Development and Delivery of a Hands-On Short Course in Adeno-Associated Virus Manufacturing to Support Growing Workforce Needs in Gene Therapy

Development and delivery of a hands-on short course in AAV manufacturing to support growing workforce needs in gene therapy

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Abstract

The manufacturing of gene therapy products is a rapidly growing industry bolstered by the tremendous potential of these therapies to provide lifesaving treatment for rare and complex genetic diseases. The industry's steep rise has resulted in a high demand for skilled staff required to manufacture gene therapy products of the expected high quality. To address this skill shortage, more opportunities for education and training in all aspects of gene therapy manufacturing are needed. The Biomanufacturing Training and Education Center (BTEC) at the North Carolina State University (NC State) has developed and delivered (and continues to deliver) a four-day, hands-on course titled Hands-On cGMP Biomanufacturing of Vectors for Gene Therapy. The course, which consists of 60% hands-on laboratory activities and 40% lectures, aims to provide a comprehensive understanding of the gene therapy production process, from vial thaw through the final formulation step, and analytical testing. This paper discusses the design of the course, the backgrounds of the nearly 80 students who have participated in the seven offerings held since March 2019, and feedback from the course participants.

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Introduction

Over the past several years, there has been a dramatic increase in the clinical applications of gene therapy and the number of companies that manufacture gene therapy products. Since Kymriah® was approved for the U.S. market in 2017, the first gene therapy approved by the United States (U.S.) Food and Drug Administration (FDA), there have been an additional 11 viral-vector-based gene therapies approved in the U.S. alone.[1] Further, with more than 200 active clinical trials for *in vivo* viral-vector-based gene therapies, the number of approved gene therapy products will grow significantly.[2]

As viral-vector-based gene therapies have moved from research laboratories to commercial manufacturing, a significant demand for a workforce skilled in all aspects of viral vector manufacturing has emerged. Recent surveys that assess talent needs have highlighted the growth of jobs in the gene therapy field.[3] The jobs required to manufacture viral vectors under current good manufacturing practice (cGMP) cover a range of functional areas, including manufacturing (operations), process development, process engineering, materials management, quality assurance, quality control - and the list goes on. Finding skilled staff is a challenge.[4] One approach that companies use to develop a skilled workforce is to recruit staff with experience in the production of more traditional biopharmaceuticals, such as monoclonal antibodies (mAbs, e.g., Humira®). However, there are a number of differences between the production of viral vectors and more traditional biopharmaceuticals that require distinct skill sets. For example, titers in viral vector processes are small compared to mAb processes, transient transfection is often used to produce viral vectors rather than the stable cell lines typically used for mAb production, and many critical quality attributes, such as product-related impurities, are different for viral-vector-based products (e.g., empty capsids) compared to mAbs. In addition,

many analytical methods used to test these quality attributes are different from those used for mAbs.

Despite the significant need to grow a skilled workforce in the area of viral vector manufacturing, to the best of our knowledge, few courses targeting professionals are designed to provide *hands-on* experiences that are integral to skill development. Such a course would benefit those directly involved in executing complex and relatively manual processes used in viral vector production. However, it would also benefit staff in other functional areas within gene therapy companies that need an understanding of the manufacturing steps and basic analytical methods utilized in viral vector production. Likewise, such a course would help those who support companies manufacturing viral vectors, including suppliers of raw materials and consumables, and validation contractors.

The Biomanufacturing Training and Education Center (BTEC) at North Carolina State University (https://www.btec.ncsu.edu/) offers a professional development program in the areas of analytical methods, process automation, manufacturing, process development, process engineering, and formulation applied to biopharmaceuticals. The program has provided training to more than 5000 professionals since its inception in 2008. (https://www.btec.ncsu.edu/industry/index.php). As part of this program, BTEC has developed and delivered multiple offerings of a short course in gene therapy vector manufacturing - Hands-On cGMP Biomanufacturing of Vectors for Gene Therapy, which provides hands-on training in the manufacture and analysis of adeno-associated virus (AAV). This paper covers the details of the design of this first-of-its kind course, as well as feedback from participants.

Overall Course Design

The Hands-On cGMP Biomanufacturing of Vectors for Gene Therapy course is a four-day, in-person course beginning at 8am and ending at 5pm daily, with 7.5 hours of contact time each day (once breaks and lunch are factored in) for a total of 30 hours of direct instructional time. The course comprises both lecture and hands-on laboratory sessions, and provides ample time for both guided and impromptu discussions between instructors and students. Lectures provide a theoretical framework for major course topics, while laboratories provide hands-on experience in the methods and operations involved. Students spend more than half of their total course time in lab conducting hands-on activities. Furthermore, enrollment in the course is limited to 12 participants to ensure that each participant had a hands-on experience.

