

### 77 Potential of denufosal as an early intervention in CF lung disease: efficacy in patients with minimal pharmacotherapy in a US phase 3 clinical trial

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**Introduction:** Early onset of lung disease in CF necessitates early intervention with disease-modifying agents. Denufosal, a novel ion channel regulator, stimulates Cl<sup>-</sup> transport, inhibits Na<sup>+</sup> absorption and increases ciliary beat frequency regardless of CFTR genotype. The potential of denufosal as an early intervention agent was explored in patients (pts) on limited background pharmacotherapy.

**Methods:** Denufosal was studied in pts on 0 to 2 concurrent pharmacotherapies for CF lung disease from a Phase 3, placebo (PL)-controlled double-blind (DB) trial (TIGER-1) in pts on US standard of care  $\geq 5$  yrs with FEV<sub>1</sub>  $\geq 75$  % pred. The 24-wk DB phase was followed by a 24-wk denufosal-only open label extension (OLE).

**Results:** The demographics of PL (n = 34) and denufosal (n = 37) pts with 91% and 93% pred. FEV<sub>1</sub> were similar to intent-to-treat population (ITT). In DB phase, the denufosal treatment resulted in net FEV<sub>1</sub> improvement over PL of 5.7% (p=0.028) or 100 ml (p=0.059) (% change from baseline [CFB] or CFB). The net improvement over PL was 6.4 % (p=0.011) in % pred. FEV<sub>1</sub>. In OLE, the pts on 48 wks of denufosal improved in FEV<sub>1</sub> by 12% (183 ml) and by 4% pred. FEV<sub>1</sub>. These treatment effects were greater than observed in ITT where >50% of pts used at least 4 pharmacotherapies. Denufosal was well tolerated with comparable safety profile to PL.

**Conclusions:** Due to its mechanism of action designed to correct the ion transport defect, denufosal may be an ideal early intervention treatment in CF lung disease. The clinically meaningful improvement in pts on denufosal who are >90% predicted and on limited medications highlights the potential of denufosal as a first line agent in CF pts.

### 78 Aerosol and pharmacokinetic properties of denufosal support its use for early intervention in CF lung disease

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**Introduction:** Early onset of CF disease in small airways calls for disease-modifying therapies that treat small airways and possess a favorable safety profile. Denufosal, an inhaled ion channel regulator, increases Cl<sup>-</sup> transport, inhibits Na<sup>+</sup> absorption and stimulates ciliary beat frequency regardless of CFTR genotype. This work examines denufosal's aerosol and PK properties and their impact on its potential as an early intervention agent.

**Methods:** Denufosal droplet size generated by PARI LC STAR was measured with the Next Generation Impactor and laser diffraction. The systemic exposure was evaluated by measuring denufosal plasma concentrations in a Phase 3, placebo (PL)-controlled double-blind (DB) trial (TIGER-1) in pts  $\geq 5$  yrs with FEV<sub>1</sub>  $\geq 75$  % pred. Efficacy was assessed by pulmonary function tests including FEF<sub>25-75</sub>.

**Results:** Denufosal's median droplet size (3.4 to 3.8  $\mu$ m, 2.4  $\mu$ m MMAD) is suitable for reaching small airways. Statistically significant improvement in FEF<sub>25-75</sub> over PL in patients <110% pred. FEV<sub>1</sub> (p=0.025) supports the *in vitro* data suggesting clinical ability to nebulize denufosal to smaller airways. Following inhalation, there was little to no systemic exposure to denufosal after the first dose, no evidence of accumulation with chronic TID dosing and a favorable safety profile.

**Conclusion:** Denufosal is an investigational treatment designed to correct the ion transport defect in CF lung disease. Denufosal nebulized in LC STAR is suitable for delivery to the smaller airways as supported by FEF<sub>25-75</sub> results. The ability to treat small airways and the lack of systemic exposure make denufosal a promising early intervention therapy for CF lung disease.

