myography. Data were compared by ANOVA. To assess translational implications, umbilical arteries were harvested from one infant diagnosed with presumed CF (meconium ileus with both parents AF508 carriers, genotype pending) and 3 control infants.

**Results:** Compared to aorta from null or wild-type pigs, aorta from AF508 pigs had decreased KCl-induced constriction (null 5.0±0.4g, wild-type 5.1±0.4g, dF/dF 3.8±0.3g, N=11-21, P < 0.01). When aorta from additional litters were incubated for 5 hours in a physiologic saline solution, those from AF508 piglets generated decreased myogenic tone (null 6.2±0.5g, wild-type 5.8±0.4g, dF/dF 3.9±0.6g, N=11-37, P < 0.001), with a greater impairment seen in dF/dF females (3.1±0.6g) then dF/dF males (4.4±0.9g). Combined inhibition of intracellular calcium channels with 2-APB (IP3R antagonist) and ryanodine (RyR antagonist) diminished aortic tone, recapitulating the phenotype seen in dF/dF piglets (null 3.3±0.8g, wild-type 3.5±0.5g, dF/dF 2.6±0.4g, N=4-9, P=0.32). While umbilical arteries obtained from all 3 control humans developed significant myogenic tone 90 minutes after passive stretch and incubation in physiologic buffer (2.4±0.6g), umbilical arteries obtained from the infant with presumed CF did not (-0.8g). Responses of control umbilical arteries were similarly diminished by preincubation in the IP3R antagonist (0.1±1.1g).

**Conclusions:** Aorta from pigs homologous for the common AF508 mutation have decreased tone. We speculate sarcoplasmic reticulum retention of CFTR-AF508 interferes with calcium channel activation, decreases vascular resistance and contributes to the lower arterial pressures seen in those carrying the AF508 mutation.

**190 STRUCTURAL ABNORMALITIES IN THE NEWBORN CF PIG LUNG**

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In newborn CF pigs we have observed tracheal cartilage ring defects, abnormal appearing airway smooth muscle bundles, and reduced tracheal and mainstem bronchi caliber. Additional studies using micro-CT based imaging of the newborn pig tracheal lobe, the porcine equivalent of the human right upper lobe, have found that the percentage of CF airway size differences were found between genotypic groups. The mean linear intercept, an estimate of the volume to surface ratio of acinar airspaces, was 65±5.3 cm3 (p < 0.05), a reduction of approximately twenty percent. A similar trend was observed when we measured total lung volume with CT in spontaneously breathing newborn pigs (81.2 ± 5.3 cm3 vs. 63.8 ± 7.1 cm3 for non-CF and CF, respectively). Following CT scanning, the tracheal lobes were processed for morphological assessment. Mean linear intercept, an estimate of the volume to surface ratio of acinar airspaces, was determined based upon tracheal lobe histology. No statistically significant differences were found between genotypic groups. The mean linear intercept length was 65±5.3 cm3 for non-CF and 60±2.1 cm3 for CF. In summary, in the newborn pig lung we have observed a reduction in caliber of the large airways and in lung size. Additional morphometric studies of alveolar size, number, and surface area will be required to fully understand the extent of these congenital abnormalities and their importance in the pathogenesis of early CF lung disease.

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**NEW THERAPIES, BIOMARKERS & OUTCOME MEASURES**

**192★ PREDICTING SUSTAINED RESPONSE TO BRONCHITOL™ TREATMENT IN PATIENTS WITH CYSTIC FIBROSIS**

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**Introduction:** Cystic fibrosis (CF) causes significant progressive pulmonary disease; identification of predictors of response to long term therapy is important given that CFTR treatment is life-long, onerous, expensive and complex. The efficacy and safety of Bronchitol™ (inhaled dry powder mannitol) has been established in two large phase III trials (CF-310, CF-302). We sought to determine the independent contribution of baseline factors to treatment response from this population, and establish whether early FEV1 response might be useful in predicting longer term impact on lung function.

**Methods:** A retrospective analysis in patients, completing 26 weeks blinded therapy. Responders were defined as patients with a sustained response during the 26-week double-blind treatment period, response was defined as ≥5% relative improvement in FEV1 (% pred) over the treatment period. Stepwise logistic regression was used to determine independently contributing baseline and on-treatment factors. Results from the week 6 on-treatment time-point were explored to assess the sensitivity and specificity of response thresholds to predict a sustained response over 26 weeks.

