Production of AAV Vectors for Gene Therapy: a Cost-effectiveness and Risk Assessment

by

Diego Rodríguez Pinhao Miessner

B.S., Chemical Engineering
Universidad Iberoamericana, Mexico City, 2010

Submitted to the Department of Chemical Engineering and the MIT Sloan School of Management in Partial Fulfillment of the Requirements for the Degrees of

Master of Science in Chemical Engineering and Master of Business Administration in conjunction with the Leaders for Global Operations Program

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Massachusetts Institute of Technology

June 2016

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Abstract

Gene therapy is a promising modality for the potential treatment of rare Mendelian diseases. To date a number of high profile proof-of-concept studies within the industry have demonstrated the significant disease-correcting promise of this therapeutic strategy. One of the major hurdles that remains for the commercialization of gene therapies is the lack of efficient manufacturing capabilities for the production of clinical-grade drug substance/drug product.

The primary goals for this project were to decrease the biological contamination and cross-contamination risk associated with the biologic manufacturing process for viral gene therapy vectors and to adjust the process in order to optimize commercial profit. The project also included documenting the different existing processes for AAV production and developing a competitive analysis using information from ongoing clinical trials in the industry pipeline.

The following process design steps were followed in order to fulfill the project objectives: (1) Define product specifications, analytical needs and market size, (2) Select production platform/process, (3) Collect data and create process flow diagram, (4) Perform material and energy balances, (5) Calculate costs: equipment and consumables, (6) Model the process in a spreadsheet, (7) Carry out sensitivity analyses, (8) Assess cost-effectiveness and risk, and (9) Develop recommendations.

Five different AAV production platforms were identified and an AAV gene therapy landscape was generated. Also, the current process that Pfizer is planning to use was documented and an initial market sizing was performed. Finally, all the data necessary to model the process was collected and the cost-effectiveness and biological contamination and cross-contamination risk assessment were completed.

This project confirmed that the use of a scalable line of single-use high cell density bioreactors for the production of AAV is cost-effective. This implies that sufficient AAV quantities can be manufactured for preclinical and clinical trials, using the process developed by Pfizer.

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A mi papá, Emilio, 
por estar siempre conmigo. Eres un ejemplo a seguir y sé que este logro sería tan especial para ti como lo es para mí.

A mi mamá, Irmgard, 
por su amor y motivación constante. Gracias por ser el pilar fundamental de mi vida. Sin ti, jamás hubiera podido lograr este sueño.

A mi familia: mi hermano, mi cuñada, mis abuelos, mis tíos y mis primos, 
por su cariño y apoyo incondicional.
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To Pfizer Inc. for the opportunity to work on a meaningful project and to grow personally and professionally. Special thanks to Martin Duballet, my supervisors: Michael Linden and Phil Jeffrey, and all my colleagues at the Genetic Medicine Institute.

To my thesis supervisors, Kris Prather and Roy Welsch, for the time invested in this project and their valuable input and direction.

To my professors, including high school and undergrad, for being an inspiration in my life.

To my friends for listening, offering me advice, and motivating me through this entire process.

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<td>Adeno-Associated Virus</td>
</tr>
<tr>
<td>CF</td>
<td>Cell Factory</td>
</tr>
<tr>
<td>cGMP</td>
<td>Current Good Manufacturing Practice</td>
</tr>
<tr>
<td>DSP</td>
<td>Downstream Processing, also known as purification</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>GMI</td>
<td>Pfizer’s Genetic Medicine Institute</td>
</tr>
<tr>
<td>GOI</td>
<td>Gene of Interest</td>
</tr>
<tr>
<td>PEI</td>
<td>Polyethyleneimine</td>
</tr>
<tr>
<td>rAAV</td>
<td>Recombinant Adeno-Associated Virus</td>
</tr>
<tr>
<td>R&amp;D</td>
<td>Research and Development</td>
</tr>
<tr>
<td>RDRU</td>
<td>Pfizer’s Rare Disease Research Unit</td>
</tr>
<tr>
<td>USP</td>
<td>Upstream Processing, also known as fermentation</td>
</tr>
<tr>
<td>VG</td>
<td>Vector Genomes</td>
</tr>
<tr>
<td>YFP</td>
<td>Yellow Fluorescent Protein</td>
</tr>
</tbody>
</table>
1. Introduction

1.1 Project Motivation

Gene therapy is a promising modality for the potential treatment of rare Mendelian diseases. To date a number of high profile proof-of-concept studies within the industry have demonstrated the significant disease-correcting promise of this therapeutic strategy. One of the major hurdles that remains for the commercialization of gene therapies is the lack of efficient manufacturing capabilities for the production of clinical-grade drug substance/drug product. In order to make gene therapy a therapeutic reality the current lack in the development and implementation of biologic manufacturing processes for viral gene therapy vectors that are amenable to industrial approaches must be addressed. This project sought to address this issue by establishing and integrating an upstream process flow map into the current developmental vector production approach as part of Pfizer’s new Genetic Medicine Institute in London, UK. The establishment of this process flow map was based on an in depth analysis of the risk/cost-effectiveness balance.

The motivation for this project was to establish a novel, high quality biomanufacturing process for the production of sufficient AAV quantities for preclinical and clinical trials.

1.2 Problem Statement and Project Goals

The primary goals for this project were to decrease the biological contamination and cross-contamination risk associated with the biologic manufacturing process for viral gene therapy vectors and to adjust the process in order to optimize commercial profit. The project also included documenting the different existing processes for AAV production and developing a competitive analysis using information from ongoing clinical trials in the industry pipeline.
The outcome of this project is an overview of the AAV gene therapy landscape and a cost-effectiveness and risk assessment of the new manufacturing process. All upstream process steps were assessed for process efficiency/robustness as well as economic viability.

1.3 Thesis Overview

Chapter 1 introduces the project by describing its context, scope and primary goals. Also, the motivation behind the project is briefly described in order to understand what the expected outcomes were. Finally, the hypothesis that the thesis attempts to test is proposed.

Chapter 2 provides an overview of the pharmaceutical and biotechnology industry and dives into some specifics about biologics, especially gene therapy. Also a brief overview about Pfizer is included with an introduction to the Rare Disease Research Unit and the Genetic Medicine Institute. Lastly, the basics of process modeling and simulation are described.

Chapter 3 contains the literature review, which summarizes previous work published on topics related to this project. The five main AAV vector production platforms are discussed and information from other studies that have attempted to scale-up the AAV production process is presented.

Chapter 4 describes the methodology followed to complete this project. Special emphasis is given to the description of the process simulation, its inputs, outputs and main variables. In addition, the main assumptions that were made are described.

Chapter 5 presents the results, including the AAV gene therapy landscape, the documented production process and its simulation.
Chapter 6 includes a sensitivity analysis to determine the operating conditions that minimize biological contamination and cross-contamination risk and optimize cost-effectiveness. The different simulation scenarios are described in this chapter.

Chapter 7 is the final chapter and it concludes the thesis with a summary of the key points and provides the recommendations derived from the research findings. Additionally, some areas for further research are highlighted.

