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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON
Non-clinical/translational tools & technologies

Guest Editor
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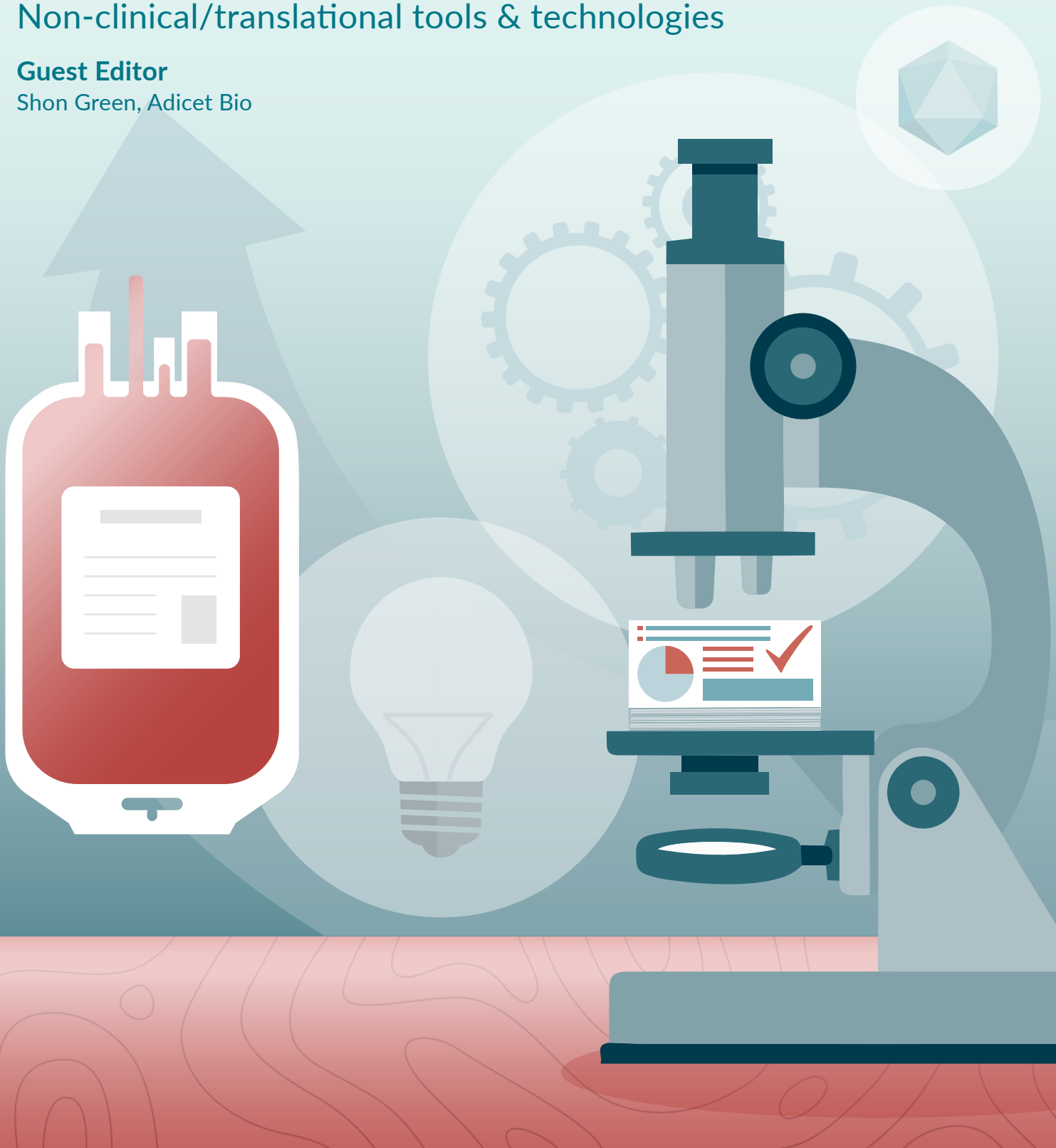

CELL & GENE THERAPY INSIGHTS

Volume 10, Issue 2

CONTENTS

SPOTLIGHT: NON-CLINICAL/TRANSLATIONAL TOOLS & TECHNOLOGIES

Latest articles

INTERVIEW: Gene therapy is having a monumental year: how can the industry keep up with supply?
Suman Subramanian

WEBINAR DIGEST: What goes into developing an in-house method for quantitation of residual host cell DNA?
Ilaria Scarfone

FAST FACTS: Dissolvable microcarriers: a modular, high-yield solution for seed train through production
Tom Bongiorno

CELL & GENE THERAPY INSIGHTS

NON-CLINICAL/TRANSLATIONAL
TOOLS & TECHNOLOGIES

SPOTLIGHT

The March 2024 issue of Cell & Gene Therapy Insights

Shon Green

"...cell and gene therapies have made significant breakthroughs against refractory cancers and rare genetic diseases..."

FOREWORD

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As drugs have evolved from small molecules to include biologics such as antibodies, gene therapies, and cell therapies, they expanded in complexity, cost to develop, and risk, but also in potential for overcoming challenging disease states. Cell therapies in particular have captured my imagination, as these living drugs can be programmed into 'machines' capable of multiple functions and responses, on top of their natural biology, making their promise as therapeutics theoretically unlimited.

Indeed, cell and gene therapies often have multifactorial mechanisms of action that involve interactions with several cells/tissues within the body, making preclinical models unable to fully capture their activity and presenting difficulties in defining their potency with simple analytics. Furthermore, these

therapies are often individualized, making characterization across patient cohorts hard to interpret. Despite these challenges, cell and gene therapies have made significant breakthroughs against refractory cancers and rare genetic diseases which has energized development and brought substantial investment to the field.

Imagine a gene therapy product that can specifically edit the genome of particular cells in the body, restoring expression of a necessary protein. Or imagine a cell therapy that can survey the body, find its target, kill it, and recruit other immune cells to help ensure all target cells are eliminated. This is not science fiction, and only the tip of the iceberg of what this field has created so far.

In this issue of Cell & Gene Therapy Insights, we focus on cell and gene therapy preclinical and translational development, to highlight the creative and novel ways in which the field is approaching developing and understating these drug modalities and bringing them to the clinic and eventually to commercial use. The following articles were assembled to capture where we are and where we are going with preclinical/translational development:

Michaela Sharp, Senior Nonclinical Director at Moare Solutions, sheds light on the current preclinical and translational space based on her many years of experience developing novel therapeutics.

Mary Ellen Cosenza, a regulatory consultant, provides comprehensive and useful advice on the unique and changing regulatory landscape for development of cell and gene therapies.

Shannon Dahl, a biotech consultant, summarizes the elements of a strong translational package and includes helpful insights into the design of successful preclinical programs.

Silvio Manfredo-Vieira, Associate Director for Correlative and Translational Studies at University of Pennsylvania, shares thoughts about preclinical and translational efforts towards making cell therapies to treat autoimmune disease.

My personal career goal is to bring impactful drugs to patients who need them, and on the way advance our collective knowledge so that new and improved drugs continue to be developed and bring hope to the many who struggle with currently untreatable diseases. I love tackling the unique challenges represented by gene and cell therapies, since nothing worth pursuing is easy, and the promise and dream of these technologies is worth it. I am pleased with how this issue turned out and hope you get some value from the varying perspectives offered, and are inspired to continue pushing the limits of innovation in this unique area of drug development.

BIOGRAPHY

SHON GREEN trained at UC Berkeley and UCSF where she developed and employed preclinical models of cancer to study tumor development and potential therapeutic approaches. She utilized these expertise to drive the preclinical and translational development of T cell-based immunotherapies targeting both solid and liquid tumors at Eureka Therapeutics, and to advance novel epigenetic editing tools to enhance cellular therapies for cancer at Altius Institute for Biomedical Sciences. She then took on the challenge of employing a gene therapy approach to realize the vision of *in vivo* T cell engineering at Umoja Biopharma. Now the Vice President of Nonclinical Development at Adicet Bio, she continues to lead at the front edge of innovation to enable effective allogeneic γδ T cell therapies for oncology.

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CELL & GENE THERAPY INSIGHTS

NON-CLINICAL/TRANSLATIONAL TOOLS AND TECHNOLOGIES

SPOTLIGHT

EXPERT INSIGHT

Preclinical trends in developing genetically altered cell therapies

Mary Ellen Cosenza

There are many scientific and regulatory considerations to be made when planning for the development of genetically altered cell therapy products. These products include different cell types (for example both T cells and B-cells) and for the treatment of diseases in different therapeutic areas (oncology, inflammation, enzyme, or protein replacement constructs). This article will present a framework for approaching these projects from a preclinical perspective and share trends in common feedback from regulatory agencies, most notably the US FDA. The intent is to share this information with other product developers at an earlier point to make planning easier.

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WAYS TO APPROACH A NEW PROJECT

Each preclinical study plan is unique, as is each product, but the goals remain the same: to recommend starting dose levels and help design a dose escalation scheme and dosing schedule. Other goals are to support the planned clinical route of administration and any devices to aid in dosing and to identify potential target tissues to evaluate product safety and determine parameters for monitoring in clinical trials [1].

Preclinical data should be adequate to support the progression of the therapy into the proposed clinical trial. The study designs and measurement parameters should be based on the product attributes, intended patient population, and clinical trial design. Sponsors should not conduct unnecessary studies just because "they can". Consideration should also be given to what data is available from similar projects. This may be data from a sponsor's other internal projects using similar or platform technologies or enhancements to products already in development. It can also include data in the literature or presented publicly on other products with the same target. Extrapolating data across products is

encouraged in a recent white paper from the Friends of Cancer Research [2]. Companies have been doing this across internal projects and platforms, but are there opportunities to do this across companies in a non-competitive manner? Publication of this data and sharing of information at consortium meetings should be considered.

Another recent trend has been to find ways to get the most data out of the studies already routinely being conducted without having to add many more animals or conduct additional studies. One key to making this successful is to plan ahead and be prepared to collect safety data in pharmacology studies by planning for this before the execution of the studies. One strategy is to look at ways to pool data across animals within a group in order to evaluate more parameters with fewer analytes. The use of historical control data from the same lab/investigators is another possibility.

GENERAL POTENTIAL SAFETY CONCERNS FOR CELL THERAPY PRODUCTS

Before discussing specifics for the different types of cell products, there are a few general issues that should be addressed early in a program. These include toxicities due to components of the formulation and administration procedure. It may also include delivery devices and scaffolds as part of cell delivery or implantation. If there is potential for an inflammatory or immune response to the administered product, then ways to evaluate or measure this should be considered early. Inappropriate cell proliferation (i.e., tumor formation) or inappropriate cell differentiation (i.e., ectopic tissue formation) should be considered. Interactions with concomitant therapies (i.e., immunosuppressive agents) or pre-treatments should be evaluated.

I like to start by asking some basic questions about the product itself. For cell therapies, this starts with these questions:

- What cell type(s) will be used?
- What is the source of the cell(s)?
- How many cells are needed to achieve a minimum/optimal biological effect?
- What happens to the cells *in vivo* following delivery?
- What is the intended mechanism of action of the cell therapy product?
- Is cell survival/engraftment necessary to achieve the desired outcome? And for how long?
- Do the cells secrete growth factors/cytokines?
- Are the cells delivered alone or with a scaffold or encapsulated?
- Combinations and devices for delivery?
- Is immunosuppression part of the administration plan?
- Will multiple administrations be needed?

Next, there are subsets of questions depending on different categories of cells. Note these are just some examples. For cells that target antigens, a traditional 'target liability' assessment may be a good place to start.

AUTOLOGOUS CELL PRODUCTS:

In vitro

- Expression profile of target: Where is the target? *In silico*/bioinformatics, expression libraries, etc. Screening with normal tissues (*in vitro* and *ex vivo*).
- Specific binding to target: where is the binding to the target (epitope)?
- Off-target binding (tissue cross-reactivity and/or proteome arrays; cancer cells vs. normal cells).
- Off-target or off-tumor, on-target activity (cytotoxicity, cytokine release, and proliferation).

- For a CAR-T cell does the binding activate the T cell? Does it kill the cancer cells directly? Are cytokines released? What happens if it binds normal cells with the target (is antigen density a factor)?
- Species specificity (similar screening as with mAbs).
- If scFv is the same as a known mAb, what is known about the pharm/tox activity of that molecule?

In vivo

- Efficacy: provide proof-of-concept for the proposed clinical study (animal disease models).
- Do the cells kill tumor cells in xenograft or syngeneic models?
- Key information for both pharmacology and toxicology may come from these studies. Consider adding safety parameters to efficacy studies. These may include survival, tumor size, cytokine expression, body weights, and clinical pathology at the end of the study. Gross and histological examination of target tissues should also be considered.
- Data should help establish appropriate dosing regimens.
- Toxicology: identify potential safety risks that inform clinical study design such as cytokine release.
- Biodistribution: identify target and non-target tissues (product-dependent, case-by-case basis); attention needs to be paid to distribution to germ cells.

PRECLINICAL CONSIDERATIONS FOR ALLOGENEIC CELLS

- Many of the same considerations as noted for autologous cells but also need to address potential alloreactivity.
- *In vivo* animal models for graft-versus-host disease.
- *In vitro* assays such as mixed lymphocyte reactions.

GENETICALLY EDITED CELLS

There are further issues to be investigated for cells that are genetically edited. The method of editing will impact which of these issues are of concern for your product. Examples of issues to consider include:

- Off-target genetic modifications;
- Risks of insertional mutagenesis;
- Insertion site profiling (risk of turning on known oncogenes);
- Oncogenesis;
- Tumorgenicity;
- Platform risks (CRISPR, TALENs, viral transduction, etc.);
- Risk of chromosomal abnormalities.

A recent FDA guidance, 'Human gene therapy products incorporating human genome editing', [3] discusses many of the points listed above as well as which of these concerns might be investigated in preclinical studies. Biodistribution is discussed in the guidance as well as in the ICH S12 guidance 'Nonclinical biodistribution considerations for gene therapy products' [4]. The European Medicines Agency also has a guidance on this topic: 'Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells' [5].

TYPICAL COMMENTS FROM THE FDA ON CELL THERAPIES

There are common or typical comments from the FDA and other international regulatory agencies on genetically altered cell therapies. I have bucketed these into a few

groupings with the hope that development teams can start thinking about these issues earlier during product development. Some of these are similar to statements in the FDA guidance on cell and gene therapies [6] and the more recent guidance on CAR-T cells [7] and human genome edited products [3]. Regulatory guidance is helpful but slow to be updated so it is important to engage with the agencies as product development progresses. The comments below have been adapted and anonymized from FDA feedback and meeting minutes across several similar products.

