genetic medicine for CF

gene and mRNA-based therapies should be agnostic regarding the patient's genotype or which of the six mutation classes these might belong to, and should be suitable for the treatment of patients with any mutation.

one gene twenty years

1989
During the day, Lap-Chee Tsui and Francis Collins were attending a gene-mapping workshop. The fax they received that night from Tsui's lab showed that **many people who have cystic fibrosis lack three base pairs from both copies of this gene,** whereas those without the disease always have at least one copy intact.

Four months later a four-year-old boy with cystic fibrosis, Danny Bessette, was shown sitting cross-legged on the cover of *Science,* framed by a rainbow of chromosomes. Inside the magazine, three papers laid out the details of the discovery of the gene responsible for Bessette's condition .

2009
Cystic fibrosis patients has a life expectancy at least ten years longer than one born in 1989 did. Such advancements help explain why Bessette —now 24, has a future at all.
But many researchers concede that relatively little of that improvement can be laid at the door of the CFTR gene.

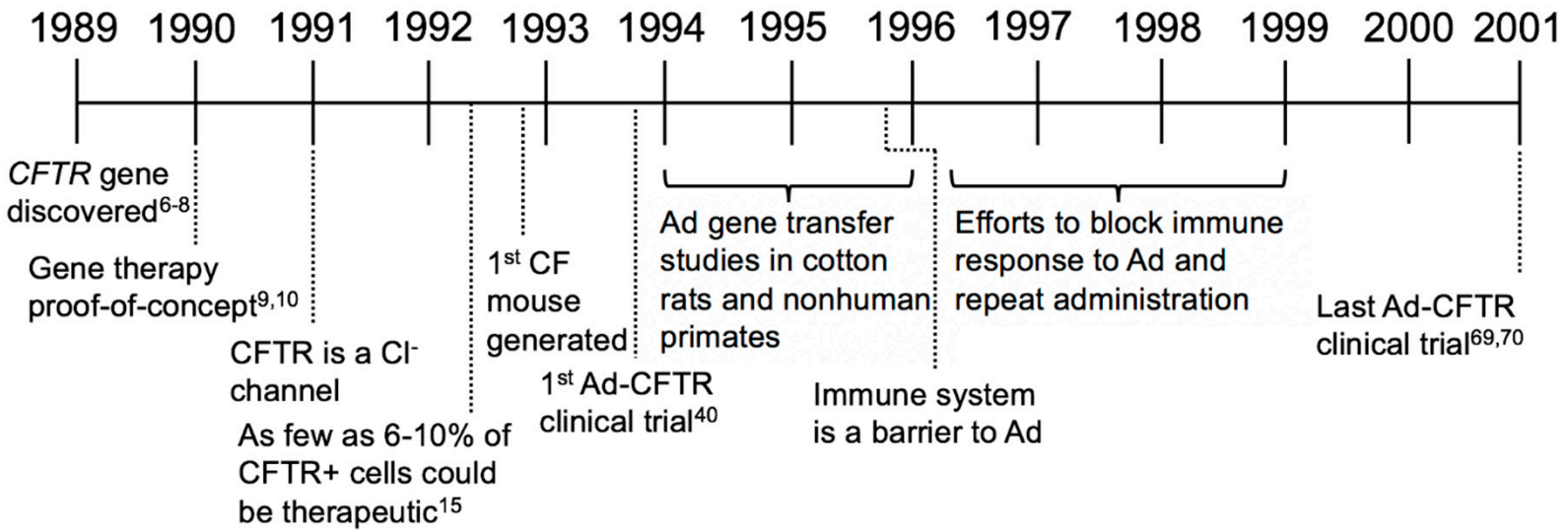


Figure 2. Timelines of CF gene therapy eras: Important milestones impacting the CF field are represented in timelines at the beginning of each era. The timelines are intended to orient the reader to new developments relative to other events and are not comprehensive of all contributions to the field (1989–2001).

CFTR gene identification and gene therapy proof of principle studies

- the CFTR was identified as responsible of CF (1989, Riordan et al)
- retrovirus-mediated gene transfer established proof of principle that cAMP-mediated chloride conductance can be corrected (Drumm et al 1990)
- adenovirus mediated CFTR cDNA transfer into cotton rats (Rosenfeld te al 1992)
- four years after CFTR cloning, non viral CFTR gene transfer was shown to partially correct the chloride transport in tracheal epithelium of CF KO mice (Hyde et al 1993)

Clinical tials

- the first trial was performed on three CF patients with first-generation adenoviral vector administrated to the nasal epithelium. partial rescue of cAMP-mediated chloride transport was shown (Zabner et al 1993)
- non-viral cDNA complex was shown to partially correct chloride transport in the nasal of CF patients (Caplen et al 1995)
- non-viral, lipid-mediated (GL67A) CFTR transfer restrored Chloride transfer in the lung of CF patients (Alton et al 1999)
- Twenty-six years after cloning of CFTR, Alton et al demonstrated that repeated administration of GL67A complexed with a plasmid DNA carrying the CFTR cDNA significantly, albeit modestly, stabilized lung function in CF patients.

Since cloning of the CFTR gene in 1989 extensive pre-clinical research led to approximately 27 clinical trials involving about 600 patients being completed.

CF gene therapy development

Identification of barriers to gene transfer into the lung
potent intra- (nuclear membrane) and extracellular barriers (airways mucus and mucociliary clearence) that have evolved to protect us from viruses, bacteria, and other inhaled particles also “protect” against inhalation and uptake of inhaled gene transfer agents (GTAs);

Identification of Gene Transfer Agents Suitable for Clinical Translation Is Challenging
Adenoviruses and adenoassociated viruses (AAV) have a natural tropism for the lungs and seemed obvious choices for early CF gene therapy trials. However, pre-existing and induced immune responses to the viral vector which effect efficacy and duration of expression, limit their usefulness for the treatment of a life-long disease such as CF.
In contrast to viral vectors, the simpler structure of non- viral formulations, which generally do not contain proteins, make them less likely to induce immune responses.

The UK CF Gene Therapy Consortium (GTC)

founded in 2001, consisting of the three groups in Edinburgh, London, and Oxford who had previously conducted CF gene therapy trials. The explicit aim was to share expertise and knowledge in a translational program to assess whether gene therapy can change the progres- sion of CF lung disease. The GTC is currently the only group conducting CF gene therapy trials and recently completed a Phase IIb multi-dose trial.

key data from GTC

finding the best DNA/lipid complexes

- Identification of the cationic lipid formulation GL67A, first used in the 1990s, as the most potent GTA for airway gene transfer
- Improvement of the first generation plasmid (pGM169) by removing the CpG islands, codon-optimizing the CFTR cDNA and incorporation of the novel regulatory element, hCEFI, consisting of the elongation factor 1a promoter coupled to the human CMV enhancer.