The course was designed with the primary objective of providing fundamental knowledge of and hands-on exposure to production-scale or scalable biomanufacturing operations–both upstream (cell culture) and downstream (recovery and purification)–and the analytical methods used in the manufacturing of vectors for gene therapy products. Introducing strategies for process development, technology transfer, and assay validation are also priorities. Further, the content was designed to appeal to a broad audience, including scientists, engineers, and other professionals from gene therapy companies, vendors who supply materials and services to the industry, and instructors from academic institutions who themselves are teaching the topic.

The course is divided into four sections: introductory concepts, upstream operations, downstream operations, and analytical methods, as shown in the schedule (Table 1). The introductory section begins with a course orientation that provides logistical and basic course information to the participants. This is followed by an overview of gene therapy products and processes that provides information on gene therapy (for example, what is *in vivo* gene therapy? *ex vivo*?), gene therapy products on the market and their indications, and basic design of processes to produce viral vectors. This lecture ensured that participants with varying backgrounds and experience levels could put subsequent course topics into context. The introductory section also provides an overview of the cGMP regulations that must be adhered to when producing viral vectors for gene therapy.

Additional details of upstream processing, downstream processing, and analytical methods of the courses are presented below.

Course Design Details

Central to the design of the course, and in particular the laboratory activities, is a scalable process that has been developed (Figure 1) for the production of AAV serotype 2 with a transgene for green fluorescent protein (AAV2-GFP).

AAV2-GFP is produced by one of two methods: triple transfection of human embryonic kidney cells (HEK293) or infection of recombinant *Spodoptera frugiperda* (Sf9) cells (F3) with engineered baculovirus. (Note that from an instructional perspective, insect cells offer the advantage of being able to execute labs in a biosafety level 1 environment versus biosafety level 2 for HEK293). Because a significant amount of AAV is intracellular in either production system, a lysis step is performed by adding Triton X-100 (1%) and $MgCl_2$ to the bioreactor and holding it for 1 h at 37°C and pH 8.0. (Note that Triton X-100 is on the European Authorisation list (i.e., Annex XIV of the Registration, Evaluation, Authentication, and Restriction of Chemicals (REACH) regulations) with a sunset date of 04 January 2021. This information is detailed in the vector harvest lecture of the course along with different methods for cell lysis. BTEC evaluated lysis with several alternative reagents and plans to use Tween 80 in the lysis step in the future). Benzonase is also added at the end of the 1 h incubation, and the lysate is incubated for an additional 30 min to digest nucleic acids for viscosity reduction and to avoid DNA-AAV complexes. The resulting lysate is clarified (i.e., cell debris removed) using a Millistak+ Depth Filter (there are several possible options), followed by a 0.22 µm polish filter (Opticap® Durapore® XL). Clarification is required because the lysate contains numerous soluble impurities (e.g., host cell proteins, host cell DNA, and capsids without the transgene) that are removed by packed chromatography beds that would clog if solids are present in the feed.

The capture (i.e., first) chromatography step mainly removes process-related impurities such as host cell proteins, host cell DNA, and surfactant. This is followed by an anion-exchange step for the enrichment of full capsids. The affinity step is performed with either of the following resins: Poros™ CaptureSelect™ AAVX affinity resin from Thermo Fisher Scientific™ or AVB Sepharose High Performance resin from Cytiva. Following chromatography, a tangential flow filtration (TFF) step may be performed to concentrate AAV2-GFP and to transfer the product into an

appropriate buffer system, typically using an Ultracel® 300 kDa ultrafiltration (UF) membrane from MilliporeSigma. The filling of drug substance into vials (to produce drug product) is typically not performed at BTEC.