General guidance

There are two main types of meetings that the FDA offers before your IND is filed. One is the INTERACT meeting which is an early meeting that focuses on preclinical and CMC issues. Your product must be clearly defined, and some proof-of-concept data is needed in order for this meeting to be granted. The pre-IND meeting is the key meeting where your preclinical and toxicology programs are discussed. Other topics, including your clinical program, can be discussed in this meeting as well. Both of these meetings can be 'live' (generally tele- or video-conference) or a written-response-only meeting. The newer Type D meeting is also a possibility when only one or two topics need to be discussed. The Center for Biologics Evaluation and Research (CBER) has a SOPP that outlines the procedures and provides guidance on these meetings [8].

CBER advice given during INTERACT meetings is informal and non-binding. Therefore, official meeting minutes will not be issued. Although the sponsor may prepare and submit to CBER a summary of their understanding of issues discussed at the meeting, this summary, if provided, will not be reviewed by CBER in any manner. No evaluation will be performed to determine if the summary is accurate. The sponsor's meeting minutes do not alter CBER's comments provided in writing or by verbal communication and they are not the official minutes of the meeting.

CBER now requires the standard for the exchange of nonclinical data (SEND) for some preclinical/toxicology studies. Note that for 'applicable' preclinical studies initiated after March 15, 2023, standardized datasets in the SEND format will be required to be submitted to your CBER IND and BLA. Details on this requirement, including the types of preclinical studies and exceptions from this requirement can be found in the Study Data for Submission to CDER and CBER Guide, and in the Study Data Technical Conformance Guide [9,10]. Repeat dose general toxicology, good laboratory practice (GLP) or non-GLP, require SEND for both CDER and CBER. Study types that need SEND continue to be updated and sponsors should continually check the FDA website for current information.

The FDA generally encourages sponsors to explore opportunities for reducing, refining, and replacing animal use in their preclinical program. For example, it may be appropriate to use *in vitro* or *in silico* testing to complement or replace animal studies. Sponsors are encouraged to submit proposals and justify any potential alternative approaches [11]. The 'three R's should always be considered when designing a preclinical plan for a new cell therapy.

Guidance on study conduct

Often it is recommended for animal pharmacology studies (as well as toxicology studies) that for all unscheduled deaths, the following be performed: comprehensive clinical pathology, gross pathology, and histopathology on a complete panel of tissues, and other analyses, as appropriate, to determine the potential cause of death. Since these efficacy studies are often conducted in mice, collecting many of these parameters may be challenging (for example blood volumes may be low for measuring multiple analytes). Also, it is recommended to provide a comprehensive discussion, with accompanying data, regarding the biological relevancy of this model to the proposed patient population.

Sometimes the feedback will ask to ensure the assessment of safety endpoints include, but are not limited to, daily clinical observations, body weights, clinical pathology parameters, immune response, complete macroscopic exams, and histopathology of selected tissues. In animal tumor models the following requests have become more common:

- Include in-life health measures during the study including but not limited to mortality, body weight, tumor volume, food consumption, clinical signs, and morbidity.
- Delineate any dose-dependent trends in toxicity following test article administration and delineate any test-article mediated effects versus non-specific toxicity (i.e., x-graft-versus-host disease) with the inclusion of appropriate controls.
- Consider additional blood and tissue collection at multiple timepoints for characterizing the expansion and persistence of the investigational product.
- Collect a comprehensive list of tissues on the day of euthanasia for macroscopic observation and histological characterization. Please address the clinical significance of any tissue abnormalities that are present.
- Provide a comprehensive discussion of any observed clinical signs to adequately inform the risk for the proposed clinical trial.

For each toxicology study performed, the sponsor should provide documentation showing that the study was conducted in compliance with GLP as per 21 CFR Part 58. If the study was not GLP-compliant, as directed by 21 CFR Part 312.23(a)(8)(iii), the sponsor should provide a brief statement of the reason for the non-compliance in your IND submission. In addition, it should be specified in the study report any areas that deviate from the prospectively written protocol and the potential impact of these deviations on study integrity. Each study should be:

- Conducted according to a prospectively written protocol;
- Performed in as nonbiased a manner as possible; and
- Have appropriate record keeping and documentation of all data.

Comments on IND preparation

A common request is to provide company product information over various stages of development and used in different preclinical studies in the IND. How do these development, training, or engineering runs compare to the intended clinical product? Providing this information in a table is often the best way to visualize the information concisely.

Requests often ask for sponsors to provide data to support the identification of the biologically active dose level range for your investigational product. If this is based on data from animal studies, please provide your method of dose extrapolation between animals to humans to support the proposed clinical dose level.

In your IND submission, please provide complete study reports for all preclinical studies used to support the safety and rationale of your proposed clinical trial. Each report should include but should not be limited to:

- a detailed description of the study design (e.g., description of the test system used, animal species/animal models, control and test articles administered, dose levels, and all parameters assessed, etc.);
- results for all assessments; and
- your analysis and interpretation of the study data.

The FDA will recommend oversight of the conduct of all non-GLP toxicology studies and each resulting final study report by a

quality assurance unit/person that is independent of the personnel responsible for the conduct of this study, as per 21 CFR Part 58.35. This quality assurance oversight is important to ensure study conduct according to sound procedures and to ensure the quality and integrity of the resulting data. This is often a challenge for small companies, so it is best to think about a plan for this early.

If a sponsor is basing this IND on data from another IND

Based on the information provided in your briefing book, the FDA may tentatively agree that data obtained from ongoing clinical studies and the scientific literature will be adequate to support the development of your new product. If so, then expect them to request a tabulated summary of the similarities and differences between your new product and the investigational product administered under the original IND. If the product is the same but for a new indication, or if there is an addition to the product of a delivery device, the requests may be similar.

If your product development data is based on published literature, then the regulatory agencies will request copies of all key publications cited that are used to support the safety and rationale for the proposed clinical trial. They will often request a comprehensive summary of each publication. This summary should include the reason for including the publication, a discussion regarding the comparability of the product(s) used in the publication (i.e., how it directly supports the safety/activity of your product), and a discussion regarding the comparability of the product(s) used in the publication to the final clinical product.

SUMMARY

In summary, there are recent trends across projects in the preclinical aspects of cell therapy development. The most obvious recent trend is to get as much information out of the studies conducted as possible. This often means measuring safety parameters in mouse efficacy models/studies. Note the use of primates should not be a default and is often discouraged unless there is no other way to investigate a potential risk. Studies that are conducted should meet regulatory requirements to support safety and INDs should include well written reports that provide regulatory agencies with sufficient information and data to properly evaluate risks. Studies should be properly designed and consider the judicious use of animals. The clinical scenario should be replicated as closely as feasible.

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CELL & GENE THERAPY INSIGHTS

NON-CLINICAL/TRANSLATIONAL
TOOLS & TECHNOLOGIES

SPOTLIGHT

Regulatory and scientific considerations for building translational cell and gene therapy packages

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"...there is a great opportunity for the sector to expand patient impact with gene editing approaches and therapies that modulate host cell responses."

VIEWPOINT

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Shannon Dahl works alongside a portfolio of companies through her consulting firm, Carve Bio, to advance therapeutics, with an emphasis on cell therapy, genomic medicines, and immunology. Her experience across a range of technologies in the cell and gene therapy

(CGT) sector and immunology, working with multiple teams to shape and build data packages to support clinical translation, situates her in a position to connect the dots between science, patients, and business considerations to build strong strategic pipelines. She works to identify synergies for partnerships, ensure that scientific programs create clear value for patients from discovery to the clinic to commercialization, set the value proposition and path to reimbursement, and provide diligence for investors.

BUILDING A TRANSLATIONAL IND PACKAGE

A strong package needs to take into account the unique mechanisms and safety risks of the product (in addition to more traditional standard testing profiles for regulatory filings). Given that the CGT class of products is evolving quickly, the datasets require rational design and often do not perfectly repeat established precedent data packages.

Developers need to define the critical safety and efficacy questions for their unique products. The composition of the IND package will then often involve a thoughtful mixture of:

1. *In vitro* human cell-based assays to demonstrate mechanistic understanding of the desired clinical pathophysiology;
2. *In vivo* studies, which may utilize humanized models and sometimes non-human primates or other large animals, and which inform biodistribution and usually inform dose selection;
3. A strong CMC dataset that demonstrates an understanding of the unique product attributes, control over those product attributes, correlation of those attributes with the *in vitro* and *in vivo* safety and efficacy datasets, and ability to maintain those attributes at a manufacturing scale that can support patient use; and
4. A clinical trial strategy.

Taking considerations for preclinical *in vivo* studies as an example, the key questions needed for successful data packages differ by product type within the sector. For example, many cell therapy INDs require safety testing for cytokine release syndrome (CRS) and tumorigenicity. Gene therapy INDs need to consider getting to the right cells and tissues, along with off-target analysis of reaching unintended cells. Gene editing INDs require a focus on off-target genomic edits within edited cells.

Preclinical model selection is often a big challenge. Human cell therapies must be tested in cross-species models. However, the immune systems in animals and humans differ, and may not have the same cellular or immunological responses as the human-to-human clinical use case. In one example, cross-species assessments can lead to the presentation of an overactive xenogenic immune response, such as testing of human cell therapies that are intended for autologous or allogeneic use in humans, but which may face rejection in the xenogenic setting. In another example, for cell therapies that are engineered to target a human antigen, the antigen must be present in the model for an adequate assessment. Further, for testing of CRS, the relevant mix of human immune cells must be present. Despite the range of immunodeficient, genetically engineered, and humanized animal models (e.g., depletion of mouse immune cells and repopulation with human immune cells), none has a complete human immune system and this may lead to challenges with efficacy readouts and prediction of risks such as CRS and on-target, off-tumor toxicity.

There is no gold standard *in vivo* model that is relevant across the sector for testing all immune responses. Developers must select the models that are most fit for purpose for their product, with attention to the components of the immune system that are

relevant to their product's target and mechanism of action. *In vitro* assays are often useful to demonstrate a product's interactions with defined human cell populations (e.g., adaptive and/or innate immune cells, and target cells). The IND package may then be developed through a thoughtful approach of identifying the mechanisms, benefits, and risks of a product, and by designing a mixture of *in vitro* and *in vivo* studies that provide data on those benefits and risks.

Another consistent area of emphasis is a strong CMC package. Product manufacturing and the impact of process parameters on critical quality attributes must be well understood and controlled to ensure that the product is reproducibly functioning as intended. A focus must be placed on CMC, product characterization, potency, and scalable manufacturing early in development to ensure that IND-supporting datasets use a version of the product that represents the clinical and commercial product.

In CGT, there is also a necessary emphasis on the interconnectedness of the parts of the IND package. For example, the tolerable range of CMC process parameters, product specifications, and potency may be assessed via qualified *in vitro* assays and *in vivo* models, leading to the selection of release criteria and dose for clinical trials. Notably, CMC parameters typically impact dose and potency. Further, *in vivo* model selection and *in vitro* assays should be considered alongside clinical endpoints to ensure bidirectionally strengthened approaches. These intersections must be managed intentionally.

Beyond translational packages for INDs, it benefits developers to look at the translational package more holistically. Often within the same data package, nuanced decisions on models and study designs (e.g., control groups), and summarizing known literature benchmarks, can provide additional data to demonstrate key differentiating benefits for patients. This approach to translational datasets additionally:

1. Supports investor and partnership interests;
2. Ensures early trials capture critical endpoints;
3. Provides early guidance on comparator Phase 3 trial designs; and
4. Paves the way for establishing a clear value proposition for payers to enable eventual commercialization.

EVOLVING REGULATORY GUIDANCE

The US FDA continues to expand the library of guidance documents for CGT to provide examples of expectations from the agency to developers [1]. These guidances focus on several consistently challenging areas for developers, such as CMC and comparability, preclinical study design, potency assays, somatic cells, gene therapies, and others. The expansion of this guidance library is useful as a starting point for developers to design their data packages and study designs and is also helpful in maintaining consistent expectations at the agency level. With this said, there are still many areas that are unique to emerging therapies and specific products that are not covered in the guidance documents, or situations in which recommended approaches may not make sense for a particular product. These scenarios are fairly frequent across CGT, and thus developers need to understand their product's unique features and propose the data package that makes the most sense.

The FDA tends to have stronger historical pattern recognition than individual companies, so the agency may suggest additional studies or push back on proposed approaches. In many cases, the agency's insights can provide a helpful added perspective, although developers should feel comfortable engaging in active respectful discussion if the developer's insights support a divergent approach to

the translation of their unique product in the targeted indication.

For those involved in preclinical trial design and data collection in preparation for IND submission, it is critical to align with the FDA on the proposed IND package scope and study designs. To accomplish this, I favor a robust and mature pre-IND meeting, in which the developer has a proposed scope of the IND package and study designs, with enough supporting data to drive meaningful discussions on the IND package.

THE NEXT DECADE OF PRECLINICAL CGT

In general, there is a great opportunity for the sector to expand patient impact with gene editing approaches and therapies that modulate host cell responses. As CGTs persist into the next decade, I see the science at an inflection point to support the development of more *in vivo* therapies. Furthermore, improvements in targeted delivery vehicles such as lipid nanoparticles (LNPs) will enable more widespread use of gene editing and *in vivo* therapies. In addition, the field of immunology is maturing at an exciting pace, and efforts such as the Human Immunome Project [2] that are aiming to map a better understanding of human immune diversity will inform and focus the future clinical targets of cell therapies and genomic medicines.

As each modality builds in clinical experience and risks are better understood, I expect that we will see more opportunities for designer therapies to address unmet patient needs. For example, do we want a durable or transient effect for the pathophysiology? What is the simplest modality to use to achieve the design goal? With an increased toolbox of modalities, we can better design solutions for unmet needs.

As we consider datasets in the next decade, the rapidly expanding clinical and commercial experience with cell therapies, gene therapies, and gene editing will provide additional insights into clinical safety and potency. I see an opportunity for clinical insights to provide feedback loops that inform future translational strategies. Clinical feedback may show that some preclinical models and assays have better predictive capabilities than others. Further, expanded clinical experience may show that some current perceived risks turn out to be minimal in practice, while other clinical risks are greater than expected. Evolving clinical safety risk profiles may change the relative emphasis on necessary translational datasets, with lower risk assessments perhaps being addressable by *in vitro* studies and higher risk with *in vivo* studies as one potential outcome. Multiple datasets to address high risk assessments and simpler quick checks for minimal risk outcomes could be another conclusion.

