

# Gene therapy for respiratory diseases; progress and a changing context

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## The UK Respiratory Gene Therapy Consortium (GTC)

The GTC was formed in 2001 from three groups at the Universities of Edinburgh and Oxford and Imperial College, London to explore gene therapy as a therapeutic option for people with cystic fibrosis (CF)<sup>1</sup>. The gene responsible for CF, *Cystic Fibrosis Transmembrane conductance Regulator (CFTR)*, was identified in 1989<sup>2</sup> and over 2000 mutations are now known<sup>3</sup>, typically classified into six groups<sup>4</sup>.

Whilst considerable progress has been made with this mutation-agnostic approach, gene therapy is not yet a clinical reality. In parallel, mutation-specific, small molecule CFTR modulator therapy has now demonstrated substantial clinical efficacy<sup>5</sup>. Here, we briefly summarise the opinions of the GTC on navigating this evolving terrain, as well as noting some opportunities for gene therapy in other respiratory diseases.

### An unmet need remains

Potentially, up to 85% of the CF population may gain benefit from small molecule modulators in the near future. However, there remains a substantial proportion either with mutations not targeted by these agents, or in whom the drugs may not be tolerated<sup>6</sup>. The modulators are currently administered orally twice daily, display significant drug-drug interaction<sup>7</sup> and access to even the earliest of these has been problematic in some regions<sup>8</sup>. Thus, clinical trials networks globally recognise the need to continue to develop alternative approaches. The North American Cystic Fibrosis Foundation recently announced a major funding initiative<sup>9</sup> around advanced therapy medicinal products to address this unmet need. We note, however, the changing context in which any such new products will need to be delivered.

### Choice of gene transfer agent

The GTC's perspective on selection of a gene transfer agent for a chronic condition such as CF has remained the key requirement to demonstrate transgene expression with that agent after three or more administrations. We hypothesise that it may be hard to access progenitor or stem cells in human CF lungs *in vivo* and therefore assume that treatment will require repeated administration, with dosing intervals being driven by the lifespan of

the target conducting airway epithelial cells. In our hands this was firstly feasible with some non-viral formulations, a finding that drove our choice of the liposome GL67A for our completed Phase 2B clinical trial<sup>10</sup>. Whilst this study encouragingly demonstrated stabilisation of lung function compared with placebo, in the context of modulators the magnitude of benefit was likely insufficient to warrant further development as a stand-alone treatment.

Secondly, with regard to viral vectors, we and others have shown airway epithelial transgene expression after repeated administration of recombinant lentiviruses<sup>11,12</sup>. This has driven the partnership formed with Boehringer Ingelheim and Oxford BioMedica with respect to our lentiviral vector pseudotyped with glycoproteins from Sendai virus<sup>13</sup>. This programme is moving rapidly through the required manufacturing and toxicology components with planning for a first-in-man trial well developed. We note that at least one other centre is also pursuing a pseudotyped lentiviral approach to the clinic at pace<sup>14</sup>. Whilst the GTC has struggled to demonstrate airway transduction by recombinant AAV vectors after three or more administrations, there are more positive reports from other centres<sup>15,16,17</sup>.

The safety of viral vectors is clearly paramount, particularly in the context of integrating vectors<sup>18</sup>. Recombinant lentiviruses have now entered clinical trials for conditions such as Parkinson's Disease and immunodeficiencies without adverse reports<sup>19</sup> and are licensed for treatment of certain lymphomas and leukaemias<sup>20</sup>. Our preclinical data indicate no additional acute toxicity compared with the clinically benchmarked GL67A, nor evidence for insertional hot spots or long-term excess mortality/oncogenesis<sup>21</sup>. Finally, this next generation of vectors that will be tested clinically need to demonstrate enhanced efficacy compared with GL67A. Our data indicate log orders of improvement in respiratory reporter gene expression<sup>22</sup> and importantly, a percentage of transduced airway epithelial cells in multiple species that is well within the 'therapeutic range' proposed for a recessive condition such as CF. Similarly encouraging data have been provided by others using recombinant lentiviral vectors<sup>23</sup>.

## Gene replacement versus gene editing

The GTC has not actively pursued gene editing for three reasons. Firstly, we envisage similar challenges for *in vivo* delivery as we have already encountered for gene replacement strategies. Indeed, until recently it could be argued that the challenge of delivering the gene editing machinery together with sufficient template DNA to favour homology-directed repair (HDR) would be even greater than for gene replacement strategies. Issues with low efficiency and specificity of this process *in vivo* likely limit its use and pose safety concerns. The advent of prime editing as an improved tool not requiring the double strand break/HDR process is a welcome development and may overcome some of these concerns. However, prime editing is still in its infancy and delivery of the large construct of RNA and enzymes will still be challenging, particularly *in vivo*. We believe this delivery issue may be a critical rate-limiting step in the development of clinically relevant gene editing strategies<sup>24</sup>.

