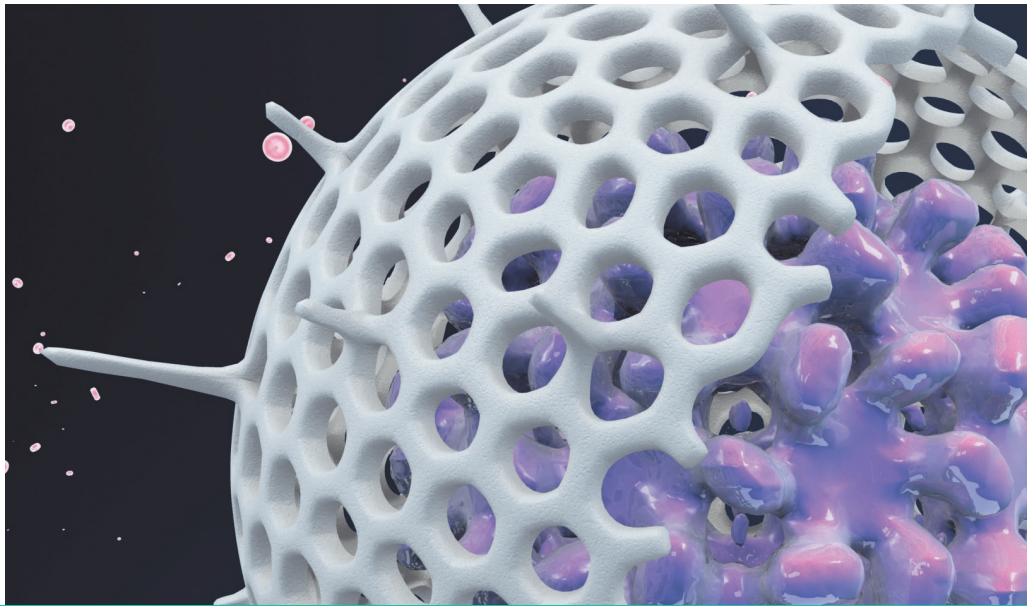


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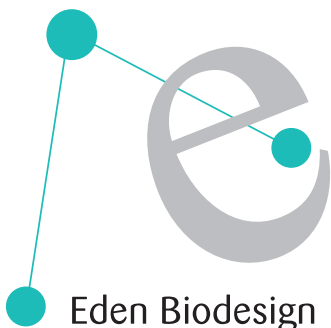
Whilst the concept of gene therapy is well established, it is still a young technology in terms of manufacturing.

There are no licensed products in routine clinical distribution and use.



Adherence to GMP Guidelines Critical for Gene Therapy

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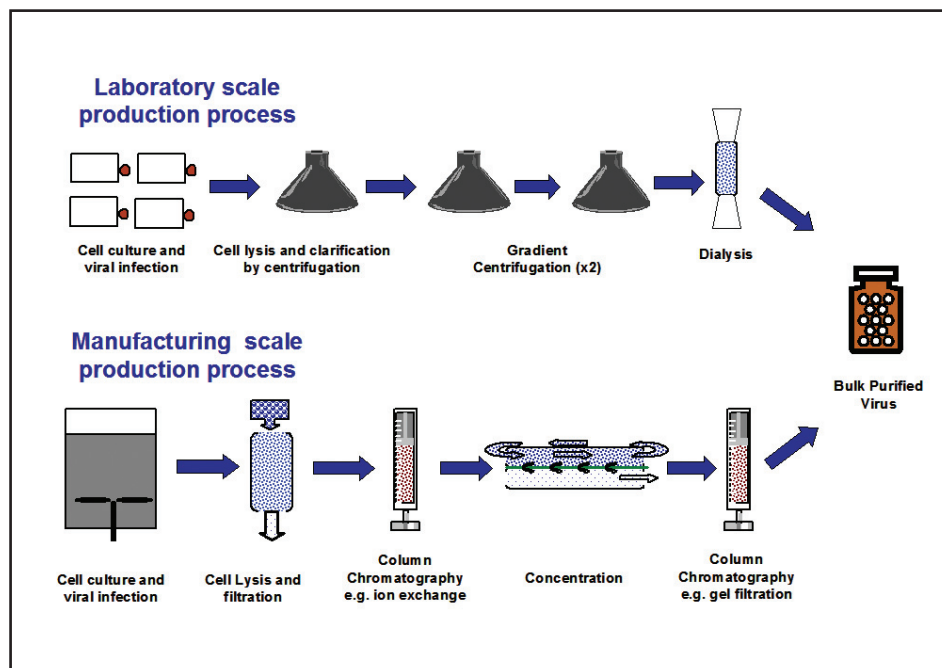
There are currently around one thousand gene transfer clinical trials in progress worldwide including 67% in America and 28% in Europe (Journal of Gene Medicine, 2004). Most of these trials are in clinical Phase I and early Phase II (around 80 %), with only 1.7% so far reaching Phase III. By far the largest indication for gene transfer is Cancer which accounts for 66% of the clinical trials, however there are still a significant number for Monogenic Disease (9%), Vascular diseases (8%) and Infectious diseases (7%).

