Translational research – from gene to treatment: lessons from cystic fibrosis

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ABSTRACT – Biomedical research is identifying a bewildering number of new targets for treatment of human disease. In order to translate this new knowledge into useful treatments, academics, charities and research councils will need to learn from the experience of large pharmaceutical companies who have been responsible for the majority of drug development over the past 50 years. Cystic fibrosis provides an ideal case study for the development of new treatments as there have been five examples in recent years and there are currently too many therapeutic targets for all to be pursued.

Traditional patterns of drug discovery may need to be replaced by new models of funding and collaboration between academics, biotech, big pharma and charities. In particular, collaboration rather than competition between different interested groups should make the process of drug discovery cheaper and quicker. This applies particularly to gene therapy where new models of research organisation are emerging.

KEY WORDS: amiloride, azithromycin, clinical trial organisation, cystic fibrosis, drug discovery, ibuprofen, nebulised tobramycin, orphan drugs, recombinant human DNase

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In his recent Harveian Oration, Sir Keith Peters praised clinical research and gave five examples of how an acute clinical observation can start a voyage of scientific discovery.[1] In his examples, science is in debt to medicine and translational research moves from the bedside to the bench. The rhetoric, however, is usually the other way round. Grant applications, scientific papers and programmes of research commonly claim that elucidation of this pathway, characterisation of that receptor or identification of another crucial gene will lead smoothly and inevitably to new treatments. While we all pay lip service to the bench-to-bedside version of clinical research, it is far from simple and seldom happens outside the R&D departments of the pharmaceutical industry. Although academic collaborations play some part, industry does the lion's share of the work, funds the programme and takes all the key decisions; decisions which, with their inevitable deadlines, are largely driven by financial and market considerations. This is efficient for new blockbuster drugs designed to treat common conditions, but the vast majority of new pharmacological compounds fail on pre-clinical testing and only one in five compounds entering clinical trials ever reaches the market. Each success takes 10–15 years and $800 million to come to fruition (Fig 1). Not all new drugs are blockbusters for common diseases, and Michael Rawlins, Chairman of the National Institute for Clinical Excellence (NICE), recently stated that the pharmaceutical industry is unable to meet the needs of people with neglected diseases such as chronic obstructive pulmonary disease (COPD), anti-bacterial drug resistance, alcoholic liver disease and stroke.[2] Furthermore, new developments in molecular and cell biology are very different from classical pharmacology and new models of

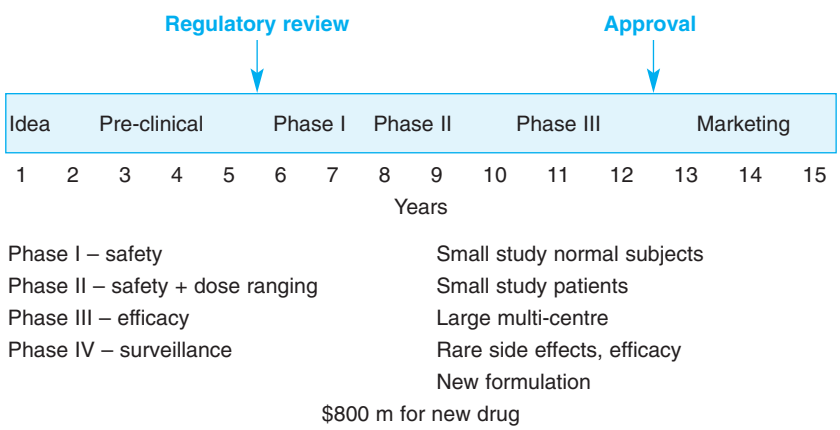

Fig 1. A typical drug development programme, taking 15 years from start to finish, at a cost of $800 million.

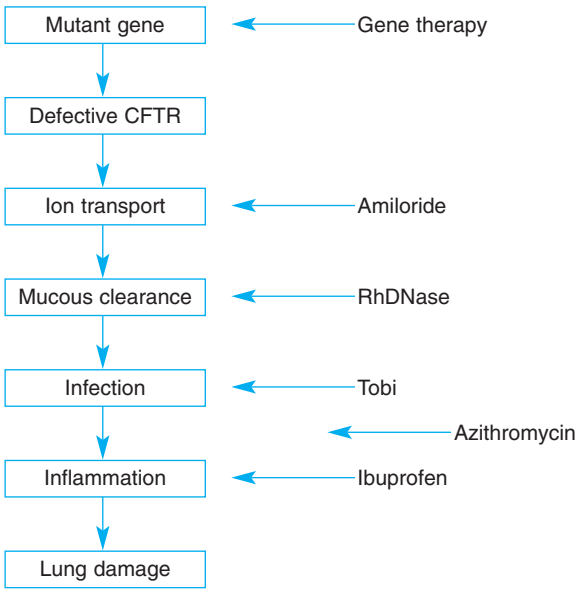

Fig 2. Cystic fibrosis pathogenesis and drug discovery. CFTR = cystic fibrosis transmembrane regulator protein.