Upstream Processing Section

Various materials were used to conduct the upstream labs of the course. The Sf9 recombinant insect cell line (F3) and shuttle plasmid pTR-Bac-UF26 were developed in the laboratory of Dr. Sergei Zolotukhin and obtained under a material transfer agreement (MTA) with the University of Florida. In the F3 cell line, rep2 and cap2 are stably integrated into the Sf9 insect cell genome[5]. MultiBac™ cells were obtained from Geneva Biotech. Sf9 cells, Sf900II cells, and fetal bovine serum (FBS) were obtained from Thermo Fisher Scientific. AAV293 cells (HEK293), viral production medium, Optimem, transfection reagent, transfection booster, transfection enhancer, and 10X lysis buffer were all components of the Thermo Fisher Scientific AAV-MAX Helper-Free AAV Production System Kit. However, the data generated used the beta version of the protocol and kit. Three plasmids, pAAV-RC2, pAAV-GFP, and pHelper, were obtained from Cell Biolabs. Baculovirus was generated from the shuttle plasmid pTR-Bac-UF26 based on a protocol from the Thermo Fisher Scientific Bac-to-Bac™ Baculovirus Expression System, with the exception of using Multibac™ cells instead of DH10Bac™. The virus was titrated using plaque assay. Adherent HEK293 cells (CRL-1573) and Eagle's Minimal Essential Medium (EMEM) were obtained from ATCC.

The upstream portion of the course consists of lectures and labs (see Table 1). Following the introductory section of the course, the first upstream lecture covers cell banking, a description of the baculovirus system, methods to produce AAV using transient transfection into HEK293 cells, and infection of insect cells with recombinant baculovirus. Other methods to generate AAV are discussed in the first upstream lecture, however the instructor choose to use the baculovirus expression system and helper virus-free transient transfection into suspension HEK293 cells as methods in the laboratory exercises. These methods are widely used in the literature. In future course offerings, lecture material will be expanded to disucss specific differences in product quality between these two systems. The lecture describes various factors, such as DNA concentration, molar ratios of the three plasmids, transfection reagents, cell density, and temperature, that can be evaluated to optimize AAV titers. The lecture also covers lentivirus generation and provides an overview of production processes for *ex vivo* gene therapy (CAR-T cells); however, there are no labs associated with these topics.

Following this lecture, students go to the lab to conduct a process design activity that optimizes AAV2-GFP production in AAV293 cells using a design of experiment (DoE) with a partial factorial design. In this study, two temperatures (34°C and 37°C), three DNA molar ratios of pAAV-GFP:pAAV-RC2:pHelper (ratio 1-1:1:1, ratio 2-1:1:3, ratio 3-1:1.5:2), and three cell densities (2.5, 3.0, and 4.0 E+6/mL) were evaluated. The effects of the addition of enhancer were also evaluated. Students counted the cultures prepared by staff, set up cultures at different cell densities, made up the DNA/transfection reagent complex, and transfected the cultures according to the conditions assigned to each group. Seventy-two hours post-transfection, the staff counted the cultures, confirmed GFP expression, and lysed the culture

using 10X lysis buffer. The samples were then analyzed by qPCR to determine the vector genomes/mL. Staff analyze the data using statistical analysis software, specifically JMP® Pro software (v15.1.0). Using a p-value cutoff of 0.05, it was shown that only temperature and cell density had a statistically significant impact on the vector titer. This finding is illustrated (Figure 2), which shows the desirability profile from the statistical analysis performed by the JMP software. L1 refers to the absence of enhancer. This is a complicated experiment to run in class, as each group executes one set of conditions in the DoE. If one group makes an error (such as forgetting to add one of the DNAs), the entire experiment is in jeopardy. With students at varying levels of general lab technique and cell culture skills, it was decided to simplify the design of the lab after running the experiment as described in six offerings. Students are now given data from the DoE described above and then set up flasks to confirm the effect of temperature and evaluate the addition of enhancer.

In the next upstream processing lecture, students are introduced to scale up in single-use bioreactors–both rocking and stirred tank. The scale-up of adherent and suspension cells is discussed. Topics include bioreactor bag films and the effect of leachables, setting up a 50 L reactor (video), as well as various tools (welders, sealers, aseptic connectors, and disconnecting devices) that are needed to connect and disconnect addition vessels to the bioreactor.

To reinforce the topics presented in the lecture, students return to the lab (day 1) and observe, under the microscope, adherent HEK293 cells growing in a 10-stack cell stack and manipulated the cell stack. A short video of harvesting cells from T-flasks and seeding the 10-stack cell stack is shown. The term scale out instead of scale-up is introduced, and students are able to fully understand why it is preferable, due to the time involved, chance for contamination, and vessel-to-vessel variability, to scale up with suspension cells where possible. Next, students open up a rocker bag (which would be used for small-scale production of AAV or to prepare inoculum for a larger production reactor), familiarize themselves with the purpose of the parts on the bag, and position the bag on the bioreactor platform. The instructor reviews the hardware and software for the rocker bag, and students, following the lab protocol, inflate the bag with air. Once the bag is inflated, the students weld the bag of viral production medium to the inflated bag and add 4.0 L of viral production medium by weight. The students also familiarize themselves with the parts of the 50 L single-use bioreactor (SUB), in which an AAV2-GFP production run using baculovirus is ongoing. In this run, the F3 cell line, which has a stably integrated Rep2 and Cap2, is used. Rep and Cap are not expressed until infection with the baculovirus.[5] The cells are scaled up to 50 L at ~1.5E+6 cells/mL and infected with GFP-ITR baculovirus infected insect cells (BIIC) at a ratio of 7 mL BIIC to 50 L.[6] Students are given the batch record, filled out to this point in the process, to review. Noteworthy observations are the cessation of cell growth and an increase in cell diameter, as shown (Figure 3).