As a field, we will continue to work on improved approaches for the discovery, design, and optimization of new therapies. Strong early design upstream of translational efforts may streamline the definition of translational model selection and success criteria for translational datasets. For example, increased ease and affordability of obtaining omics data (genomics, proteomics, transcriptomics, etc.), along with complementary computational biology expertise, may enable greater confidence with *in vitro* data summarizing a product's mechanisms of action. Likewise, as genetic screens that demonstrate gain or loss of function become more widely available, these will improve the early design for genetically modifying therapies for a desired clinical effect. With omics and genetic screens, it is critical to go beyond standard approaches that summarize data trends and focus on study design and data analysis to identify key success criteria for future products. Such approaches to improve product design and characterization of the mechanism may drive improved clarity for selection of the most relevant *in vitro* assays and *in vivo* models for IND submissions.

The FDA is working on a guidance for the Platform Technology Designation Program [3,4], which will hopefully make

the alignment of datasets and regulatory processes more predictable for products that arise from platform technologies. However, it remains to be seen if this will disproportionately favor follow-on products within a given company's platform, or if this will apply in a more widespread manner for a given modality across the sector.

In conclusion, we expect to see CGT therapies advance into the clinic and to commercialization via the purposeful design of therapies and datasets, feedback loops from clinical experience, and novel therapeutic modalities, all of which serve the goals of expanding treatment options and improving outcomes for patients.

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BIOGRAPHY

SHANNON DAHL founded Carve Bio, through which she currently consults as Chief Scientific Officer and Strategic Advisor with a portfolio of companies and investors in CGT and immunology (autoimmune, inflammation, and immuno-oncology) who are seeking to translate therapeutics into the clinic, build pipelines or partnerships, assess new technologies, scope scientific datasets for regulatory submissions, navigate reimbursement, and maximize value creation from science or platforms for broad range of stakeholders. Dahl also serves on strategic and scientific advisory boards, and previously served as CSO at Cell Care Therapeutics, Adjunct Faculty at Duke, and co-founder of Humacyte where she advanced an innovative allogeneic regenerative medicine platform from academic research into the clinic and through global Phase 3 trials with a path to reimbursement. In 2018, PharmaVOICE named Dahl as one of the 100 most transformational leaders in the global life sciences industry. Dahl received a PhD in biomedical engineering from Duke University, performed research in orthopedic surgery at Harvard Medical School, and received an SB from MIT in materials science and engineering with a concentration in economics.

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CELL & GENE THERAPY INSIGHTS

NON-CLINICAL/TRANSLATIONAL
TOOLS & TECHNOLOGIES

SPOTLIGHT

INTERVIEW

The preclinical journey: transitioning a novel cell therapy into early phase clinical trials

Abi Pinchbeck, Commissioning Editor, *Cell & Gene Therapy Insights*, speaks to **Silvio Manfredo-Vieira**, Associate Director for Correlative and Translational Studies at the Department of Dermatology, University of Pennsylvania, about his role directing the translational research to move novel cell therapies from the lab into early phase clinical trials and pioneering a chimeric autoantibody receptor to target B-cell-mediated diseases.

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Q What are you working on right now?

SMV: I am working on clinical trial correlative studies of a Phase 1 first-in-human cell therapy to treat autoimmune disease, i.e., pemphigus vulgaris (PV). Recent successful CAR-T therapies to treat blood cancers have achieved durable efficacy. Our team used the principle of that technology to bioengineer T cells to target specifically pathogenic B cells and spare healthy ones in mouse models of PV and myasthenia gravis. In brief, this last technology approach includes the use of a chimeric autoantibody receptor (CAAR) where an epitope is fused to intracellular domains in the T cell, rather than a single-chain variable fragment, to recognize

and eliminate autoantibody-producing B cells selectively. The immune response before and after CAAR T cell therapy on patients enrolled in the clinical trials is being evaluated by single-cell multiomics.

Q From your experience, can you distill any essential advice for those looking to move novel cell therapies into those early clinical phase trials?

SMV: Before moving novel cell therapies into early-phase clinical trials, testing safety, efficacy, pharmacokinetics, and pharmacodynamics in the known preclinical studies is fundamental. It can take several years to collect enough data during the preclinical studies before an Investigational New Drug (IND) application can be filed so the regulatory authorities can carefully review and allow progression to a clinical trial.

During the preclinical studies, the use of *in vivo* and *in vitro* studies is important to determine the cell therapy product specificity, affinity, and avidity for the target cells and to evaluate the potential for on-target and off-target toxicities. Assays may include:

1. Cytotoxicity and cytokine release using human primary cells, cell lines, and induced pluripotent stem cell-derived organs and tissues;
2. Protein arrays;
3. Tissue cross-reactivity studies; and
4. Animal models.

Referring to the last, literature has shown that animal models present limitations and an inability to make reliable predictions for human clinical trials. Indeed, several limitations due to species specificity, xenogeneic graft versus host response, and the difficulties in developing human immune responses in animals can limit the animal model and its use in preclinical studies. Despite these limitations, *in vivo* testing can help establish proof-of-concept data for cell therapy functionality, and murine xenograft models can provide information on the trafficking and proliferation profile of cell therapy products.

Q Can you describe the journey and challenges commonly faced when translating a cell therapy approach from proof-of-concept to Phase 1?

SMV: Numerous preclinical assays generated data to support an IND application to the US FDA for the DSG3-CAAR T cell therapy.

"Following up on the preclinical studies, a pre-IND meeting was scheduled to discuss considerations specific to this non-oncology indication...The FDA reviewed the application and allowed progression to a clinical trial."

Starting with the proof-of-concept, we assessed CAAR T cell-specific cytotoxicity toward primary human anti-DSG3 B-cells by coculturing with non-transduced T cells, DSG3-CAAR T cells, or anti-CD19 CAR T cells (CART19) with primary human B cells from PV patients or healthy donors, followed by ELISpot to detect and enumerate B cells secreting antigen-specific IgG (anti-DSG3 IgG) and B cells secreting IgG irrespective of antigen specificity (total IgG).

Next, we studied CAAR T cell activity and efficacy in dose-related pharmacology and toxicology assays using a PV model composed of polyclonal anti-DSG3 hybridomas engrafted into NSG mice. Good features of this model were that the human clinical product could be examined, as opposed to models demanding murine DSG3-CAAR or T cells; hybridomas produced anti-DSG3 antibodies that cause a PV phenotype; anti-DSG3 BCRs targeted physiologically important epitopes; bioluminescence allowed sensitive longitudinal measurements for B-cell eradication; DSG3-CAART engraftment happened in NSG mice; and off-target interactions with DSG3 ligands would be observed if they occur, as human DSG3 shows 86% sequence homology to mouse DSG3 and functionally rescues loss of mouse DSG3.

In addition, we evaluated DSG3-CAART in a modified active immune PV model involving immunization of DSG3-deficient mice with recombinant human DSG3 ectodomain (rhDSG3), followed by splenocyte transfer into $RAG2^{-/-}$ mice. Transferred splenocytes contained B cells and $CD4^+$ and $CD8^+$ T cells. $RAG2^{-/-}$ recipient mice developed mucocutaneous erosions with suprabasal acantholysis. DSG3-CAART treatment improved mucocutaneous erosions and decreased serum anti-DSG3 antibodies.

We also assessed the pharmacologic and toxicologic effects of soluble anti-DSG3 antibodies derived from PV patients at physiologic levels, and we observed that PV IgG stimulated a titer-dependent increase in DSG3-CAART IFN-γ production. However, IFN-γ production induced by soluble PV IgG is lower than levels caused by target cell encounter.

Among other assays, to evaluate CAAR T cell cytotoxicity, we tested off-target interactions of DSG3-CAART by pursuing an unbiased, high-throughput approach to identify potential off-target interactions by screening a cell-based array of more than 5300 membrane proteins with a soluble Fc-tagged DSG3EC1–4 CAAR ectodomain. We tested whether CAAR T cells can cause redirected autoantibody-mediated lysis of Fc receptor-expressing primary human monocytes, natural killer cells, and Fc receptor-overexpressing K562 cells. To evaluate cytokine release syndrome risks, we ran *in vitro* assays where the CAAR T cell was cocultured with target cells and patient autoantibodies, increasing concentration till maximum found in the patient serum.

Following up on the preclinical studies, a pre-IND meeting was scheduled to discuss considerations specific to this non-oncology indication. Then, we submitted this first-in-human

"...the early positive results with systemic lupus erythematosus patients triggered hope and, concomitantly, a race to novel applications for the CD19 CAR T cells in other diseases..."

therapy to treat autoimmune diseases as an IND Application. The FDA reviewed the application and allowed progression to a clinical trial.

Q How do you see the application of CAR T and other cellular immunotherapy approaches to autoimmune diseases evolving?

SMV: Recent reports have shown that CD19 chimeric antigen receptor (CAR) T cell has presented preliminarily outstanding signs of clinical efficacy in autoimmune disease therapy. In this context, systemic lupus erythematosus (SLE) is a chronic autoimmune disease where the immune system attacks healthy tissues throughout the body, driving end-organ damage and an increased risk of death. The Department of Internal Medicine of the Friedrich Alexander University Erlangen-Nurnberg recruited 5 SLE patients and, in 2022, reported notable clinical data where those patients showed expansion of the CD19 CAR T cells *in vivo*, B-cell depletion, and an improvement of SLE symptoms and markers of end-organ damage. Astonishingly, just a few months after CD19 CAR T cell therapy, patient follow-up showed naïve B cells in peripheral blood, and patients still did not present SLE symptoms.

The results from this small clinical trial has led to the rationale for more patients to be enrolled in clinical trials of CD19 CAR T cell therapy to treat B-cell mediated autoimmune diseases and a longer time to evaluate the clinical outcomes to understand and elucidate the long-term immune response to the cell therapy. Nonetheless, the early positive results with SLE patients triggered hope and, concomitantly, a race to novel applications for the CD19 CAR T cells in other diseases, such as idiopathic inflammatory myositis, myasthenia gravis, and systemic sclerosis.

Q When we last spoke, you voiced your planned use of single-cell RNA sequencing to study the cellular and molecular profiles of DSG3-CAAR T. Can you explain this approach further and any findings you may have?

SMV: The approach focused on determining phenotypic and transcriptional profiles of DSG3-CAART cells that promote immune tolerance induction. In this context, we will be

analyzing the relative ratios of various B- and T-cell subsets and activation status in the infused and engrafted product. We will correlate these data with clinical outcomes and also identify immunophenotypic and transcriptomic features of DSG3-CAART in the infused product that are selected for acute expansion and long-term engraftment.

In addition, we will investigate if there are cellular determinants of immune tolerance in host T and B cells induced by DSG3-CAART. We will focus on defining B-cell immunophenotype and single-cell transcriptomic profiles of total B cells before and after DSG3-CAART. Moreover, we plan to investigate global T-cell immunophenotypes and single-cell transcriptomic profiles before and after DSG3-CAART infusion.

Q How are/will next-generation sequencing and single-cell analysis tools be used within nonclinical cell therapy development?

SMV: Deeper sequencing is more readily available than ever, with next-generation sequencing giving more information and readings from a single cell. This plays a vital role in supporting the development of a better understanding of a precise, wide-ranging view of innate and adaptive immune cell diversity by detecting rare cell types and biomarkers. In this context, next-generation sequencing (NGS) and single-cell analysis support identifying models at the single-cell level associated with relevant disease subtypes and mechanisms.

The S100 calcium binding protein A4 (S100A4) was recently identified as a potential target for glioblastoma immunotherapy in an integrated single-cell sequencing analysis of thousands of gliomas, stromal and immune cells from human glioma samples [1]. In this study, the authors demonstrated that survival was improved in nonclinical models by deleting S100A4 from non-cancer cells and observed that removing S100A4 had reprogrammed the immune landscape.

Cytoskeleton-associated protein 4 (CKAP4) and runt-related transcription factor 1 (RUNX1) are other exciting targets defined by target identification. The first one, CKPA4, was identified by scRNA-seq from a comparison between hearts from ischaemic and healthy animals, implying a possible role in diminishing cardiac fibrosis risk through inhibiting fibroblast activation [2]. In contrast, RUNX1 appears to be an essential transcription factor and target to block myofibroblast differentiation determined by scATAC-seq target identification in a mouse model of kidney fibrosis [3].

Recently, Lareau *et al.* discussed the map charting potential new tumor antigens and the discovery of antigens for precision therapies via single-cell genomics [4]. In this context, chimeric antigen receptor T cells are used for hematological cancers, and the recent data provided by single-cell genomics on potential new tumor antigens open the possibilities for developing novel cell therapies to be examined in nonclinical models.

The potential of using NGS and single-cell analysis to pinpoint disease-relevant cell types and targets can be explored by using single-cell differential expression studies and confirming

them in functional genomics single-cell studies (e.g., CRISPR-seq). The nonclinical model setting offers an excellent opportunity to discover new essential disease targets in conjunction with NGS and single-cell analysis.

Q What are your key goals and priorities over the next 12 to 24 months?

SMV: We are currently performing correlative studies to investigate the patients responding and not responding to the DSG3-CAAR T cell therapy. We will define T- and B-cell immunophenotypes and transcriptional profiles before and after therapy and correlate profile changes with clinical outcomes.

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BIOGRAPHY

SILVIO MANFREDO-VIEIRA is the Associate Director for Correlative and Translational Studies at the Department of Dermatology of the University of Pennsylvania, where he is responsible for direct translational research necessary to move novel cell therapies developed in the lab into early-phase clinical trials under an academic-industry alliance. He received his PhD at the School of Medicine of Ribeirao Preto, Sao Paulo, Brazil, followed by postdoctoral training at Harvard Medical School and Yale School of Medicine, where he discovered that gut bacteria translocate to distal tissues and induce autoantibodies typical of lupus and autoimmune hepatitis, indicating a critical role for intestinal microbes in initiating or perpetuating local or systemic autoimmunity.

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INTERVIEW

Evolution in the landscape of non-clinical safety testing for advanced therapies

Abi Pinchbeck, Assistant Editor, *Cell & Gene Therapy Insights*, speaks to **Michaela Sharpe,** Senior Nonclinical Director at Moare Solutions Ltd, about the evolving regulatory guidance surrounding non-clinical safety assessments for cell and gene therapies, as well as enabling the transition into the clinic and beyond.

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Q What are you working on right now?

MS: I am working on a range of projects in the cell and gene therapy space, supporting products as they move through their non-clinical development. I work on projects in the early stages of preclinical development, all the way through to those close to transitioning into clinical trials. I enjoy having the opportunity to work on such a variety of different products. It is great to have a small part in helping to realize the potential of these cutting-edge therapies.

Q Over your 18 years of experience in the biotech and pharma sector, can you distill any key learnings and advice for therapy developers working on the translation of cell and gene therapies into the clinic and beyond?

