WHITEPAPER

Accelerate innovation and manufacturing to better serve patients

PRESENTED BY:

GenScript PROBIO

CONTENTS

INTRODUCTION

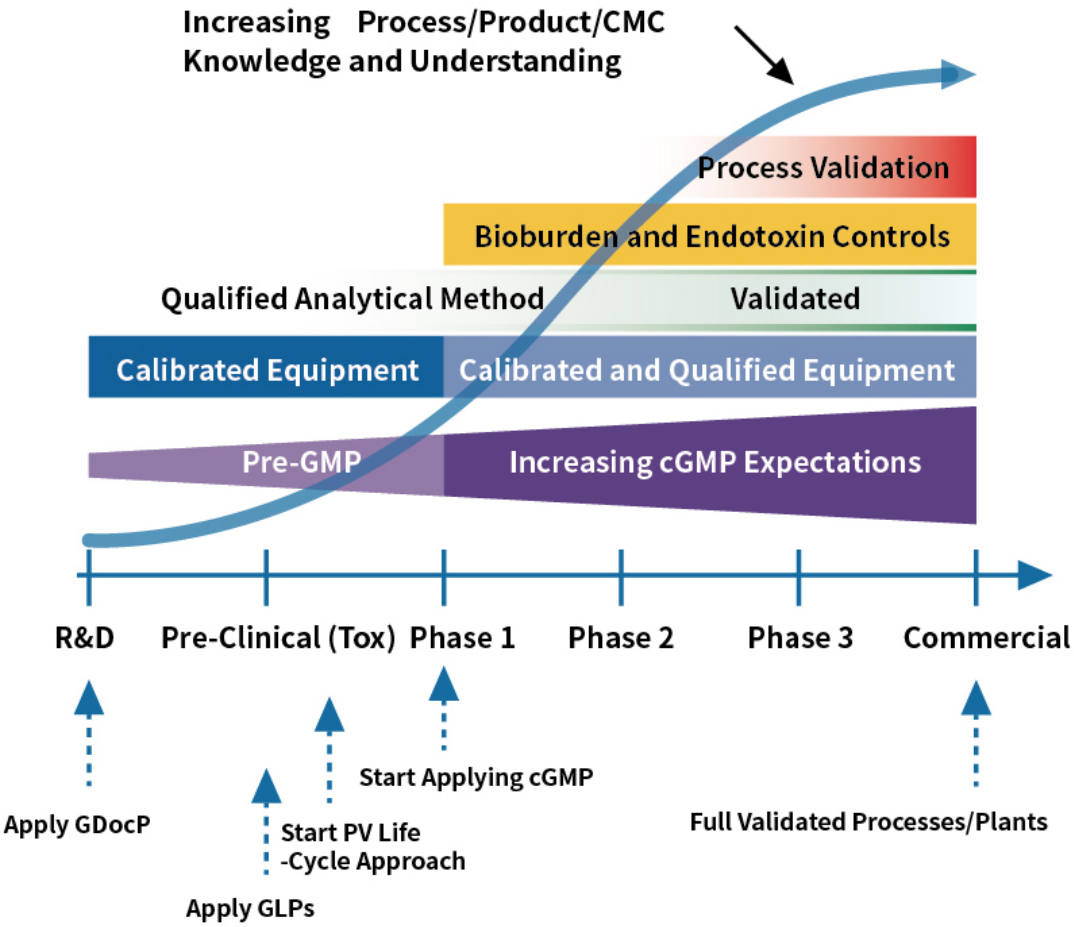
Many advancements are ongoing in science and technology, and advanced medicinal products are essential for the treatment and prevention of diseases. Gene and cell therapy, as well as immunotherapy products, belong to the advanced medicinal products category, and a wide variety of product platforms involve new technologies in development, production and control. A significant number of new treatments have been approved so far, but some manufacturing and regulatory guidelines pose challenges for advanced therapies, leading to poor production yields.

Rapid expansion of the gene and cell therapy pipeline created constraints to accessing contract capacities around the globe. Innovation in gene and cell therapy expanded many drug development pipelines, and startups that are lacking internal production capacities heavily rely on contract manufacturing organization (CDMO).

GENE AND CELL THERAPY (GCT)

Gene therapy emerged as a new treatment option for untreatable genetic diseases, and the techniques in gene therapy are aimed at mitigating disease features of recessive and dominant disorders along with several cancers. The field of gene therapy has made significant progress over the last several decades, and while numerous clinical trials for various indications are in progress, only five therapies have reached FDA and/or EMA approval since 2017 (Luxturna, Kymriah, Yescarta, Zolgensma and Tecartus) for clinical use.

Cellular therapies have let to huge advancements in curing many diseases, especially the liquid and solid cancers. First, two CAR-T therapies that emerged as the new pillars for B-Cell (Acute lymphoblastic leukemia) achieved FDA approvals in USA – one from Novartis and the other from Gilead's Kite Pharma, following recent approvals by EMA. CAR-T therapy is a landmark for pharmaceutical industry and is expected to dominate the global market with over 1000 clinical trials and billion dollars of investments. Several pharma collaborations, mergers and acquisitions also support CAR-T therapy. Despite the growth, there are hurdles to overcome, particularly in viral vector manufacturing, one of the major bottlenecks in the whole industry. The successful business cooperation with CDMO in therapeutic antibody can provide inspiration to drug developers in CAR-T space.