1.4 Hypothesis

The hypothesis of this thesis is that the process based on adherent mammalian cells using transient transfection is economically viable and does not represent a limitation to large-scale production of AAV vectors for gene therapy. The expected outcome is to confirm if the use of a scalable line of single-use high cell density bioreactors for the production of AAV is cost-effective and has a low biological contamination and cross-contamination risk. If the hypothesis is confirmed, the lack of efficient manufacturing capabilities for the production of clinical-grade drug substance will be addressed and sufficient AAV quantities could be manufactured for preclinical and clinical trials.
2. Background

2.1 Pharmaceutical Industry Overview

The Pharmaceutical industry is distinguished by a high level of concentration. Worldwide prescription drug sales were estimated at $748.4 billion in 2014 and they forecast an average growth of five percent per year from 2014 to 2020. The top 20 Pharma companies account for 64.4% of the total Pharma revenue and 63.9% of the total R&D spend.

Table 1: Worldwide Prescription Drug Sales and R&D Spend (2014): Top 20 Companies and Total Market [1]

<table>
<thead>
<tr>
<th>Rank</th>
<th>Company</th>
<th>2014 WW Rx Sales (Sbn)</th>
<th>2014 Pharma R&amp;D (Sbn)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Novartis (Switzerland)</td>
<td>46.1</td>
<td>9.3</td>
</tr>
<tr>
<td>2</td>
<td>Pfizer (U.S.)</td>
<td>44.5</td>
<td>7.2</td>
</tr>
<tr>
<td>3</td>
<td>Roche (Switzerland)</td>
<td>40.1</td>
<td>8.6</td>
</tr>
<tr>
<td>4</td>
<td>Sanofi (France)</td>
<td>38.2</td>
<td>6.2</td>
</tr>
<tr>
<td>5</td>
<td>Merck &amp; Co. (U.S.)</td>
<td>36.6</td>
<td>6.5</td>
</tr>
<tr>
<td>6</td>
<td>Johnson &amp; Johnson (U.S.)</td>
<td>30.7</td>
<td>6.2</td>
</tr>
<tr>
<td>7</td>
<td>GlaxoSmithKline (U.K.)</td>
<td>30.3</td>
<td>5.1</td>
</tr>
<tr>
<td>8</td>
<td>AstraZeneca (U.K.)</td>
<td>25.7</td>
<td>4.9</td>
</tr>
<tr>
<td>9</td>
<td>Gilead Sciences (U.S.)</td>
<td>24.5</td>
<td>2.7</td>
</tr>
<tr>
<td>10</td>
<td>AbbVie (U.S.)</td>
<td>19.9</td>
<td>3.3</td>
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<tr>
<td>11</td>
<td>Amgen (U.S.)</td>
<td>19.3</td>
<td>4.1</td>
</tr>
<tr>
<td>12</td>
<td>Teva Pharmaceutical Industries (Israel)</td>
<td>17.5</td>
<td>1.5</td>
</tr>
<tr>
<td>13</td>
<td>Eli Lilly (U.S.)</td>
<td>16.5</td>
<td>2.5</td>
</tr>
<tr>
<td>14</td>
<td>Bayer (Germany)</td>
<td>16.4</td>
<td>4.4</td>
</tr>
<tr>
<td>15</td>
<td>Novo Nordisk (Denmark)</td>
<td>15.8</td>
<td>2.5</td>
</tr>
<tr>
<td>16</td>
<td>Boehringer Ingelheim (Germany)</td>
<td>13.4</td>
<td>3.1</td>
</tr>
<tr>
<td>17</td>
<td>Takeda (Japan)</td>
<td>13.0</td>
<td>3.5</td>
</tr>
<tr>
<td>18</td>
<td>Bristol-Myers Squibb (U.S.)</td>
<td>12.0</td>
<td>3.9</td>
</tr>
<tr>
<td>19</td>
<td>Actavis (Switzerland)</td>
<td>11.1</td>
<td>1.1</td>
</tr>
<tr>
<td>20</td>
<td>Astellas Pharma (Japan)</td>
<td>10.4</td>
<td>1.9</td>
</tr>
<tr>
<td>Total Top 20</td>
<td></td>
<td>481.9</td>
<td>88.5</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>266.5</td>
<td>50.0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>748.4</td>
<td>138.5</td>
</tr>
</tbody>
</table>
Biopharmaceuticals is the fastest growing segment of the Pharmaceutical industry. In 2014 it accounted for revenues of $178.5 billion, which represent 23.9% of the worldwide prescription drug sales. The Pharmaceutical industry has significantly shifted its focus from a historical concentration on small molecules to incorporate an increasing number of biologies. Figure 1 shows this shift for many of the big global Pharmaceutical companies. It is important to mention that the R&D strategy of many of these firms has shifted in the same direction by allocating more budget to Biotechnology.

![Change in percentage of revenues from Biotechnology 2000-2014, percentage points](image)

**2014 total revenue percentage of Biotechnology**

<table>
<thead>
<tr>
<th>Company</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>AstraZeneca</td>
<td>3</td>
</tr>
<tr>
<td>GlaxoSmithKline</td>
<td>13</td>
</tr>
<tr>
<td>Johnson &amp; Johnson</td>
<td>35</td>
</tr>
<tr>
<td>Novartis</td>
<td>11</td>
</tr>
<tr>
<td>Merck</td>
<td>22</td>
</tr>
<tr>
<td>Bayer</td>
<td>37</td>
</tr>
<tr>
<td>Pfizer</td>
<td>24</td>
</tr>
<tr>
<td>Bristol-Myers Squibb</td>
<td>31</td>
</tr>
<tr>
<td>Sanofi</td>
<td>42</td>
</tr>
<tr>
<td>Roche</td>
<td>77</td>
</tr>
</tbody>
</table>

Figure 1: Change in percentage of revenues from Biotechnology 2000-2014 [2] (Adapted from [3])

2.2 Bioprocessing

A bioprocess makes use of microorganisms, cells or enzymes to manufacture products. There are three main product groups manufactured through bioprocesses: biopharmaceuticals, diagnostics and biomass-derived small molecules/products.
Bioprocessing is divided into two phases: upstream and downstream. Upstream Processing (USP) involves the creation of the bulk bio-product through cell culture or fermentation. Downstream Processing (DSP) refers to the recovery and purification of protein therapeutics for biopharmaceuticals, vaccines, and other biologics.

2.3 Gene Therapy

"Gene therapy is the use of nucleic acids as a pharmaceutical agent to treat disease. It derives its name from the idea that DNA can be used to supplement or alter genes within an individual’s cells as a therapy to treat disease” [4].

Genes can be delivered using viral and non-viral vectors. A vector is a vehicle or carrier that encapsulates the genes for delivery to cells. Non-viral approaches for gene delivery can be divided into two groups: physical methods and chemical methods.

"The purpose of physical methods for gene transfer is to facilitate entry of nucleic acids into the cells essentially by modifying the properties of biological membranes using physical forces such as pressure or electricity. Instead, chemical methods are aimed at modifying the properties of nucleic acids themselves, by promoting their association with molecules able to reduce their hydrophilicity and neutralize their charge, ultimately leading to an increased cellular uptake” [5].

There are five main classes of viral vectors for human gene therapy: vectors based on Gammaretroviruses, vectors based on Lentiviruses, vectors based on Adenoviruses, vectors based on the Adeno-Associated Virus (AAV) and vectors based on the Herpes Simplex Virus (HSV). The focus of this project is AAV-based gene therapy.
There has only been one gene therapy approved in the Western world: Glybera. Glybera is a medicine that contains an active substance called alipogene tiparvovec. It is a gene therapy product used to treat adults with lipoprotein lipase (LPL) deficiency. Glybera was designed to restore the activity of the LPL enzyme, which is responsible for breaking down fats. It was approved in 2012 by the European Commission for marketing in all European Union member states. The drug is manufactured by the Dutch biotech firm UniQure and Chiesi, an Italian pharmaceutical company, has exclusive rights to commercialize it [6] [7] [8].

