimicked by the iron chelator/2-oxo-glutarate analogue DFO (10 mM) and DMOG (1 mM) and blocked in a concentration-dependent manner by the protein synthesis inhibitor cycloheximide. In preliminary experiments hypoxia also independently reduced the capacity of monocyte-derived macrophages to phagocytose apoptotic eosinophils as seen previously with neutrophils. Most strikingly, hypoxic incubation also reduced the normal pro-apoptotic effect of dexamethasone (1 µM) (% apoptosis at 24 h: N + Dex 27.6±3.9%, H + Dex 11.2±2.9%). qPCR analysis of the glucocorticosteroid-dependent gene GILZ and the hypoxia-HIF-1α-dependent gene GLUT1 demonstrated that the above suppression of dex-induced eosinophil apoptosis was not a consequence of the inhibition of steroid-induced transcriptional activity (mRNA fold change at 16 h: GILZ N 12.89±3.6, GILZ H 23.9±9.2; GLUT1 H (control) 28.5±7.2, GLUT1 H (Dex) 45.5±13.5, n=5).

These data suggest that hypoxia can cause a profound effect on eosinophil longevity and macrophage uptake, and render these cells partially resistant to the pro-apoptotic effects of dexamethasone. This may impede the effective resolution of eosinophil inflammation in vivo.

**Rationale**
The asthma susceptibility gene ADAM33/Adam33 is associated with bronchial hyperresponsiveness (BHR) in humans and mice. Soluble ADAM33 is increased in bronchoalveolar lavage-fluid (BALF) of allergic asthma patients (Lee JY et al, AJRCCM 2006 Apr;178(7):729–35). Its levels correlate with declining FEV₁%, suggesting a role in airway remodelling in asthma. Maternal allergy or endogenous IL-13 suppresses Adam33/ADAM33 mRNA expression but enhances ADAM33 protein processing in human embryonic and juvenile mouse lungs (Hatchi HM et al, JACI. 2009 Sep;124(3):590–7, 597). We hypothesise that conditional expression of IL-13 in mouse lungs induces the enzymatically active, soluble form of ADAM33 in BALF, which is associated with BHR.

**Methods**
IL-13 expression was induced using Doxycycline in C57BL/TgOet/Il-13 double-transgenic (dTG) mice. Methacholine challenge and lung function measurements were performed and lungs were harvested for mRNA extraction and immunohistochemistry (IHC). BALF was obtained for Western-blotting for ADAM33 and testing of ADAM33 enzymatic activity using a fluorescence resonance energy transfer (FRET) peptide assay.

**Results**
There was significant increase in BHR to Methacholine in IL-13 expressing double transgenic mice. IHC showed an increase in bronchial smooth muscle in lungs of double transgenic mice. Similar to the RTqPCR findings in human embryonic and juvenile mouse
lungs, IL-13 suppressed Adam33 mRNA but no difference in α-smooth muscle actin (αSma) was evident. Immunoblotting for ADAMA33 in BALF demonstrated a 76kDa band, consistent with the ADAMA33 ectodomain and processed forms at 58/44kDa in dTg animals. ADAMA33 enzymatic activity was also significantly increased.

**Conclusion** The data suggest that allergic inflammation induced by IL-13 suppresses Adam33 mRNA expression but induces the release of soluble forms of ADAMA33, yielding enzymatically active forms. The release of soluble forms may play a role in airway remodelling, potentially leading to BHR. We next propose to test the effect of specific ADAMA33 inhibitors on airway remodelling in this allergic mouse model to assess their potential as novel treatments for asthma.

**Evaluation and treatment of Cystic Fibrosis**

The UK CF GTC has been working for several years to determine the clinical benefit of CFTR gene therapy. Our premise was that for such a therapy to achieve clinical benefit, repeated administration would be required, and that therefore a non-viral approach was needed. We demonstrated in laboratory and preclinical models that GL67A (Genzyme Corp) was the optimal gene transfer agent, and designed a plasmid, pGM169, completely depleted of pro-inflammatory CpG motifs and driven by the novel hCEFI promoter, designed for prolonged expression. In a longitudinal observational study (the Run-in) we measured the variability of multiple outcome measures, both conventional and novel. These data have allowed us to perform power calculations and a) choose our primary outcome (FEV1), b) secondary efficacy outcomes (lung clearance index, various parameters on CT scan, Quality of life questionnaire (CFQ-R), exercise capacity and activity, and selected sputum and serum inflammatory markers), and c) safety measures (clinical findings, exacerbation rate, gas transfer, sputum culture, serum inflammatory markers, renal and hepatic markers). We have recently completed a single-dose safety and dose ranging study.

In this trial, 150 patients, aged 12 years and above were randomised in a 1:1 fashion to active treatment or placebo and will receive the nebulised agent at monthly intervals for 12 doses. The group size was determined on the basis of a 6% relative improvement in FEV1. An adaptive design will be used for additional safety; the first 20 patients will receive 3 doses ahead of the rest of the cohort. Patients will be invited to participate in either one or two substudies, being conducted to explore mechanisms; a) nasal administration followed by nasal potential difference (PD) and brushings for mRNA expression and b) pre- and post-treatment bronchosopies for lower airway PD, gene expression and histology. The double-blinded nature of the trial means that final outcome data will only be available upon completion of the study. The trial was initiated in April 2012; here we will update on recruitment, projected time-lines and progress.

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**Roles of TLR3, TLR4 and TLR7-9 in Interferon Induction in Bronchial Epithelial Cells and Peripheral Blood Mononuclear Cells from Asthmatic and Non-Asthmatic Subjects**

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**Introduction** Defective rhinovirus (RV) induced interferon (IFN)-β and IFN-λ production has been reported in primary human bronchial epithelial cells (HBECs) and peripheral blood mononuclear cells (PBMCs) from asthmatics. The mechanisms of defective IFN induction in asthma are unknown. Virus infection can induce IFNs through Toll like Receptors (TLR)3, TLR4 and TLR7-9 and TLR agonists have been identified as potential therapeutic options for asthma. The role of these TLRs in IFN induction in asthma is unclear.

**Objective** To investigate IFN responses to TLR stimulation in HBECs and PBMCs from atopic asthmatic and non-asthmatic individuals.

**Methods** HBECs and PBMCs from atopic asthmatic and non-asthmatic subjects were stimulated with agonists to TLR3, TLR4 & TLR7-9 and type I and III IFN responses assessed by qPCR and ELISA.

**Results** TLR3 and TLR7, but not TLR4, 8 or 9, stimulation induced IFN protein and mRNA expression in HBECs and PBMCs. IFNs induced were IFN-β and predominantly type III IFN-λ in HBECs and type I (–α and –β) with no IFN-α in PBMCs. TLR function was not defective in asthmatic compared to non-asthmatic subjects.

**Conclusions** TLR3 & TLR7 were the predominant TLRs involved in IFN induction in HBECs and PBMCs. Defective IFN induction to TLR agonists was not observed in these well controlled asthmatic subjects. TLR3/7 agonists could be effective in inducing IFNs in more severe/less well controlled asthmatics who may have deficient virus induced IFN production.