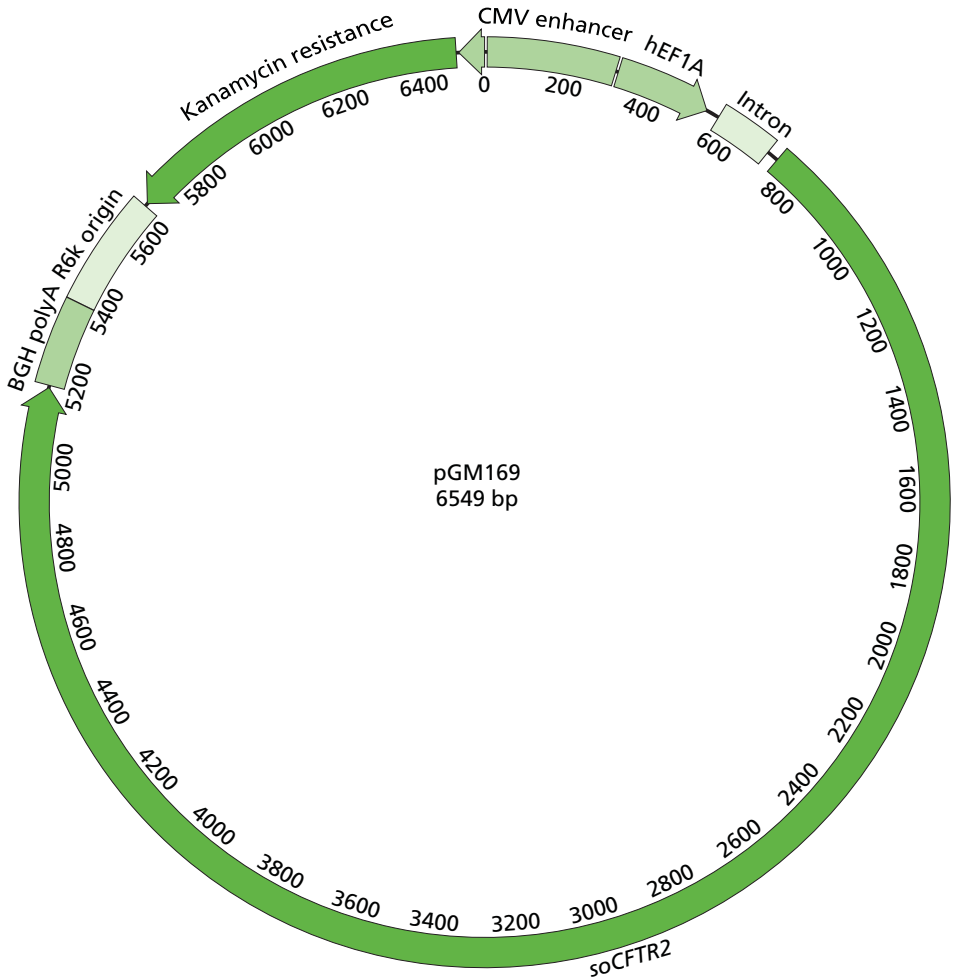
key data from GCT: finding the most efficient dosing

- Multi-dose toxicology studies in mice and sheep were undertaken: repeated aerosolization of pGM169/GL67A to mice led to cumulative dose-related expression on repeat dosing, reaching 94 +/-19% of endogenous murine Cftr levels after 12 deliveries. **These data further supported progression into a multi-dose clinical trial.**

- A single administration, dose-escalation (5, 10, and 20ml of pGM169/GL67A). Phase I/IIa safety trial showed that despite CpG-depletion of the plasmid, patients receiving the 10 and 20 ml dose still developed mild flu-like symptoms including a fever. Both the volume administered to the lung, and the lipid contribute to the inflammatory response (in addition to CpG sequences)**. The 5 ml dose (containing 12.5 mg plasmid DNA) was chosen for the multi-dose trial.**

A double-blinded, placebo-controlled multi-dose trial was undertaken

Plasmid pGM169



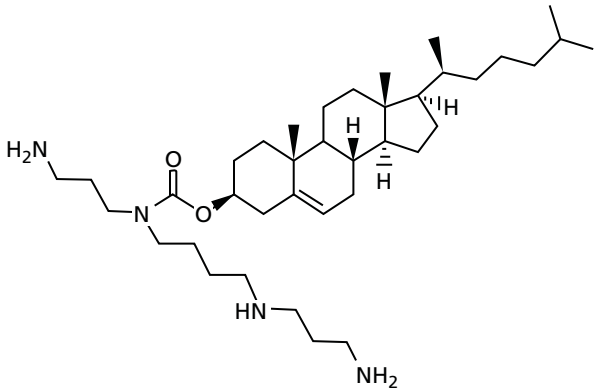
Plasmid pGM169. The basic features of pGM169 (proceeding clockwise from 0 base pairs) are the CpG-free human cytomegalovirus enhancer/elongation factor 1 alpha enhancer/promoter; a CpG-free synthetic intron sequence to enhance mRNA splicing; a CpG-free version of the CFTR coding sequence termed soCFTR2; a CpG-free version of the bovine growth hormone polyadenylation sequence; a CpG-free version of the R6K bacterial plasmid origin of replication; a CpG-free version of the kanamycin resistance gene; and a CpG-free synthetic bacterial promoter sequence termed EM7. bp, base pair; BGH, bovine growth hormone.

plasmid preparation

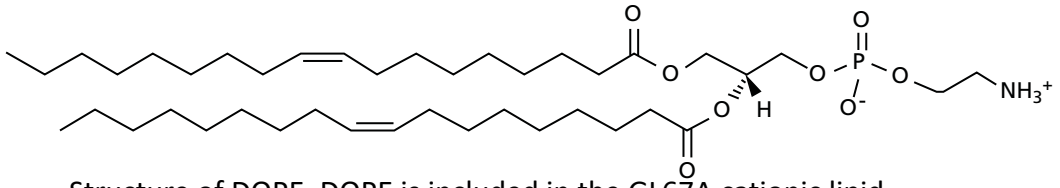
Good manufacturing practice (**GMP**) manufacture of pGM169 was conducted by VGXi Inc. (The Woodlands, TX, USA).
Bacteria containing the plasmid were fermented to a high density and harvested. The bacteria were then lysed to release their contents, including the plasmid, into solution.
The lysate was subjected to three significant purification steps: (1) solid/liquid separation, (2) ion-exchange chromatography and (3) hydrophobic interaction chromatography. Subsequently, the purified plasmid was concentrated and desalted by ultrafiltration/diafiltration into a sterile 8 mM sodium chloride (NaCl) solution and finally subjected to aseptic filtration to provide the bulk drug substance. This bulk was aseptically filled into single-unit vials and stored at ≤ –70 ° C. To prepare the final drug substance, single or multiple pooled lots of bulk drug substance were, if necessary, diluted to 5.3 ± 0.3 mg/ml with sterile 8 mM NaCl and then filled into 10-ml clear glass vials at a fill level of 5.2 ± 0.2 ml. Vials were stored at –80 ° C. The material is stable for at least 3 years.