Originally, the main objective of my internship was to assess the profitability and biological contamination and cross-contamination risk of the AAV production process. However, during the first project scope discussions, it was decided to change the profitability assessment for a cost-effectiveness assessment given the huge implications of pricing. The only price reference to date is Glybera, which will be sold at a price close to $1 million per treatment [9].

To set a price for gene therapy there are several considerations, including the specific attributes of the therapy and the selected general pricing scheme. Given that the process that is being developed at Pfizer is intended to create a stable vector production platform and is not yet targeting a specific disease, it would be hard to set a price for the profitability analysis.

Unlike rare disease treatments that are often administered over long timeframes (up to decades), gene therapy would only be administered once, with the expectation to provide a lifelong clinical benefit. This makes pricing a challenging problem for gene therapies. However, discussions about price tend to fall into three general schemes. The simplest approach is an up-front, one-time payment, such that UniQure is seeking for Glybera in Europe. This approach could generate significant financial burden to the healthcare system, particularly if reimbursement is not thoughtfully structured. Another alternative
would be an annuity model that spreads the payment over a number of years. Finally, a pay-for-performance, risk-sharing model based on the development of efficacy metrics that rewards manufacturers for maintaining patients' health over a period of time could be a solution. For a pay-for-performance scheme it would be key to have the ability to transfer prices between succeeding insurers [9] [10].

2.4 Pfizer

Pfizer Inc. is a global pharmaceutical and biotechnology company whose mission is "to be the premier innovative biopharmaceutical company" [11]. Pfizer was founded by cousins Charles Pfizer and Charles Erhart in 1849 and currently employs more than 97,000 people around the world (as of September 2015, after completing the acquisition of Hospira) [12]. It is the world’s second largest drugmaker by revenue.

Pfizer’s revenues in 2014 were $49.6 billion, which represents a 3.8% decline compared to 2013. This revenue decrease can be attributed to the continued erosion of branded Lipitor due to generic competition, products that have come off patent and unfavorable foreign exchange. Increased revenue from new products and growth of certain products has not offset the decline in other revenues [13]. Pfizer’s R&D expenses increased 26% in 2014, compared to 2013 (from $6.7 billion to $8.4 billion), which reflects their commitment to innovation.

Historically, Pfizer generated almost all their prescription pharmaceutical sales from small molecule pharmaceutical products. The merger/acquisition of Wyeth by Pfizer in 2009 significantly increased the company’s participation in biologics and vaccines. In 2014, biologics represented 24% of Pfizer’s prescription drug sales.
Pfizer’s Rare Disease Research Unit was created in 2010 as a new research unit within its Worldwide Research and Development division focused on rare diseases. In December 2014, Pfizer announced the establishment of the Genetic Medicine Institute (GMI) in London for the development of gene therapies. Additionally, in order to expand the company’s rare disease R&D, Pfizer signed an agreement with Spark Therapeutics to develop a program for the potential treatment of Hemophilia B through a bio-engineered AAV vector [14]. The GMI is headed by Michael Linden, Ph.D., former Professor of Virology at King’s College London and Director of the University College London Gene Therapy Consortium. Initially, the GMI initiative’s goal is to build expertise in vector design, manufacture and standardization for AAV-based therapeutics.

2.5 Process Modeling and Simulation

Models can be built either in a spreadsheet or in process simulation software. Tools such as Aspen Plus or SuperPro Designer allow efficient modeling for large processes in which calculations are complex given the number of unit operations involved [15].

Given that the simulation for this project was only performed for the upstream portion of the process it was easily built in Microsoft Excel. However, it would be recommended to implement the complete process model (including DSP) in one of the mentioned process simulators due to the complexity that multiple unit operations imply. DSP typically includes thermodynamics and physical chemistry constraints that make the process simulators particularly useful.
3. Literature Review

3.1 Gene Therapy using Adeno-Associated Virus Vectors

AAV belongs to the dependovirus genus of the Parvoviridae family and it represents one of the most attractive vectors for in vivo gene therapy. “The unique life cycle of adeno-associated virus (AAV) and its ability to infect both nondividing and dividing cells with persistent expression have made it an attractive vector. An additional attractive feature of the wild-type virus is the lack of apparent pathogenicity. Gene transfer studies using AAV have shown significant progress at the level of animal models; clinical trials have been noteworthy with respect to the safety of AAV vectors. No proven efficacy has been observed, although in some instances, there have been promising observations” [16].

The main goals of gene therapy are either the correction of an intracellular defect or the synthesis of a secreted protein, which is active at an extracellular level. In the last few years, the popularity of AAV vectors for use in human clinical trials has increased considerably. This reflects the appreciation of the long-term transgene expression observed in animal models and the relative lack of immune response and other toxicities in the models. In addition, the discovery of new serotypes and the appreciation that matching the tissue specificity of the serotype with the presumptive target tissue can greatly enhance the potential effectiveness of therapy have also encouraged the use of AAV vectors [16].

3.2 AAV Production Processes

AAV has a linear single-stranded DNA genome with 145 nucleotide-long inverted terminal repeats (ITR) at both ends. The virus does not encode a polymerase and therefore relies on cellular polymerases for genome replication. The ITRs flank the two viral genes - rep (replication) and cap (capsid), encoding non-structural and structural proteins, respectively [17]. In order to produce AAV
Vectors three components need to be supplied to cultured cells: the rAAV vector composed of the transgene expression cassette flanked by the AAV terminal repeats, the AAV rep and cap coding sequences, and the helper virus functions [18].

Various strategies based on different principles are currently being used for AAV vector production. Standard methods are based on the co-transfection of cells with two or three plasmids that provide the sequences of the rAAV vector, the rep and cap genes, and the adenovirus helper functions. Variations from this standard protocol use helper activities from other viruses to make sure new viral particles can be packaged. The HEK293 and plasmids production system (in adherent cell lines or in suspension) is also known as helper-virus–free transient transfection method because it avoids the use of a helper virus altogether. Other systems that use an alternative kind of virus are the following: Sf9 and baculovirus (or baculovirus system), mammalian cells and HSV-1 (or herpes simplex virus based), and packaging/producer cells and adenovirus (cell lines derived from HeLa) [18] [19].

"The most widely used [method] is based on the helper-virus–free transient transfection method with all cis and trans components (vector plasmid and packaging plasmids, along with helper genes isolated from adenovirus) in host cells such as 293 cells. [...] A second strategy is the recombinant herpes simplex virus (rHSV)-based AAV production system, which utilizes rHSV vectors to bring the AAV vector and the rep and cap genes into the cells. The third method based on baculovirus system was developed in recent years and requires simultaneous infection of insect cells with several baculovirus vectors to deliver the AAV vector cassette and the rep and cap genes. For both the rHSV- and baculovirus-based AAV production systems, it is inconvenient to prepare large quantities of helper and vector viruses and maintain their purity and stability. The fourth method is based on the AAV producer cell lines derived from HeLa or A549, which stably harbored AAV rep/cap genes. The AAV vector cassette was either stably integrated in the host genome or introduced by an adenovirus that contained the cassette. Although the cell line method is easy to scale up and produces relatively high titers of AAV
vectors comparable to transient transfection method, these cell lines required wild-type adenovirus as the helper. Contamination of wild-type adenovirus in the final vector preparations is highly undesirable in view of vector safety” [20].

3.3 Other Studies about large-scale AAV production

The objective of the literature review is to gather information from other studies that have attempted to scale-up the AAV production process, while minimizing biological contamination and cross-contamination risk and ensuring cost-effectiveness.