MS: First and foremost, the goals of non-clinical programs are the same as ever—to acquire data to support efficacy, to select potential clinical dosages, and to establish product feasibility and safety. This remains the same for all types of products in development. For cell and gene therapies, it is likely that a standard set of non-clinical studies will not be used, so one must consider how to design the non-clinical program to obtain the information needed to support clinical translation. For a number of these products, this can pose challenges and *in vivo* studies might not be feasible for specific assessments of efficacy and safety, so any models used must be carefully considered.

One must have a strong scientific rationale for choosing a particular approach. Many people believe that regulators expect an *in vivo* model. In reality, regulators expect that you are performing the right study to address a specific question. An *in vivo* model may not be the right study. It is critical to think about how to maximize the information gained from the development studies, in order to move through the preclinical phase as efficiently as possible.

Another point to consider is that your product may change. When starting to perform the non-clinical program for a given product, in an ideal world this will be your final clinical product, but the reality is that it probably won't be. You need to consider how any changes in manufacturing may affect what you have been doing in a non-clinical program and how you can use the data you have already generated. It is not necessary to repeat your studies if you have a sound scientific rationale not to. *In vitro* approaches can be particularly helpful. If you have tests that can show product comparability, there may not be the need to repeat all or any of the studies.

The most important thing is to engage with regulators early because having regulatory input is key, particularly if you are using novel methods or approaches. In addition, it helps to understand if there are any aspects that the regulators want you to look at or are unsure of so you can make sure these requirements are fulfilled.

Q Can you outline the current key considerations for the non-clinical safety assessment of cell and gene therapies?

MS: The safety factors fall into several broad categories related to the biology of products and how they function. This includes their biodistribution, immunogenicity/immunotoxicity, safety, and persistence, and in the case of gene therapy, where their gene expression is being observed and the risk of insertional mutagenesis. These factors will not be the same for each therapy, so it is necessary to determine the specific risks for each particular product. When thinking about the key safety considerations, a scientific data-based approach can be taken. There is no one-size-fits-all strategy so you must consider how your product is made, its biological characteristics, the target patient population, and the available models to assess product risks.

A risk-based approach to designing your non-clinical package is also recommended, as it allows you to assess whether alternative *in vitro* or *in silico* testing could be scientifically

> "Within the cell and gene therapy space, how non-clinical studies are evolving is about how much data is acquired from a study."

justified. The risk-based approach is a series of generic scientific questions that apply to any advanced therapy medicinal product based on factors relating to the quality, manufacturing of the product, biological activity, and clinical application. By determining the risks for each product based on these characteristics, one can begin to determine the extent of the non-clinical package needed, including the relevant studies and the mechanisms for acquiring data. This may be through *in vivo* studies, *in vitro* studies, or paper-based exercises from existing knowledge in the literature. Published literature can be underestimated as a valuable tool here—there is a lot of data out there to be used. There are also opportunities for the replacement of animal studies with well-designed *in vitro* alternatives.

Q How are non-clinical *in vivo* testing requirements evolving?

MS: Fundamentally, the development of any program is about doing the right study to address a specific issue. Regulators are looking for well-designed, appropriate studies to address a specific risk. Within the cell and gene therapy space, how non-clinical studies are evolving is about how much data is acquired from a study. Historically in pharmaceutical development, there has been a distinct delineation between the different non-clinical phases but for many advanced therapy medicinal product studies, we are not separating efficacy, pharmacokinetics, and safety, and instead potentially testing all of these within a given study. Acquiring more information from one study will limit the number of animals needed, and biologically relevant models can be used where possible.

There is also the challenge, particularly with gene therapies, that a product may only be active in humans and potentially primates. The risk is that we begin to do more non-human primate studies. Investigating whether you can obtain the same information from an animal equivalent product or an *in vitro* assessment is important. As a field, we need to limit the use of non-human primates to only those studies where there is no alternative.

Q Do you see regulators coming around to less animal testing in practice? If so, in what specific circumstances?

MS: Yes—there has been clarification that non-animal testing methods can be accepted. It has always been the case that regulators would consider non-animal testing, but they have become more explicit about it. In 2021, the European Medicines Agency implemented new measures to minimize animal testing during medicines development, and specifically measures sought to promote the 3R principles. In 2022, legislation was enacted in the US to replace a stipulation that drugs had to be tested in animals as part of the US FDA Modernization Act 2.0. Other regulatory agencies are either bringing in similar measures or providing greater

clarification and being more explicit on the topic. Regulators will look to developers to use the most appropriate study to assess patient risk, whether *in vitro* or *in vivo*.

The key driver is that the tests must be relevant and must be shown to work. The measures must be in place to show that that test can address the questions asked and that the sensitivity and robustness of the assay have been established. There are a variety of tests that are coming into development to do this, such as organ-on-chip technologies, novel genomic technologies, and *in silico* modeling methods. There are potentially many biologically relevant surrogates out there.

One challenge is that some safety aspects are complex and are the result of multi-parameter, multi-organ effects. There is the risk that some *in vitro* tests will not detect specific safety issues, but it is also true to say that animal studies do not always identify safety issues either. We must always be aware there may be knowledge gaps and consider model limitations. This may mean additional monitoring may be needed to go into clinical trials, and/or a panel of tests may be needed to address different safety aspects.

Q What does the 'umbrella IND' mean for cell and gene therapy non-clinical development? How much can you leverage from one advanced therapy product or platform to another?

MS: The umbrella IND allows a sponsor to evaluate multiple versions of an investigational product. The similarities in terms of the products and biology will determine which non-clinical data could be considered for a platform. I certainly think there is great potential but it will be product specific.

If you have a gene therapy that is similar to another except for some small differences in sequences, you may be able to develop a scientific position on the commonality of the products and hence determine that existing studies are relevant, which could reduce animal use in testing. This is also not limited to the umbrella IND. There is the potential for opportunities to utilize published data for related products, potentially both preclinical and clinical data to assess risks, and thus minimize the required studies.

As an example, extensive data has been published on the distribution and persistence profile of multiple routes of administration of mesenchymal stem cells. There may be a strong scientific case for not repeating a study with a new product that is perhaps for a different indication but uses a standard route of administration. It may be possible to utilize existing published data on specific aspects, such as clinical dose levels or safety risks, without repeating the studies. In general, looking at what has been published and sharing safety information will help to minimize animal use in some of these programs.

Q What will the non-clinical safety space for advanced therapies look like in a decade?

MS: The space will evolve due to the acquisition of knowledge of the long-term risks of products. Hopefully, some of the risks that are currently considered theoretical will not materialize. In addition, we will know of any risks that do develop as reality and their risk-benefit profile. As we get more long-term data with gene therapies, we will understand any long-term

potential risks and the longer-term efficacy of the products. Based on that, the types of non-clinical programs will evolve.

I also believe we will see an increase in the use of alternative testing methods due to recent advancements, particularly organ-on-a-chip and genomic technologies that enable more specific assessments. An example of this is the change in the way to assess the potential for teratoma formation for pluripotent stem cell-derived products. This involves looking for the presence of residual pluripotent cells, which could be a teratoma risk. Previously, this was done in 6 to 12-month studies in immune-compromised mice. Now, technologies like qPCR and flow-based assays to look for the presence of rare cells will allow you to do these assessments in days, utilizing no animals.

Within the industry, groups are working together to identify where the needs are for some of these alternative methods. Organizations such as the Health and Environmental Sciences Institute and others are looking to collaboratively develop these types of technologies. Supporting information from collaboration studies that show that these technologies work will lead to greater acceptance to enable the rapid deployment of some of these alternative technologies in valid circumstances. The general principle of the right study to address the right question will remain. We will become more confident within the field that in some cases, the right study may be an alternative *in vitro* approach.

Q What are your own goals and priorities in your work for the next 12–24 months?

MS: My goal is primarily to help the programs I am working on, that are currently in their preclinical development phase, move into clinical trials. As I move forward, my priority will be to continue to work with new and innovative products and keep abreast in terms of the methodologies and ways to assess these types of products.

I would like to bring in these new technologies to help accelerate programs as they move forward and continue to work with peers across the industry to bring the technologies and the knowledge surrounding them to a wider audience going forward.

BIOGRAPHY

MICHAELA SHARPE is a Senior Nonclinical Director at Moare Solutions Ltd. She has specialized in the nonclinical development of cell and gene-based therapies, and has designed and implemented nonclinical development programs for a broad range of cell and gene therapy products. She has supported numerous academic teams through the nonclinical development pathway, as well as global small and medium-sized enterprises, and pharma organizations.

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INTERVIEW

Quantitive viral vector characterization: prioritizing genome vector integrity

David Dobnik

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ANALYTICS CHANNEL

CHANNEL CONTENT

INTERVIEW

Quantitative viral vector characterization: prioritizing genome vector integrity

Abi Pinchbeck, Commissioning Editor, *Cell & Gene Therapy Insights*, speaks to **David Dobnik**, Senior Research Associate at Biotechnological Hub of the National Institute of Biology, Slovenia. They discuss the viral vector characterization by different methods, the importance of measuring vector genome integrity in AAV analysis, and the advancement of methods in the quantification of residual host cell DNA.

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Q What are you working on right now?

DD: A few months ago, the relocation of the National Institute of Biology (NIB) to the Biotechnology Hub (BTH-NIB) was completed. The project resulted in a whole new building for our institute. The numerous activities taking place at the NIB are related to the environment, agriculture, food, and more recently and increasingly so, human health. From the gene therapy perspective, we are currently running an applicative project on the development of new approaches for the characterization of viral vectors funded by the Slovenian Research

and Innovation Agency, which will conclude this year. We have collected various interesting results and data, some of which have already been shared with the scientific community at conferences.

We are also applying for new projects to continue this research. Currently, we are not able to produce any of the research material on our own, so we are relying on collaborations with outside partners to perform the research. However, with new lab space, we have a plan to establish our own production capabilities for AAVs to enable continuation of our research in a way that will best support our ideas.

Q Can you expand on your work exploring transmission electron microscopy (EM) for evaluating viral capsid integrity, sample purity, and the ratio of full and empty viruses?

DD: We began our work in the field of gene therapy in 2016. Partially, this was because of our previous expertise in virology and molecular biology, which could easily be translated to viral vectors. EM is the only technique that allows you to visualize the viral vectors. Rather than having them in a solution and performing indirect observations or characterizations, you can actually see the particles. Any capsid integrity in terms of damaged capsids or other things that might appear and are present in the sample become visible. However, long-standing experience in EM is important to distinguish between the actual properties of the sample and any artefacts introduced during sample preparation.

At first, we used transmission EM (TEM) technique to observe any differences between the particles that are present in the sample and to evaluate the ratio of full and empty viruses. Later within the research project, we also compared TEM to three other commonly used methods that evaluate full and empty particles. We have seen that the results correlate well with other methods, though this does depend on what is being observed. For empty particles, this correlation is clearly seen. However, for full particles, some discrepancies were observed. By TEM, we can see if there are slightly damaged particles that may not be considered empty or full, and we can consider these as a separate population. If we consider all particles other than empty as full, then the values correlate better for full particles. These visual distinctions between particle populations, with the definition of partially full or damaged particles (or any other observed population), distinguishes EM from other methods.

Recently, we have developed an immunolabeling TEM approach to detect and semi-quantify DNA impurities. We have seen that free DNA can still be present in highly purified AAV material, and it can be difficult to evaluate whether this is bound to particles on the outside or is free-floating. With this new approach, we can show visually whether nuclease-resistant DNA is present and whether it is on the surface of the capsids or not. Surprisingly, we have seen this nuclease resistant DNA mostly present around the capsid rather than free-floating. This means that it may be bound somehow, and thus the nucleases are not able to access it to degrade it to a full extent.

"Moving towards the evaluation of vector genome integrity, i.e., the full-length genome in the capsids, is currently the main challenge."

A few years ago, we also got access to cryo-EM. We are looking forward to doing more work on the cryo-EM because it preserves the native hydrated state of the samples, leaving no artefacts of sample preparation, as might happen with TEM samples after negative staining and drying. I am excited to see how cryo-EM might help us in viral vector characterization in the future, not only on the level of full and empty capsids.

Q What are the biggest challenges currently facing the field of quantification and characterization of viral vectors for gene therapy?

DD: I am mostly involved in the quantitative aspect of viral vector characterization. Over the last year, I have seen more people realizing that vector genome titer, as determined by PCR-based techniques targeting a small region, might not be an accurate measure for the actual full-length vector genome quantity. Moving towards the evaluation of vector genome integrity, i.e., the full-length genome in the capsids, is currently the main challenge. We began implementing the idea of vector genome integrity 2 years ago, and, in the last year, there has been a shift in the field in prioritizing this analysis. More companies and developers are interested in this idea of genome integrity.

Related to that, one challenge refers to the evaluation of the specific content of individual capsids. Currently, some techniques measure particles and group them. The results are averaged in terms of content: full, empty, and partial. Currently, we cannot obtain exact particle-specific content information. I believe it may be possible to evaluate each individual capsid separately in much more detail in the future. The development of such analytical methods presents a big challenge. Although I am not sure such a detailed level of information is currently needed, it is interesting to support these kinds of developments as we are all striving towards the best-characterized drug products. When broadly introduced in regular process development operations in the future, such a method would allow scientists to better characterize their viral vector products as well as direct the process development towards viral vector populations with desired content (i.e., full-length genomes).

One other challenge that we now have, since we know how to measure integrity, is finding a correlation to potency. As potency is the ability of the product to achieve a given result and should reflect clinical efficacy, it is expected that potency will be affected by the number of full-length genomes present in the capsids. In theory, we should have better potency if we have more of the full-length genomes in the product. However, other fragments of the genomes can to some extent also contribute to the potency. The challenge now is to connect these

different fragment populations and different amounts of full-length genomes to the actual potency outcomes.

Q What are the primary throughput-related and other challenges currently faced in vector manufacturing assays/analytical tools?

DD: There are two perspectives here. For certain large companies and CDMOs, throughput is crucial due to the large number of samples and batches. However, for developers at the initial stages, throughput is not so important. There have been numerous developments in the field to support higher throughput of analytical methods, yet an additional challenge remains in the need for more techniques to monitor parameters in real-time, during the manufacturing process.

This is particularly important when producing viral vectors for gene therapy, as it is not possible to freeze the process for a day to analyze if everything goes according to plan. The production process flows continuously. We might get the results of the analyses long after some process is already completed. By having such fast on-line analytical tools available, the manufacturing process could be adjusted and optimized in real-time. Beyond throughput, time efficiency and conducting analysis in real-time must also be considered.

Q How are methods for the detection and quantification of residual DNA evolving and becoming optimized?

DD: Over the last 7 years of using residual DNA detection methods, one thing we have seen is a shift from the human host cell DNA kits to more specialized, host cell-specific ones, such as HEK kits.