GL67A cationic lipid

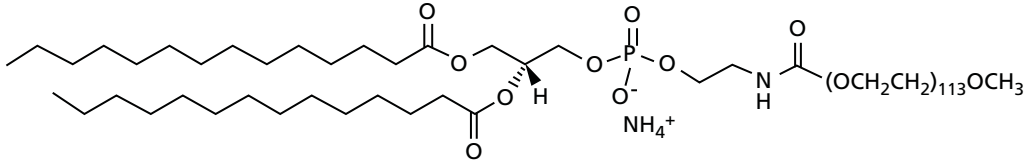
The cationic lipid mixture GL67A is an excipient, consisting of a mixture of three components (the structure of which is shown in Figures 2–4; GL67, DOPE, and DMPE-PEG5000) formulated at a 1 : 2 : 0.05 molar ratio. Good manufacturing practice-grade GL67 was manufactured by Sanofi-Genzyme (Haverhill, UK).
GL67-to-DNA ratio of 0.75 : 1 was termed **GL67A.**



Structure of GL67. GL67 is included in the GL67A cationic lipid mixture for its DNA-binding properties



Structure of DOPE. DOPE is included in the GL67A cationic lipid mixture for its endocytosis- inducing properties.



Structure of DMPE-PEG5000. DMPE-PEG5000 is included in the GL67A cationic lipid mixture for its charge-shielding properties that facilitate final preparation of the pGM169/GL67A drug product and allow efficient nebulisation.

Repeated nebulisation of non-viral *CFTR* gene therapy in patients with cystic fibrosis: a randomised, double-blind, placebo-controlled, phase 2b trial

Lancet Respir Med 2015

Published Online
July 3, 2015
http://dx.doi.org/10.1016/S2213-2600(15)00245-3

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Lung delivery of plasmid DNA encoding the CFTR gene complexed with a cationic liposome is a potential treatment option for patients with cystic fibrosis. We aimed to assess the efficacy of non-viral CFTR gene therapy in patients with cystic fibrosis.

Methods: We did this randomised, double-blind, placebo-controlled, phase 2b trial in two cystic fibrosis centres with patients recruited from 18 sites in the UK. Patients (aged ≥12 years) with a forced expiratory volume in 1 s (FEV1) of 50–90% predicted and any combination of *CFTR* mutations, were randomly assigned, via a computer-based randomisation system, to receive **5 mL** of either **nebulised pGM169/GL67A** gene–liposome complex or 0·9% saline (**placebo**) **every 28 days** (plus or minus 5 days) for 1 year. Randomisation was stratified by % predicted FEV1 (<70 *vs* ≥70%), age (<18 *vs* ≥18 years), inclusion in the mechanistic sub-study, and dosing site (London or Edinburgh). Participants and investigators were masked to treatment allocation. **The primary endpoint was the relative change in % predicted FEV** . The primary analysis was per protocol. This trial is registered with ClinicalTrials.gov, number 1 NCT01621867.

Trial profile

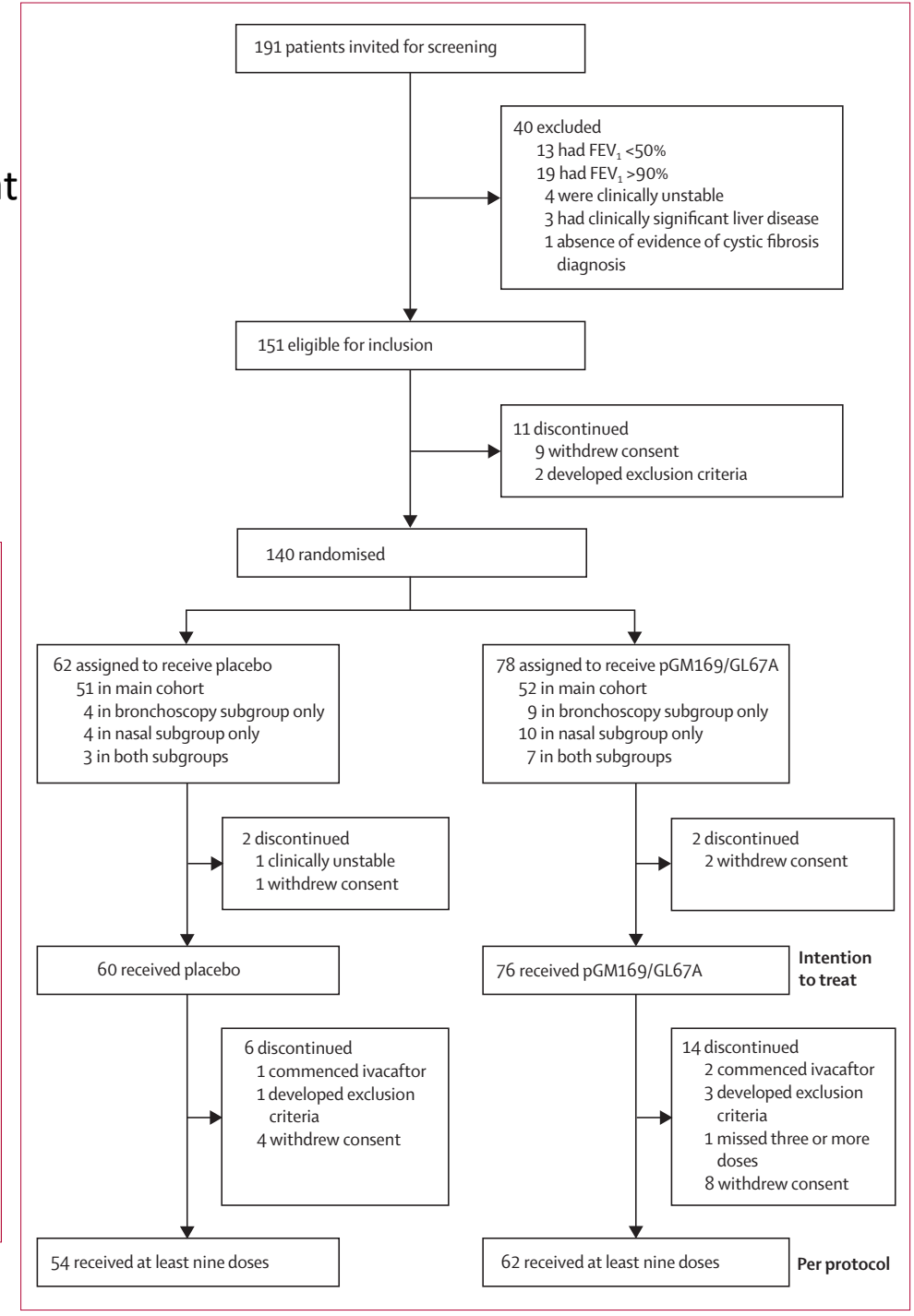
Numbers of patients in the intention-to-treat population.
FEV1=forced expiratory volume in 1 s.