**Results:** Baseline Predictors: Two baseline factors were significantly associated with response; treatment with Bronchitol™ vs control (p=0.0038) and the baseline rate of %/yr decline in FEV1 (p=0.0068). Sensitivity and specificity tests showed moderate utility but not high enough to be clinically useful. At a cut off of 50%, the sensitivity and specificity for predicting a sustained response were 35% and 83% respectively.

On-treatment Predictors: All thresholds explored provided clinical utility as changes from baseline after 6 weeks were similarly predictive of
response over the whole 26 weeks eg. 41% of patients had ≥6% response; sensitivity was 87% and specificity 83% [Table]. Improvement in % pred FEV1 >0% after 6 weeks of Bronchitol™ was significantly correlated with sustained response over 26 weeks (p=0.0001).

Conclusion: Although baseline rate of decline in FEV1 independently predicts sustained response to Bronchitol™, its inadequate sensitivity excludes too many responders to be clinically useful. While baseline treatment on patient phenotype does not appear to be useful, the utility of a range of response thresholds predicting long term response after 6-weeks of treatment with Bronchitol™ provides clinicians with an approach that improves the certainty of response, minimises unnecessary exposure and risk, and is consistent with an individualised treatment strategy for patients with CF.

Table 1. Sensitivity and specificity of different response thresholds at Week 6 predicting overall response in the Bronchitol ITT population

<table>
<thead>
<tr>
<th>Threshold</th>
<th>% predicted</th>
<th>Wk 0</th>
<th>Wk 6</th>
<th>Utility of week 6 data to predict a treatment effect over weeks 0, 4, 12, 24, 36</th>
<th>Sensitivity%</th>
<th>Specificity%</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥12%</td>
<td></td>
<td>11.2</td>
<td>8.0</td>
<td></td>
<td>78.2</td>
<td>71.5</td>
</tr>
<tr>
<td>≥12%</td>
<td></td>
<td>7.0</td>
<td>7.0</td>
<td></td>
<td>78.0</td>
<td>71.5</td>
</tr>
<tr>
<td>≥14%</td>
<td></td>
<td>8.0</td>
<td>7.0</td>
<td></td>
<td>78.5</td>
<td>71.0</td>
</tr>
<tr>
<td>≥15%</td>
<td></td>
<td>10.5</td>
<td>10.0</td>
<td></td>
<td>78.0</td>
<td>71.5</td>
</tr>
<tr>
<td>≥15%</td>
<td></td>
<td>12.0</td>
<td>11.5</td>
<td></td>
<td>80.5</td>
<td>75.5</td>
</tr>
<tr>
<td>≥15%</td>
<td></td>
<td>15.5</td>
<td>15.0</td>
<td></td>
<td>80.5</td>
<td>80.5</td>
</tr>
</tbody>
</table>

Ataluren enables readthrough of premature termination codons to produce full length, functional CFTR in patients with nonsense mutation CF (nmCF), who comprise ~10% of all patients with CF.

Methods: A Phase 3, randomized, double blind, placebo controlled clinical trial evaluated the efficacy and safety of ataluren in patients with nmCF. Eligible patients aged 6 yrs of age with nmCF and %pred FEV1h between 40-90% were randomized to receive ataluren 10, 10, 20 mg/kg orally TID or placebo for 48 wks. Stratifications included chronic inhaled antibiotic use, age, and %pred FEV1. Primary and secondary endpoints were %pred FEV1 change from baseline at Wk 48 and pulmonary exacerbations (PEX), respectively. Tertiary endpoints included nasal potential difference.

Results: In all, 238 patients were randomized; the intent-to-treat population comprised 232 patients (116 ataluren, 116 placebo). The primary endpoint compared mean relative changes in % pred FEV1h at Wk 48, and demonstrated a treatment difference of 3% favoring ataluren (-2.5% change on ataluren vs. -5.5% change on placebo; p=0.124, primary analysis); the average treatment effect across all post-baseline visits demonstrated a treatment difference of 2.5% favoring ataluren (-1.8% change on ataluren vs. -4.3% for placebo, p=0.048). Over 48 wks, the PEX rate was 23% lower for ataluren versus placebo (p=0.099). Among the a priori stratifications, only the interaction of treatment with chronic inhaled antibiotic use was statistically significant (nominal p=0.007). In patients not being treated with inhaled antibiotics, a 6.7% difference in the relative change in % pred FEV1h at Wk 48 favoring the ataluren treatment group was seen (nominal p=0.015); the average treatment effect was 5.5% in favor of ataluren across all post-baseline visits (nominal p=0.009). Similarly, a 43% lower PEX rate over 48 wks (nominal p=0.012) favoring ataluren was observed in this subgroup. These effects appeared to be driven by patients receiving chronic inhaled tobramycin and concurred with in vitro data which shows that aminoglycosides antagonize ataluren-induced readthrough, suggesting an explanation for the observed subgroup effect. The tertiary endpoint of nasal potential difference did not demonstrate a treatment difference in this study. Safety profiles were similar for ataluren and placebo, other than cases of reversible Grade 3-4 creatinine elevations, which were associated with the combination of nephrotoxic antibiotics with ataluren. Preliminary data from the ongoing open-label extension study are encouraging.