The vast majority of these products use viruses as the vector for gene transfer (70%), the technology focus for this article.

Gene therapy is presenting major headaches for manufacturers

As with any new therapeutic approach there have been achievements and disappointments, and the ultimate success of gene therapy will depend on the efficacy and safety of its products.

There has been increased regulatory vigilance for all medicinal products over the last decade to ensure the safety of patients recruited into clinical trials.



In Europe the Clinical Trials Directive (2001/20/EC) has dictated that all investigational medicinal products (IMP) should be manufactured to GMP and released by a Qualified Person for clinical use. Whilst active ingredients (drug substance) are in theory not included in the legislation, in practice most European regulatory agencies are applying the legislation to biological actives. Good manufacturing practices (GMP) were born out of adversity with tragedies such as SV40 in polio vaccines and thalidomide and are written into legislation. Gene therapy IMPs are also the subject of increasing scrutiny in the USA after the death of a patient in 1999

The main focus of GMP is patient protection, it encompasses all aspects of routine manufacture and demands that all critical parameters are controlled:

The facility and equipment must be of suitable quality and have be thoroughly tested in place to show it is operating correctly; All operating procedures and testing must be rigorously documented and thorough records kept to ensure traceability through all stages of the manufacture; GMP also emphasises the continuous training of personnel to ensure all procedures are understood and complied with; All testing procedures performed must be validated to ensure they provide consistent and true results; so good project management is crucial to bind the process together.

This all sounds straightforward if you say it quickly, but in practise a lot of effort, expertise, specialist facilities and resources are needed to get it right, and this necessarily increases the expense of the manufacture of investigational products for early phase clinical trials.

Further legislation applies to the safe working with live virus necessary for manufacture of viral vectors, with substantial consequences for facility design and working practices such as sterile manufacture and segregation.

The majority of current clinical trials are indicated for the prevention or treatment cancer which can affect up to 1/3rd of the population at sometime in their lives and therefore, are clinically and commercially attractive. However, many gene transfer medicines are targeted for rare disorders that affect less than 0.1% of the population but are never the less severe and life threatening. These products are often developed by small organisations which lack the resources of large pharmaceutical companies and can make the unique challenges of developing gene transfer medicines a daunting prospect.



For the research groups, charities and other small organisations focused on alleviating these diseases, there is not only the problem of the resource needed to conduct the clinical trials, but also a lack of funding, expertise and appropriate facilities to make the product to the required quality. Many of these activities will require outsourcing to other organisations such as contract manufacturers and testing organisations which in itself requires extensive understanding of the technical issues and exhaustive project management by the sponsor. Given that gene therapies are often targeted at rare disorders, the ability to produce a product which is commercially viable, even as an orphan drug, is a challenge requiring expert development know how and an attention to cost of goods from day one.

The technical transfer of the production method and analysis to the manufacturing unit needs to be well managed and controlled. The concept of providing the viral seed and cell bank and letting the manufacturer 'get on with it' is not a viable option since there may be particular requirements for that specific cell and vector system which require specialist knowledge transfer. Proper management is essential if the process is to survive the scrutiny of the licensing authorities.

Early manufacturing development and characterisation is crucial to the success of the ultimate product.

It is all too common for laboratory based processes to be employed for the manufacture of early phase material in which vital parts of documentation are inadequate or missing leading to