patients demographic and genetic and clinical characteristics

	Placebo group (n=54)	pGM169/GL67A group (n=62)
Age (years)	26·0 (13·0)	23·6 (10·8)
<18 years old	17 (31%)	23 (37%)
≥18 years old	37 (69%)	39 (63%)
Sex		
Female	25 (46%)	31 (50%)
Male	29 (54%)	31 (50%)
Centre distribution number		
Edinburgh	24 (44%)	22 (35%)
London	30 (56%)	40 (65%)
Height (cm)	165·0 (10·6)	163·6 (10·9)
Weight (kg)	61·6 (15·6)	61·0 (15·7)
FEV_1 (% predicted)	69·0 (9·9)	69·9 (11·1)
Body-mass index (kg/m²)	22·4 (4·4)	22·4 (4·5)
Mutation class		
Phe508del/Phe508del	26 (48%)	31 (50%)
Phe508del/class 1–6	22 (41%)	23 (37%)
Not Phe508del/class 1	1 (2%)	3 (5%)
Heterozygous/homozygous class 3–6	2 (4%)	2 (3%)
Phe508del/unknown class	3 (6%)	3 (5%)

Data are mean (SD) or n (%), unless otherwise indicated.

***Table 1*: Baseline and demographic characteristics**

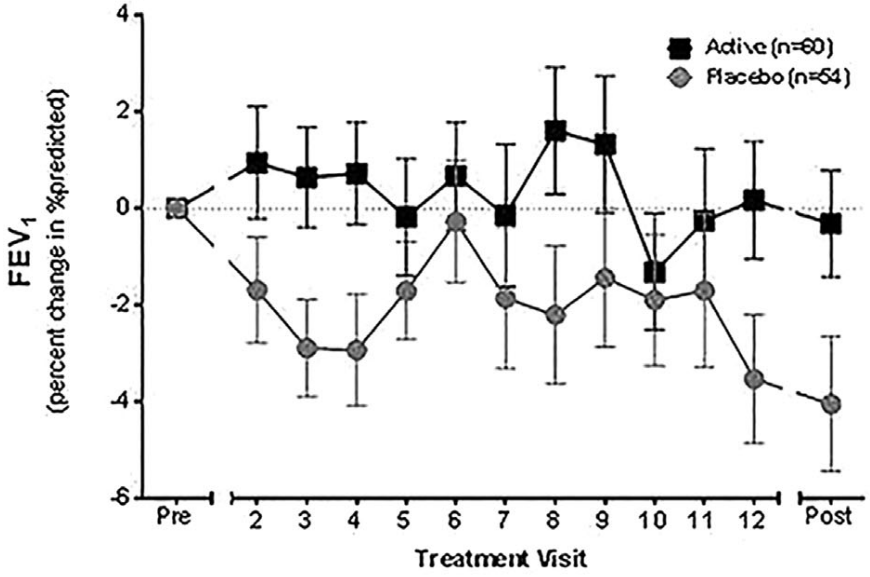


outcomes

Between June 12, 2012, and June 24, 2013, we randomly assigned 140 patients to receive placebo (n=62) or pGM169/GL67A (n=78). We noted a significant, albeit modest, treatment effect in the pGM169/GL67A group versus placebo at 12 months' follow-up (3·7%, 95% CI 0·1–7·3; p=0·046). This outcome was associated with a stabilisation of lung function in the pGM169/GL67A group compared with a decline in the placebo group. We recorded no significant difference in treatment-attributable adverse events between groups.

Stabilization of lung function after repeated administration of the non-viral formulation pGM169/GL67A : all patients

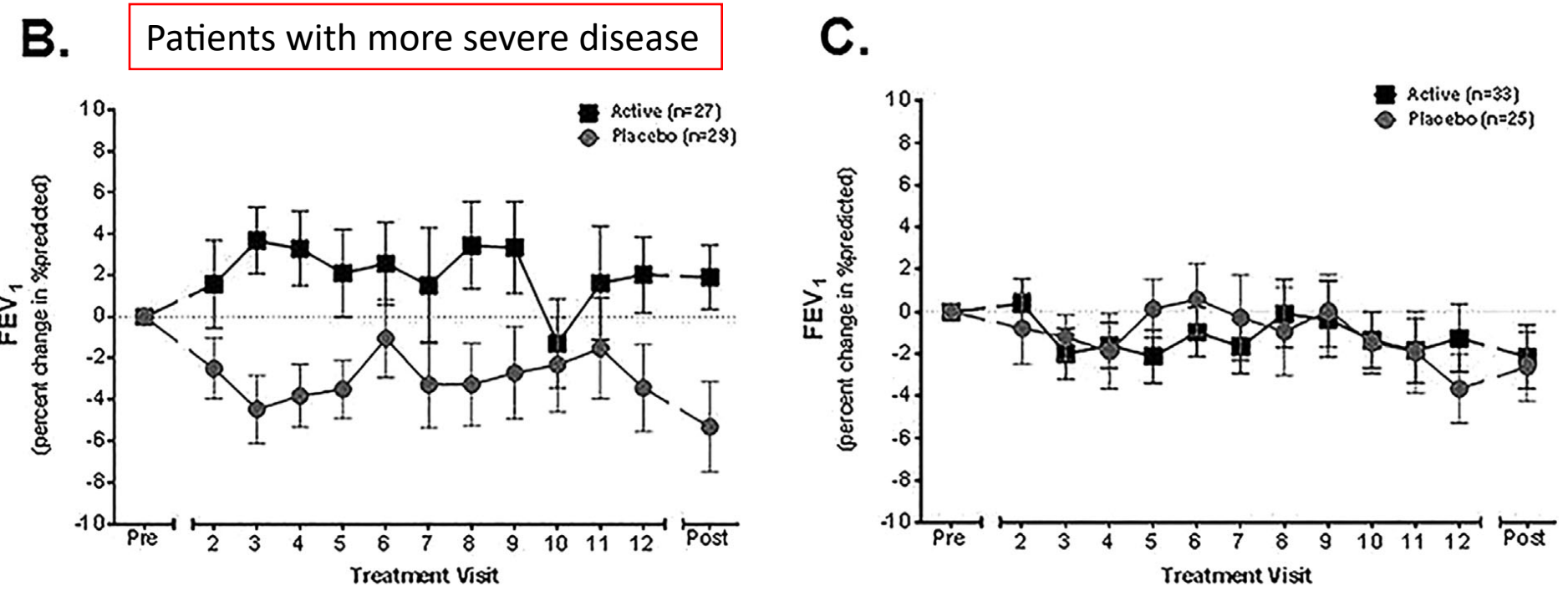
A.