Expedite Gene and Cell Therapy product development

Advanced medicinal products have considerable therapeutic value and need support from intermediate agencies and manufacturers in order to accelerate product development. GenScript ProBio is the CDMO segment of the world's leading CDMO/CMO (Contract Development and Manufacturing Organization/Contract Manufacturing Organization), providing a one-stop biological drug research and development platform. Our professional plasmid team has developed a well-established plasmid manufacturing process, which not only guarantees the high quality and high purity of clients' products, but also offers the clients lower cost and a shorter turn-around-time compared to other CDMOs. With our viral vector platform, GenScript ProBio is assisting clients from plasmid to viral vector development and manufacturing, dedicated to providing one-stop solutions for gene and cell therapy programs, and covering the whole product lifecycle. As the demand for lentiviral vector is becoming larger, GenScript ProBio is making effort to meet market need and ready to manufacture lentiviral vectors using both adherent culture and suspension culture. Our team has collaborated with several clients to support their projects and has assisted several clients to get IND clearance from FDA and NMPA since 2018 (1st Legend Biotech IND case). Our mission is to be the professional CDMO partner to the global gene and cell therapy industry, by delivering best-in-class product plasmid and viral vectors to promote life-changing gene and cell therapy programs.

GenScript ProBio has expertise in regulatory compliance

cGMP compliance is a key component of the pharmaceutical industry and is necessary for ensuring the quality of drugs or medicinal products. Pharmaceutical and biotech companies adhere to regulatory guidelines for the patient safety. Changes in cGMP guidelines can create challenges and a high workload for any company affected, as a response to new regulations needs to be organized and implemented. Integrating changes that have a relevant process change in one department can also affect associated departments. Integrated quality systems with independent quality control and quality assurance department can help to better manage the quality of each client's project.

- In line with ICH/GMP guidelines/regulatory compliance
- Use **PAC (Phase-Appropriate Compliance)** to perform quality assurance process specifically in each phase of drug development
- **One-to-one QA** for each project to ensure quality throughout the project

Figure 1. GMP plasmid manufacturing facility

TECHNOLOGIES THAT ACCELERATE PLASMID MANUFACTURING

Strains used for plasmid manufacturing

Because of its long history in laboratory culture and ease of manipulation, *E. coli* plays an important role in modern biological engineering and industrial microbiology. Large-scale production using *E. coli* is rather economical, and it is therefore the first choice for plasmid production. The most widely used cell lines for plasmid DNA manufacture are derivatives of K12; for example, DH5α, DH10B, JM108, Stbl3 and Stable-NEB, etc.

Key points in selecting strains

- ☐ Select a K12 strain with clear source
- ☐ Solve the IP issue when submitting IND/BLA/NDA
- ☐ Screen for strains with high yield

Regulatory and quality solutions

- ☐ Create documents to record the history of each strain
- ☐ Reach agreement with strain owner regarding the commercial use of the strains and also for sub-license authority. Solve IP issue of strains in BLA/NDA and commercialization
- ☐ Strains we adopted are efficient in producing plasmids with high purity and high supercoiled plasmid content.

Typical GMP plasmid manufacturing process

Typical GMP plasmid manufacturing process can be summarized in the flowchart (Figure 2). The whole process procedure begins from master cell bank (MCB) or working cell bank (WCB), continues to upstream fermentation and downstream purification, and ends up with fill and finish. Each step involves specific process control to ensure the quality of the final product.

Process Development Platform

Figure 2. Typical GMP plasmid manufacturing process

Cell banking

Lentiviral vectors are generated using two, three or four plasmid constructs for each vector, with two or three helper plasmids and a single therapeutic transgene. In cell therapy, lentivirus systems are widely used, and 4-plasmid system is currently the most used in lentivirus system due to its high safety standards. For gene therapy, Adeno Associated Virus (AAV) is used extensively, and 2 packaging plasmids are required for the AAV system.

MCB is regarded as the starting point of a GMP plasmid DNA by the regulators, yet the initial plasmid construct is used to generate MCB. The plasmid DNA is transformed in *E. coli* and expanded to generate PCB, MCB, and WCB. To guarantee the quality of plasmid DNA and to manufacture a viral vector for the intended therapeutic benefit, the MCB must be thoroughly tested to remain homogeneous. The cell bank must also be large enough to provide a stable source for a long-term large-scale production to ensure the consistency of the plasmid DNA. For manufacturing of DNA plasmid from bacterial cell banks, FDA recommends MCB testing to include:

- Bacterial host strain identity
- Plasmid presence, confirmed by bacterial growth on selective medium, restriction digest, or DNA sequencing
- Bacterial cell count
- Bacterial host strain purity (no inappropriate organisms; negative for bacteriophage)
- Plasmid identity by restriction enzyme (RE) analysis
- Full plasmid sequencing. We recommend fully sequencing plasmid vectors and submitting an annotated sequence for the vector, as described in more detail in the section below on viral vector banks
- Transgene expression and/or activity

Fermentation process

Fermentation is usually run in shaking flasks or bio-reactors. When compared to shake flask fermentation, fermentation in bio-reactors usually shows advantages in high yield and feasibility for scaling up. Up-scaling is not just a question of using bigger devices but of carefully adjusting each process step in a way that can be reproduced. The most commonly adopted fermentation process is fed-batch, as it is likely to be free of antibiotics to reduce the risk of the viral vectors to animals or human. By adjusting and monitoring the operating parameters, such as pH, oxygen uptake rate, specific growth rate, etc., the fermentation rate and yield can be optimized to find the best fermentation conditions.