Recently, a journal article was published in the Biotechnology Journal that demonstrates substantial bioprocess optimizations leading to more efficient and scalable production processes suggesting a promising way for flexible large-scale AAV production [21]. Suspension cell-based processes have low productivity compared to adherent cell lines. However, the main drawbacks of adherent cell lines are manpower and space requirements due to the large number of flasks, roller bottles or cell factories that need to be manipulated in each run [22]. “[This] […] increases risk due to the number of steps that include open manipulation during aseptic processing” [23]. Researchers showed successful process transfer from plate format to a single-use bioreactor system, addressing the previously mentioned disadvantages of adherent cell lines. Among the most popular fixed-bed single-use bioreactor systems are: CellCube (Costar), iCELLIS (Pall) and Celligen (NewBrunswick Scientific). It is worth mentioning that the process that Pfizer is planning to use is an adherent cell-based process.
4. Methodology

The content of this document is based on work performed during a six month internship completed at Pfizer’s Genetic Medicine Institute (GMI) in London. As described earlier, the purpose of this internship was to assess the biological contamination and cross-contamination risk and cost-effectiveness of the process under development for the production of AAV Gene Therapy clinical-grade drug substance. This work was conducted in three phases: (1) understanding and documentation of the existing processes for AAV production, (2) efficiency/robustness assessment of the process that Pfizer is planning to use, and (3) cost and risk analysis.

For phase 3, the following process design steps were followed in order to fulfill the project objectives (Figure 2): (1) Define product specifications, analytical needs and market size, (2) Select production platform/process, (3) Collect data and create process flow diagram, (4) Perform material and energy balances, (5) Calculate costs: equipment and consumables, (6) Model the process in a spreadsheet (or process simulator - e.g., SuperPro Designer), (7) Carry out sensitivity analyses, (8) Assess cost-effectiveness and risk, and (9) Develop recommendations.
The main assumptions that were made while performing the risk and cost assessment were the following:

- Use of an industrial scale fixed bed bioreactor with surface area of 133 m²
- Prices as of December 2015 (in GBP)
- ~40-60% recovery rate of downstream processing product
- Expected transfection productivity of 10,000 - 20,000 VG/cell
- Costs of analytics and/or quality control not included
- USP final step is the harvest/lysis
5. Results

5.1 AAV Gene Therapy landscape

According to the Journal of Gene Medicine, over 137 clinical trials using rAAV are currently open, have been completed, or are being reviewed around the world (Data as of July 2015) [24]. Statistics about these trials are shown in Figures 3-5. The most popular diseases under investigation for treatment by AAV gene therapy are monogenetic (caused by a mutation in one gene), cancer and neurological, at a total of 76%. The first clinical trials were conducted in the early 90’s to treat cystic fibrosis and hemophilia B with AAV2 vectors. Currently, many other diseases are being tested for treatment using AAV and serotypes other than AAV2 are being utilized (including AAV1, AAV5, AAV6, AAV8, and AAVrh10).

Figure 3: Percentage of clinical trials using rAAV in different categories of disease (n=137) [24] (Adapted from [25])
While there are several AAV gene therapy clinical trials worldwide, there are relatively few manufacturing facilities capable of producing AAV Vectors. The consequence of this is that many companies rely on the vector core facilities from universities for their production. Pfizer believes that
having their own manufacturing facility they will possess a competitive advantage. Appendix A: Clinical Studies [33] on page 57, defines what a clinical trial is and describes the five phases that clinical trials undergo according to the Food and Drug Administration (FDA).

Figure 6 on the next page shows most of the players in the AAV gene therapy landscape and maps them according to the production platform that they use and the rare diseases that they are targeting.
<table>
<thead>
<tr>
<th>Transient Transfection</th>
<th>Adherent</th>
<th>Suspension</th>
<th>HSV-Based System</th>
<th>Baculovirus System</th>
<th>HeLa-Based System</th>
<th>Unknown</th>
<th>No production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-specific</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemophilia A</td>
<td>Spark</td>
<td>asklepios</td>
<td></td>
<td>uniQure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemophilia B</td>
<td>Spark</td>
<td>asklepios</td>
<td></td>
<td>uniQure</td>
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<td></td>
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<tr>
<td>AAT deficiency</td>
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<tr>
<td>LPL deficiency</td>
<td></td>
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<tr>
<td>Heart failure</td>
<td></td>
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<tr>
<td>Alzheimer's dis.</td>
<td></td>
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<tr>
<td>Parkinson's dis.</td>
<td></td>
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<tr>
<td>Huntington's dis.</td>
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<tr>
<td>Friedreich's ataxia</td>
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<tr>
<td>ALS¹</td>
<td></td>
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<tr>
<td>DMD²</td>
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<tr>
<td>Pompe disease</td>
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<tr>
<td>LCA³</td>
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<td></td>
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<tr>
<td>Choroideremia</td>
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<td></td>
</tr>
<tr>
<td>AMD⁴</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Ocular diseases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Amyotrophic lateral sclerosis, 2. Duchenne muscular dystrophy, 3. Leber's congenital amaurosis, 4. Age-related macular degeneration

Source: Company websites, ClinicalTrials.gov

Figure 6: AAV gene therapy landscape
5.2 Market size

As mentioned earlier, in order to support the accelerating pace of clinical progress for AAV gene therapy, it is critical to develop manufacturing capacity.

According to J. Fraser Wright, “cGMP lots in the range of $10^{16}$ to $10^{18}$ VG will be required to meet the requirements of late-stage clinical development and product licensure for many recombinant AAV products, especially those aimed at the most commercially viable disease applications” [26].

In order to validate those numbers, the total amount of AAV needed for the treatment of a representative disease example for each type of disorder (eye, brain, liver and musculoskeletal) was calculated using the average dose per patient in clinical trials and the global prevalence. Appendix B: AAV Vector therapeutic applications: Clinical Trials [34] [35] provides some details about clinical trials using rAAV.

Table 2: Projected AAV Vector need in vector genomes

<table>
<thead>
<tr>
<th>Type of disorders</th>
<th>Average dose per patient (VG)</th>
<th>Prevalence (Patients globally)</th>
<th>Total amount needed (VG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye (Leber's congenital amaurosis)</td>
<td>$5.82 \times 10^{10}$</td>
<td>135,000 [27]</td>
<td>$7.86 \times 10^{15}$</td>
</tr>
<tr>
<td>Brain (Parkinson's disease)</td>
<td>$6.46 \times 10^{11}$</td>
<td>8,500,000 [28]</td>
<td>$5.49 \times 10^{18}$</td>
</tr>
<tr>
<td>Liver (Hemophilia B)</td>
<td>$5.65 \times 10^{13}$</td>
<td>80,000 [29]</td>
<td>$4.52 \times 10^{18}$</td>
</tr>
<tr>
<td>Musculoskeletal (DMD)</td>
<td>$1 \times 10^{15}$</td>
<td>250,000 [30]</td>
<td>$2.5 \times 10^{20}$</td>
</tr>
</tbody>
</table>

The results shown on the table indicate that lots in the range of $10^{16}$ to $10^{20}$ VG would be required, which is in line with the statement from Fraser Wright in the journal Human Gene Therapy.
5.3 Current Process used at the Genetic Medicine Institute

Figure 7 shows a high-level overview of the process that is currently being developed at Pfizer's Genetic Medicine Institute. There are seven main steps and all of them require quality control analyses. The first four steps constitute the Upstream Processing and the last three steps are part of the Downstream Processing.