Cystic fibrosis patients were treated monthly for 12 months with either active drug or the placebo. Lung function (FEV1 1⁄4 forced expiratory volume in 1 sec) was measured at each treatment visit before administration of study drugs. Data are expressed as relative change from baseline in percent predicted FEV1. Error bars show the standard error of the mean. (A) All patients.

There was a significant, albeit modest, treatment effect in the pGM169/GL67A group versus placebo at 12 months' follow-up (3 7%, P=0.046).

Stabilization of lung function after repeated administration of the non-viral formulation pGM169/GL67A: patients with more (B) or less (C) severe lung function decline



Cystic fibrosis patients were treated monthly for 12 months with either active drug or the placebo. Lung function (FEV1 1⁄4 forced expiratory volume in 1 sec) was measured at each treatment visit before administration of study drugs. Data are expressed as relative change from baseline in percent predicted FEV1. Error bars show the standard error of the mean. (B) Patients with more severe lung function at start of treatment (Baseline FEV1=50–70%), (C) Patients with less severe lung function at start of treatment (Baseline FEV1=70–90%).

conclusions from the phase IIb clinical trial

- Monthly application of the pGM169/GL67A gene therapy formulation was associated with a **significant, albeit modest, benefit in FEV compared with placebo at 1 year**, indicating a stabilisation of lung function in the treatment group.
Further improvements in efficacy and consistency of response to the current formulation are needed before gene therapy is suitable for clinical care; however, our findings should also encourage the rapid introduction of more potent gene transfer vectors into early phase trials.

FEV stabilization is effectively due to exogenous CFTR expression?

The significant effect on lung function shown for the first time in this trial was paralleled by only minimal changes in the ion transport assays and no detectable vector-specific mRNA. This discordance may relate to the timing and sensitivity of the assays, the site of measurement, and/or the relatively small area of airways assessed when using molecular assays and further questions the use of these assays as go/no-go decision points in the development of CF gene therapy. It also raises the possibility of non- specific effects of the gene transfer complex on airway function, although this is difficult to rationalize with current knowledge of airway biology.

The outcome of the trial raises a number of questions

Could the Dose Be Increased? The 5 ml dose was well tolerated when administered repeatedly and a follow-on trial might include a higher dose, supported by preliminary data from our single administration Pilot Study.

Was the Right Dosing-Interval Chosen? Although animal studies have shown that gene expression persists for more than a month, it is conceivable that more frequent administration may further increase efficacy. However, moving from monthly to fortnightly or weekly dosing will clearly increase the treatment burden.

Was the Appropriate Placebo Used? The use of lipid alone as a placebo is a poor choice because charge, pH, tonicity, and chemical composition are very different compared to lipid/DNA complexes. The alternative could have been to use an empty plasmid or a plasmid carrying a mutant CFTR sequence. However, both these strategies are risky as it would not have be able to rule out expression of an immunologically active peptide or novel non-coding RNA molecules with deleterious biological functions. Thus, 0.9% saline, which has not been shown to negatively affect lung function, is likely the optimal placebo from a range of non-ideal options.

What Is the Best Primary Endpoint? Spirometry (FEV1) is a variable and effort-dependent measurement and, therefore, is less than ideal. However, we spent approximately 2 years studying the longitudinal progression of numerous validated and more novel markers of disease severity in about 200 patients and were unable to identify a more appropriate, regulatory- compliant endpoint.

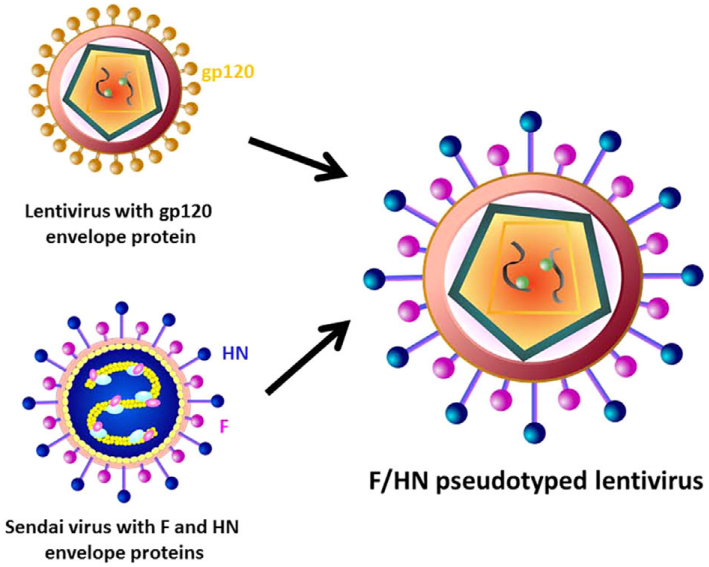
Alternative Gene Transfer Agents Suitable for CF Gene Therapy

Lentiviral vectors, which integrate into the genome, are able to transduce dividing and non-dividing cells and might, therefore, be suitable for targeting differentiated cells in the lung.