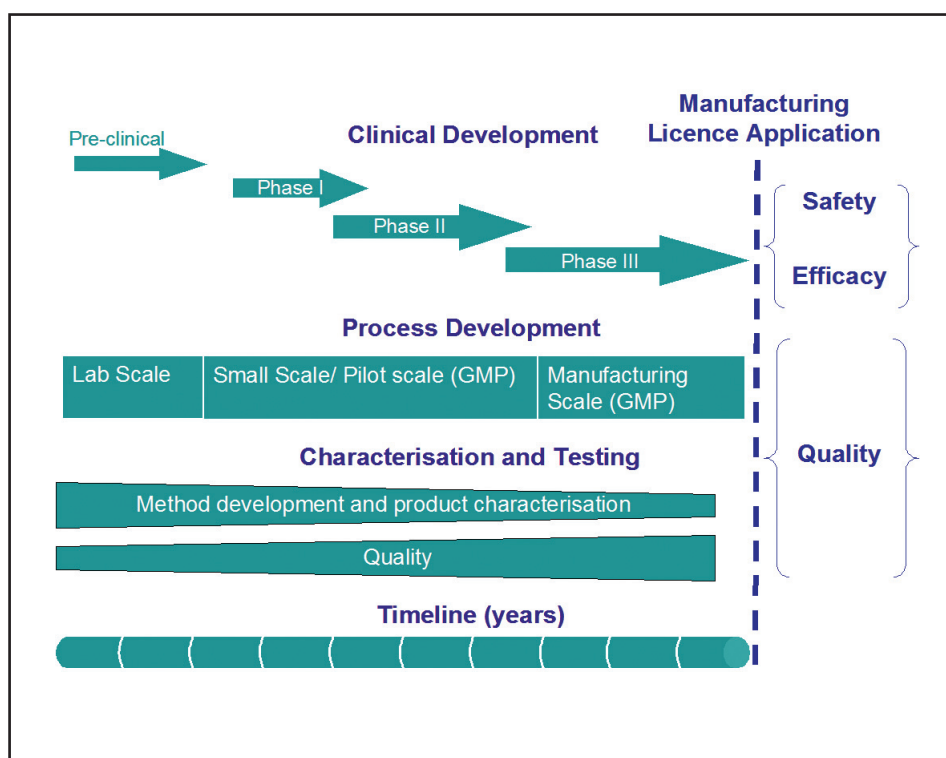
results that are not reproducible. Many development u-turns, repetitions of work, and even product failures can be traced to this.

Documentation is critical

One of the key areas is the construction of the vector and the cell line. Although there are some well defined expression systems available commercially, the price of these systems can be a major hurdle for research organisations with limited R&D budgets. Naturally, they are reticent to spend money on proprietary expression systems, and either create their own or obtain a system from another source. This can create traceability issues since each step of the history of construction may not be documented adequately and hence provide concern regarding the origin of the materials used in construction.

Yield and purity always pose problems

Vectors for gene transfer products are required at high titres and there are a number of factors which may influence production. Vectors are designed so that they are unable to replicate in the target tissues, this requires construction of vectors which are lacking in the essential genes needed for replication and release from the cell. This necessarily reduces the number of cell types that the vector will replicate in and can also affect the yield. Novel methods of manufacture and raw materials may be required in order to create a viable production process.



The techniques required to obtain high purity required of products resulting from recombinant DNA technologies will also further reduce the final vector yield. It is important that impurities derived from the production process such as serum and host cell proteins are reduced to acceptable levels whilst still retaining the activity of the virus.

It is also important to ensure that the process is scalable to ensure adequate supply.

Density gradient centrifugation is often used in research laboratories for production of virus stocks; however, this method is difficult to scale up and results in impure preparations.

Chromatography methods, widely used in protein purification, have successfully been introduced for the production of adenoviral vectors yielding products of high purity with reduced defective particle concentrations. Although this approach is challenging for the more labile viruses such as retroviral vectors it is an important consideration in the development of a new product as it will reduce the cost and improve the consistency of manufacture.

The basis of product analysis is good characterisation

The analysis of products at early stage process development is often limited to the titre of the virus, the presence of the gene of interest and the potency. Although these are key parameters for any vector, it is also important that a characterisation program is considered during early development with regard to purity, identity, potency, stability and physicochemical properties of the viral preparation

So, in conclusion, gene therapy poses many both old and new challenges to manufacturing:

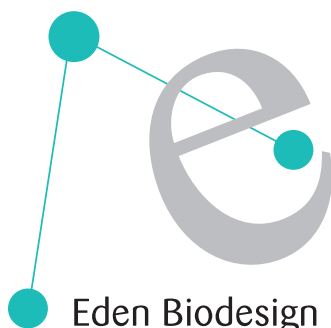
Inadequate early stage documentation and traceability continues to cause problems and delays many approvals, not only for gene therapy products but many other biopharmaceuticals. Starting off on the right track is crucial.

Since gene therapy offers radical treatments for low prevalence gene disorders many small organisations are joining up with contract manufacturing organisations to generate products –

and the challenge in these cases is to ensure that proper and informed project management is steering the process through the correct stages.

The complexity of systems, and facilities needed to properly enforce GMP particularly with reference to live virus handling makes it difficult to undertake in anything other than purpose designed facilities. So vector manufacture may become concentrated into a handful of well accredited institutions.

And finally gene therapy, because of its infancy, is still to some extent venturing into the unknown and so from time to time will throw up unique problems not encountered elsewhere. This can be evidenced by the increased scrutiny of the regulatory agencies.



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Dr Anita Bate is Director of Science for Eden Biodesign, the operator of the UK National Biomanufacturing Centre. Eden Biodesign designs and delivers valuable biopharmaceutical medicines by the application of good science from day one. The remit of the NBC is to provide expert guidance, state of the art facilities and financial assistance for biopharmaceutical development programmes.