![Diagram of the process]

**Figure 7: Overview of the process**

### 5.3.1 Upstream Processing (USP)

The upstream portion of the process starts by a Working Cell Bank (WCB). The cells used to produce the virus for gene therapy are Human Embryonic Kidney 293 (HEK-293) cells. Cell banks are frozen for long term storage and therefore require thawing to be cultured. The amplification of the cells is
performed in three separate phases: Cell Amplification in T-Flasks, Cell Amplification in Cell Factories, and Cell Amplification in a Bioreactor. Cells are grown using Dulbecco's Modified Eagle Medium (DMEM) culture media, supplemented with Fetal Bovine Serum (FBS).

After the amplification, DNA-PEI complexes are used for efficient transfection of the mammalian cells. Finally the cells are lysed and the fixed-bed washed with Phosphate-buffered saline (PBS).

5.3.2 Downstream Processing (DSP)

The downstream portion of the process starts with concentration by TFF (Tangential Flow Filtration). The steps that follow are clarification, purification, concentration/diafiltration by TFF, and fill and finish. Due to confidentiality and given that the main focus of this project was the upstream process, no further details are given about the manufacturing process.

5.4 Simulation of the Production Process

The simulation of the production process consisted of the identification of four different possible process maps: one for the base case and three for sensitivity testing. The differences between all four processes will be described in section 6.

For each one of the different steps in the four process variants, the following elements were identified:

- Consumable bioprocessing products needed
- Detailed operations
- Price for every consumable
- Number of aseptic open operations
For the purpose of this thesis, aseptic open operations were defined as aseptic operations realized in an open system (i.e., under a biocontainment hood). An open system is a process system that exposes the product to the room environment. The opposite of an open system is a closed system in which the product is not exposed to the room environment. Sterile connectors are commonly used to avoid exposure of the product to the room environment.

The full details of the process simulation are confidential and cannot be included in the thesis but a simple example is presented below (Figure 8). This example shows how the number of aseptic operations was determined for the harvesting procedure from two CF’s in the cell amplification step of the process. Operations are shown in black when they pertain to one single cell factory and they are shown in red when they comprise both cell factories. A similar analysis was performed for every step in the AAV manufacturing process.

**Figure 8: Aseptic open operations when harvesting two CF’s**
Cost was calculated using the prices of each of the bioprocessing products needed. Biological contamination and cross-contamination risk was calculated as a percentage using the equation below:

\[ R = \frac{Number \ of \ aseptic \ open \ operations \ in \ process \ i}{Number \ of \ aseptic \ open \ operations \ in \ base \ case \ process} \times 100 \]

Since the three cases for sensitivity testing include changes that decrease the number of aseptic open operations, the risk of the base case process is 100% and should be lower for the other three alternatives.
6. Analysis

6.1 Base Case

The base case process follows all the steps mentioned in section 5.3 without the use of sterile connectors. Specifically, for the cell amplification in T-Flasks and cell amplification in Cell Factories cells are split with a 1:3 split ratio. This process happens as follows (Figure 9):

![Diagram of cell amplification in base case process (Process A)](image)

Figure 9: Cell amplification in base case process (Process A)

6.2 Sensitivity Testing

The first change proposed for sensitivity testing was the use of two CF’s instead of 15 T-225 Flasks for the amplification step before seeding in 6 CF’s. Reducing 15 T-225 Flasks into two CF’s decreases the number of aseptic open operations and consequently minimizes contamination risk.

However, CF’s require a larger amount of media, which represents a higher cost. The sequence for cell amplification in this process variant is as follows (Figure 10):
Figure 10: Cell amplification replacing 15 T-225 Flasks with two CF’s (Process B)

On top of the T-Flask vs. CF variation, both processes were evaluated with the use of non-sterile connectors and sterile connectors. The resulting cases are the following:

- Process A (15 T-225 Flasks) with non-sterile connectors
- Process A (15 T-225 Flasks) with sterile connectors
- Process B (two CF’s) with non-sterile connectors
- Process B (two CF’s) with sterile connectors

After running the calculations, the following results were obtained:
As shown in Figure 11, if process B is selected (with sterile connectors) the contamination risk decreases by ~40% while only increasing costs ~3%. This can be attributed to the fact that the elements of the process that cost the most are the media, the DNA, the bioreactor and the transfection agent. Sterile connectors and replacing T-Flasks by CF’s only represent a small portion of the total cost per run.

Due to the proprietary nature of the information, the total cost per run cannot be disclosed but the order of magnitude is around the hundreds of thousands of GBPs.
7. Conclusion

7.1 Recommendations regarding research findings

The main conclusion from the analysis performed is that the contamination risk reduction obtained from the use of sterile connectors in the bioreactor and cell factories for the cell amplification portion of the process outweighs its costs.

In absolute terms, the purchase of sterile connectors, different bioprocess containers in which those connectors can be used, and more media for the amplification in cell factories instead of T-Flasks might seem expensive. However, once the amount that it represents is compared with the total cost per run it becomes clear that it is almost insignificant. In contrast, the contamination risk reduction achieved by doing this is quite relevant.

According to Forbes magazine, health technology is projected to be the most profitable industry in 2016 [31]. Major pharmaceutical companies such as Pfizer and Merck are described as “superstars of profitability” within the healthcare technology category. These high profit margins have been maintained for many years and give companies very little incentive to control their costs, including R&D expenditure. Process development in the Pharmaceutical Industry seems to be used in a less rigorous way to optimize the design and operation of integrated process than in other less profitable industries, such as the chemical and oil industries.

This project demonstrated how simulation tools, Computer Aided Process Design (CAPD) or other tools can benefit the Pharmaceutical Industry. Pharmaceutical price controls have been a hot topic in the last couple of years with the purpose of improving people's access to medicines. Whether they will actually be implemented or not is still uncertain, but if it happens drug manufacturers will have to employ
better mechanisms to manage their costs. It is understood that being first to market is really important in the industry and that argument sometimes justifies investments without computer simulations. However, money is often spent on products that eventually fail during laboratory and pilot plant efforts, which are costly and time-consuming.

7.2 Remaining questions for further research

In addition to the use of sterile connectors in the bioreactor and the use of cell factories for the cell amplification portion of the process in order to reduce biological contamination and cross-contamination risk, Pfizer should perform a similar analysis for the downstream portion of the process. The process that was modeled assumes that the recovery rate of downstream processing product is ~40-60%. However, elements such as the chromatography resin performance have a big impact on the results obtained and that’s why the outcome for the downstream analysis would be so important.

The biological contamination and cross-contamination risk could be reduced further if the number of aseptic open operations in the CF amplification phase is reduced. Pfizer could potentially look at sterile connectors designed for small-scale connections in the 1/8” to 1/4” ID range. Colder Products Company (CPC) offers AseptiQuik® S Connectors for small-flow applications and they could potentially diminish risk. CPC also designed the first all-in-one single use connection technology that offers both a sterile connection and a sterile disconnection: AseptiQuik® DC Connectors [32]. Those connectors could replace the Kleenpak sterile connectors and disconnectors that were considered for the bioreactor portion of the process and reduce the cost while maintaining the same contamination risk level.