On the days the students are present in the cell culture lab, F3 cells are infected for 72 h (day 1 of the course) and 96 h (day 2 of the course). A group of students samples the reactor. First, the process values are recorded from the controller. The dissolved oxygen (DO) level is controlled at 50%, pH 6.0, with the addition of 1 M sodium carbonate and a temperature of 27°C. We briefly discuss bioreactor control with an in-depth discussion of this topic in the next

lecture. Students are taught how to take a sample using a CLAVE® connector and a needless syringe. After the sample is obtained, the pH, pCO_2 and pO_2 are determined using a Beckman Coulter Vi-CELL MetaFLEX™ bioanalyte analyzer. Students are urged to do this quickly because cells continue to consume oxygen and produce metabolites, resulting in inaccurate results. Viable cell density (VCD), percent viability, and diameter are determined using a Beckman Coulter Vi-CELL BLU cell viability analyzer according to the trypan blue exclusion method to determine live and dead cells. Vi-CELL cell viability analyzers are used extensively in industry; thus, students are gaining experience with equipment they would encounter in a process development or manufacturing environment. Next, the samples are analyzed for glucose, lactate, glutamine, glutamate, ammonia, and lactate dehydrogenase using a Roche Custom Biotech Cedex BioHT analyzer. The osmolality of the medium is determined using an Advanced Instruments osmometer. The students examine the cells using a Thermo Fisher Scientific EVOSM5000 microscope fitted with a GFP cube. Using the display monitor, the instructor and students are able to see the same image simultaneously. On Day 2 of the course, the above monitoring activities are repeated with a different group of students.

To recover all AAV2-GFP (both intracellular and AAV2-GFP that had been released into the medium), the cells are lysed in the bioreactor. To prepare for lysis, the temperature is increased to 37°C, and the pH is adjusted to 8.0 with 1M NaOH and $MgCl_2$ is added to 2mM. Triton X-100 is then added at a concentration of 1% (as mentioned earlier, BTEC plans to replace Triton X-100 with Tween 80 in future course offerings). Lysis is monitored over time for one hour and then benzonase is added to 20 units/mL and incubated for 30 min. The lysate is processed using a depth filter. The various steps in the production process executed in the upstream portion of the course are illustrated (Figure 4).

On the second day, while one group of students monitor the 50 L bioreactor, the other group of students sample the AAV293 inoculum prepared in a shake flask by the lab instructor and determine the VCD, percent viability, and cell diameter. According to the lab protocol, the seeding density is 0.55 x 10E+6 cells/mL. The students use the following formula: concentration 1 × volume 1 = concentration 2 × volume 2 to determine the volume of culture and additional fresh medium that need to be added to the rocker reactor to achieve the target initial cell density. Prior to cell addition, a before-inoculation (BI) sample is taken and analyzed using the analytical equipment described above.

The Upstream portion of the course concludes with a lecture discussing cell growth in bioreactors, media formulations, mode of bioreactor operation (batch, fed-batch, and perfusion), critical process parameters, and bioreactor process control.

Downstream Processing Section

Following the upstream processing section of the course, students begin downstream. As shown (Table 1), four lectures and three labs are conducted as part of the downstream section of the course, and the order of the lectures and labs follows the sequence of unit operations used in the AAV2 process (Figure 1). The key materials used in the labs are detailed throughout this discussion.

The downstream section opens with two lectures: an overview of downstream processing and vector harvesting methods. This overview provides a brief summary of all unit operations common to downstream processes for viral vector manufacturing. The lecture on vector harvesting discusses the steps required to separate the viral vector from the host used for production. The lecture content includes a discussion of intracellular vs. extracellular vectors and their impact on the design of the harvest stage of a downstream process. The lecture also provides a survey of cell lysis methods, focusing on those most suitable for intracellular vector recovery in a manufacturing process, and covers solid-liquid separation methods (i.e., centrifugation and depth filtration) used to clarify cell culture broths or lysate streams.