Secondly, our preferred lentiviral delivery platform has been tailored for gene replacement and is unlikely to be the choice for gene editing, given the desirability for short-term expression of the gene editing machinery. Finally, gene editing would be particularly advantageous as a 'single-hit' approach. The implication is either the need to target progenitor or stem cells *in vivo*, or the delivery of *ex vivo* transduced cells which are then able to repopulate the conducting airways. Currently, we have not seen sufficient progress in either area to suggest an advantage over gene replacement approaches. We note the considerable and welcome effort in the field of gene editing for CF and speculate that this may serve as a therapeutic option further downstream<sup>25,26</sup>.

## Choosing the most suitable trial population

The European CF Society Clinical Trials Network (ECFS CTN) and the CFF Therapeutics Development Network (TDN) have played pivotal roles in the rapid progress of the trials pipeline to date<sup>27,28</sup>. However, the networks recognise that designing and conducting trials for next generation genetic therapies will pose new challenges once a substantial proportion of the CF population is receiving modulators<sup>29</sup>. There are two broad possible approaches, each with different challenges which require different mitigation strategies.

Firstly, given the likely mutation-agnostic characteristic of gene therapy, clinical trials with broad inclusion criteria could be undertaken. The size of any pivotal study required to detect an additional independent treatment effect of gene therapy over and above the efficacy of modulators will be governed by the magnitude of effect and the likelihood of synergy with modulators; the latter is both theoretically likely and has been shown by the GTC to occur *in vitro*. However, given that modulators are capable of inducing significant improvements in health and well-being<sup>30</sup>, people with CF may be less motivated to take part in the early phase trials needed to establish safety, magnitude and duration of effect. Withdrawing modulator treatment is unlikely to be ethical or acceptable to patients.

Secondly, gene therapy trials could be directed at the population with the greatest unmet need, namely the estimated 15% of those with CF either unsuitable for, or unable to access modulators<sup>29</sup>. For such patients, the perceived risk-benefit ratio for a novel approach may be more favourable and may facilitate rapid approval of trials by ethics committees and consideration of licensing applications by regulatory agencies. Data from this group of people with CF may then inform studies in the broader population. However, it is important to note that the numbers in such groups will be small in individual centres. Thus, multicentre and/or multinational studies will likely be required with their attendant logistical and trial cost implications. Some countries are already adapting to these challenges by establishing referral networks and hub-and-spoke models.

### Selecting trial outcome measures

Assessing the effect of CF gene therapy in trials has included both proof-of-concept molecular surrogate markers as well as clinically relevant markers of efficacy<sup>31</sup>. The former has proved a difficult area across the community. Endogenous levels of CFTR mRNA are well recognised to be low (perhaps 1-2 copies per cell<sup>32</sup>) in the airways and given this, together with the relatively inefficient gene transfer agents used to date, trials have unsurprisingly encountered issues with assay sensitivity<sup>10,33</sup>. To address this challenge, the GTC is placing considerable emphasis both on refinements to assay sensitivity, as well as developing new single cell-based assays. In addition, assessment of CFTR protein levels are widely seen to be hampered by the relative non-specificity of anti-CFTR antibodies which

although useful in cell line studies, have proven to be less so in *ex vivo* patient samples<sup>34, 35</sup>.

In terms of measuring CFTR function as a chloride channel, *in vivo* nasal potential difference has been assessed in multiple studies<sup>36, 37, 38</sup>. Given this assay is assessing continuous rapid physiological changes it unsurprisingly shows a considerable degree of intra-subject variation<sup>39</sup>. Whilst globally agreed standardised performance procedures are in place<sup>40</sup>, the required numbers to power a study are high and typically of the order of around 40 in each group to detect a 'clinically meaningful' difference. We note that in early trials of modulators this assay showed disproportionally lower benefit than was subsequently evident in the clinic and the assay has not been incorporated into more recent trials. Measurement of lower airway potential difference, whilst feasible in the context of a clinical trial, requires the procedure to be undertaken using general anaesthesia (local anaesthetics interfere with ion transport), measures from only a tiny proportion of the treated airway mucosa and requires substantial expertise<sup>10, 41</sup>.

At later stages of gene therapy development, signals of clinical efficacy will be required. The most widely used and accepted outcome is assessment of lung function and specifically the Forced Expiratory Volume in the first second (FEV<sub>1</sub>)<sup>42</sup>. At the time of writing, it is the efficacy outcome approved by regulatory agencies globally and, as such, is likely to continue to be required as a major outcome in future trials. However, it is well established that this measurement lacks sensitivity in the context of milder lung disease, being within the 'normal range' in the presence of structural lung disease on imaging and detectable inflammation/ infection on bronchoalveolar lavage. These observations have largely arisen from paediatric studies, but as the general health of the CF population improves, FEV<sub>1</sub> is also likely to be less useful in the older populations. In our opinion, more sensitive measures such as Lung Clearance Index (LCI)<sup>43, 44</sup> and/or imaging will become increasingly useful. Although the former has been standardised globally and accepted as a primary outcome in paediatric modulator trials, it requires further work before being fully validated as an outcome measure by regulatory agencies; this work is ongoing amongst the international LCI network<sup>45</sup>. We note the rapid progress with Magnetic Resonance Imaging (MRI) of the lung<sup>46, 47</sup> and the advantages this modality offers in terms of being

radiation-free and therefore frequently repeatable. In our opinion, MRI holds considerable promise and work to further expand on the currently small data sets should be encouraged.