Also, additional variables such as transfection efficiency, harvest method, bioreactor size/surface area, etc. could be incorporated in the assessment of cost-effectiveness and risk. For example, currently the transfection productivity is expected to be only between 10,000 and 20,000 VG/cell (based on past
experiments). Recent experiments have shown that the use of different plasmid systems or other production platforms could achieve enhanced vector yields (productivities up to 270,000 VG/cell have been reached) [21]. Increased transfection productivities could significantly decrease the cost per run and positively impact Pfizer’s process and the industry in general. In the short-term, Pfizer will also have to evaluate the impact of changing the GOI and/or serotype. Those changes could impact the performance of the entire process since until now all runs have been made using YFP expression cassette and one single serotype.

One final opportunity that could be addressed in the future is to translate the cost-effectiveness assessment into a profitability assessment. As mentioned in section 2.3, there are three general pricing schemes for gene therapies and selecting either one of them has several implications that could translate into different possible profits. To accomplish this, the costs of utilities and labor should also be incorporated into the model.
References

[21] V. V. Emmerling, A. Pegel, E. G. Milian, A. Venere-Sanchez, M. Kunz, J. Wegele, A. A. Kamen, S. Kochanek, and M. Hoerer, “Rational plasmid design and bioprocess optimization to enhance...


Appendix A: Clinical Studies [33]

A clinical study involves research using human volunteers (also called participants) that is intended to add to medical knowledge. There are two main types of clinical studies: clinical trials (also called interventional studies) and observational studies.

Clinical Trials

In a clinical trial, participants receive specific interventions according to the research plan or protocol created by the investigators. These interventions may be medical products, such as drugs or devices; procedures; or changes to participants' behavior, such as diet. Clinical trials may compare a new medical approach to a standard one that is already available, to a placebo that contains no active ingredients, or to no intervention. Some clinical trials compare interventions that are already available to each other. When a new product or approach is being studied, it is not usually known whether it will be helpful, harmful, or no different than available alternatives (including no intervention). The investigators try to determine the safety and efficacy of the intervention by measuring certain outcomes in the participants. For example, investigators may give a drug or treatment to participants who have high blood pressure to see whether their blood pressure decreases.

Clinical trials used in drug development are sometimes described by phase. These phases are defined by the Food and Drug Administration (FDA). There are five phases:

- **Phase 0:** Exploratory study involving very limited human exposure to the drug, with no therapeutic or diagnostic goals (for example, screening studies, microdose studies)
- **Phase 1:** Studies that are usually conducted with healthy volunteers and that emphasize safety. The goal is to find out what the drug's most frequent and serious adverse events are and, often, how the drug is metabolized and excreted.
• Phase 2: Studies that gather preliminary data on effectiveness (whether the drug works in people who have a certain disease or condition). For example, participants receiving the drug may be compared with similar participants receiving a different treatment, usually an inactive substance (called a placebo) or a different drug. Safety continues to be evaluated, and short-term adverse events are studied.

• Phase 3: Studies that gather more information about safety and effectiveness by studying different populations and different dosages and by using the drug in combination with other drugs.

• Phase 4: Studies occurring after FDA has approved a drug for marketing. These include postmarket requirement and commitment studies that are required of or agreed to by the sponsor. These studies gather additional information about a drug's safety, efficacy, or optimal use.

Observational Studies

In an observational study, investigators assess health outcomes in groups of participants according to a research plan or protocol. Participants may receive interventions (which can include medical products such as drugs or devices) or procedures as part of their routine medical care, but participants are not assigned to specific interventions by the investigator (as in a clinical trial). For example, investigators may observe a group of older adults to learn more about the effects of different lifestyles on cardiac health.
Appendix B: AAV Vector therapeutic applications: Clinical Trials [34] [35]

Given that the list of the more than 137 clinical trials that have been or are being conducted is too extensive, some illustrative examples are discussed below.

Cystic Fibrosis [36] [37] [38] [39] [40]

- The goal of a gene therapy for Cystic Fibrosis is to deliver the **CFTR** (Cystic Fibrosis transmembrane conductance regulator) cDNA into the epithelial cells in the conducting airways of the **lung**. The lung is the primary target for Cystic Fibrosis gene therapy, but the **maxillary sinuses** also reflect some aspects of lung disease.

<table>
<thead>
<tr>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase IIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>25 adult and adolescent CF patients with mild to moderate lung disease (17 males and 8 females)</td>
<td>37 CF patients with mild lung disease</td>
</tr>
<tr>
<td>Serotype</td>
<td>AAV2</td>
<td>AAV2</td>
</tr>
<tr>
<td>Route of administration</td>
<td>Nasal epithelium through catheter and bronchoscopy to right lower lobe</td>
<td>Aerosol dose delivery to the lower respiratory tract</td>
</tr>
<tr>
<td>Production process</td>
<td>N/A</td>
<td>tgAAVCF was produced under cGMP guidelines at Targeted Genetics Corporation</td>
</tr>
<tr>
<td>Sponsor</td>
<td>National Institute of Diabetes and Digestive and Kidney Diseases</td>
<td>Targeted Genetics Corporation</td>
</tr>
<tr>
<td>NCT Number</td>
<td>NCT00004533</td>
<td>NCT00073463</td>
</tr>
</tbody>
</table>

- **Note**: In March 2011 Targeted Genetics Corporation announced the change of the Company's name to AmpliPhi Biosciences Corporation.
**Hemophilia B [41] [42] [43] [44]**

- Additional ongoing trials by Spark Therapeutics with AAV2 and AAV8 (NCT00515710, NCT01620801), UniQure Biopharma B.V. with AAV5 (NCT02396342) and Baxalta US Inc. (NCT01687608)

<table>
<thead>
<tr>
<th>Phase I</th>
<th>Phase I</th>
<th>Phase I AAV8</th>
</tr>
</thead>
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<tr>
<td><strong>Patients</strong></td>
<td><strong>Patients</strong></td>
<td><strong>Patients</strong></td>
</tr>
<tr>
<td>8 subjects were enrolled, 3 in both the low- and medium-dose cohorts and 2 in the high-dose cohort</td>
<td>7 subjects with severe hemophilia B</td>
<td>6 adult male subjects</td>
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<tr>
<td><strong>Serotype</strong></td>
<td><strong>Serotype</strong></td>
<td><strong>Serotype</strong></td>
</tr>
<tr>
<td>AAV2</td>
<td>AAV2</td>
<td>AAV8</td>
</tr>
<tr>
<td><strong>Route of administration</strong></td>
<td><strong>Route of administration</strong></td>
<td><strong>Route of administration</strong></td>
</tr>
<tr>
<td>Injection at multiple sites in muscles of one or two legs</td>
<td>Hepatic artery infusion to target the liver</td>
<td>Peripheral vein infusion</td>
</tr>
<tr>
<td><strong>Production process</strong></td>
<td><strong>Production process</strong></td>
<td><strong>Production process</strong></td>
</tr>
<tr>
<td>Using a triple transfection procedure in 293 cells</td>
<td>By helper virus-free transient transfection of HEK-293 cells</td>
<td>In phosphate-buffered saline (PBS) supplemented with 0.25% human serum albumin by the Children’s GMP, in Memphis, Tennessee</td>
</tr>
<tr>
<td><strong>Sponsor</strong></td>
<td><strong>Sponsor</strong></td>
<td><strong>Sponsor</strong></td>
</tr>
<tr>
<td>Avigen</td>
<td>Avigen</td>
<td>St. Jude Children's Research Hospital</td>
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<td><strong>NCT Number</strong></td>
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<td>N/A</td>
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</table>

**Leber's congenital amaurosis (LCA) [45] [46] [47] [48]**

- Additional ongoing trials by Spark Therapeutics (NCT00999609, NCT01208389)