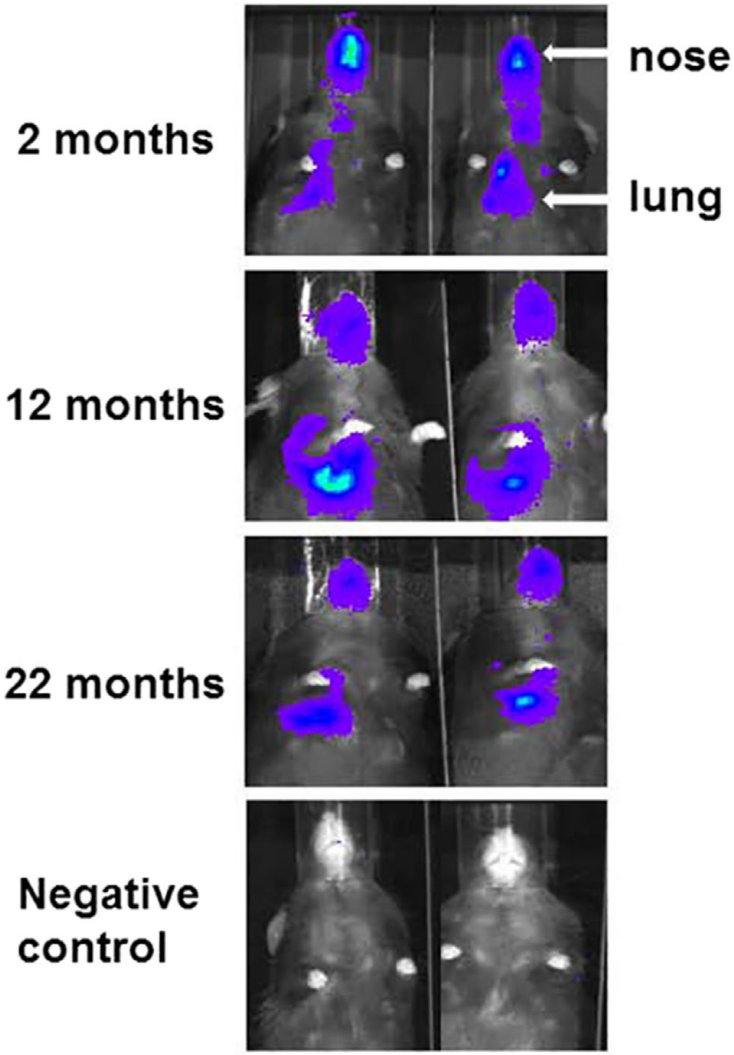
Lentiviral vectors have no natural lung tropism and, therefore, require pseudotyping with appropriate envelope proteins to facilitate lung gene transfer. The vesicular stomatitis virus G (**VSV-G**) protein, commonly used for this purpose and works well for bone marrow transduction ex vivo, is not suited for transduction airways epithelium.

Other envelope proteins including the **baculovirus protein GP64**, proteins from **Ebola or Marburg filoviruses, the HA protein from influenza virus and the F and HN protein from Sendai virus,** which are viruses that either have a broad tissue tropism (baculovirus), or a natural tropism for the lung (influenza and Sendai virus), have been investigated.

F/HN pseudotyped lentiviral vector



Generation of F/HN-pseudotyped lentiviral vector. Molecular techniques enable the replacement of the gp120 envelope glycoprotein, which supports lentivirus entry into T-cells but is not suitable for entry into airway epithelial cells, with the F (fusion) and HN (hemagglutinin-neuraminidase) proteins from Sendai virus which support efficient entry into airway epithelial cells. This process of pseudotyping leads to the generation of a chimeric pseudotyped F/HN lentiviral vector. The Sendai virus F and HN proteins were chosen because they are, in part, responsible for the high transduction efficiency of Sendai virus in lungs.



F/HN-pseudotyped lentivirus transduction leads to persistent gene expression in mouse airways. Mice were transduced with F/HN-pseudotyped lentivirus expressing a luciferase reporter gene by nasal sniffing (or received PBS (negative controls). Luciferase expression was visualized using bioluminescence imaging, 2–22 months after transduction.

Preparation for a first-in-man lentivirus trial in patients with cystic fibrosis

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To cite: Alton EWFW, Beekman JM, Boyd AC, *et al*. *Thorax* 2017;**72**:137–147.

What is the key question?

▸ Is a lentiviral vector, which was pseudotyped to achieve efficient gene transfer into airway epithelial cells, suitable for progression into a first-in-man gene therapy trial in patients with cystic fibrosis (CF)?

▸ The data support the progression of the F/ HN-pseudotyped lentiviral vector into a first-in-man CF trial in 2017 for which funding has been obtained.

▸ In contrast to other viral vectors, lentiviral vectors hold substantial promise for the development of gene therapy for a range of diseases, including chronic conditions due to their high efficacy, duration of expression and the fact that pre-existing and acquired immune responses do not interfere with vector efficacy on repeated administration.

In preparation for a first-in-man CF trial using the lentiviral vector

we have undertaken key translational preclinical studies:

•Regulatory-compliant vectors carrying a range of promoter/enhancer elements were assessed in mice and human air–liquid interface (ALI) cultures to select the lead candidate;

•cystic fibrosis transmembrane conductance receptor (CFTR) expression and function were assessed in CF models using this lead candidate vector.

•Toxicity was assessed.

•Integration site profiles were mapped and transduction efficiency determined to inform clinical trial dose-ranging.

•The impact of pre- existing and acquired immunity against the vector and vector stability in several clinically relevant delivery devices was assessed.

we found:

-hybrid cytosine guanine dinucleotide (CpG)- free CMV enhancer/elongation factor 1 alpha promoter (hCEF) consisting of the elongation factor 1α promoter and the cytomegalovirus enhancer was most efficacious in both murine lungs and human ALI cultures (both at least 2-log orders above background).

- efficacy (at least 14% of airway cells transduced), toxicity and integration site profile supports further progression towards clinical trial

-pre-existing and acquired immune responses do not interfere with vector efficacy.

The lead rSIV.F/HN candidate expresses functional CFTR and the vector retains 90–100% transduction efficiency in clinically relevant delivery devices. The data support the progression of the F/HN-pseudotyped lentiviral vector into a first-in-man CF trial in 2017.