After the fermentation process, the crude product is obtained through cell lysis. The plasmid DNA stays in the solution and can be separated from genomic DNA and other floccules by filtration or centrifugation.

Key points in fermentation

- ☐ The fermentation process is supposed to lead to high plasmid yield through high density fermentation, animal free and antibiotic free with pilot scale production
- ☐ The fermentation process is supposed to be easy to scale up options 5L to 200L
- ☐ The fermentation process is supposed to have high stability of plasmids

Purification process of plasmid DNA

The purity of the plasmid DNA is one of the most relevant specifications regarding the quality of the plasmids and even final efficacy and toxicity of the final cell products. For this reason, the purification process plays an important role in removing impurities and contaminants. Chromatographic methods are widely applied for plasmid purification due to their high efficiency in removing undesired components. Another reason for the application of chromatographic purification methods is the feasibility in large-scale

production. Chromatography methods are usually stable, reproducible, and easy to scale up.

A certain chromatographic step is responsible for removing host cell residuals, including host cell proteins, host cell DNA and host cell RNA, and certain chromatographic steps can eliminate endotoxin and other contaminants. Some steps, such as hydrophobic-interaction chromatography (HIC), work well for separating open and closed-circle plasmid forms. By reasonably combining various purification steps, the purity and the quality of the plasmid DNA can be improved significantly.

Key points in purification

- ☐ Improve the stability of the process, especially in lysis
- ☐ Improve recovery rate to get high yields and 3-step purification process
- ☐ Improve the efficiency in removing impurities, especially critical quality attributes such as HCP (host cell proteins) and HCD (host cell DNA), to improve the content of supercoiled plasmid

High-quality solutions

- ☐ Recovery rate has reached between 20%-50% to get a higher yield of plasmid after purification
- ☐ Established 3-step purification based on experience with several projects
- ☐ Adopted proprietary automated closed continuous lysis system to improve the stability of the process

Quality control

Through the purification process, impurities are removed and controlled for obtaining products that are high in purity and quality. As a core part in the whole manufacturing process, the quality control of a batch release should be carefully studied, and the best suitable assay methods validated to ensure the final plasmid quality. Due to the specialty of plasmid DNA, it can either be used to produce viral vectors, or to be the drug substance or drug product itself. With respect to different use of plasmid DNA, the critical quality attributes and the release standards are different. However, the basic rules remain the same: The quality of the plasmids is highly related to the final products, either viral vector, cells or plasmid, and the quality of the plasmid DNA should be strictly controlled in terms of identity, purity, and safety. The following quality items may be listed in QC: sterility, endotoxin, purity (including percent of supercoiled form and residual cell DNA, RNA, and protein levels), and identity testing (restriction digest and sequencing if sequencing was not performed on the bacterial bank). In addition, reasonable assay methods and release standards should be studied.

Key points in quality control

- ☐ Difficulties in development and qualification of analytical methods

GenScript ProBio's solutions

- ☐ GenScript ProBio recognizes the critical quality attributes of products based on industrial experience and regulation information. The safety and efficacy properties of products, such as the product characterization, process-related impurity detection and safety related items will be assessed.
- ☐ GenScript ProBio designs and develops assay procedures scientifically and systematically to meet the varied analytical requirements. The assay performance applied in GenScript ProBio meets or exceeds industry standards in the field of quality characterization.
- ☐ GenScript ProBio follows the guidelines of the Chinese Pharmacopoeia (ChP) Technical Guideline 9101, ICH Q2 and the United States Pharmacopoeia (USP) General Rules 1225/1226 to confirm the performance of the quality analysis method and prove that the method is suitable for testing requirements.

GenScript ProBio's plasmid offerings for GCT

GenScript ProBio offers plasmid DNA in different degrees and different amounts. From pre-clinical through clinical to commercial supply, we are always ready to assist you in making breakthroughs.

Plasmids from research grade to GMP manufacturing

Different grades of plasmids are available to meet expectations during GCT development cycle. Plasmid manufacturing services are generally featured with:

- **Animal free, antibiotic free,** low risk to animal health
- High yield with **600-800 mg/L** in helper plasmids from GenScript ProBio
- High supercoiled plasmid content: **>90%**
- Strict quality controls and appropriate quality assurance process
- **Cost-effective with short lead time**

ProPlasmid is an option suitable for the use in non-clinical stages. It is manufactured in well-controlled environments under critical quality assurance.

GMPro Plasmid is manufactured from WCB under comprehensive quality oversight, is applicable in early clinical trials and is cost-effective to enable faster transition to clinic.

GMP Plasmid can meet all needs throughout the development cycle. No matter if it is used as raw materials for production of viral vectors or used as the drug product, GMP plasmid meets all of these requirements.

VIRAL VECTORS IN GENE AND CELL THERAPY

Understanding of lentivirus

Lentiviruses (LV) are RNA viruses that belong to the *Retroviridae* family. The best-known lentivirus is the Human Immunodeficiency Virus (HIV), which causes AIDS. Lentiviruses can integrate a significant amount of viral cDNA into the DNA of the host cell and can efficiently infect non-dividing cells, so they are one of the most efficient methods of gene delivery.