Following these lectures, the students take part in a two-hour lab on the design of a depth filtration step for lysate clarification in the production process. Single-use depth filtration is a commonly used method in viral vector manufacturing to clarify cell culture broths and lysate streams. As discussed previously, a clarification step is required to prevent solids from clogging the subsequent chromatography columns used to remove the soluble impurities from the product stream. Bench-scale studies are typically required as part of process development to screen different depth filter media and generate data for sizing the filters used in a production train. In this lab, students executed these screening/scale-up studies to clarify the lysate generated from F3 cells in BTEC's AAV2-GFP production process. From the data generated, students select which of the three filter media tested is the best choice for a production process, and then use their data to design a clarification train for the scenario in which 1000 L of lysate is processed.

To conduct the study, 12 students are divided into three groups, each testing one of the designated filter media (Table 2). Lysate from an F3 culture that produced AAV2-GFP is fed with a peristaltic pump to small 23 cm^2 test filters (one for each media type) at a constant feed flux of 300 LMH (L per m^2 of filter area per h). As the lysate is pumped across the depth filter, cell debris accumulates on and in the filter, resulting in an increase in pressure. The pressure drop across the filter is monitored over the course of filtration along with filtrate volume and filtrate turbidity. Students plot the pressure drop against the throughput, defined as the volume (in L) of lysate fed per unit area of filter (23 cm^2). Representative data generated in this lab are shown (Figure 5).

To define the capacity of a filter, a pressure drop endpoint is selected based on system capabilities at large scale. In the case of the filters used in this study, 15 psig is the endpoint based on filter supplier recommendation. The throughput for each depth filter at the 15 psig pressure endpoint represents the capacity of the filter for the F3 lysate used in the study. Students determine the capacity of their depth filtration media for F3 lysate from the generated plot and share their data (including both capacity and filtrate turbidity) with the other two groups. From the aggregated data, the best filter is selected by considering the filter capacity (high is desirable) and filtrate clarity (low turbidity is desirable) of each filter medium. Based on the data shown (Figure 5), Millistak+® C0SP gives the best filter performance in terms of filter capacity, with a value of approximately 66 L/m^2, compared to 40-41 L/m^2 for Millistak+® C0HC and Millistak+® D0HC. In addition to having the highest capacity, Millistak+® C0SP also produced a

filtrate with the lowest turbidity,~ 9 nephelometric turbidity units (NTU) among the three filters. Note that for this study, the feed turbidity was 411 NTU.

Following data generation, students independently estimate the filter area and number of depth filtration modules required for a filter train used to process larger volumes (e.g., 1000 L) of the lysate using the filter that provided the best performance. The minimum depth filtration area, A_{min}, required to filter 1000 L of clarified F3 lysate is calculated as follows, using the data given (Figure 5):

$$A_{min} = \frac{V_{feed}}{Capacity\ at\ 15\ psig\ endpoint} = \frac{1000\ L}{66\ L/m^2} = 15.15\ m^2\ filter\ area \qquad \text{equation (1)}$$

where V_{feed} is the volume of the feed lysate. Once this calculation is complete, the concept of a safety factor (SF) for filter area estimates is introduced to account for variability in the feed stream from batch to batch in a production process[9] and a calculation to determine the actual number of filtration modules for a production run is performed as follows:

$$\#\ filter\ modules = \frac{A_{min} \times SF}{A_{module}} = \frac{15.15\ m^2 \times 1.5}{0.77 m^2\ per\ module} = 30\ modules \qquad \text{equation (2)}$$

where A_{module} is the filtration area in a single production-scale depth filtration module.

Following these design calculations, the instructor leads a group discussion on the impact of process parameters (i.e., parameters directly controllable in a manufacturing setting) such as feed flux on the design. For example, if a higher or lower feed flux than that used in the bench-scale study is chosen, what impact will this have on the design of the filter train for production?

Following this lab, students hear lectures on chromatography for purification of viral vectors and UF for concentration and buffer exchange of vector solutions. The chromatography lecture starts with a general introduction to the purpose of chromatography in the purification of viral vector products, highlighting the types of impurities present and the physical/chemical properties of AAV vectors. An introduction to chromatography principles, types, and modes of operation is presented next. The lecture then focuses on chromatographic media commercially available for viral vector capture and for impurity removal. While affinity chromatography is covered in depth during the lab, details on the use of anion exchange chromatography for enrichment of full capsids is given in the lecture. Literature data for the separation is also shared with the students, moreover, information about membrane adsorbers and monoliths – two chromatography alternatives to traditional packed beds that offer the advantage of small diffusional resistance–is presented. Finally, the principles of scale-up of chromatography systems are presented. The next lecture, on the topic of ultrafiltration, discusses the underlying principles of ultrafiltration, differences in using UF for concentration and buffer exchange, UF membranes, operating procedures, and process and performance parameters.