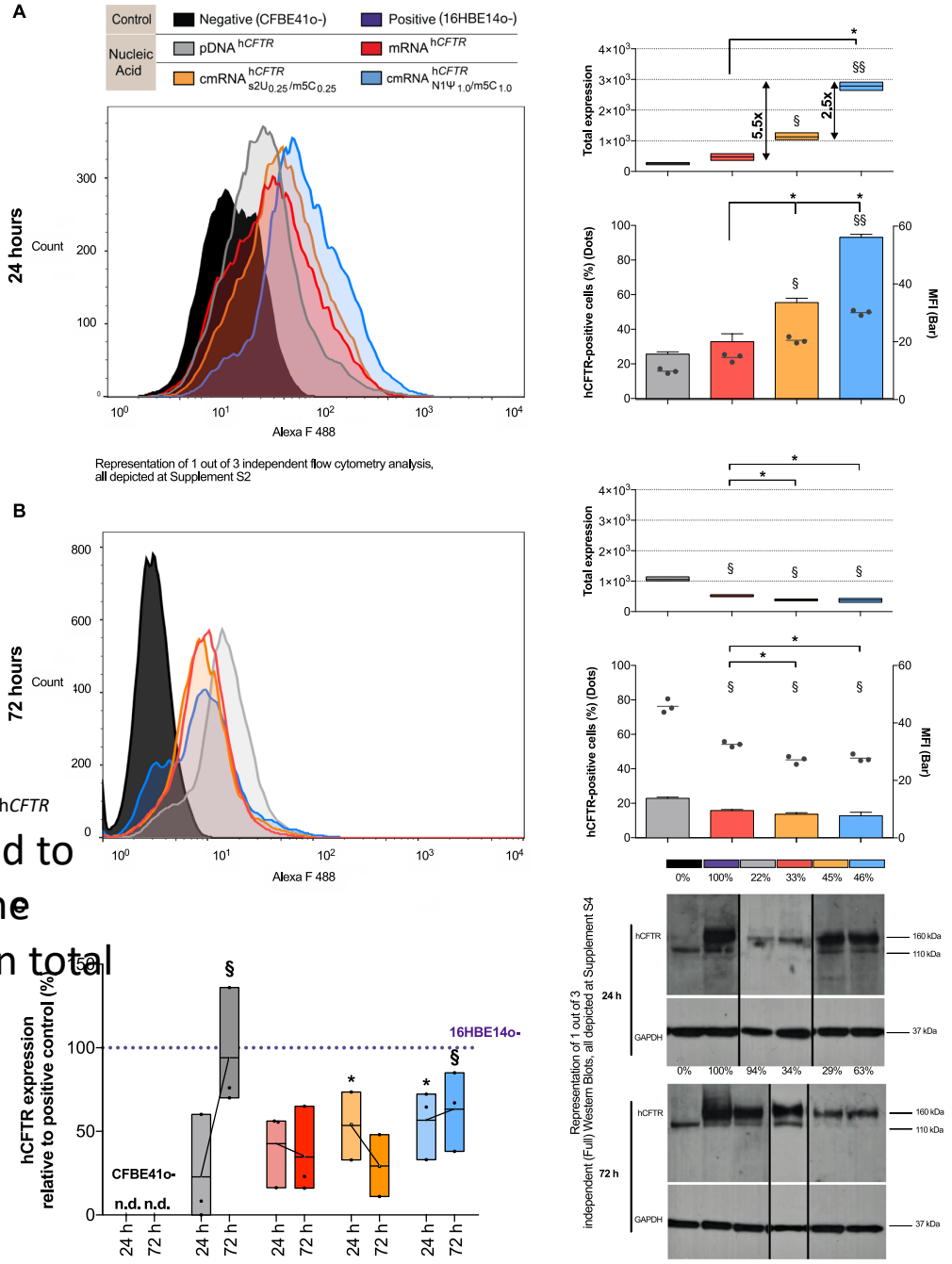
New approaches

Chemically modified h*CFTR* mRNAs recuperate lung function in a mouse model of cystic fibrosis

A. K. M. Ashiqul Haque[1], Alexander Dewerth[1], Justin S. Antony[1,7], Joachim Riethmüller[2], Georg R. Schweizer[1], Petra Weinmann[1], Ngadhnjim Latifi[1], Hanzey Yasar[3], Nicoletta Pedemonte[4], Elvira Sondo[4], Brian Weidensee[1], Anjali Ralhan[5], Julie Laval[5], Patrick Schlegel[1], Christian Seitz[1], Brigitta Loretz[3], Claus-Michael Lehr[3,6], Rupert Handgretinger[1,7] & Michael S. D. Kormann[1]

expression profile of plasmid-encoded hCFTR (pDNAh*CFTR*), unmodied h*CFTR* mRNA (mRNAh*CFTR*) and two well-defined nucleoside modifications (cmRNAh*CFTR* and h*CFTR* s2U0.25/m5C0.25 cmRNAN1Ψ1.0/m5C1.0) which have been described to exert state-of-the-art stability/expression *in vitro* or lung-specific cell contexts *in vivo*

After 72 h from transfection (c) mRNA$_{hCFTR}$ expressed significantly lower compared to pDNA$_{hCFTR}$ transfected cells, reflecting the percentage of positive cells, MFI and in total hCFTR expression



A

Invivo lung function measurements in cmRNA$_{hCFTR}$ and pDNA$_{hCFTR}$ treated *Cftr*$_{-/-}$ mice

		CS-PLGA NPs	µg
Controls	*Cftr* $^{-/-}$	—	n.a.
Controls	*Cftr* $^{+/+}$	—	n.a.
Mock	1 cmRNA $^{DsRed}_{s2U_{0.25}/m5C_{0.25}}$	+	40 i.v.
Mock	2 Nanoparticles (NPs) i.v.	+	n.a.
Nucleic Acid	pDNA ^{hCFTR}	+	40 i.v. ⊘
Nucleic Acid	cmRNA $^{hCFTR}_{s2U_{0.25}/m5C_{0.25}}$	+	40 i.v.
Nucleic Acid	cmRNA $^{hCFTR}_{N1\Psi_{1.0}/m5C_{1.0}}$	+	40 i.v.