The lentivirus genome usually comprises three open reading frames (ORFs):

- Group-specific antigen (gag): Encodes structural proteins that form the viral capsid
- Polymerase (pol): Encodes the reverse transcriptase, protease and integrase enzymes
- Envelope (env): Encodes the viral envelope (surface and transmembrane glycoprotein) proteins

Further, these viruses have additional genes called accessory genes, which encode proteins that are responsible for various replicative functions.

Advantages and limitations

In the past, many clinical trials based on the use of other retroviral vectors – murine leukemia viruses (MLV) – were successful. Although these vectors are still used, the use of LV vectors is generally preferred for the following reasons:

1. LVs are able to transduce non-dividing cells because they can translocate across the nuclear membrane
2. Their integration patterns seem to be less risky: These vectors stably integrate the transgene into the target cell without transferring the sequences that encode for proteins that are derived from the packaging virus. This reduces the risk of setting off an adverse immune response inside the patient's body
3. Lentiviral vectors can be pseudotyped to broaden their tropism
4. They can be produced at high vector titer

There are also some limitations in the use of lentiviral vectors:

5. Lentiviral vectors may induce oncogenesis through insertional mutagenesis
6. These vectors have potential of generating replication competent lentivirus (RCL)

LV vector system(s)

Lentiviruses that have been developed as gene transfer vectors include mainly HIV-1 and HIV-2, but HIV-1 is the one that is very well-studied. Most of the others have not yet reached the clinical study stage.

Considering the safety issue of the pathogenicity of HIV-1 in humans, different generations of LV vector systems have been developed by modification of the helper plasmids and the existing genes.

Two-plasmid systems

In two-plasmid systems, all helper functions (gag-pol, rev, tat and VSV-g) are on one plasmid. Though such a production system is easier to produce and less expensive in its application and leads to higher vector titer than the three or four plasmid systems, the presence of all helper genes located on one plasmid might be a concern with respect to the formation of replication-competent lentivirus (RCLs).

Three-plasmid systems

Two helper plasmids coding for the gag-pol and the env functions and one transfer plasmid comprise three-plasmid systems. All the accessory genes are removed to improve the safety of the virus.

Four-plasmid systems

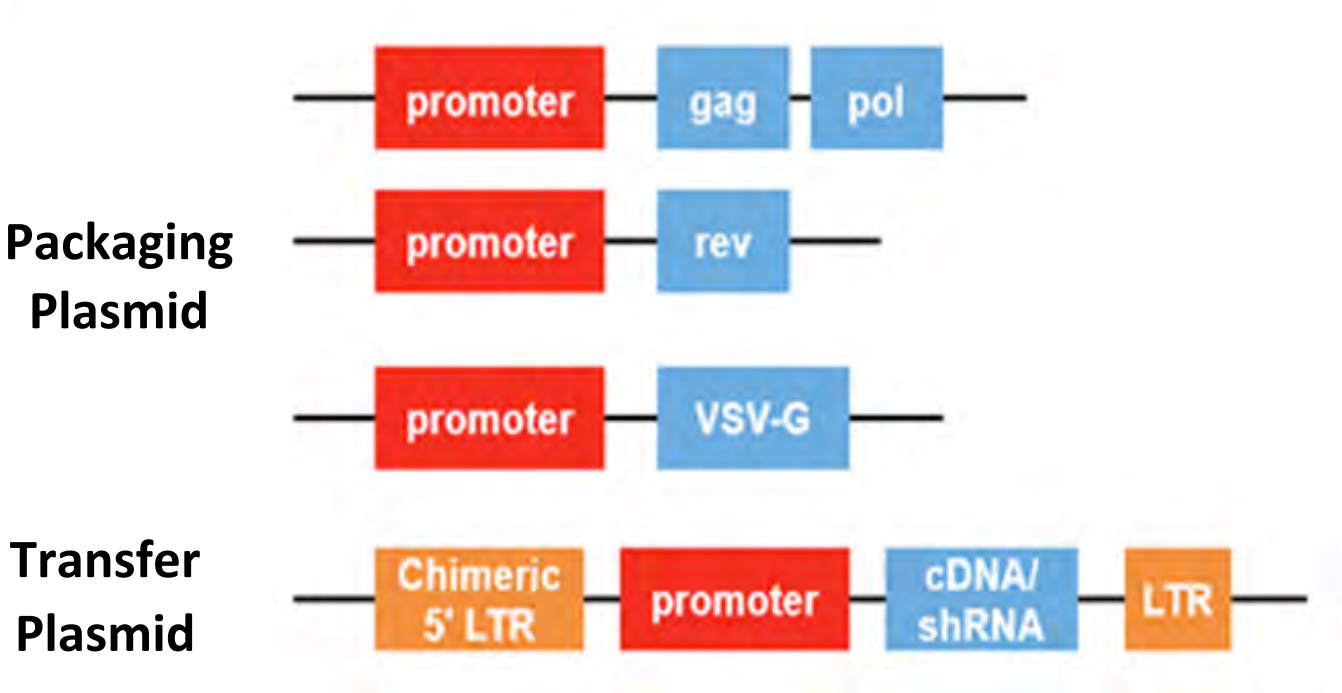
The third generation of the LV vector system is widely used and guided by safety considerations of the potential generating of RCLs. All accessory genes of HIV-1 (vif, vpr, vpu, and nef) have also been removed because they are not necessary. The rev gene is placed on a helper plasmid independently to improve the safety of the system. The regulatory tat gene, present in the second-generation LV, has been eliminated because its transacting function is dispensable. The whole system with 3 helper plasmids and one transfer plasmid reduces the possibility of formation of RCLs and improves safety.