Following these lectures, students take part in a two-hour lab involving affinity chromatography for the purification of AAV2-GFP in a simulated cGMP environment. The students are divided into three groups, and each makes use of bench-scale fast protein liquid chromatography

(FPLC) systems (AKTA pure systems from Cytiva). An affinity column packed with either AVB Sepharose High Performance resin from Cytiva or POROS™ CaptureSelect™ AAVX Affinity Resin from Thermo Fisher Scientific is used to purify the AAV2-GFP clarified lysate. The AVB Sepharose HP columns are a 1 mL prepacked HiTrap and an 8 mL column packed in house, while the AAVX column is a 1 mL prepacked POROS™ GoPure™ AAVX column. A complete chromatographic cycle that includes equilibration, load, wash, elution, regeneration, sanitization, and storage steps is performed with each lab group following instructions from a batch record, similar to the type that would be used in a cGMP manufacturing environment, with data from the run documented using good documentation practices.

A typical chromatogram for a run using POROS™ CaptureSelect™ AAVX Resin is shown (Figure 6) with equilibration, sample application (i.e., load), wash, elution, cleaning, and storage steps. Capsids are eluted by decreasing the pH, as shown in the chromatogram. The flow rate during the load step is 0.33 mL/min (~3 min residence time), while the elution flow rate is at 0.5mL/min.

Owing to time constraints, analysis of the product from the chromatography run is not possible; however, participants are given data from a previous chromatography run for AAV2 capsid concentration measured for the load, load flow-through (FT), wash flow-through, elution fractions (not shown in Figure 6), and the cleaning (i.e., the CIP or clean-in-place) step of the chromatography run. Capsid concentrations were determined using an enzyme-linked immunoassay (ELISA, specifically the Progen AAV2 titration ELISA). The students use the data to calculate step yield, as required by the U.S. Code of Federal Regulations (21 CFR 211.103)[10] and a mass balance, with the following equations:

$$\%\ step\ yield = \frac{\sum_{i=fraction\ 1}^{i=fraction\ N}(fraction\ volume\ (mL)_i \times AAV2\ concentration\ in\ fraction\ (capsids/mL)_i)}{load\ volume\ (mL) \times AAV2\ concentration\ in\ load\ (capsids/mL)} \qquad \text{equation (3)}$$

$$\%\ mass\ balance = \frac{\begin{array}{c}(load\ FT\ volume\ (mL) \times AAV2\ concentration\ in\ load\ FT\ (capsids/mL) + \\ wash\ FT\ volumn\ (mL) \times AAV2\ concentration\ in\ wash\ FT\ (capsids/mL) + \\ eluate\ volume\ (mL) \times AAV2\ concentration\ in\ eluate\ (capsids/mL) + \\ cleaning\ FT\ volume\ (mL) x\ AAV2\ concentration\ in\ cleaning\ FT\ (capsids/mL))\end{array}}{load\ volume \times AAV2\ concentration\ in\ load\ (capsids/mL)} \qquad \text{equation (4)}$$

The data provided to the course participants results in a >95% step yield and a mass balance of just > 100%. However, based on our experience, we see that step yields vary between 50 and 95%. This variability suggests that capsids could be partially degraded (denatured) by low pH elution conditions, although this has not been proven for the BTEC process. Typically, no capsid is detected in the load or wash flow-through samples. Furthermore, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of load and elution samples (not shown here) and the elevated ultraviolet (UV) absorbance at 280 nm during the load step clearly indicate that significant purification (e.g., separation of AAV2 capsids from host cell proteins) occurs during this chromatography step.

The final laboratory in the downstream section of the course covers ultrafiltration (UF) for the concentration and buffer exchange of viral vectors. It is performed in TFF mode using a bench-top PendoTECH filtration unit that houses a Millipore Sigma Pellicon® 3 UF cassette with an

Ultracel® membrane of a molecular weight of 30, 100, or 300 kDa. The lab explores the impact of certain process parameters on UF performance. For example, the effect of transmembrane pressure (TMP) on permeate flux is measured, and the relationship between diafiltration volumes (DVs) – the amount of diafiltration buffer fed relative to the product retentate in the system – and the extent of buffer exchange determined. The data gathered are then discussed in the context of scaling up to manufacturing.