The second shift has been a transition from qPCR to digital PCR for residual DNA testing. The results of qPCR are given in mass (ng or pg/µL) and there is a need for a standard curve (hopefully prepared from a certified reference sample, which should show the same amplification efficiency as tested samples). With digital PCR, you perform the absolute measurement of DNA copies without any standard curves. This requires the implementation of conversion factors to obtain a mass-based result to be compliant with regulations. Digital PCR is becoming a new standard for quantification in gene therapy, including for host cell or residual DNA analyses.

Many people are starting to consider the different lengths of fragments that they are amplifying because of the FDA regulation regarding a 200-base pair limit. However, since any PCR method gives you a view of the specific target only, this may not be entirely informative of the whole population of impurities present. Other methods such as sequencing may be more informative here. With short-read sequencing (such as Illumina), you can easily see the present impurities besides your viral vector genome. With recent developments in long-read sequencing, you can see the longer fragments, including those that may pose concerns according to the regulators

"Identification and faster evaluation of important CQAs would strengthen understanding of process parameters and could reduce technical development timelines..."

(e.g., oncogenes). Both sequencing technologies can detect the nucleic acids that are present in the sample, thus providing another level of information compared to the targeted PCR-based approaches. Nevertheless, sequencing still lacks the quantitative aspect.

Q Looking to the future, where specifically would you like to see efforts focused on improving the value of the analytical toolkit for AAV and lentiviral manufacturers?

DD: As mentioned before, the industry needs analytical capabilities that can increase throughput and deliver results on CQAs faster, as these attributes are directly linked to process performance or product quality and potency. Technologies are evolving to give more information.

Identification and faster evaluation of important CQAs would strengthen understanding of process parameters and could reduce technical development timelines by reducing the number of full end-to-end runs required to support development. It would also enable more efficient manufacturing platform development through a focus on specific unit operations most critical to viral vector quality and potency.

I have seen many presentations showing results on optimization of manufacturing processes, where they have used viral genomes (determined by simplex dPCR) and capsid concentration as measures to show that better yield of full particles have been achieved. These kinds of results might be misleading, as the process perhaps only produced more fragments of the genome, but the concentration of full-length genomes stayed the same. The problems of current vector genome titering approaches might be improved if the focus shifts to prioritizing vector integrity rather than quantifying one small fragment as a vector genome. Replacing older technologies with new ones could mean a significant improvement for manufacturing, especially if we were able to focus on the attributes that are the most relevant.

Q What do you expect the analytical toolkit of a viral vector manufacturer to look like in a decade?

DD: I hope that there will be many more multiplex technologies, bringing the ability to evaluate several different parameters in one system. This would be a big improvement over

the current system of assessing vector genomes by one machine, the capsid titer by another, impurities with another assay, and so on. A multiplex, all-in-one assay system is a development I would like to see soon, even sooner than in a decade.

Q What are your main priorities over the next few years in your work at the NIB?

DD: The main priority is to acquire additional funding for our research project ideas. We have an idea as to how we could add value to the production of viral vectors by improving vector genome integrity. We recently applied for a national project to support this improvement of the integrity of viral vectors. Since we do not have the production capabilities in-house, the goal for this year is to build these research production capabilities. We are working with human cell cultures and multiplying a few other human viruses, but not yet recombinant AAVs.

Once we have those in-house capabilities, together with the results of our current projects, we aim to generate new research ideas and evolve further in the field.

BIOGRAPHY

DAVID DOBNIK has been developing new nucleic acid detection/quantification approaches since 2007. From 2016, he has been closely working with companies in the field of gene therapy (such as AveXis [now Novartis Gene Therapies]) to develop and apply methods for precise quantification of viral vectors (e.g., dPCR), observation of viral particles (electron microscopy), and for identification and quantification of impurities (e.g., nucleic acids by high-throughput sequencing [HTS/NGS] or PCR-based methods). David has been leading the development and tech-transfer projects for pharmaceutical companies focused on the field of gene therapy, helping their process development efforts with characterizations of viral vectors. Lately, most of his work has been focused on new approaches for genome integrity evaluation.

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AUTHORSHIP & CONFLICT OF INTEREST

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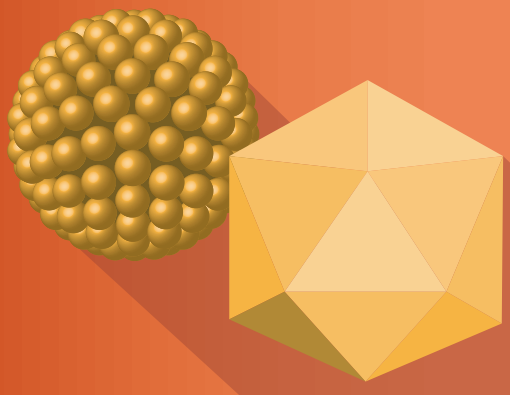

CELL & GENE THERAPY INSIGHTS

VECTOR CHANNEL EDITION
Scalability

MARCH 2024
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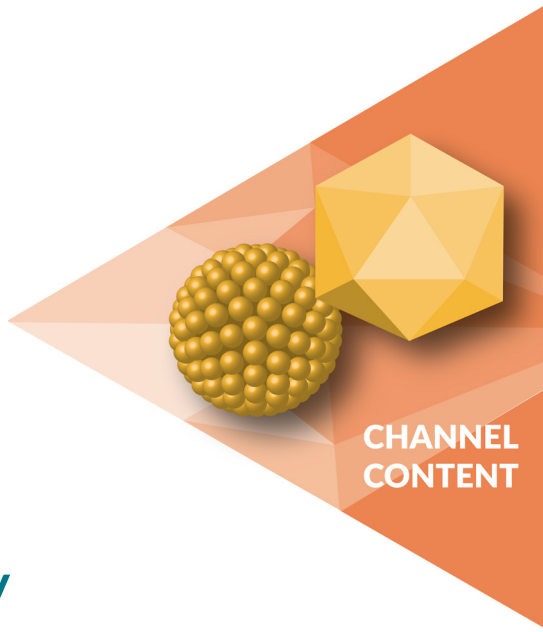
CELL & GENE THERAPY INSIGHTS



SCALABILITY

INNOVATOR INSIGHT

Advancing gene therapy development with a multi-serotype AAV affinity resin

Nicolas Laroudie and Quentin Bazot

AAV purification poses a number of unique challenges to viral vector manufacturers including the need for scalability, a significant impurity burden, and ensuring a good recovery yield. In this article, a multi-serotype AAV affinity resin will be described and its use illustrated by relevant experiences and case studies from a CMDO developing an AAV purification platform for application across various AAV serotypes and client processes.

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DOI: 10.18609/cgti.2024.036

AAV DOWNSTREAM PROCESS CHALLENGES

When considering a typical AAV process for viral vector manufacture, the downstream purification process normally includes one or two chromatographic steps. Typically, this consists of an affinity capture step followed by a polishing step. This approach has been developed to address the challenges associated with the purification of complex molecules such as viral vectors and has been made possible by the development of modern tools such as immunoaffinity resins.

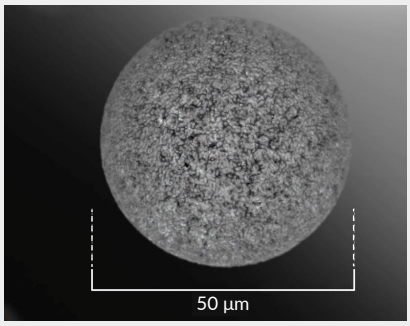
One challenge is the significant impurity burden in the feedstock due to cell lysis. AAVs are not secreted viruses, and it is necessary to break the cells to release the vectors, resulting in a very high amount of impurities. It is also necessary to develop

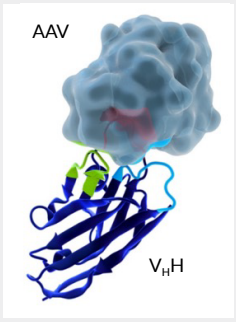
process steps that are robust, reproducible, provide a good recovery yield, and that are easy to scale up.

Most AAV manufacturers will eventually work with various serotypes, and it is therefore extremely convenient to have a platform tool that enables capture of most of the serotypes commonly used in gene therapy without having to redesign the process each time. This is what the POROS™ CaptureSelect™ (CS) AAVX resin has been designed for, and over the last decade it has become the most commonly used tool for AAV purification. However, an immunoaffinity resin such as AAVX is not able to distinguish between full and empty capsids

FIGURE 1

POROS CS affinity solutions to support AAV purification.



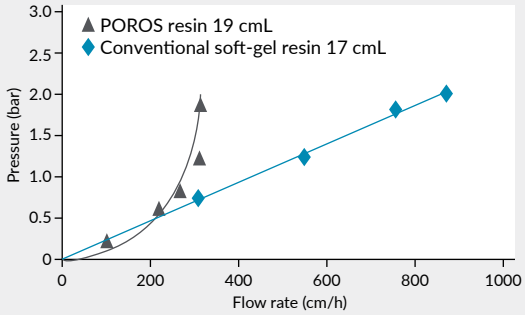


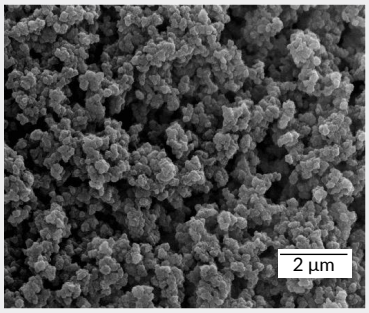
Thermo Scientific™ resin	Serotype affinity
POROS™ Capture Select™ AAV8 resin	AAV8
POROS™ Capture Select™ AAV9 resin	AAV9
POROS™ Capture Select™ AAVX resin	AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, Rh10, recombinant and chimeric vectors

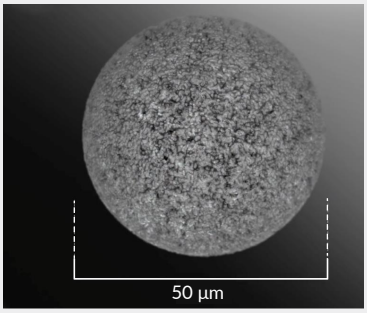
Products come with full regulatory support (RSF) enabling use in commercial manufacturing

FIGURE 2

Features of the POROS bead.







Poly(styrene-divinylbenzene) backbone

- Linear pressure flow curve
- Rigid, linear, and scalable performance
- Easy handling
- Highly robust and chemically stable

Large throughpores

- Reduced mass transfer resistance
- Capacity and resolution well maintained over a wide range of linear velocities
- More efficient purification

50 micron bead size

- Superior resolution
- Improved capacity through novel surface chemistries
- Excellent pressure-flow properties
- Fully scalable

and therefore, a second polishing step is necessary for this purpose. Here, the use of an anion exchanger—the POROS HQ 50, for example—is recommended.

Thermo Fisher has designed and released three immunoaffinity resins for the specific capture of AAV vectors (Figure 1). Along with POROS HQ CS AAV8 and 9, for the capture of serotypes 8 and 9, The POROS CS AAVX has been developed with the capability to target a broader range of serotypes, and for possible use as a platform tool. All three resins utilize CS technology, which involves the use of ligands derived from a camelid single variable domain on a heavy chain (VHH) nanobody which is then grafted onto a POROS base bead. These VHH ligands are synthetically produced in an animal-free system and at large scale.

All three resins are manufactured in a GMP-compliant environment allowing for their use in commercial manufacturing and come with a regulatory support package.

The CS ligands are supported by POROS base beads (Figure 2), with a unique pore structure for the efficient purification of large molecules such as viral vectors.

CONSIDERATIONS FOR PLATFORM AAV AFFINITY CAPTURE

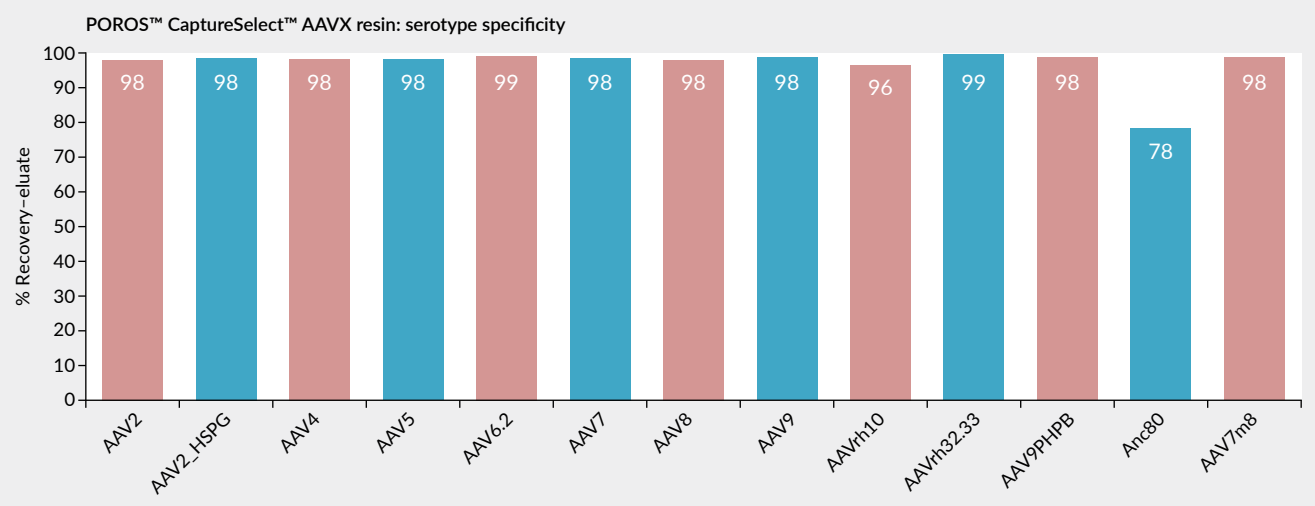
In order to develop a platform for AAV capture there are a number of requirements to be considered, including:

- Broad specificity to different AAV serotypes;
- High dynamic binding capacity;
- High purity and recovery;
- Scalability; and
- Reusability

The system must allow for the recognition of any serotype, whether naturally occurring or engineered. There is a need for high binding capacity to reduce column size requirements and maximize productivity. High purity and high recovery are two characteristics one can expect from immunoaffinity technology, and they can both be achieved via finetuning of intermediate washes and elution buffer optimization. Scalability, with consistent performance upon scale-up, is also key. Reusability

FIGURE 3

Broad specificity of POROS CS AAVX. Batch binding experiments performed and data provided by Massachusetts Eye and Ear.