developmental research are emerging. One such is the Geneva-based 'drugs for neglected diseases initiative' and another the collaboration between Bill and Melinda Gates, the Rockefeller Foundation and the World Health Organization to improve treatment for tuberculosis (TB).

In the USA, where venture capital is abundant, it is increasingly common for clinical scientists to start biotechnology companies based on their own intellectual property, and the aim of such companies is to develop a new treatment to the point where a large pharmaceutical company buys it. In contrast, the European approach is for universities to encourage start-up companies with an academic/commercial mix. A third model, pioneered in neglected diseases such as TB or rare diseases like cystic fibrosis (CF), is to build an academia/charity/industry consortium aimed again at developing a treatment to a point that large drug company takes it on.

Cystic fibrosis provides is an ideal case study for the development of new treatments. Over the past 15 years the path from mutant gene to disease has been well mapped (Fig 2) and many new treatments proposed; some are now widely prescribed,

Table 1. Clinical trial program for RhDNase (biotech company).

Date	Phase	n	Outcome	Authors
1992	I	14	No adverse effect	Aitken *et al*[3]
1993	I + dose range	32	No adverse effect	Hubbard *et al*[4]
1993	II	181	FEV_1 +10–14%	Ramsey *et al*[5]
1994	II	71	FEV_1 + 13%	Ranasinha *et al*[6]
1994	III	968	FEV_1 + 5.8% Fewer exacerbations	Fuchs *et al*[7]

Key Points

Biomedical research is providing an increasing number of targets for new treatments

Traditional methods of drug discovery are too costly for all except blockbuster drugs for common diseases

New models of collaborative research involving charities, academics and government funding are developing

These new models will be particularly appropriate for the development of gene therapy

some have failed and many are still being investigated. Indeed, there are now so many potential new treatments in the pipeline that obvious losers may get in the way of potential winners. So priorities need to be set, and some academics will have to abandon their favourites to allow limited resources to go to the most promising. This article reviews the development of new treatments for CF, focusing on different ways that academics, clinicians and industry can work together, and suggests a way forward for gene-based treatments.

Drug treatments

RhDNase (pulmozyme)

The development of RhDNase was dependent on new biotechnology but followed a conventional path for a new drug. The idea of liquefying sputum with DNase had been around for a long time and there was prior evidence of clinical efficacy. However, previously DNase came from a crude extract of pig pancreas and was allergenic, impure and irritant. Genentech, then a small biotech company, saw that pure human DNase might overcome these problems and set out to manufacture it using recently developed recombinant protein technology. Formal phase I–III trials (Table 1)[3–7] were fast tracked; these showed that within a few days of starting treatment lung function and symptoms improved, so pleasing patient and prescriber alike (Fig 3).

All pre-clinical tests and regulatory issues were done and paid for by Genentech. Clinicians in selected centres were approached and asked to join the clinical trial programme and were generously funded for doing so. The whole process shows industry working at its best. The programme was well planned and clinical trials professionally organised and staffed. The drug was quickly marketed and, although expensive, was soon widely prescribed.

Amiloride

Academic clinicians in North Carolina identified deranged airway ion transport as central to the pathogenesis of CF and went on to show that the epithelial sodium channel was overactive and could be blocked by topical (not systemic) amiloride.[8] After some elegant pre-clinical studies, an uncoordinated

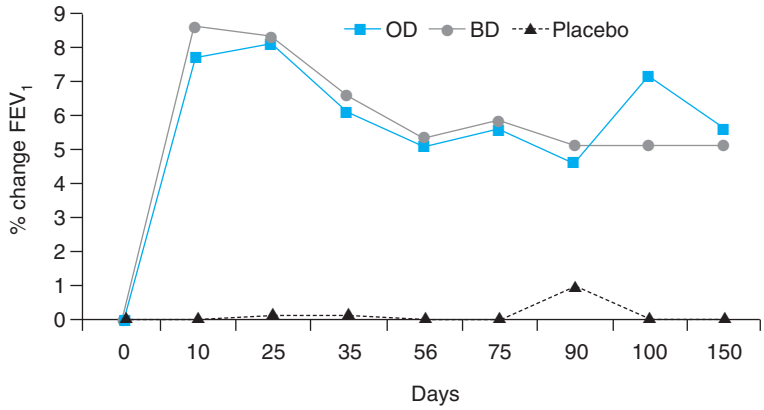

Fig 3. Phase III trial of RhDNase in cystic fibrosis. BD = twice daily; OD = once daily.

