Development, Production and Evaluation of clinical grade CFTR Expression Plasmid for CF Lung Gene Therapy


On behalf of the UK CF Gene Therapy Consortium (www.cfgenetherapy.org.uk)

Introduction

The UK CF Gene Therapy Consortium is undertaking clinical studies to evaluate a non-viral gene therapy for CF lung disease.

For delivery to the lungs of CF patients, a single dose of the cationic lipid GL67A is complexed with a novel plasmid pGM169. This plasmid contains an improved enhancer/promoter (hCFII) [Hyde 2008 Nature Biotechnology 26:549] and is capable of persistent, high-level expression of the human CFTR (hCFTR) gene product in vivo.

Quantitative Assay of hCFTR Expression

We developed quantitative TaqMan PCR and RT-PCR assays to measure plasmid DNA delivery and hCFTR mRNA expression from clinical plasmid pGM169.

Persistent Expression in Mouse Lungs

The performance of pGM169 complexed with GL67A was evaluated following aerosol delivery to the lungs of BALB/c mice using a Pari LC+ nebuliser. Bulk plasmid DNA levels in the lung after aerosol delivery, fell by approximately three orders of magnitude from day 1, to a steady state level by d14 onwards.

Consistent levels of hCFTR mRNA were expressed in the lungs for at least 2 months (range 9-20% V/E) and was not significantly different at d1, d14, d28 and d56 (P>0.05; Kruskal wallis).

Clinical Studies with GL67A/pGM169

A single-dose, clinical study to evaluate the safety of the GL67A/pGM169 non-viral formulation in the lungs is underway in CF patients. The persistence of hCFTR expression is being evaluated via nasal Potential Difference measurements.

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Clinical Manufacturing Process

Following bacterial cell fermentation, harvest and lysis, a 2-step chromatographic process consisting of an anion exchange membrane and hydrophobic interaction chromatography was used (VGXI, Houston Texas).

Tangential Flow Filtration and further concentration resulted in GMP production of high purity plasmid (<2% containing contaminating RNA, DNA, Protein) with a very low endotoxin standard (<5 EU/mg).

Expression in Human Airway Cells

To confirm hCFTR expression in human primary airway cells, plasmid pGM169 was complexed with Lipofectamine 2000 (Invitrogen) to transfet ex vivo cultures of nasal cells grown at an air-liquid interface (ALI) (Epithelix, Switzerland).

CFTR mRNA from pGM169 was detected in 8/8 airway cell samples on day 2 (median 28.6 %VE at day 2) and 5/8 samples on day 7. In comparison, hCFTR expression from first generation clinical plasmid (pCF1-CFTR) did not persist (0/7 positive on day 7) and hCFTR expression was generally lower.

Preparation of Clinical Formulation

An automated, pneumatic mixing device, the LMD2, was developed to facilitate the standardized production of non-viral gene therapy formulations [Davies 2010 Biotechniques 49:666].

Vials of GMP grade GL67A lipid and GMP grade pGM169 plasmid DNA can be combined, using a dual-lumen syringe, to prepare virtually any volume of formulation required. The device is used for production of the gene therapy formulation in our current Phase I clinical study.

Preparation of GMP Plasmid DNA for Clinical Studies

Multi-gram quantities of GMP-grade plasmid pGM169 were required for clinical use. This was achieved via bacterial cell fermentation at the 100L scale by VGXI (Houston Texas).

Reproducibility of GMP manufacture was assured via validated master and working cell banks.

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