AAV quantified by qPCR [1].

can reduce the COG in particular at the small- and pilot-scale or in routine manufacturing for the same serotype and same transgene. Reusability is therefore crucial for reducing cost and maximizing productivity.

Specificity

The POROS CS AAVX medium has the capability to capture a very broad range of serotypes, which is a unique feature in the current market. In a set of experiments designed and conducted by the Massachusetts Eye and Ear Institute in the US, researchers took different AAV serotypes and placed them in tubes with AAVX resin. The supernatant was incubated and then removed and the resin was washed. The bound material was eluted by adding acidic solution. The amount of AAV recovered was then measured and compared to what was initially added.

As shown in Figure 3, all of the serotypes evaluated were recognized and captured on the resin. There is currently no identified AAV serotype that is not recognized by the AAVX ligand. This makes the POROS CS AAVX resin an ideal candidate for the establishment of a platform purification tool.

Binding capacity

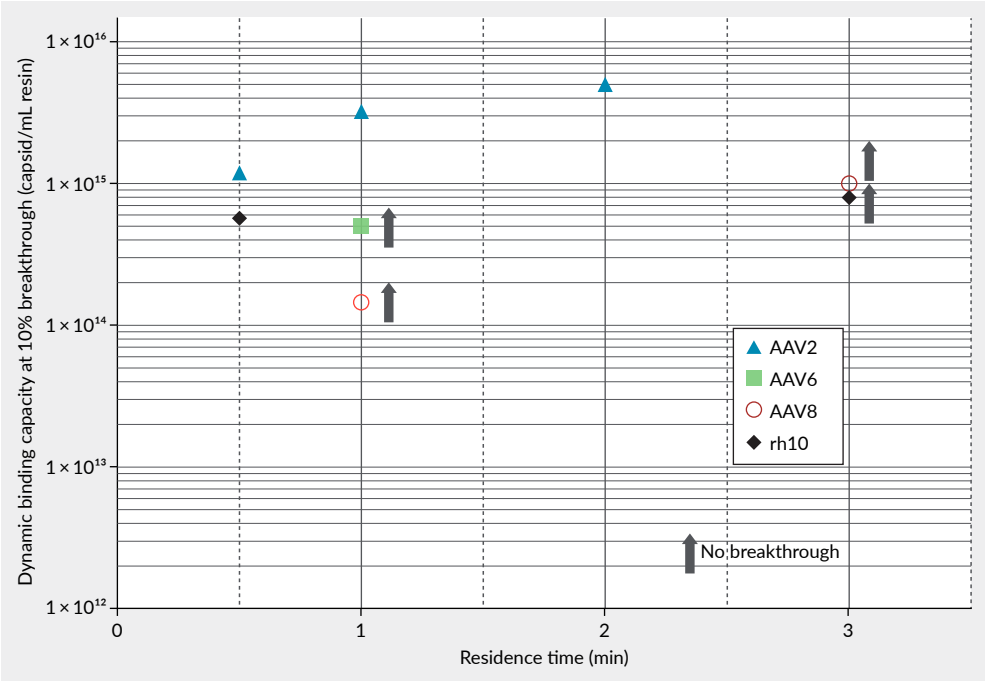
The POROS CS AAVX resin exhibits a high dynamic binding capacity for many serotypes as shown in Figure 4. For AAV2, the capacity at 10% breakthrough was measured as higher than 1×10^{15} capsids/mL for a residence time as short as 30 seconds, and at least 1×10^{14} capsids/mL for serotype rH10 for the same residence time. For AAV8, it was not possible to saturate the resin due to the shortage of material, and this is indicated by the grey arrows that suggest a higher dynamic binding capacity than measured is expected.

Performance overview

A rigid chromatography medium means that the pressure evolves linearly with the flow

FIGURE 4

The POROS CS AAVX resin dynamic binding capacity or multiple serotypes, including at short residence times.



Data sources: AAV2, AAV6: internal work; AAV8: [2]; rh10: [3].

increase, as compared to a semi-soft material such as agarose, for which the pressure increases exponentially with the flow rate when it exceeds a certain value. Figure 5 illustrates an example with POROS CS AAV9; the behavior is identical with AAV8 or AAVX.

With rigid material, the backpressure relies on the flow and on the bed height but not on the column diameter. This makes it simple to anticipate the expected backpressure when scaling up a process. In addition, backpressure remains limited even at high velocities, allowing processes to be run at high speed, resulting in increased productivity with linear and predictable scalability.

Reusability

To address the question of reusability, an internal cycling study with an AAV2 feedstock was performed (Figure 6). No significant decrease in recovery yield was observed over 35 cycles, and the performance of the resin in terms of purity was consistent from run 1 to 35.

Polishing step: separation of full and empty AAV capsids with POROS HQ

As mentioned previously, one capability the immunoaffinity resin lacks is the ability to distinguish between full and empty capsids, and therefore the utilization of an affinity column requires a polishing step after capture to separate empty capsids from full ones. Ion exchange chromatography has shown to be most suited for this application, making use of the difference in pI of full and empty AAV particles. Since the difference in pI is only 0.4 units, a high resolution ion exchange resin with superior selectivity is required.

Thermo Fisher's portfolio includes two strong anion exchangers—POROS HQ50 and POROS XQ—that have been successfully used for the separation of full and empty capsids, and which share the same POROS backbone.

Data from Lavoie *et al.* demonstrates capsid enrichment using POROS AEX resins. A

FIGURE 5

POROS CS AAV9 pressure flow curve. Low back pressure: <3.0 bar at 700 cm/h in 22 cm length column.

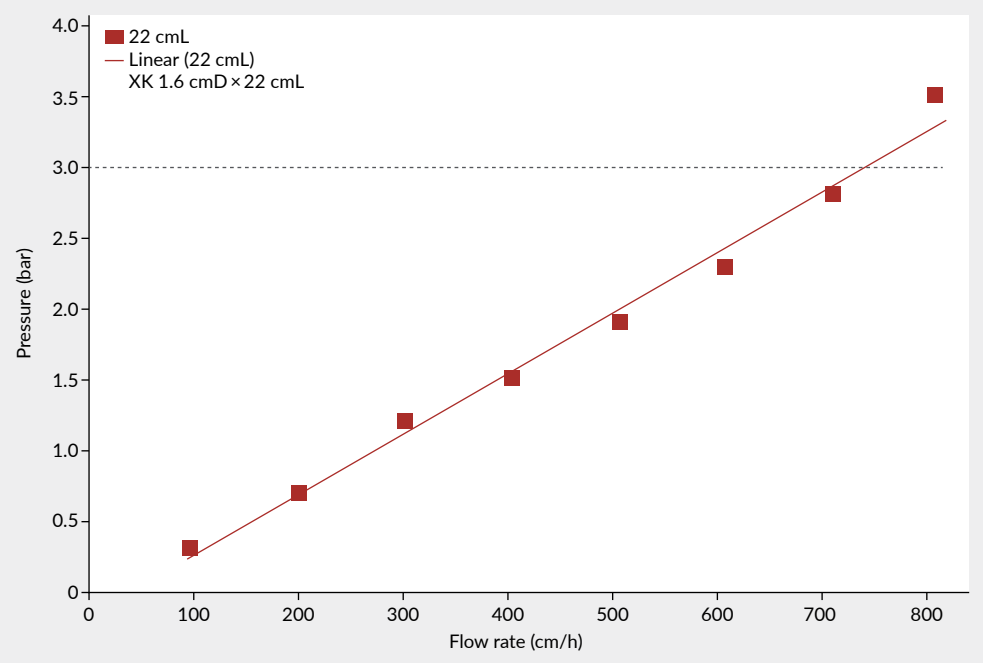
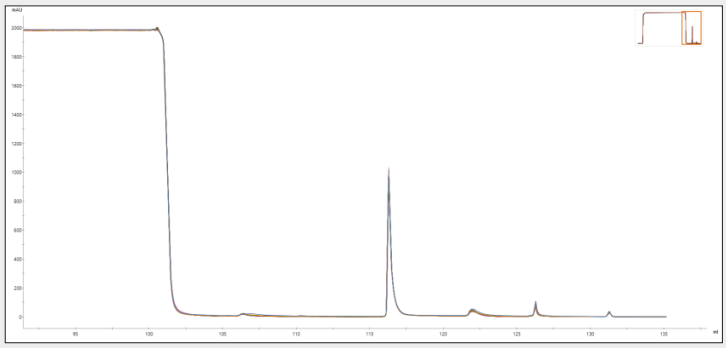
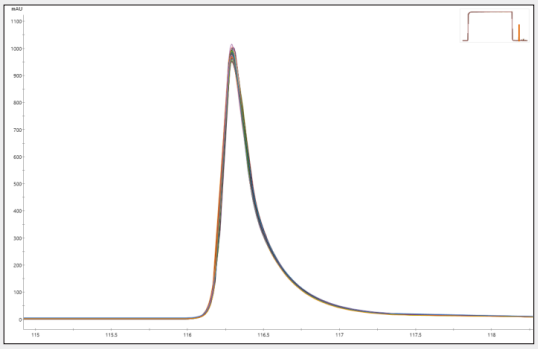
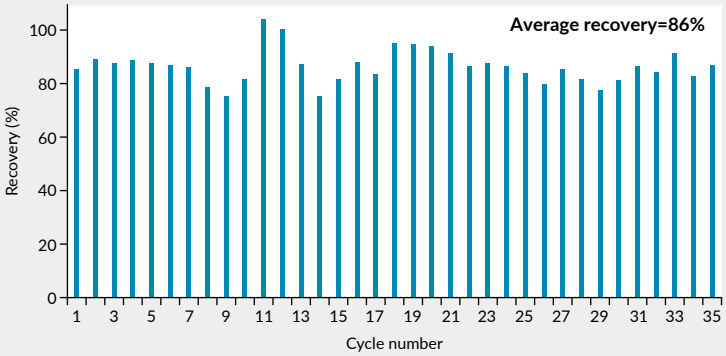


FIGURE 6

POROS CS AAVX reuse study with AAV2.



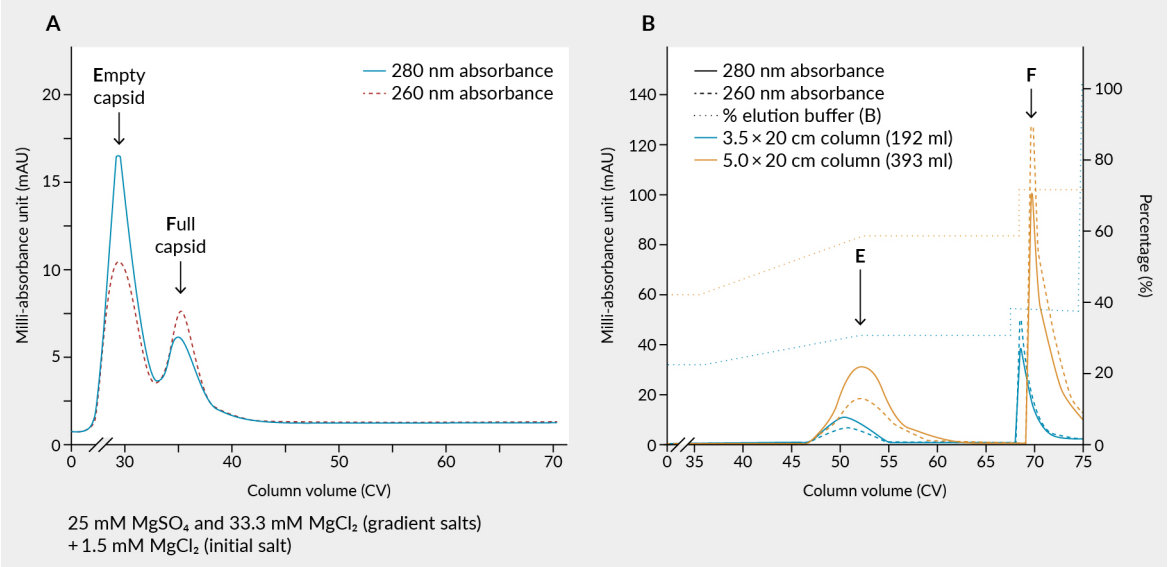




Study format	
Sample	Clarified lysate AAV2
Load preparation	Concentration and buffer exchanged
Resin	POROS CaptureSelect AAVX
Column details	1 mL (0.5 cmD × 5 cmL)
Loading/cycle	6.6×10^{14} cp/mL resin
Regeneration	0.2 M phosphoric acid (15 min)
Cleaning	6 M guanidine–HCL (15 min)

FIGURE 7

Optimized elution profiles of AAV5 on POROS HQ using 'dual' salts.



Percentage full was determined by AUC, measuring a 75% and 79% full respectively. Data from Lavoie et al. [4].

dual salt gradient was utilized to separate full and empty capsids in AAV5, as shown in the chromatogram in Figure 7A. A gradient was used, then converted into a more elaborate purification process to achieve baseline separation of full and empty capsids (Figure 7B).

TABLE 1

Performance of elution of AAV5 on POROS HQ at various scales.

Scale	vg yield(%)	cp yield (%)	Eluate % full (vg/cp)	Enrichment factor
(0.66 cm × 20 cm) 6.8 mL	69	22	80	4.0
(3.5 cm × 20 cm) 192 mL	80	22	80*	4.0
(5.0 cm × 20 cm) 393 mL	63	24	63*	3.2

cp: capsid; vg: vector genome.

Using POROS HQ, the process was then scaled up to different column sizes and geometries, as shown in Table 1. Notably, this approach enriched percentage of full capsids in purified feedstock by a factor of three to four, reaching up to 80% full capsids.

CASE STUDY SERIES: INTEGRATION OF THE POROS AAVX AND POROS HQ50 INTO AN AAV PURIFICATION PLATFORM

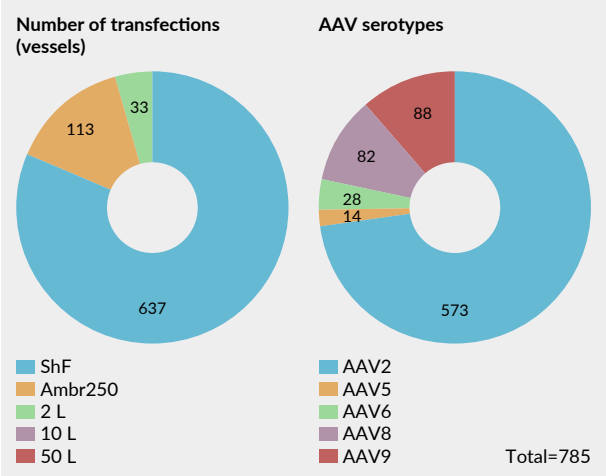
ABL Europe, now Oxford Biomedica, is a pure-play CDMO specialized in viral vector production that has manufactured a broad range of viral vectors for various applications, including vaccines, oncolytic virotherapies, and gene therapies.