<table>
<thead>
<tr>
<th>UCL</th>
<th>Spark</th>
<th>AGTC</th>
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<td><strong>Patients</strong></td>
</tr>
<tr>
<td>3 young adults (17 to 23 years of age) with early-onset, severe retinal dystrophy caused by missense mutations in RPE65</td>
<td>3 consecutive patients who had LCA2 and were between the ages of 19 and 26 years</td>
<td>3 young adults (ages 21–24 years) with RPE65-LCA</td>
</tr>
<tr>
<td><strong>Serotype</strong></td>
<td><strong>Serotype</strong></td>
<td><strong>Serotype</strong></td>
</tr>
<tr>
<td>AAV2</td>
<td>AAV2</td>
<td>AAV2</td>
</tr>
<tr>
<td><strong>Route of administration</strong></td>
<td><strong>Route of administration</strong></td>
<td><strong>Route of administration</strong></td>
</tr>
<tr>
<td>Subretinal injection</td>
<td>Subretinal injection</td>
<td>Uniocular subretinal injection</td>
</tr>
<tr>
<td><strong>Production process</strong></td>
<td><strong>Production process</strong></td>
<td><strong>Production process</strong></td>
</tr>
<tr>
<td>Produced by Targeted Genetics Corporation according to GMP guidelines</td>
<td>Manufactured by the Center for Cellular and Molecular Therapeutics at the Children’s Hospital of Philadelphia</td>
<td>HEK-293 cells (adenovirus serotype 2 [Ad2], E1A+E1B+) were cotransfected with the pDG packaging plasmid</td>
</tr>
<tr>
<td><strong>Sponsor</strong></td>
<td><strong>Sponsor</strong></td>
<td><strong>Sponsor</strong></td>
</tr>
<tr>
<td>University College, London</td>
<td>Spark Therapeutics</td>
<td>Applied Genetic Technologies Corp</td>
</tr>
<tr>
<td><strong>NCT Number</strong></td>
<td><strong>NCT Number</strong></td>
<td><strong>NCT Number</strong></td>
</tr>
<tr>
<td>NCT00643747</td>
<td>NCT00516477</td>
<td>NCT00749957</td>
</tr>
</tbody>
</table>
Lipoprotein Lipase Deficiency (Glybera - First approved gene therapy) [49][50][51][52]

- The goal of a gene therapy for Lipoprotein Lipase Deficiency is to deliver the LPL (Lipoprotein lipase) cDNA into muscle cells

**Glybera**

**Patients**
- 8 patients in an initial Phase I/II trial, 14 patients in a subsequent trial. Additional trial in 5 patients

**Serotype**
- AAV1

**Route of administration**
- Intramuscular injection

**Production process**
- Initial trials: AAV1-LPL vector (AMT 010) was produced by transfection in mammalian HEK-293 cells. For all subsequent trials and the commercial launch: vector manufactured in the insect cell (Sf9) baculovirus system

**Sponsor**
- Amsterdam Molecular Therapeutics. Please note that as of April 2012, uniQure B.V. has taken over the gene therapy business of Amsterdam Molecular Therapeutics

**NCT Number**
- NCT00891306, NCT01109498

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Alpha-1 Antitrypsin Deficiency [53][54][55]

- The goal of a gene therapy for Alpha-1 Antitrypsin Deficiency is to deliver the AAT cDNA
- Additional ongoing trials by University of Massachusetts, Worcester (NCT00430768, NCT00377416)

**Phase 1 AAV2**
- 12 AAT-deficient adults, 10 of whom were male

**Phase I AAVrh10**
- Estimated enrollment: 20

**Phase 1 AAV1**
- 9 AAT-deficient subjects

**Phase II AAV1**
- 9 AAT-deficient individuals

**Patients**
- University of Massachusetts, Worcester, AGTC

**Serotype**
- AAV2

**Route of administration**
- Intramuscular injection

**Production process**
- Using a published cotransfection technique in HEK-293 cells

**Sponsor**
- University of Massachusetts, Worcester, AGTC

**NCT Number**
- NCT00377416

---

**Phase I AAV2**
- University of Massachusetts, Worcester, AGTC

**Phase I AAVrh10**
- AAVrh10

**Phase I AAV1**
- AAV1

**Phase II AAV1**
- AAV1

**Serotype**
- AAV2

**Route of administration**
- Intraperitoneal administration

**Production process**
- Using a published cotransfection technique in HEK-293 cells

**Sponsor**
- University of Massachusetts, Worcester, AGTC

**NCT Number**
- NCT02168686

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**Phase 1 AAV2**
- University of Massachusetts, Worcester, AGTC

**Phase I AAVrh10**
- AAVrh10

**Phase I AAV1**
- AAV1

**Phase II AAV1**
- AAV1

**Serotype**
- AAV2

**Route of administration**
- Intramuscular injection

**Production process**
- Using a published cotransfection technique in HEK-293 cells

**Sponsor**
- University of Massachusetts, Worcester, AGTC

**NCT Number**
- NCT01054339

---

**Phase 1 AAV2**
- University of Massachusetts, Worcester, AGTC

**Phase I AAVrh10**
- AAVrh10

**Phase I AAV1**
- AAV1

**Phase II AAV1**
- AAV1

**Serotype**
- AAV2

**Route of administration**
- Intramuscular injection

**Production process**
- Using a published cotransfection technique in HEK-293 cells

**Sponsor**
- University of Massachusetts, Worcester, AGTC

**NCT Number**
- NCT01054339
**Heart Failure** [56] [57] [58] [59] [60] [61]

- The goal of a gene therapy for Heart Failure is to deliver the gene encoding the sarcoplasmic reticulum calcium ATPase (SERCA2a) in order to normalize SERCA2a protein levels and enzymatic activity in the *myocardium*
- Additional ongoing trials by Imperial College London (NCT00534703) and University of Florida (NCT00976352)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Patients</th>
<th>Serotype</th>
<th>Route of administration</th>
<th>Production process</th>
<th>Sponsor</th>
<th>NCT Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>9 patients with advanced HF (NYHA Class III/IV)</td>
<td>AAV1</td>
<td>Single intracoronary infusion</td>
<td>AAV1/SERCA2a (tgABG12, MYDICAR) was manufactured by Targeted Genetics Corporation (Seattle, WA)</td>
<td>Celladon Corporation</td>
<td>NCT00454818</td>
</tr>
<tr>
<td>II</td>
<td>39 patients with advanced HF (NYHA Class III/IV)</td>
<td>AAV1</td>
<td>Antegrade epicardial coronary artery infusion</td>
<td>AAV1/SERCA2a was manufactured by Targeted Genetics Corporation (Seattle, WA) and Celladon Corporation (La Jolla, CA)</td>
<td>Celladon Corporation</td>
<td>NCT01966887</td>
</tr>
<tr>
<td>IIB</td>
<td>250 patients with moderate-to-severe HF and NYHA Class II to IV</td>
<td>AAV1</td>
<td>Percutaneous intracoronary administration</td>
<td>Estimated completion: January 2016</td>
<td>Celladon Corporation</td>
<td>NCT01643330</td>
</tr>
</tbody>
</table>

**Note:** In March 2011 Targeted Genetics Corporation announced the change of the Company's name to AmpliPhi Biosciences Corporation