clinical trial programme began (Table 2).[9–13] There was a series of pilot trials, none of which formally fitted the phase I–II definitions; one American trial showed that the drug worked and two European trials showed it did not. These were followed by two multi-centre phase III studies, one funded by the French CF Charity AFRM and one by Glaxo, but these failed to show any benefit. This was, perhaps, not surprising as the North Carolina group had shown that amiloride has a very short half-life on the airways. In 2004, a comparison of airway pharmacodynamics of sodium channel blockers was published by the group who had had the original idea 23 years earlier.[14]

This programme was started by academic clinicians and funded by charitable donations. Amiloride was already licensed for human use and the advantages of developing new indications for off-the-shelf treatments are many. Only minimal preclinical work was needed, toxicology had already been done (although some had to be repeated in the airways), regulatory hurdles were low or non-existent and the clinical programme could have been short and cheap. In practice, this did not happen. First, there were no formal phase II dose ranging studies. A second problem was the lack of a formal coordinated programme which resulted in duplication of effort with a number of independent under-powered studies. It is interesting to speculate whether a pharmaceutical company would have abandoned the compound early in the programme in view of this short half-life, or would have done the comparative pharmacodynamic studies earlier in order to develop a chemical with a better chance of success. If this had happened, then there is a good chance that a new and effective treatment would have emerged 20 years ago. Instead, research continues in a few academic centres but lack of intellectual property and poor prospects of profit mean that the level of investment is low.

Table 2. Clinical trial program for amiloride (academic groups).

Date	Phase	n	Outcome	Authors
1986	Pilot	8	Lung clearance	Kohler *et al*[9]
1990	Pilot	18	Spirometry + clinical	Knowles *et al*[10]
1992	Pilot	9	Spirometry + clinical	Reidler *et al*[11]
1993	Pilot	14	Spirometry + clinical	Graham *et al*[12]
2000	III	137	Spirometry + clinical	Pons *et al*[13]
?	III	?	Spirometry + clinical	Glaxo unpublished

Table 3. Clinical trials of ibuprofen in CF (academic group).

Date	Phase	n	Outcome	Authors
1991	II	13	Safety + pharmacokinetics	Konstan *et al*[16]
1995	III	85	Decline in FEV_1 + clinical	Konstan *et al*[17]

Ibuprofen

Airway inflammation is the main pathway leading to lung damage in CF, and the idea of limiting this damage with anti-inflammatory drugs remains an important focus of research. Oral corticosteroids have been shown to work but the doses used caused unacceptable side effects. An academic group therefore assessed ibuprofen as a non-steroidal anti-inflammatory drug aimed at the airways (Table 3). Inflammation was reduced in a rat model of Pseudomonas infection[15] and so, following a dosing safety study in children,[16] a single multi-centre trial was done and showed a reduction in rate of decline in lung function at least in the younger subjects in the trial.[17] This was a new and somewhat ambitious outcome measure and questions have been raised about the unusually rapid decline seen in the placebo group. Nevertheless, a recent phase IV surveillance study appears to confirm the benefit, albeit to a much smaller degree. Perhaps because there was no short-term symptom gain to encourage patients and prescribers, perhaps because of concerns about side effects, and perhaps because blood drug levels are needed to monitor treatment, ibuprofen has not been very widely prescribed. There is, however, little, if any, money to be made and so no drug company has funded a marketing campaign and this has probably contributed to the low uptake of ibuprofen. In this context, it will be interesting to see which of two new ways of improving muco-ciliary clearance wins; one, dry powder mannitol, involves intellectual property, while the other, hypertonic saline, does not.

