The following commercially available jet nebulisers were tested:

- Pari LC+
- Pari Sprint Star
- Trudell AeroEclipse II
- Sprint Junior
- Sprint Star

The animal model and delivery method chosen for evaluation are crucial, as lung delivery of a bolus of fluid via tracheal instillation or nasal sniffing can report misleading toxicity and efficiency.

The UK CF Gene Therapy Consortium has selected GL67A cationic liposomes complexed with plasmid DNA for clinical evaluation after aerosol lung delivery.

The clinical nebuliser system used previously for aerosol delivery of pDNA/GL67A formulation to CF patients has been discontinued.

Five nebulisers were evaluated for their compatibility with the formulation and their aerosol output characteristics.

The aerosol droplet mass median aerodynamic diameter (MMAD) suitable for delivery to the small airways of the CF lung is estimated to be 2-4 µm.

The MMAD was measured for each nebuliser following inertial impaction of aerosol in a chilled (7-8°C) Next Generation pharmaceutical Impactor (NGI) operating at 15 L/min.

The MMAD for each nebuliser was dependent upon the operating pressure with the smallest droplets produced at 50 psi.

The Sprint Junior (3.43 ± 0.07 µm), Sprint Star (3.37 ± 0.09 µm) and AeroEclipse II (3.40 ± 0.1 µm) generated suitably sized droplets.

The Sprint Star nebuliser was prone to “sputtering” with pDNA/GL67A formulations and was excluded from further studies.

The five test nebulisers were used to spray the pDNA/GL67A formulation.

Aliquots of nebulised material were collected from the reservoir (A) and after aerosol delivery (B), and subjected to agarose gel electrophoresis.

Nebulisation of naked (non-complexed) pDNA resulted in degradation after as little as 5-10 minutes of nebulisation.

Complexing pDNA with lipid preserves formulation integrity after aerosol delivery with all five nebulisers tested.

The five test nebulisers were used to deliver the pDNA/GL67A formulation (10ml; 25mg pDNA) to mice (n=6) via the whole body exposure chamber.

Luciferase reporter expression was measured in homogenised lung tissue and expressed as Relative Light Units (RLU).

All nebulisers were capable of generating detectable reporter gene expression.

The aerosol delivery rate (nebuliser output) was measured with our pDNA/GL67A formulation, to confirm clinical acceptability.

A human breath simulator (with a sinusoidal breathing pattern at 15 breaths/min; inspiratory : expiratory ratio of 1:1; tidal volume 500 ml) was used (A).

The Sprint Junior nebuliser was faster than the AeroEclipse II nebuliser indicating a 1-2h delivery time for clinically relevant (50mg dose) (B).

The efficiency of the breath-actuated AeroEclipse II was significantly higher (83 ± 2% compared with 55 ± 1%) as determined by collection of aerosol on the inspiratory arm of the breathing circuit (C).

Both the Sprint Junior and AeroEclipse II nebulisers would be suitable, but the AeroEclipse II was selected for clinical studies (D).

CONCLUSIONS

- pDNA/GL67A formulation can be aerosolised with only minimal loss of plasmid integrity and generated aerosols with MMAD suitable for human delivery.

- The breath actuated Trudell AeroEclipse II jet nebuliser has been selected for the aerosol delivery of the pDNA/GL67A formulation to CF patients.