**Muscular Dystrophy** [62] [63]

- Additional ongoing trials for DMD by Nationwide Children's Hospital with AAV1 (NCT02354781) and with AAVrg74 (NCT02376816) and Genethon (NCT01385917). For LGMD by Genethon (NCT01344798)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Patients</th>
<th>Serotype</th>
<th>Route of administration</th>
<th>Production process</th>
<th>Sponsor</th>
<th>NCT Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duchenne</td>
<td>6 DMD boys ranging in age from 5 to 11 years and ranging in mass from 16 to 57kg, each with unique and defined dystrophin mutations</td>
<td>AAV2.5</td>
<td>Injection into the bicep muscle</td>
<td>Using the standard triple transfection method using the XX6-80 adenoviral helper plasmid with a packaging plasmid (either AAV1, AAV2 or a modified packaging plasmids) and an inverted terminal repeat plasmid</td>
<td>Nationwide Children's Hospital</td>
<td>NCT00428935</td>
</tr>
<tr>
<td>Limb-girdle</td>
<td>Six potential LGMD2D subjects met inclusion criteria</td>
<td>AAV1</td>
<td>injection into the extensor digitorum brevis (EDB) muscle</td>
<td>Produced at the Harvard Gene Therapy Initiative according to cGMP. Vector production followed previously published methods using plasmid DNA tritransfection of HEK293 cells, followed by iodixanol and anion exchange column chromatography purification.</td>
<td>Nationwide Children's Hospital</td>
<td>NCT00494195</td>
</tr>
</tbody>
</table>

**Note:** AAV2.5 was generated from the AAV2 capsid with five mutations from AAV1
### Parkinson’s Disease [64] [65] [66] [67]

- The gene therapy approaches for Parkinson’s Disease are either dopaminergic or non-dopaminergic.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Phase I/II AAV2-AADC</th>
<th>Phase I AAV2-GAD</th>
<th>Phase I AAV2-GDNF</th>
<th>Phase I/II AAV2-NTN</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 patients with moderate to advanced PD</td>
<td>11 men and 1 woman with Parkinson’s disease</td>
<td>Estimated enrollment: 100</td>
<td>12/58 patients aged 35-75 years with a diagnosis of PD for at least 5 years</td>
<td></td>
</tr>
<tr>
<td>Serotype</td>
<td>AAV2</td>
<td>AAV2</td>
<td>AAV2</td>
<td>AAV2</td>
</tr>
<tr>
<td>Route of administration</td>
<td>Bilateral intraputaminal infusion</td>
<td>Injection into the subthalamic nucleus</td>
<td>Surgery to infuse AAV2-GDNF into the brain</td>
<td>Intraputaminal injections or sham surgery</td>
</tr>
<tr>
<td>Production process</td>
<td>Manufactured by Avigen and provided by Genzyme</td>
<td>N/A</td>
<td>N/A</td>
<td>CERE-120 was provided by Ceregene</td>
</tr>
<tr>
<td>Sponsor</td>
<td>Genzyme, Jichi Medical University</td>
<td>Neurologix, Inc.</td>
<td>National Institutes of Health Clinical Center (CC)</td>
<td>Ceregene</td>
</tr>
<tr>
<td>NCT Number</td>
<td>NCT00229736, NCT02418598</td>
<td>NCT00195143, NCT00643890</td>
<td>NCT01621581</td>
<td>NCT00252850, NCT00400634, NCT00985517</td>
</tr>
</tbody>
</table>

### Canavan Disease [68] [69] [70]

- The goal of a gene therapy for Canavan Disease is to deliver the ASPA (Aspartoacylase) cDNA into the central nervous system.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Phase I</th>
<th>Long-Term Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pediatric population of 10 subjects with CD (age between 3 and 96 months)</td>
<td>28 patients, with a subset of 13 patients being treated with AAV2-ASPA</td>
<td></td>
</tr>
<tr>
<td>Serotype</td>
<td>AAV2</td>
<td>AAV2</td>
</tr>
<tr>
<td>Route of administration</td>
<td>Intracranial infusions via six cranial burr holes</td>
<td>Intraparenchymal delivery at six brain infusion sites</td>
</tr>
<tr>
<td>Production process</td>
<td>Recombinant AAV was produced by transient transfection of HEK 293 cells followed by harvesting and purification involving heparin sulfate affinity column chromatography. The final formulation was as recombinant AAV2-NSE-ASPA-WPRE-bGHVa, in 1X phosphate-buffered saline</td>
<td>N/A</td>
</tr>
<tr>
<td>Sponsor</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>NCT Number</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
### Batten Disease [71] [72]
- The goal of a gene therapy for Batten Disease is to deliver the **CLN2** gene into the whole brain

<table>
<thead>
<tr>
<th>Phase I AAV2</th>
<th>Phase I AAVrh10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients</strong></td>
<td><strong>Patients</strong></td>
</tr>
<tr>
<td>10 children (6 boys and 4 girls) with late infantile neuronal ceroid lipofuscinosis</td>
<td>Two studies will run in parallel: one with 8 children and one with 16 children</td>
</tr>
<tr>
<td><strong>Serotype</strong></td>
<td><strong>Serotype</strong></td>
</tr>
<tr>
<td>AAV2</td>
<td>AAVrh10</td>
</tr>
<tr>
<td><strong>Route of administration</strong></td>
<td><strong>Route of administration</strong></td>
</tr>
<tr>
<td>Intracranial (12 cortical locations delivered through 6 burr holes)</td>
<td>Direct intracranial administration</td>
</tr>
<tr>
<td><strong>Production process</strong></td>
<td><strong>Production process</strong></td>
</tr>
<tr>
<td>The AAV2CLN2 vector was produced by a two-plasmid cotransfection procedure in the Belfer Gene Therapy Core Facility (Weill Cornell Medical College) under current Good Manufacturing Practice conditions</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Sponsor</strong></td>
<td><strong>Sponsor</strong></td>
</tr>
<tr>
<td>Weill Medical College of Cornell University</td>
<td>Weill Medical College of Cornell University</td>
</tr>
<tr>
<td><strong>NCT Number</strong></td>
<td><strong>NCT Number</strong></td>
</tr>
<tr>
<td>NCT00151216, NCT00151268</td>
<td>NCT01161576, NCT01414985</td>
</tr>
</tbody>
</table>

### Rheumatoid Arthritis [73] [74] [75] [76]
- The goal of a gene therapy for Rheumatoid Arthritis is to deliver the human TNFR:Fc fusion gene (tgAAC94)

<table>
<thead>
<tr>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients</strong></td>
<td><strong>Patients</strong></td>
</tr>
<tr>
<td>15 subjects with inflammatory arthritis (14 with rheumatoid arthritis and 1 with ankylosing spondylitis)</td>
<td>127 subjects received the first blinded administration and 95 subjects received the second, open-label dose</td>
</tr>
<tr>
<td><strong>Serotype</strong></td>
<td><strong>Serotype</strong></td>
</tr>
<tr>
<td>AAV2</td>
<td>AAV2</td>
</tr>
<tr>
<td><strong>Route of administration</strong></td>
<td><strong>Route of administration</strong></td>
</tr>
<tr>
<td>Intra-articular injection</td>
<td>Intra-articular administration</td>
</tr>
<tr>
<td><strong>Production process</strong></td>
<td><strong>Production process</strong></td>
</tr>
<tr>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Sponsor</strong></td>
<td><strong>Sponsor</strong></td>
</tr>
<tr>
<td>Targeted Genetics Corporation</td>
<td>Targeted Genetics Corporation</td>
</tr>
<tr>
<td><strong>NCT Number</strong></td>
<td><strong>NCT Number</strong></td>
</tr>
<tr>
<td>NCT00617032</td>
<td>NCT00126724</td>
</tr>
</tbody>
</table>