Tobi

This is an example of a highly successful new treatment developed by a small company which took a previously tested form of therapy and redesigned it. Here the regulatory hurdles were higher than with off-the-shelf treatments but clinical trial design and outcome measures were conventional and it was marketed successfully and remarkably quickly. The drug (tobramycin) was on the shelf, nebulised delivery systems were available, and numerous previous studies had shown that nebulised antibiotics worked. Pre-clinical work focussed on developing a preservative-free formulation to reduce airway irritation and matching the variables of drug dose, nebulised volume and nebuliser characteristics.[18] These refinements required regulatory approval and a more complex programme of clinical trials (Table 4).[19–22] Interestingly, the company took considerable trouble to assess the optimal dose – something that previous nebulised antibiotic studies had ignored. There were intellectual property incentives and the prospect of profit. A small start-up company, Pathogenesis, helped by the Orphan Drug Act 1983 and financial support from the US Cystic Fibrosis (CF) Foundation, developed the treatment and can be justly proud that it is the only antibiotic treatment specifically formulated for nebulised use.

The drug development path for Tobi was conventional except that clinical efficacy had already been shown with similar compounds. Surprisingly, nebulised antibiotics had already passed the meta-analysis test without a single trial initiated by a drug company. The development of Tobi was particularly interesting as there was intimate association between Pathogenesis, a small pharmaceutical company, and the CF Foundation, a charity, involving financing, staffing and the clinical trial programme. The drug reached the market quickly, was aggressively marketed and, in spite of a cost of approximately $10,000 per annum, was widely prescribed. On the back of this success, Pathogenesis was bought by the larger Chiron and both the company and the CF Foundation made money. Again, a dose ranging trial, trial programme coordination and subsequent marketing were crucial. Tobi is now the lead nebulised antibiotic for CF in spite of the fact that there were 20 years experience and more than 10 controlled trials using other drugs – perhaps at too low a dose. No trial has shown conclusively that Tobi is better than the competition but it has been expertly tested and marketed. Industry is better at planning and marketing than clinicians. Programmes of post marketing development include a dry powder formulation and clinical trial in non-CF bronchiectasis and ventilator-associated pneumonia.

Table 4. Clinical trials of Tobi in CF (small pharma + charity).

Date	Phase	n	Outcome	Authors
1997	II	68	Safety + sputum levels	Eisenberg *et al*[18]
1993	II	71	Safety + FEV_1	Ramsey *et al*[19]
1997	III	520	Spirometry + clinical	Ramsey *et al*[20]
2002	IV	115	Spirometry	Hodson *et al*[21]
2004	IV	63	Hospitalisation + spirometry	Murphy *et al*[22]

Table 5. Clinical trials of azithromycin in CF (academic + charity).

Date	Phase	n	Outcome	Authors
2002	II	60	Spirometry + safety	Wolter *et al*[23]
2002	II	41	Spirometry + safety	Equi *et al*[24]
2003	III	185	Spiromety + clinical	Saiman *et al*[25]

Azithromycin

This most recent example shows how well the previous lessons have been learned. Clinicians knew that erythromycin was effective in Japanese panbronchiolitis and noticed that azithromycin seemed to provide benefit in CF even in the presence of resistant bacteria, so they set up a number of small clinical trials. These trials confirmed an increase in lung function and reduction in infective exacerbations. A large multi-centre trial was then set up by the North American CF Foundation with similar results. No pre-clinical work was needed, there were no regulatory hurdles, no new intellectual property and the clinical trial program was simple (Table 5).[23–25]

Lessons learnt

Perhaps the single most important lesson from the recent history of drug development in CF is that a charity can make an enormous contribution not only by funding the trials but by coordinating academics, clinicians and clinical trial centres in a way that would normally be done by a drug company. Where there is no profit, altruism must take over.

Gene therapy

The concepts are simple: isolate and manufacture the normal human gene, use a gene transfer agent (GTA) to get it to the cell nucleus and switch on protein synthesis. The CF protein will then normalise cellular function and the disease will be corrected. The 1990s saw academics, clinicians and a few biotechnology companies racing towards the finishing line, while big pharma looked on waiting for the moment to pounce.

Pre-clinical work involved identifying and cloning the gene and attaching promoters (academics), growing it in bacteria (biotech), developing potential GTAs (everyone) and selection of the best gene-GTA combination (no-one). Inevitably, a range of academic/industrial groupings grew up, driven by competition more than coordination; each grouping developed their own gene-GTA combination and there was no attempt to choose the best or to join up for clinical trials. As a result there were many toxicology programmes, thousands of pages of regulatory submissions and more than ten similar proof of principle clinical trials.