Analytical Methods Section

The analytical portion of the courses comprises 3.5 hours of lectures, 2.75 hours of hands-on labs, and 1.25 hours of data analysis discussion and workshop, as detailed (Table 1). This format provides course participants with a theoretical framework for understanding gene therapy analytical methods and with hands-on experience generating and analyzing data from real AAV samples.

The initial lecture covers where, in a biopharmaceutical process, analytical testing is taking place and what aspects of AAV are to be analyzed to evaluate product identity, purity, safety, and potency. This lecture also covers the ICH guidelines for assay validation. The next two lectures focus on the specifics of assays for AAV titer and SDS-PAGE for AAV purity, which participants conduct during the hands-on portion of the course. The lecture on AAV titer gives particular attention to quantitative polymerase chain reaction (qPCR) because it is an important dosing assay that includes technical aspects of the assay and considerations for sample preparation for AAV viral genome quantification. There is also a discussion of droplet digital PCR (ddPCR) in this lecture, and a comparison between ddPCR and qPCR. The final lecture focuses on assays for safety, purity, and potency. This lecture addresses many of the complicated product-related impurities that are unique to gene therapy and pose significant analytical challenges, including empty capsids, encapsidated host or plasmid DNA, and noninfectious particles. The goal of this portion of the course is to ensure that participants have a foundation in analytical method development, unique analytical aspects of AAV, and an overview of the assays used to characterize and assess the quality of AAV. To date, students do not perform potency assays or other advanced characterization techniques (transmission electron microscopy, analytical ultracentrifugation, etc); however, BTEC offers a three day short course entitled Analytical Methods for Gene Therapy Vector Characterization and Testing in which these methods are covered in detail.

In order to prepare course participants not only for some of the unique assays in gene therapy but more broadly for analytical testing of biopharmaceuticals, the hands-on portion of the analytical section seeks to highlight method validation principles using gene therapy assays. The participants receive hands-on training in two assays: qPCR and SDS-PAGE. These methods were chosen because they cover different aspects of AAV analysis (quantity and purity), and they can be completed in sufficient time to allow for data analysis. While these methods are currently used, as newer methods are adopted, such as ddPCR and capillary electrophoresis, the newer methods will be incorporated into the course.

In the qPCR lab, participant groups prepare AAV-containing samples to evaluate DNase-treatment effectiveness, linearity, intermediate precision, and matrix effects. These aspects were chosen because they teach important lessons in sample preparation, assay development and optimization, and assay validation. A similar strategy is utilized during the SDS-PAGE lab, where students prepare samples and run SDS-PAGE to evaluate validation concepts such as linearity, precision, and accuracy.

The samples and qPCR plate prepared by the students in the lab are run on an ABI 7500 Fast qPCR instrument while students attend the lecture. The data are retrieved and quickly analyzed by the instructor for a data analysis lab in the afternoon. In this lab, the students begin by evaluating the aspects of system suitability in qPCR. This includes assessing the standard curve to determine amplification efficiency, linearity, and contamination in the no-template control. To prepare the qPCR plate, all samples are loaded in triplicate, and the relative standard deviation (RSD) of the triplicate is calculated. This allows for a discussion of the importance of the pipetting technique on assay precision. The topics discussed in the data analysis for each laboratory group are presented (Table 3). The SDS-PAGE data are also discussed, with particular attention to the pharmacopeial considerations in assessing SDS-PAGE.

Participants and Participant Profile

Since the first Hands-On cGMP Biomanufacturing of Vectors for Gene Therapy course in March 2019, seven offerings have been held with nearly 80 participants.

Course participants represented over 30 different organizations – industrial and academic (both universities and community colleges), and supplier/service providers–and traveled from 10 different states, with about half of the participants based in North Carolina. The background of participants has varied and includes academic (both researchers and teachers), research (in industry), manufacturing, operations/planning, engineering, sales/marketing, and quality assurance.

Program Evaluation

Upon the conclusion of the on-site classroom and laboratory activities, participants are asked to provide their opinions on the relevance, organization, and delivery of and their satisfaction with the course using a web-based evaluation form. A summary of feedback from these post-course evaluations is provided in Table 4. From the seven course offerings held to date, 96% of survey respondents indicated overall satisfaction or extreme overall satisfaction with the course. Eighty-seven percent thought the level of the course content was appropriate (i.e., not too easy and not too difficult), which indicates that the course design suits the audience. Eighty percent of respondents agreed or strongly agreed that the course provided the information and skills needed to effectively carry out job responsibilities, while 91% indicated that they could transfer learnings from the course to their current position. Further, more than 90% of respondents agreed or strongly agreed that they have a greater understanding of the subject matter and the ability to explain what they learned to a coworker.