Conclusion: Ataluren treatment over 48 wks resulted in trends towards improved lung function and pulmonary exacerbation rate compared to placebo in patients with nmCF; a larger treatment benefit was observed in the subgroup of patients not receiving chronic inhaled aminoglycoside therapy, commensurate with in vitro data.

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THE SYNTHETIC AMINOGLYCOSIDE NB124 SUPPRESSES CFTR PREMATURE TERMINATION CODONS MORE EFFECTIVELY THAN GENTAMICIN AND PRIOR SYNTHETIC DERIVATIVES

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Certain aminoglycosides induce translational readthrough of premature termination codons (PTCs). However, toxicity and lack of efficacy deter treatment with clinically available aminoglycosides for genetic diseases caused by PTCs, including CF. Previously, our laboratories showed that using a structure-based approach, the novel aminoglycoside derivative NB54 exhibited reduced cellular toxicity while also demonstrating efficacy that equaled that observed with gentamicin in vitro and in vivo. In this study, we provide new evidence that a second generation of aminoglycoside derivatives, NB74, NB84, and NB124, further enhance efficacy and reduce toxicity, resulting in translational readthrough that exceeds gentamicin and other prior aminoglycoside derivatives.

NB derivatives were first screened in a dual luciferase reporter system transiently transfected into CFB4E14o- cells encoding several relevant CFTR PTC contexts. In R162X, NB124 caused dose dependent readthrough of nonsense transcripts, achieving 2.5-fold over control at 100 μM, and exceeding gentamicin (1.4-fold, P<0.0001); other NB derivatives were intermediate. Similar efficacy was observed in W1282X (1.9-fold NB124 over control vs. 1.3-fold gentamicin, P<0.0001) and G542X (1.5-fold NB124 over control vs. 1.1-fold gentamicin, P<0.0001) constructs.

CBE4E14o- cells stably transfected with the R162X transgene demonstrated partial restoration of CFTR-dependent cAMP stimulated current following 48 hr incubation with aminoglycosides at 250 μg/mL. Stimulated Isc was 2.8 μA/cm² in NB124 treated monolayers vs. 0.5 and 0.3 in gentamicin and vehicle control, respectively. Western blotting confirmed translation of full-length CFTR C-band that was superior with NB124 treatment (5.5-fold, P<0.005) vs. vehicle or gentamicin (each 2-fold over control). Similar results were observed in CBE4E14o- transfected with W1282X CFTR, with NB124 providing greater CFTR dependent Isc (15.9 μA/cm²) NB124 vs. 5.2 and 3.9 following vehicle and gentamicin treatment, respectively, P<0.0005). Dose-dependent rescue of CFTR currents were observed in both CBE4E14o-line lines with NB124 treatment, with peak effects at 250 μg/mL. In primary HBE cells expressing G542X/P508del, NB124 treatment rescued forskolin-dependent (20 μM) Isc to 3.1 μA/cm² compared to 1.2 μA/cm² in control treated cells (P<0.01). NB124 treatment improved CFTR dependent Isc activity to ~12% of WT-CFTR and was greater than that observed with gentamicin, NB84, and other derivatives. In toxicity studies using coczcella monolayers, NB124 exhibited 4.3 fold lower LD50 than gentamicin, indicating an efficacy/toxicity ratio at least 15X greater than gentamicin. The synthetic aminoglycoside NB124 significantly enhances readthrough of PTCs and rescues CFTR activity as shown by several complementary assays. The current results support further research efforts to optimize synthetic aminoglycosides for translational readthrough, and could result in agents suitable for long-term treatment of the basic CF defect found in ~10% of CF patients and many other genetic diseases.