Production of lentivirus

Cell lines

Due to the difficulties in establishing stable LV producer cell lines, transient transfection of HEK293 or HEK293-derived cells with vector and helper or packaging plasmids is the most widely used method to generate lentiviral vectors.

Compared with HEK293 cells, HEK293T cells may be preferred in producing LV because the presence of SV40 T-antigen in the producer cells can lead to more efficient production of vectors. Besides, HEK293T cells show increased cell growth and transfection efficiency in comparison to HEK293 cell line. In spite of this, the parental HEK-293 cell line may present an advantage in terms of safety since SV40 large T antigen is an oncogene.



Key points in selecting cell lines

- ☐ Virus titer: Select high-yield cell line; developing cell strains on your own will be a time-consuming and labor-intensive process
- ☐ Cell lines with clear source: Traceability is a critical issue for regulatory compliance. Therefore, the cell line used should have a clear source.

Cell culture systems

Currently used cell culture systems are predominantly adherent or suspension-cell-based systems. The key difference between these two kinds of cell culture systems is the way of passaging the cells. In case of suspension system, the cells can be directly diluted into a fresh medium to easily scale up the process. It can also be as complicated as detaching the adherent cells from a surface and plating them onto a new surface in a fresh medium. And in this system, the scale-up is limited due to the surface available for the cell growth.

Vector production using adherent cultures

Adherent cells are usually cultured in multilayer tissue culture flasks such as CFs or Cell STACKS for research or development purposes, because they are easy to manipulate and cost-effective. With respect to larger-scale LV production in clinical trials, the production process needs to be scaled up. With the use of adherent cells in CF systems, a scale-out approach should be performed by adding supplementary production units. Generally, the 10-stack CF and the largest 40-stack CF or 36 HYPER Stack are the most widely used. The 40-stack device is a semi-closed system, which provides improved safety for the operator and the environment, as well as the final product. However, it also requires a specific handling system due to its semi-closed architecture and the elevated weight of the CF-40. Another method for larger-scale LV production that is easier for scale-up is the use of suspension cultures in large bioreactors.

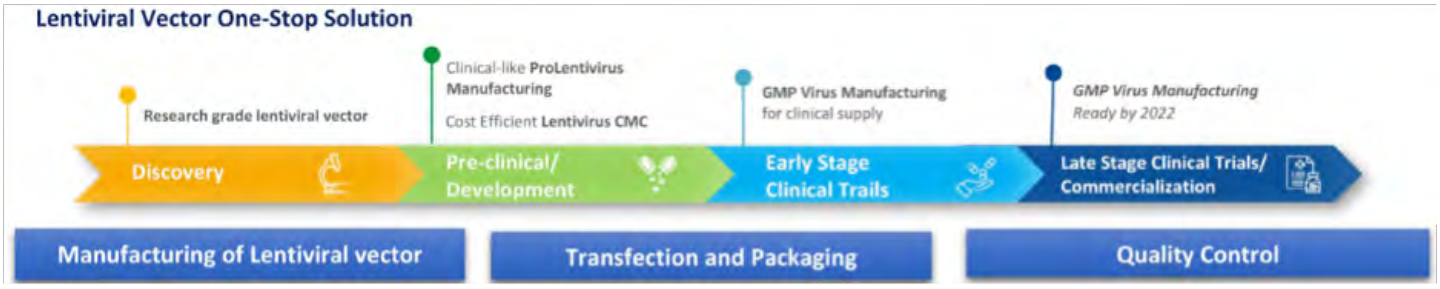
Key points in adherent systems

- ☐ Difficulties in large-scale manufacturing
- ☐ Stable and well-established manufacturing process

Suspension cell culture system

Expansion of suspension cells in large-scale bioreactors is the first step in the suspension culture system. Adaptation procedures will be needed for choosing cell lines. Several cell lines used for lentivirus production (293T, 293FT, and 293SF-3F6) have been described to grow readily in suspension with no need for microcarriers, which means expansion of suspension cells is easier than that of adherent cells. In addition, unlike in the adherent culture system, the suspension cell culture system usually uses serum-free media. The absence of bovine serum and animal origin components in the culture media is the most suitable situation for clinical manufacturing as this decreases the risk of contamination by adventitious agents.

However, choosing a transfection reagent for a suspension cell culture system is quite complex. Due to continuous culture stirring, DNA precipitation using calcium phosphate is expected to be less efficient. Thus,



other transfection agents like cationic polymers (eg. PEI) are mostly used. But the drawback of PEI is also obvious. To transfect suspension cells, enormous amounts of plasmids are needed at large scale; this leads to a high cost of raw materials and residual elimination in downstream processing. Another problem associated with PEI is the absence of an analytical method to detect and quantify the molecule. It is therefore unable to evaluate the risk that residual PEI may carry.

Finally, the harvesting cell in suspension is another issue compared to adherent cells. The LV culture supernatant can be harvested at least twice a day, which is efficient in reducing costs while in suspension, this approach is hard to implement because the culture medium cannot be collected independently from the cells. A perfusion system has been introduced and has suggested the feasibility of multiple harvest in suspension culture, though the system has also proven to be complicated and costly in large scale.