When the dust had settled a few lessons had been learned. First, there was no show-stopping toxicity, although dose-dependent inflammation was a problem with some viral vectors. Second, repeated application of viral vectors effectively vaccinated against the virus making each subsequent dose less effective. Third, most but not all trials showed some limited efficacy, judged by the whole field to be inadequate for therapy. The consequences were damaging. Biotech companies ran out of venture capital, academics ran away to more fertile fields, charitable funding ran round in circles looking for better bets, and gene therapy research ran into the ground. Perhaps the most important lesson, not fully appreciated at the time, was that we had all gone about it in the wrong way. Big pharma might have done it better. More collaboration would have allowed the best product to be identified, so saving money and time on toxicology and regulatory paperwork, as well as fewer clinical trials with larger numbers of volunteers and better defined outcome measures.

Table 6. A matrix of candidate outcome measures for CF gene therapy trials showing clinical trial numbers for statistical power for each outcome measure.

Assay	Mean & SD (normal)	Mean & SD (CF)	10%	30%	50%	100%
Chloride transport	9.5 ± 3.5	–4.6 ± 2.6	100	26	6	4
Airway surface liquid height	4.68 ± 0.42	4.14 ± 0.28	1109	107	37	11
Bacterial adherence	5.0 ± 1.8	9.8 ± 3.1	605	74	28	8
Goblet cell number	64 ± 16.2	86.7 ± 10.3	780	90	34	10

This brings us to where we are today. The three UK groups who worked independently in the 1990s have joined up and formed the UK CF gene therapy consortium. Core facilities, techniques, data and strategy are all shared. Collaboration has replaced competition and has brought major benefits. First, each group plays to its strengths: molecular biologists improve vector design while clinicians concentrate on nebulised delivery and clinical outcomes; whereas previously everyone did everything. Second, strategic planning is more formal and with more experts in the room the decisions are better informed. Third, difficult areas of research are better staffed and resourced; and fourth, routine work such as grant writing, industrial links, regulatory paperwork etc is no longer duplicated.

Naturally, there are also disadvantages. Academics can no longer follow their enthusiasms and many projects have to be abandoned. For example, the three research groups were previously working with three different gene/GTA combinations; these have to be compared and the losers discarded. Second, the shift from discovery research into product development does not suit all researchers and some may feel that they are giving up academic freedom without getting any more money – so why not apply for a better paid job in industry. Third, publications and career development may be inhibited; there is only one first author on every paper. Finally, while giving due respect to many powerful egos, diplomacy and sometimes a little coercion are needed. But perhaps the single most important motive for making the consortium work comes from the patients: all 50 researchers see what the patients have to endure and see exactly what their research is trying to do.

The UK CF Trust helped to set up the consortium and has provided the money. There has naturally been some resentment from CF researchers outside the consortium who fear that there will not be enough money to go round but fortunately the consortium has proved a uniquely successful fund-raising platform (dedicated CF gene therapy money was even allocated by government in 2004). The charity convenes an independent panel annually to review progress against plans and to approve future milestones. Big pharma have not been formally involved so far, but there are many collaborative projects with biotech and the fundamental aim of the consortium is to develop gene therapy to the point where big pharma want to take it over.

One major problem that will be shared by many, if not all, gene-based therapies in the future is that of intellectual property. The gene is free for all and so a patentable GTA, manufacturing process or delivery system may be needed as bait.

Future research

Gene therapy and many other treatments are being worked on in parallel and in particular the US CF foundation has invested heavily in high throughput screening of compounds with chloride channel activity. One major problem for all is the choice of clinically relevant outcome measures. The conventional outcomes, used in both COPD and CF trials are FEV_1, and frequency of exacerbations. Rates of decline of FEV_1 have also been proposed but carry certain problems: first, there is no immediate symptom relief and so changes may not be detected by patients or prescribers; and second, rates of decline are slow and variable and trials need to enrol large numbers of subjects for a long time – difficult in the absence of symptom gain. In the past, CF was a rapidly progressing disease and FEV_1 used to decline fast but now the fall is <1% per year so this outcome measure may be out of date. The UK consortium has assessed many outcomes including airway ion transport, bacterial numbers, histopathology and inflammatory markers in sputum and exhaled breath to inform clinical trial design (Table 6). Such information is essential for testing any new treatment to ensure that a trial can provide an answer.

Conclusions

Low-hanging fruit have been harvested and new treatments will be increasingly difficult to develop. The experience of CF allows the following conclusions:

1 There are too many therapeutic targets for all to be pursued.
2 Traditional patterns of drug discovery and testing may no longer work well.

3 New models of funding and collaboration between academics, biotech, big pharma and charities are evolving; no size fits all.

4 Academic researchers need to learn from industry and apply a more focused strategic approach to translational research.

5 Simple rapid-response outcome measures may need to be replaced by panels of surrogate outcomes to inform clinical trial design.

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