These case studies will focus on AAV vectors and the development of an AAV platform based on HEK293 suspension cells, using transient transfection. The goal of this work was to create a scalable platform that could easily be implemented for different AAV serotypes and at various stages of a project in order to meet client needs.

The transfection step is key to producing AAV, and ABL Europe's process development and innovation lab screened a number of different HEK293 suspension cell lines and media, along with various transfection reagents and production systems, including one-, two- and classical three-plasmid systems. In terms of production scale, all projects start with transfection in shake flasks, and can go up to 50 L in the process development lab. Optimization work was done using serotypes AAV2, 5, 6, 8, and 9. A summary of the various vessels and AAV serotypes utilized is outlined in Figure 8.

FIGURE 8

Summary of AAV upstream platform development work undertaken by ABL Europe's development and innovation laboratory.



For optimizing the downstream process, various detergents were screened for the cell lysis step, along with filters and tangential flow filtration (TFF) cassettes, and chromatography media for the capture and polishing step. An additional goal was to retain an optional first TFF step before capture and after clarification, for client processes that required it.

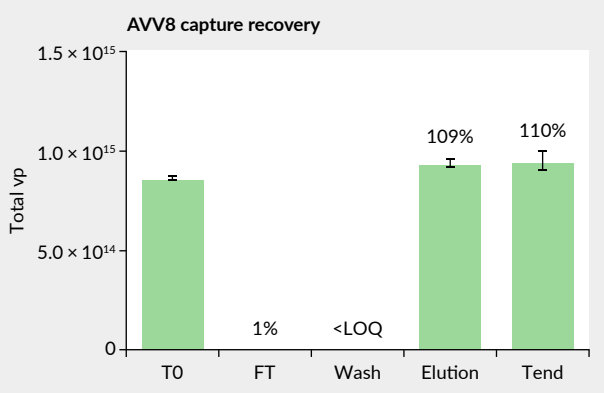
AAV PLATFORM DEVELOPMENT: ASSESSING THE AAV CAPTURE STEP

For the AAV capture step, the AAVX resin was an obvious candidate for evaluation when developing a pan-serotype platform.

Evaluation began with AAV8, and the material was taken directly from clarification, with no TFF step. Capture recovery was assessed as shown in Figure 9, showing very high recovery with AAV8 using the AAVX resin.

FIGURE 9

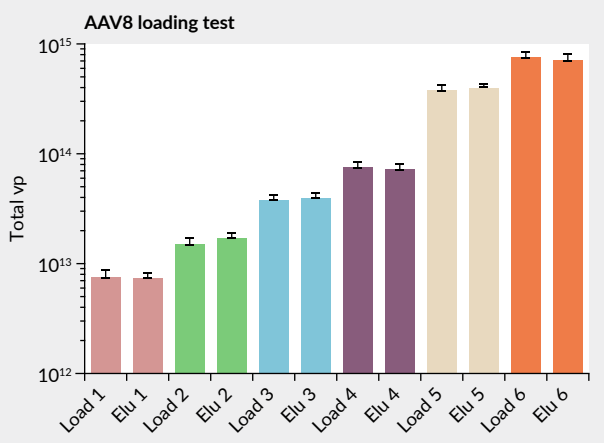
Evaluation of AAV8 vp recovery using the POROS AAX resin.



vp: viral particle.

FIGURE 10

Evaluation of loading capacity and recovery with the POROS AAVX resin using AAV8.



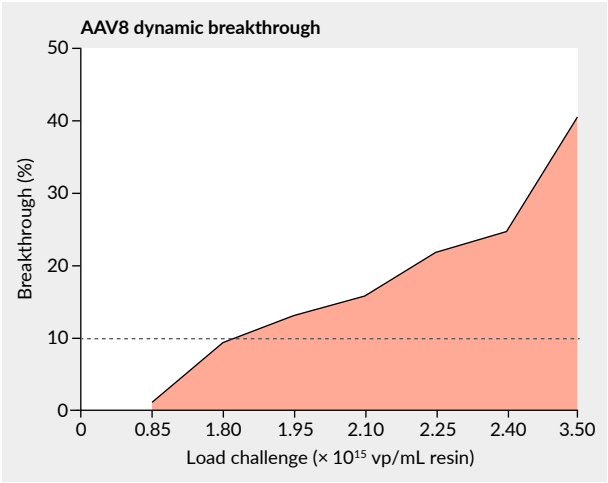
Run	Load total vp/ml resin	Recovery (%)
1	8.0×10^{12}	98
2	1.6×10^{13}	112
3	4.0×10^{13}	105
4	8.0×10^{13}	95
5	4.0×10^{14}	104
6	8.0×10^{14}	94

Next, the loading capacity of the resin with AAV8 was evaluated, as shown in Figure 10. Experiments were carried out with different amounts of AAV8 per mL of resin. Recovery of above 90% was achieved in all experiments, with high recovery of >2 log difference.

Next, resin dynamic binding capacity was determined (Figure 11). Loading of 1.7×10^{15} viral particles (vp) of AAV8 per mL

FIGURE 11

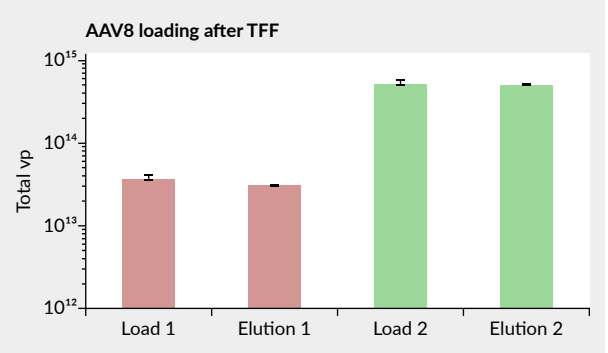
Determination of AAVX resin dynamic binding capacity for AAV8.



Loading of 1.7×10^{15} vp of AAV8 on AAVX resin leads to 10% breakthrough. Breakthrough curve realized with 1 min residence time. vp: viral particle.

FIGURE 12

Impact of concentration/diafiltration step on AAVX resin performance.



Run	Load total (vp/mL resin)	Recovery (%)
1	4.0×10^{13}	83
2	5.5×10^{14}	98

of resin led to a 10% breakthrough, indicating that no more than 1×10^{15} vp per mL of resin should be loaded.

As noted above, retaining TFF as an optional process step was one of the goals of the platform development process. Various TFF cassettes were screened before one with good recovery was selected. The impact of the TFF step on resin performance was then assessed. TFF was performed after clarification, then a capture step was carried out with two different amounts of AAV8 loaded

FIGURE 13

Characterization of protein impurities during the capture step using the POROS AAVX resin.

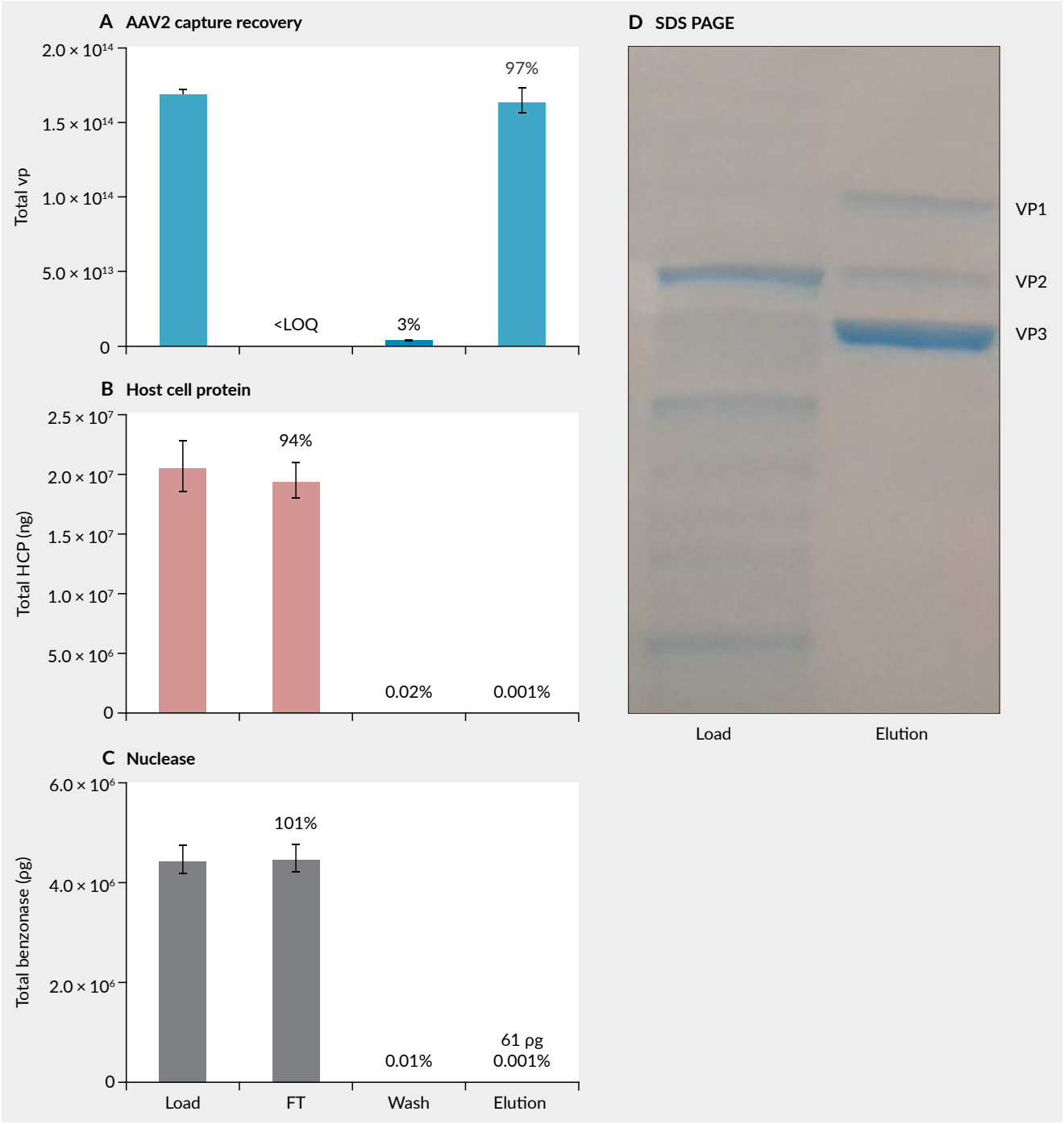
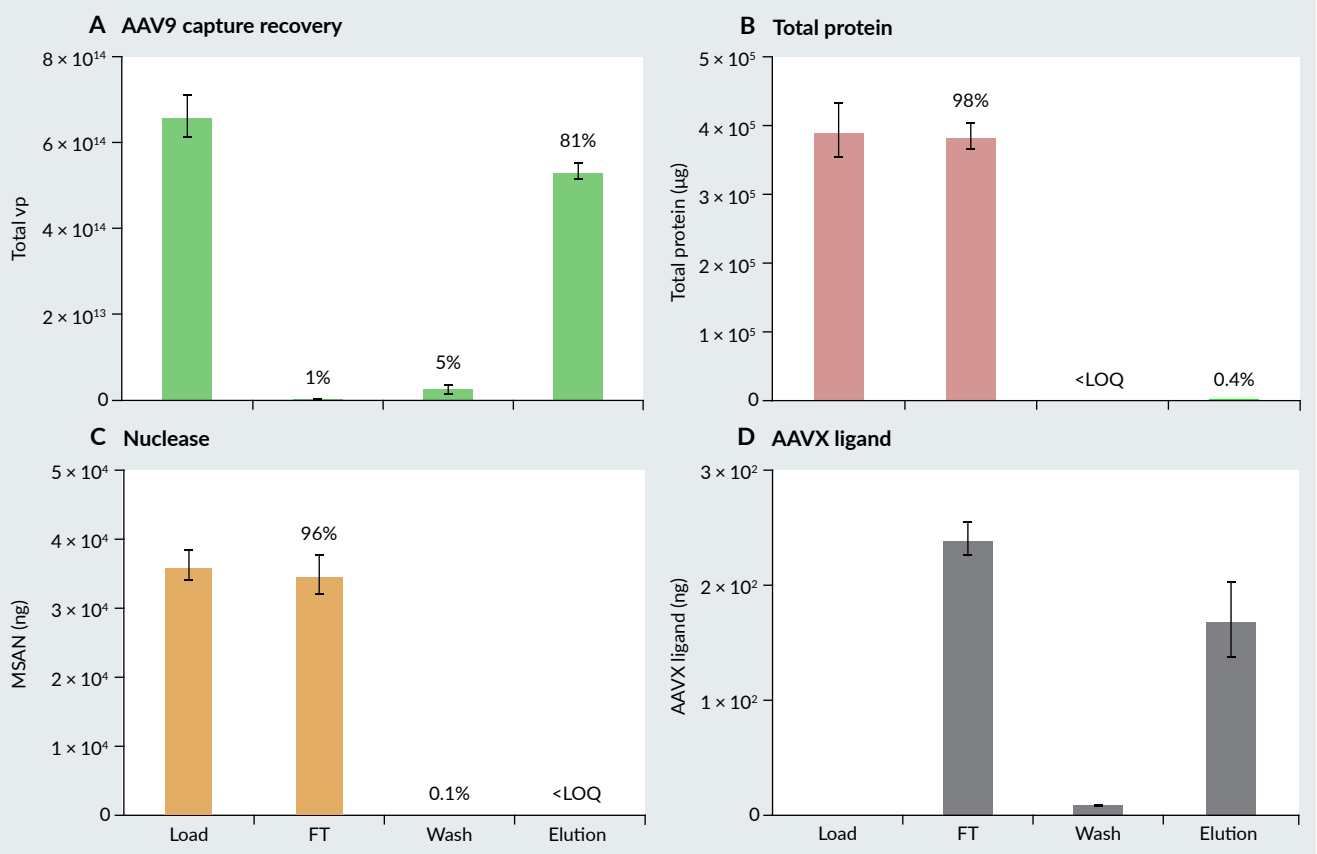


FIGURE 14

Characterization of protein impurities and of AAVX ligand during the capture step using the POROS AAVX resin.



(Figure 12). It was observed that the concentration step appears to slightly reduce recovery at low load, such as 1×10^{13} vp. However, 83% would still be considered a highly satisfactory recovery.

Another question to be addressed was whether the AAVX resin would perform similarly for different AAV serotypes. After working on elution parameters using AAV8, the AAVX resin was tested and evaluated on a number of additional serotypes (AAV2, 9, and 6), and good overall recovery of total vp was achieved for all serotypes tested.