Downstream process

Downstream process aims to remove impurities from the product to ensure its safety, purity, identity, and quality. And purification methods are required to remove any contaminants of potential adverse effect when vector preparations are used *in vivo*. In addition, when used for clinical or industrial applications, the purification methods involved are supposed to be scalable in large-scale processes.

In small-scale purification, centrifugation methods are usually applied. But these methods lack scalability and may lead to partial vector inactivation due to long process time. The purity of the final products is insufficient for an *in vivo* application. Therefore, other methods have been developed in large-scale purification.

Generally, four phases can be distinguished in large-scale purification. First, a capture step is the initial purification process for eliminating major contaminants. In this step, membrane filtration is usually applied for clarification purposes. A concentration step based either on TFF/ultracentrifugation or ion-exchange chromatography follows.

To eliminate residual cellular contamination and in particular, plasmid-derived DNA contaminants, a benzonase step is often applied, either in the capture step or afterwards. In different protocols, there are different considerations in the benzonase step. When it takes place in the capture step, large DNA pieces are easily reduced in their size and following purification steps may eliminate residual benzonases; however, the disadvantage is that large quantities of benzonases are used. On the other hand, the late use of the benzonase step can reduce the amount of benzonases that have to be applied, but residual purification steps must ensure that residual benzonases are reduced to acceptable levels. What's more, if the benzonase step is not applied in the early capture step, the large size DNA pieces might lead to the formation of aggregates which may capture vector particles and therefore result in vector loss. In the next phase of the downstream process, an intermediate purification step may be taken to remove specific impurities. In most cases, diafiltration/ concentration is applied, which is followed by diafiltration or size exclusive chromatography steps as polishing steps, to remove trace contaminants and impurities and vector is sterile filtered with 0.2 µm membranes followed by TFF (tangenial-flow filtration). This is a standard regulatory requirement to mitigate the risk of microbial contamination of the final product in GMP conditions. Although sterile filtration is strongly recommended, it is possible to skip it provided that the process can be certified as being fully aseptic.

Key points in large-scale purification

- ☐ Scalability of purification methods
- ☐ The effect of sterilization on the yield is large, and the entire downstream purification process should have a high efficiency of removing impurities to improve the safety of the final product

Solution in DSP of adherent system

- ☐ Reagents in purification process: all reagents applied in the downstream process are pharmaceutical grade and compliant with regulatory requirements
- ☐ Key consumables: chromatography resin and hollow fiber applied for each single project are disposable, eliminating the risk of cross contamination
- ☐ Fill/finish: After aseptic filtration, all processes are performed in a sterile isolator. Closed semi-automatic dispensing equipment is used to ensure the aseptic filling process and aseptic filling verification

LV product quality control

In the process of manufacturing LV, contaminants from the production medium, producer cells or the process may constitute a risk when using the product in patients. Therefore, the downstream process is extremely important to improving product safety. Characterization of the specific vector contaminants and product identification also guarantee the quality of the final product. The LV products may be characterized in terms of identity, potency, purity, and safety. Identity assays are applied to identify the product and distinguish it from other products.

Purity assays may include a variety of characterizations of impurities, including proteins, DNA, cell debris, etc. One of the process byproducts is Benzonase, which is supposed to be eliminated in the late steps of purification and should also be controlled at product release. Another constituent, bovine serum albumin, which usually exists in viral production medium, should be controlled in the final product to keep it at a low value. The SV40 large T antigen, which is a specific product of the producer cells, is oncogenic for many cell types. And in the product that is to be applied in patients, such impurities must be controlled in the final product as well. In addition to these safety measures, the absence of RCL must be demonstrated under permissive conditions using a sensitive assay.

Key points in quality control

- ☐ Difficulties in development and qualification of analytical methods

ADENO-ASSOCIATED VIRUS (AAV) IN GENE THERAPY

AAV belongs to the Parvovirus family and is one of the most useful and actively investigated gene therapy vehicles. Initially it was discovered as a contaminant while preparing adeno virus. AAV has a protein shell surrounding and protecting small, single-stranded DNA genome 4.8 kb and has single stranded genome that contains three genes: Rep (replication), Cap (Capsid), and aap (assembly). These genes rise at least 9 gene products through three promoters and are flanked by inverted terminal repeats (ITRs) that are required for replication and packaging (Rep78, 68, 52 & 40; VP1, VP2 & VP3).

AAV has many serotypes and AAV2 was the one first identified and characterized, including the sequence of its genome. AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8 and AAV9 are currently used in gene therapy product developments for various indications such as CNS, heart, kidney, liver, lung, pancreas, and skeletal muscle.

AAV vector GMP manufacturing

Initially, cell and viral plasmid banking is produced prior to start the process development. Process development has process parameters, a scale-up process, and analytical method development followed by GMP readiness.

In GMP AAV vector manufacturing, tech transfer runs are recommended in small scale followed by verification runs (1/2 scale). Both adhesion and suspension systems are available to produce AAV vectors similar to lentiviral vectors. Full scale or engineering run is critical to achieve the maximum output with the available production capacities. In majority of cases, 50L to 2000L production capacities are available. Full GMP run along with many quality assurance (QA) methods. Approximately more than 20 methods are available for quality control and assurance of quality of the AAV vectors. Biodistribution and toxicology studies are done with engineering runs with vector release and testing in rodent and non-human primates. GMP AAV vector lot is released after fill, finish and biorepository.