Ratings were likewise high on various aspects of course delivery, with greater than 90% of survey respondents agreeing or strongly agreeing that the learning environment, course materials, lab activities, and lectures were effective. In addition, feedback on course instructors' instructional skills (not shown in the table) has been extremely positive. Overall, post-course evaluation results from the seven courses offered so far has been very encouraging, especially considering the varied backgrounds and experiences of participants, and confirms the success of the program.

The strengths of the course that were commonly cited in the post-course evaluation comments included hands-on lab sessions, the flow of topics, and the integration of lectures and labs. The main weakness cited by the participants was that some information presented in the course was already known to individual participants. However, a number of participants also mentioned the need for more time for both lectures and labs. These comments reflect the varied level of participants' understanding of the gene therapy field prior to entering the course.

Lessons Learned

Several challenges have been addressed during the development and delivery of this course. Notable among these is the challenge of designing a course that is appropriate for a wide range of backgrounds and expertise of course participants. The lectures and labs that make up the course have undergone revision after almost every offering to ensure content meets the needs of a diverse group of participants, regardless of the background or experience coming into the course. In addition, as important as lecture and lab design are to the quality of the course, short courses require instructors who are capable of answering the very specific and applied questions asked by an audience of professionals. Based on BTEC's experience, the depth and specificity of questions are key differentiating factors between teaching professionals and university students. Instructors with at least some experience working in the biopharmaceutical industry are needed.

Conclusions and Future Opportunities

BTEC has developed and delivered a hands-on course (Hands-On cGMP Biomanufacturing of Vectors for Gene Therapy) that covers numerous topics related to viral vector manufacturing. These topics include an overview of gene therapy products, fundamental concepts underlying the operations used to manufacture these products (both upstream and downstream), process parameters that must be controlled, assays used for product intermediate and drug product testing, and validation of these assays. Since its inception in May 2019, nearly 80 participants have attended the course's seven offerings. Post-course evaluations show that overall participant satisfaction with the program is high and that the course provided participants with the information and skills needed to effectively carry out their jobs. Demand for the course remains high, and BTEC will continue to offer it as part of its professional development program in the foreseeable future.

Because of BTEC's success with the course, the gene therapy training network (GTTN) has been initiated with support from the National Institute for Innovation in Biopharmaceutical

Manufacturing (NIIMBL) to organize efforts to transfer this and other gene therapy courses to other institutions. To date, the Hands-On cGMP Biomanufacturing of Vectors for Gene Therapy course has been transferred to the National Center for Therapeutic Manufacturing (NCTM) at the Texas A&M Engineering Experiment Station. They piloted the course in October 2021 and have since offered it to 25 participants, with more than 100 participants anticipating to take the course (or an abbreviated lecture-only version) by the end of 2022. Similar to the BTEC version of the course, the response to the NCTM program has been extremely positive. Both BTEC and NCTM continue to work with partners interested in joining GTTN to exchange course content on topics related to viral vector manufacturing. Furthermore, BTEC has also incorporated elements of the course into its academic programs for undergraduates and graduate students to prepare them for opportunities in the rapidly expanding gene therapy industry.

Finally, we believe that more focused training in any of the areas covered in this course is warranted. BTEC has recently completed the development and initial delivery of a course titled Analytical Methods for Gene Therapy Vector Characterization and Testing, which focuses on analytical methods for viral vector process intermediate and final product testing. Likewise, individual courses that look specifically at upstream or downstream processing, including the design and development of these processes, are needed and are likely to see high demand based on our experience in professional development programs. Based on the feedback BTEC received from course participants, we believe that training on the topic of plasmid design and plasmid production (required for transient transfection to produce viral vectors) would also be of great interest.

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Authorship Confirmation/Contribution Statement

Author 1: Conceptualization (equal); methodology (equal); investigation (equal); writing-original draft (equal).
Author 2: Conceptualization (supporting); investigation (equal); writing-review and editing (equal).
Author 3: Investigation (equal); writing-review and editing (equal).
Author 4: Conceptualization (equal); methodology (equal); investigation (equal); writing-original draft (equal).
Author 5: Funding acquisition (supporting); project administration (equal); writing-original draft (supporting).
Author 6: Project administration (equal); writing-original draft (supporting).
Author 7: Funding acquisition (lead); conceptualization (equal); methodology (equal); supervision (lead); writing-original draft (equal).

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Ethics Statement

Development of this course did not include studies on human subjects, human data or tissue, or animals.

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