Clearly, signal finding for the majority of the above noted assays will be more difficult against a background of modulator-enhanced CFTR function, again suggesting that the 15% of the population with unmet need may provide the optimal entry point for new approaches such as gene therapy. Finally, we note in terms of safety, that this population is enriched for those with null mutations thereby raising the theoretical possibility of an immune response to the therapeutic transgene protein<sup>48</sup>. To date, this has not been observed in our studies but will continue to be closely monitored.

### **Prevention versus treatment**

CF lung disease starts early after birth. Some features, such as mucus plugging may be reversible, whereas others such as distortion of the airways (bronchiectasis) are likely not. Clearly, any treatment initiated later in life, once irreversible disease has occurred, may be limited in its impact. However, measuring improvement from an abnormal baseline is, in general, much easier than measuring slowing of disease progression which requires large numbers of participants and lengthy periods of observation.

CF gene therapy trials have conventionally only recruited adults for reasons of safety and ethics related to informed consent. This is very different to treatment of lethal childhood disorders such as severe combined immunodeficiency syndromes<sup>49</sup>, in which the unmet need has mitigated the risks of the new therapy despite some of these being serious. It seems unlikely, given the clinical course of CF in childhood and the conventional therapies already available, that gene therapy trials will be conducted in children at an early stage. However, once an effective and safe intervention has been established in adults, there is an argument to be made; topical delivery of any drug is better in a clear, non-plugged airway than one with more established disease. Long-term monitoring is mandated by the regulatory authorities for genetic therapies. This will be particularly pertinent for an integrating viral vector<sup>50,51</sup> but may be less of a challenge in CF, given the well-established networks of specialist centres across all age groups.

## Beyond CF; secreted versus membrane proteins

In contrast to the membrane resident CFTR relevant to CF, we and others have previously described the potential advantage of targeting diseases amenable to treatment through secreted proteins. This approach is largely agnostic as to transduced cell type secreting the therapeutic protein which is, therefore, likely to accumulate to much higher levels.

Relevant genetic respiratory conditions might include  $\alpha$ 1-antitrypsin deficiency and the group of surfactant deficiencies, whilst multifactorial conditions such as alveolar proteinosis, interstitial lung disease and COPD may also benefit<sup>52,53,54,55,56</sup>.

Using our lentiviral vector, we have been able to demonstrate apparently therapeutic levels of human  $\alpha$ 1-antitrypsin for up to 2 years in normal mice<sup>57</sup> and in a murine model of alveolar proteinosis were able to correct the clinically relevant phenotypic markers of the disease<sup>58</sup>. Further, we note that when sufficiently high levels of secreted proteins can be produced within the lungs, there is spill-over into the circulation. Thus, apparently therapeutic levels of human Factor VIII can be produced for up to 2 years from pulmonary application of our lentiviral vector<sup>59</sup>. Finally, the use of the vector to secrete antibodies is a means of providing passive immunity<sup>60</sup>. We have shown protection against an influenza lethal challenge in mice (unpublished data), also of obvious relevance to COVID-19 and other infectious lung diseases.

## Cost-of-goods

Despite its apparently curative impact, the gene therapy product Glybera struggled to establish a market presence, largely related to cost-per-dose<sup>61</sup>. Similar considerations have underpinned lengthy discussions about the introduction of CFTR modulators in the UK and elsewhere<sup>62,63</sup>. Whilst at this early stage of development the market cost of our lentivirus-based product is not yet established, it is clear that such therapies are increasingly being manufactured at scale, a key factor in reducing costs<sup>64</sup>. Further, if the lengthy duration of effect (months to years) that we observe in preclinical studies after a single administration is verified in man, we anticipate infrequent dosing requirements. Finally, CF is at the commoner end of the rare disease spectrum with perhaps around ~100,000 patients



worldwide<sup>65</sup> suggesting a substantial market for these products. The GTC is closely focused on this key issue as development of our lentivirus programme proceeds towards the clinic.

## Conclusions

The GTC has made substantial progress since we began working together 20 years ago. We have established proof-of-concept that repeated dosing of the CFTR gene to the lung can stabilise lung disease in a large, placebo-controlled double-blind Phase 2B trial<sup>10</sup>. Our pseudotyped lentiviral programme is partnered with both big pharma and arguably the world leaders in lentiviral manufacturing and is on target for a first-in-man trial. We have taken our experience in developing gene therapies for CF and are now applying it to other respiratory diseases. This progress has been made against the welcome changing context of CF lung disease and this needs to be clearly recognised in the development of the next new therapy for CF. Above, we have outlined some of the issues and solutions we foresee as we look forward to introducing gene therapy into the CF therapeutic armoury.

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