GenScript ProBio's solution

- ☐ GenScript ProBio recognizes the critical quality attributes of products based on industrial experience and regulation information. The safety and efficacy properties of products, such as the product characterization, functional titer testing, process-related impurity detection and safety-related items will be assessed.
- ☐ GenScript ProBio designs and develops assay procedures scientifically and systematically to meet the varied analytical requirements. The assay performance applied in GenScript ProBio can meet or exceed the industry standards in the field of quality characterization for lentivirus.
- ☐ GenScript ProBio follows the guidelines of the Chinese Pharmacopoeia (ChP) Technical Guideline 9101, ICH Q2 and the United States Pharmacopoeia (USP) General Rules 1225/1226 to confirm the performance of the quality analysis method and prove that the method is suitable for testing requirements.

INNOVATE AND CO-CREATE BIOLOGICS INNOVATION

Outbreaks of emerging infectious diseases have been increasing risk in recent times. Many liquid and solid cancers, as well as auto immune diseases, are also progressively increasing. Timelines from early discovery to clinical evaluation of novel recombinant antibodies to produce advanced biologics products requires a long time. Potential life-saving therapies and early clinical testing need to be translated into pivotal trials with maximal patient benefit. The latest discovery and development technologies in antibody or protein productions can make substantially faster timelines with the acceptance of business risks and costs in patient clinical trials.

Cutting-edge discovery platforms

Advanced discovery platforms have made breakthroughs in treating many diseases, and traditional hybridoma technologies have been used to develop therapeutic antibodies. Later, display technologies and B-cell technologies accelerated early discovery research that enabled faster IND approvals to carry early clinical trials. Early discovery proof-of-concept reduced to 5-6 months' time from a traditional timeline of 10-12 months. During the pandemic, early discovery is ongoing in many therapeutic modalities like immunoglobulin G (IgG) and monoclonal antibodies (mAbs). Until recently, production capacity was a bottleneck to accelerating the production and manufacturing of these molecules, but recent advancements in discovery lead mAb identification to phase I IND in 10-12 months in many pipelines. A few years ago, this timeline was 18-24 months. The combination of recent technical advancements and regulatory acceptance offers further acceleration of drug development.

Rapid immunization procedures, transgenic mice, alternatives to hybridoma technologies such as naïve, synthetic and immune libraries, and single B-cell technologies, efficient, high-throughput screenings (sequencing, anti-idiotype and bioassays) make remarkable speed in early discovery of therapeutic antibody development. Fully human antibodies are the mainstream of therapeutic antibody development because of the increasing concern on immunogenicity and safety issues. Among the techniques that develop fully human antibodies, Antibody Library and Phage Display have been proven to be an effective platform to pave the way to the fully human candidates. Single B cell screening platform, also known as single B cell cloning technology, is a microsystem-based screening method. Transgenic animals are one of the major developments in biotechnology that transformed discovery research.

Interchanging DNA with genetic manipulation able to produce various transgenic animals such mice, rat and chicken, that accelerated antibody discovery research and animal models. This platform conducts isolation, screening and evaluation on the B cells, avoiding the cell fusion and library construction steps. Also, single B cell screening platforms usually integrate high throughput platforms, therefore assays could be carried out in a highly efficient manner.

Replacing everything but the complementarity determining region (CDR) reduces the degree to which an antibody drug itself acts as an immunogen. Immunization against an antibody drug lowers efficacy through reduction in circulating half-life and/or neutralization. The engineering and screening procedures are integrated, allowing for simultaneous antibody humanization and stabilization.

Affinity is one of the key parameters of an antibody drug which will affect the function and efficacy of the antibody. Generally, antibody candidates from hybridoma platform has already acquired high affinity, but it may not exactly fit in the practical needs in research. In affinity maturation, antibodies increased its affinity, avidity and activity through multiple rounds of somatic hyper mutation (SHM) in germinal centers. Successive generations of B cells mutate and present to the antigen that recognize the antigen with high affinity will survive and the low affinity ones are eliminated. Affinity maturation process more often applied to antibody leads that are selected from library approaches using a phage display technique and the affinity of the leads have 10 –100 nM affinity levels to the target. In antibody drug discovery, affinity maturation is applied to antibody leads to increase the affinity at least 10 to 50 folds.

Therapeutic antibody drugs have recently experienced explosive growth. Within oncology research alone, more than 20 therapeutic antibody drugs received FDA approval for new treatments or indications during 2015 to 2017. Additionally, more than 300 therapeutic antibody drugs are in ongoing clinical trials. Most recently the success of cancer immunotherapy, by blocking immune checkpoint proteins such as PD-1 and CTLA-4, created a hope for “cancer cure” and triggered a second revolution that the majority of pharmaceutical companies are devoting major research force and investment in antibody therapeutics. Amidst fierce competition together with many unmet medical needs, there is an urgent demand for new therapeutic strategies, such as combination therapy, and novel modalities, such as bispecific antibodies. In this interactive webinar, we will first give an overview on therapeutic antibodies, and focus on the opportunities and challenges of current monotherapy. Aiming at addressing unmet medical needs, we will then discuss the major benefits of bispecific antibodies, and review current platforms for that. Bispecific antibodies (bsAb) have become a focus of interest for therapeutic applications with nearly 85 commercial candidates entering the clinical trials and 3 having been approved to market. SMAB (Single-domain antibody fused to monoclonal Ab) platform naturally combines the single domain antibody and the monoclonal antibody to make a bispecific antibody in symmetric format. With the design concept of “Keeping Natural,” SMAB platform gives good developability and biosuperiority which are comparable to monoclonal antibody.