### 79 Phase III study of inhaled dry powder mannitol (Bronchitol™) in cystic fibrosis – results from the 6 and 12 month open label phase

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**Background:** Inhaled dry powder Mannitol (DPM) is an osmotic agent that increases water content of the ASL, improves mucociliary clearance and cough clearance [1]. A 6 month-double blind (DB) phase III trial of DPM (400 mg bd), in a broad representative CF population, demonstrated a sustained clinically meaningful FEV<sub>1</sub> improvement (6.5% from baseline) irrespective of concomitant rDNase use [2]. This open label phase (OLP) aimed to further assess safety and efficacy of DPM for 12–18 months.

**Methods:** 198 patients (112 DPM, 86 control) who completed the DB phase were given the option of entering the OLP and receiving DPM at 400 mg bd. The OLP followed patients at week 38, 52, 64 and 78 to assess lung function, safety and review pulmonary exacerbations.

**Results:** 85.9% of patients who completed the DB phase elected to continue into the OLP. Preliminary data showed that patients on DPM maintained the increase in FEV<sub>1</sub> seen in the DB phase throughout the OLP (Table 1). Control group patients had an absolute mean FEV<sub>1</sub> % predicted improvement of 5.4% (p < 0.05) after switching to DPM. The final 12 month OL patient visit (wk 78) will be March 2010 and additional data from this OLP will be presented.

Table 1. Preliminary FEV<sub>1</sub>% predicted OLP results

Group	Start of the first OLP (week 26)	End of the first OLP (week 52)
Mannitol–Mannitol	67.6% (SD±16.3) (n=97)	67.8% (SD±17.3) (n=82)
Control–Mannitol	61.7% (SD±17.0) (n=71)	67.8% (SD±25.5) (n=52)

**Conclusions:** DPM shows sustained improvement in FEV<sub>1</sub>. Control subjects who changed to DPM in the OLP showed a similar improvement in FEV<sub>1</sub> as seen for patients on DPM in the DB phase, further confirming the efficacy and safety of DPM therapy in patients with CF.

#### Reference(s)

- [1] Robinson M, et al. Eur Resp J 1999.  
 [2] Bilton D, et al. Eur Resp J 2009.

### 80 Commercial garlic preparations inhibit quorum sensing *lasB* and *pqsA* gene expression in *Pseudomonas aeruginosa* clinical strains

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**Background:** There are no new antibiotics in development to address infections caused by *Pseudomonas aeruginosa* (PA). PA is an environmental organism that causes chronic destructive lung infection in patients with CF. Quorum sensing (QS), an intercellular signalling mechanism coordinating gene expression has a key role in PA's virulence, resistance and immune system evasion. Inhibiting QS may reduce the virulence of this devastating pathogen.

**Aim:** To determine the *in vitro* efficacy of a garlic supplement upon the inhibition of QS in clinical strains of PA.

**Methods:** A laboratory strain (PA01) and clinical isolates from the sputa of 5 children with CF and chronic pulmonary PA infection were tagged using chromosomal transcriptional *lux*-based fusions (CTX::*lux*) to the QS controlled genes *pqsA* and *lasB*. Isolates were grown in control media or media infused with garlic oil. Growth and luminescence of the transcriptional fusions were measured. The reduction in expression was calculated between the difference in expression in garlic and expression in control media.

**Results:** See the table.

Strain	Mean reduction of QS target gene expression when grown in garlic-infused media [absolute value; relative luminescence units $\times 104$ (standard error of mean)] <sup>a</sup>	
	<i>lasB</i>	<i>pqsA</i>
PA01	8.44 (1.53)	10.57 (1.86)
PA03	1.86 (0.21)	4.06 (0.34)
PA06	4.27 (0.43)	4.06 (0.23)
PA09	8.91 (0.62)	27.53 (2.54)
PA15	5.82 (0.41)	7.48 (0.30)
PA21	0.91 (0.08)	2.10 (0.16)

<sup>a</sup>Experiment repeated in triplicate – all values significant, p < 0.05.

**Conclusion:** Garlic oil reduces the expression of two genes which represent major loci of control of QS in all clinical strains tested. This further advances the potential for translation as a therapeutic candidate.