⊘ pDNA has been equalised to mRNA by nmols instead of µg

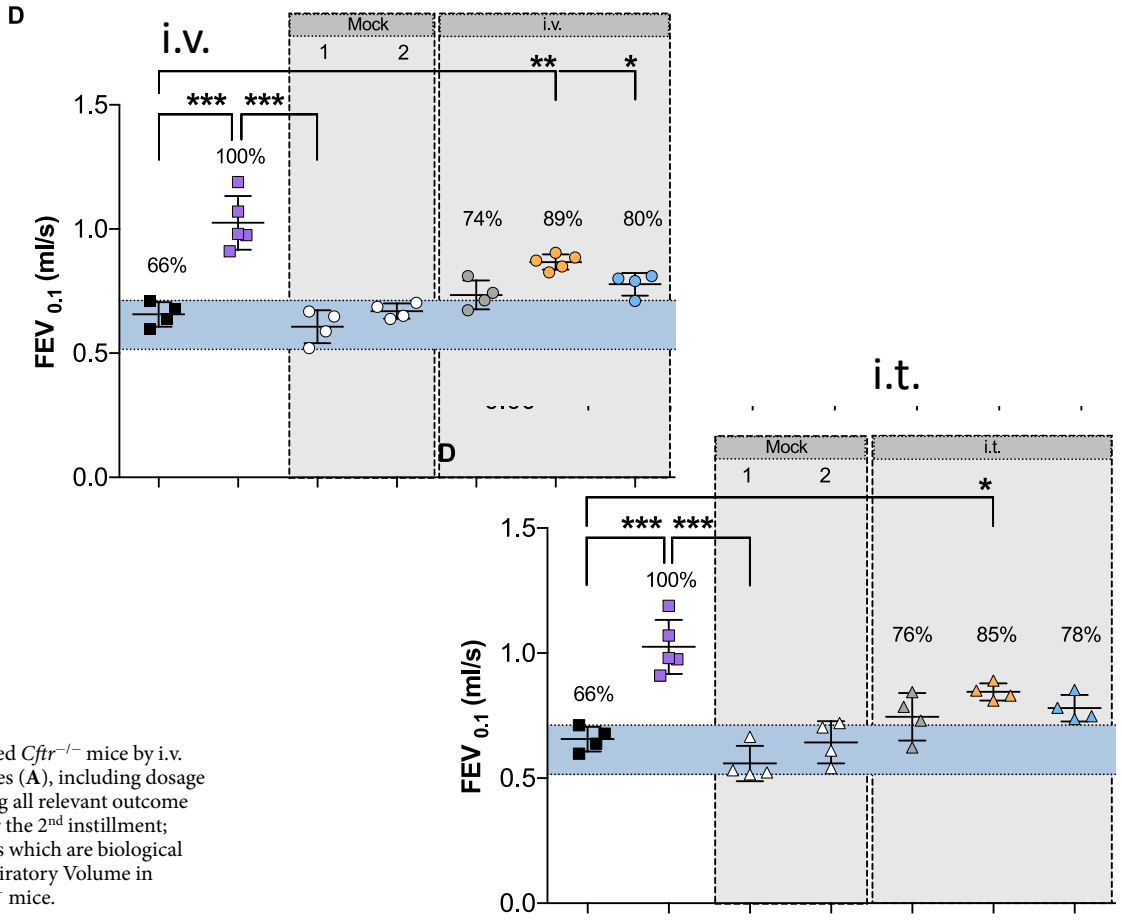
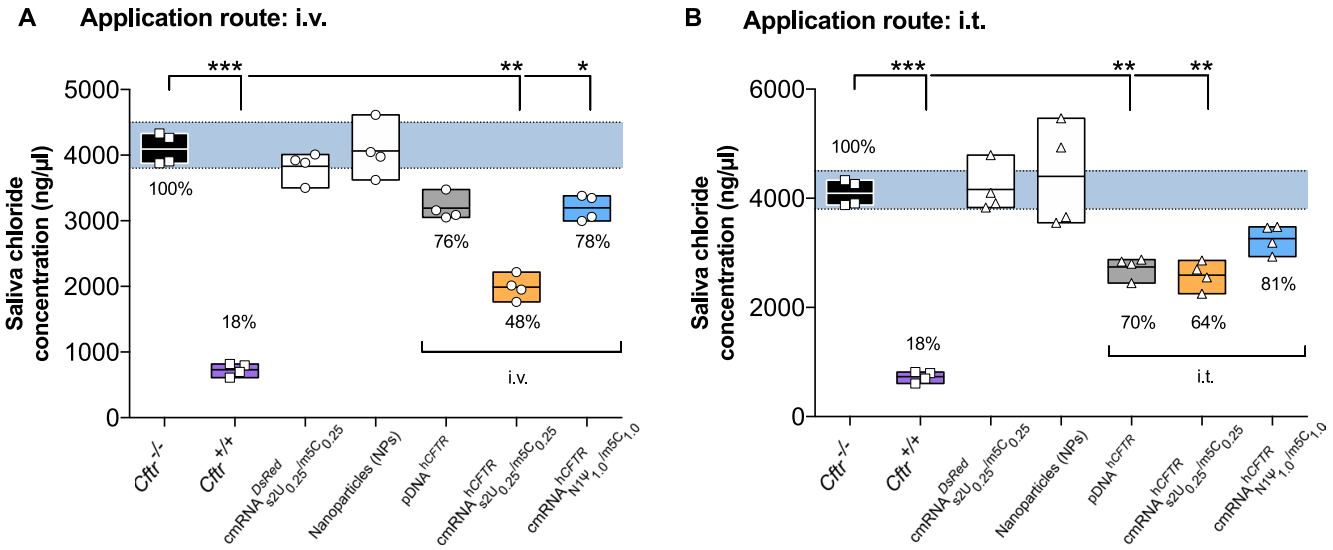


Figure 3. *In vivo* lung function measurements in cmRNA^{hCFTR} and pDNA^{hCFTR} treated *Cftr*$^{-/-}$ mice by i.v. route. All mouse groups utilized in (**B–D**) are color-coded for their treatment schemes (**A**), including dosage and application routes. (**B–D**) Precision *in vivo* lung function measurements covering all relevant outcome parameters on in *Cftr*$^{-/-}$ mice treated twice via i.v. route and measured 72 hours after the 2nd instillment; $n = 4–7$ mice per group. The blue area represents the variance of the negative controls which are biological replicates. Data represent the means ± SD on compliance, resistance and Forced Expiratory Volume in 0.1 seconds ($FEV_{0.1}$). *$P \leq 0.05$; **$P \leq 0.01$ and ***$P \leq 0.001$ versus untreated *Cftr*$^{-/-}$ mice.



conclusion

Taken together, this study is the first proof of concept of efficient application of NP-cmRNA^{hCFTR} *in vivo* to restore lung function in a *Cftr*-deficient mouse model. Importantly, we could neither detect immune responses *in vivo* nor in a more defined setting *ex vivo*. Applying cmRNA^{hCFTR} to *Cftr*$^{-/-}$ mice could efficiently restore lung function close to levels of healthy control mice. In addition, our study compared - apart from two well-known mRNA modifications and pDNA^{hCFTR} - also two different delivery routes, demonstrating that systemic administration of cmRNA targets lung cells more efficiently at lower dosages. This study provides a proof of concept for alternative treatment of patients suffering from CF. cmRNA^{hCFTR} transcript supplementation may be broadly applicable for most *CFTR* mutations, not only in adults but already in the postnatal state, thereby protecting the lungs from exacerbations from the very beginning of life.

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Pediatric Pulmonology 51:S5–S17 (2016)

Reviews

Genetic Medicines for CF: Hype Versus Reality

Eric W.F.W. Alton, FMedSci,[1] A. Christopher Boyd, PhD,[1] Jane C. Davies, FRCPCH,[1] Deborah R. Gill, PhD,[1] Uta Griesenbach, PhD,[1]* Patrick T. Harrison, PhD,[2] Noreen Henig, MD,[3] Tracy Higgins, BSc,[1] Stephen C. Hyde, DPhil,[1] J. Alastair Innes, FRCPE,[1] and Michael S.D. Korman, PhD[4]

Review

Cystic Fibrosis Gene Therapy: Looking Back, Looking Forward

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Genes **2018**, *9*, 538; doi:10.3390/genes9110538