Accelerate biologics development

Cell line development plays an essential role in drug development. It is the bridge connecting drug discovery and development, and good cell line development service can always save valuable time and lower cost. Some antibody candidates discovered in early stages will involve issues such as post translational modification (PTM) hotspots or poor physiochemical stability, which will lead to potential risk during preclinical CMC development, resulting in huge time and financial loss to researchers. Taking advantage of bioinformatics tools and a series of quality study instruments, developability assessment used to identify the inherent risk in antibody discovery stage, which seamlessly connects antibody discovery with preclinical CMC development. In

therapeutic antibody development, superior commercial stable cell lines made huge progress to achieve high yields in a short time frame. Gene synthesis to single clone production can be achieved in a 14-18 week timeline. Rapid clinical production capacity has benefited from development of highly productive commercial stable cell lines, and huge single-use bioreactors produced thousands of doses of therapeutic antibody from single batch of over 5 or more kilograms. Pandemic outbreak made the fastest path to produce mAbs for clinic within 5-6 months rather than 12 months. Along with key technology drivers, regulatory compliance is essential with rich experience.

Many IgG1 therapeutic antibodies are approved and are commercialized, and many are in clinical trials currently. Among many therapeutic antibodies, IgG1 mAb has many safety profiles are low risk. In biologics production, IgG1 mAbs are broadly established under cGMP production. In clinical development, Chinese Hamster Kidney (CHO) cell lines are used and alternative hosts are available such as *E. Coli*, yeast and other plants. New technologies and approaches can save 2 months or more in lead identification to cell line suitable for phase I clinical studies. In PK/PD toxicological studies, the timelines are also shortened in CMC studies to IND filing. For Biologics, the lead sequence will be determined after early research of developability assessment and pre-toxicity study, and then enter CMC stage to provide preclinical data for IND application. GenScript is capable of providing all the services involved in pre-IND study, including stable cell line development, process development of cell culture and purification, quality study and method validation, formulation development, GMP pilot production, bioassay development, PK/PD, and toxicology study. The comprehensive analysis platform usually equipped with state-of-the-art instruments to fulfill the need of quality study for biomacromolecule characterization and process impurity analysis. In addition, cell-based bioassays have been established for many hot immune checkpoints and are customizable for client's specific target. The qualified CMC service supports the IND application under FDA, cFDA and EMA. It generally takes 14-18 months for the whole CMC progress, from gene to preIND.

Best-in-class timeline for clinical studies

Many biopharma or innovator companies produce best-in-class discovery targets in lead identification to IND timeline up to 12 months. Large pharmaceutical and CDMOs produce various sizes of production batches with a small to large number of doses for distribution. Large number of doses can be produced using 2000L bioreactor for clinical manufacturing and the need for large production batches have high demand due to its usage in clinical trials and the production of the batches for patients who are in need of help with their treatments.

GENSCRIPT PROBIO DISCOVERY AND DEVELOPMENT OFFERINGS

GenScript provides an integrated biologics discovery and development solutions from target to IND. With our cutting-edge technology platforms in therapeutic antibody discovery and development, we are able to deliver functional antibody leads with good developability and safety in the discovery phase, as well as reliable, productive and regulatory-compliant process and drug production for IND filing in development phase, which significantly save our clients time and money.

GenScript ProBio is the bio-pharmaceutical CDMO segment of the world's leading biotech company, proactively providing end-to-end service from drug discovery to commercialization with professional solutions and efficient processes to accelerate drug development for customers.

GenScript ProBio's innovative solutions for antibody drug development include antibody drug discovery (hybridoma, antibody library, fully human transgenic mice, bispecific antibodies technologies, and single B-cell screening technology), antibody engineering (antibody humanization, affinity maturation, Fc Engineering) and antibody characterization (analytics and bioassays). In terms of biologics development service, GenScript ProBio has built a regulatory-compliant platform, from stable cell line development to clinical manufacturing services, providing high quality material for IND and clinical trials and accelerating drug development process. GenScript ProBio's total gene and cell therapy solution covers CMC of plasmid and virus for IND filing as well as clinical manufacturing and commercial manufacturing. Our quality management systems ensure phase appropriate compliance, data integrity and traceability.

GenScript Biotech Corp., has 17 years of CRO experience, and continues to develop and optimize the process for AVV, Vaccinia virus and lentiviral vector manufacturing through GenScript ProBio. Our production processes are developed, defined and controlled to ensure compliance. Our records are clear and accurate, and any deviations are investigated and documented. With the optimization of manufacturing processes and the tighter relationships between the pharma and CDMO, the bottlenecks will be overcome step by step to reach mature commercialization for safe, efficacious, and accessible CAR-T therapy.

Toward the mission of "Innovation through Collaboration," GenScript ProBio is committed to helping customers shorten the timeline for the development of biological drugs from discovery to commercialization, significantly lowering R&D costs and building a healthier future.