IMPURITY REDUCTION CASE STUDIES

Characterization and reduction of protein impurities during the capture step was evaluated. Two case studies were performed to evaluate purity reduction. The first was an AAV2 run where a capture step was performed. As shown in Figure 13A, nothing was detected in the flowthrough, 3% of AAV2 was detected in the wash, and 97% in the elution. Host cell protein was then evaluated using analyzer kits specialized for HEK293. Almost all host cell protein was detected in the flowthrough, with small traces in the wash and the elution (Figure 13B).

Finally, this case study looked at another impurity introduced at the beginning of downstream processing: nuclease (in this case, Benzonase). ELISA kits were used for the Benzonase and as for the host cell protein, Benzonase was found in the flowthrough, with traces in the wash and in the elution (Figure 13C).

Additionally, SDS-PAGE was performed during these experiments (Figure 13D). In the load a number of impurities can be seen, but for the elution, mainly VP1, VP2, and VP3 are observed, i.e. the capsid protein.

A second case study was performed with AAV9. In this case, 81% recovery was achieved with the capture step (Figure 14A). Total protein was assessed using a Micro BCA Protein Assay kit, and 98% was found in the flowthrough, an amount below the limit of quantification in the wash, and 0.4% in the elution (Figure 14B). In this case, salt active nuclease was used and traces were found in the wash (Figure 14C). In this set of experiments, the AAVX ligand—an impurity introduced during the capture step—was assessed using an analyzer kit. As shown in Figure 14D, nothing is detected in the load, which is to be anticipated as there is no resin before this step. AAVX was detected in the flowthrough, wash, and elution. One important point to note is that this experiment was continued onto the polishing step. AAVX ligands were tested for at the end of the polishing step and were below the limit of quantification.

FIGURE 15

Effect of glycine- and citrate-based elution on polishing step with AAV9.

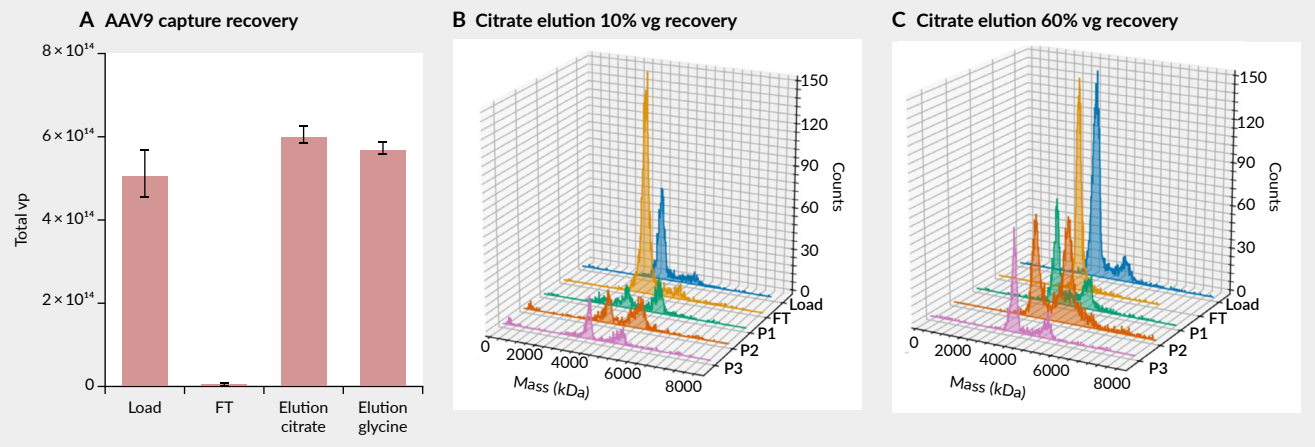
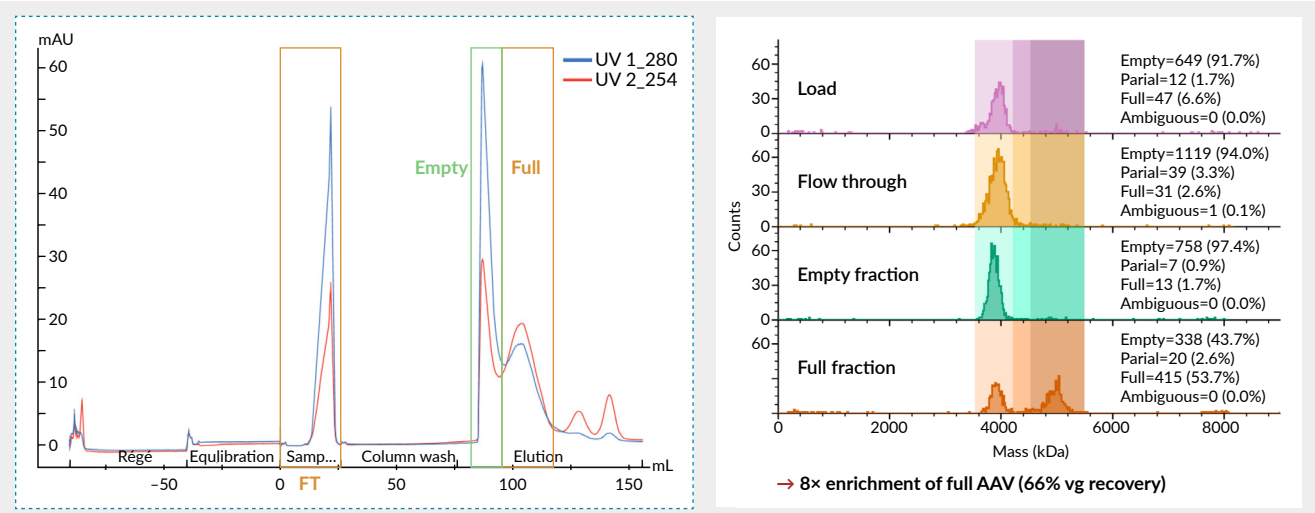


FIGURE 16

Poros HQ resin used for AAV full enrichment.



AAV POLISHING STEP

The polishing step is arguably the most technically challenging step in an AAV manufacturing process, and one that is quite specific to AAV vectors. The relationship between the capture and polishing step, and more specifically the choice of the elution buffer for the capture step, may have an important impact. In this set of experiments, the effect of glycine and citrate-based elution on the polishing step was studied. Utilizing AAV9 capture runs, both citrate-based and glycine-based elution buffers were tested. As shown in Figure 15A, both buffers worked well with up to around 100% recovery. Via chromatographic analysis, it was observed that around 10% vector genome (vg) recovery was achieved with citrate, as compared to glycine where 60% recovery was achieved (Figure 15B and C). Glycine-based elution was therefore found to have a positive relative impact on the polishing step.

USE OF POROS HQ RESIN FOR AAV POLISHING STEP

In collaboration with a client, ABL Europe developed an AAV2 polishing step using the POROS HQ 50 resin. An example chromatogram of the optimized process is shown in Figure 16. A challenge of this project was starting with a low amount of full AAV capsids (4–8%).

Looking at the flowthrough fraction, this was comprised mainly of empty AAVs, with around 97% being empty particles. The full fraction shows 53% full AAV (i.e. 8 × enrichment of full AAV) with 66% vg recovery.

CONCLUSION

To summarize, the pan-affinity ligand POROS resin AAVX offers high capacity, high yield, and high purity for different AAV serotypes. It is the AAV affinity resin of choice for ABL Europe, as it can be used for a variety of projects and performs well across different serotypes. The POROS HQ resin is efficient at enriching full AAV capsids. While data on AAV2 has been outlined in this article, internal work has also been performed on AAV9 and AAV8, which has also performed well and enriched for full fractions.

Q&A

Nicolas Laroudie (left) and **Quentin Bazot** (right)

Q Did you compare the performance of the AAV9- or AAV8-specific resin against the AAVX?

QB: I don't think we have done AAV8, but we have compared AAV9. The AAV9-specific resin works well. However, for us, the binding capacity was better with the AAVX resin for AAV9 compared to the AAV9-specific resin.

Q What analytical assay did you use to assess the total vector particles?

QB: We used ELISA methods. For AAV2, AAV6, and AAV8 we used an automated ELISA, and for AAV9, we used a classical ELISA kit.

Q Are you producing AAV on microcarriers or free suspension cultures?

QB: In the work I presented, we are only using suspension cells. I've worked with micro-carriers and AAV previously, before my time at ABL Europe, and it works well. Obviously, there are different challenges, and it requires different optimization because you are working with adherent cells. You need to find the right microcarrier, and look at the confluency and the cell growth. In my opinion, the process is easier with suspension cells, especially when you scale up and when considering GMP processes.

Q Should the process parameters be adapted to each serotype or is there a universal protocol used with AAVX?

NL: That is the beauty of the AAVX resin—it can be used as a platform as demonstrated by Quentin. The parameters that you have developed for one serotype can be used for other serotypes with very minimal adaptation. For exotic serotypes, you may have to play a little bit with the elution buffer to improve the efficiency of elution. You also have to be careful with some serotypes like AAV2, for instance, because it tends to aggregate somewhat. To prevent this, some salt must be added to the elution buffer. Therefore, some very small changes must be applied. Otherwise, yes, it is a universal protocol.

Q How should the CS with AAVX resin be scaled up?

NL: Resins based on POROS material are rigid and not compressible, so they are easy to scale up because the pressure versus flow curve is linear. It is really easy to predict the back pressure generated for a specific flow rate, whatever the scale of the column. Because it is rigid, back pressure does not depend on the column diameter but only on the bed height.

Therefore, when you want to scale up such a process, you maintain the bed height constant and simply increase the diameter of your column. The idea is to keep the velocity or the residence time constant when you increase your scale, maintain the number of CVs for each step and if possible, maintain the ratio between the volume of loading material to the volume of resin that is used.

Q Were you able to define a platform buffer to wash and elute all the AAV serotypes on AAVX?

QB: We worked on different process parameters and elution buffers. As mentioned above, for some serotypes it is better to add or to reduce the concentration of some buffer components. We have buffers that we prefer for different serotypes, especially AAV2, where we alter some things in order to avoid any problems such as aggregation.

NL: A recommendation and a good practice would be to have a little bit of surfactant and especially Pluronic F-68 in all the different solutions and buffers used for AAV purification. AAV tends to stick to plastics and to the walls of tubes. We sometimes see bad recoveries due to this, but the AAV is not still on the resin or on the column itself. I recommend paying special attention to this and to the analytics as well. Always take care to close the mass balance.

Q Was impurity or vector carryover quantified during resin reuse?

NL: On reusability, we have run some experiments where we use the resin up to 35 times. We have data regarding the potential carryover over 14 cycles, with the resin cleaned for each cycle with phosphoric acid and guanidinium HCl. We have not seen carryover of capsids in the eluate. Even after 14 cycles, it is below the detection limit.

QB: It is the same for us—we did some work on that, and we don't see any specific carryover of AAV between the runs.

Q Regarding POROS resin stability over time, there is no stated expiration date. Is there an effect of resin storage on its binding performance?

NL: We do not provide an expiry date with our POROS resins, but we support this with stability data that we provide to our customers when available. Typically, we provide stability data for three years with POROS CS AAVX, but that does not mean that the resin is not stable for a longer time. It is up to the customers—with our support, of course—to generate more stability data to fit with their specifications.

Q What is the capacity of POROS HQ resin for AAV?

NL: We don't have numbers for every serotype, but typically, we use the same column size for POROS HQ and AAVX when we have a full purification process. Nevertheless, for development purposes, we recommend loading much less on the POROS HQ: in the range of 3×10^{12} vg/mL for development, and then once your parameters are well established, you can increase the loading up to 2×10^{14} vg/mL, the same as on the AAVX.

Q What are your recommendations for cleaning the resin?

NL: AAVX or CS resins are not alkaline-resistant, so please don't use caustic to clean them—use acids, and especially, phosphoric acid or citric acid (0.1 M for cleaning after each cycle). This works pretty well. Guanidinium HCL up to 6 M can be used as well to improve the cleaning. POROS HQ is resistant to caustic so it can be cleaned with sodium hydroxide.

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BIOGRAPHIES

NICOLAS LAROUDIE worked at Généthon, France between 2001 and 2011 as Head of Downstream Development. He led a team developing and scaling-up purification processes for AAV, retroviral, and lentiviral vectors used for gene therapy treatments. He then joined Merck Millipore as a BioManufacturing Engineer and supported European customers for all DSP technologies with a strong focus on chromatography. In particular, he took an active role in the establishment of a fully continuous, large-scale disposable DSP process for the purification of a monoclonal antibody, within the framework of a large multi-company European consortium. Next he joined ThermoFisher Scientific in 2019 as Field Application Specialist for purification, supporting the technical implementation of POROS and CS chromatography products in south-western Europe.

QUENTIN BAZOT is a molecular virologist. He received his PhD from the École Normale Supérieure (ENS) de Lyon in 2012. After a postdoctoral training at the Imperial College London, he joined the CGT Catapult where he led viral vector process and manufacturing development activities. In 2020 Quentin joined ABL where he is currently leading AAV process development and innovation programs working towards the optimization of AAV production and purification.

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FASTFACTS

Transient and suspension: scalable yet flexible single-use process for multiple AAV serotypes

Johanna Viiliäinen, Scientist, Protein and Virus Production, Cytiva

The growing need for AAV in the gene therapy sector has increased the demand to develop better upstream processes, as this is still one of the biggest bottlenecks in AAV manufacturing. This FastFacts poster illustrates a process to scale-up HEK293T suspension cell culture in a single-use bioreactor. This process is suitable for multiple serotypes adopting different production media and transfection protocols.

AAV TRANSFECTION PROTOCOLS

Cytiva offers two different HEK293 production media, each with unique capabilities. HyCell™ TransFx-H medium is designed for micro- to large-scale transfection and production applications and for high cell yield and recombinant protein production. It is manufactured from traceable components according to cGMP guidelines, supports high transfection efficiency, and allows for direct or sequential adaptation. Alternatively, HyClone™ peak expression medium is a regulatory-friendly medium that is compatible with a variety of PEI- and lipid-based transfection reagents. It is designed for small- to large-scale transfection and production applications, as well as for high cell yield and robust virus production, making it the recommended virus production medium. It supports high transfection efficiency and allows for direct adaptation.

Figure 1. Transfection protocols.

Protocol 1 TransFx-H
VCD=1 × 10^6 cells/mL
DNA (µg/mL)=0.75
DNA ratio=1:1:2
(Rep/cap=helper: transgene GFP)
DNA:PEI MAX ratio=1:2

Protocol 2 peak expression
VCD=2 × 10^6 cells/mL
DNA (µg/mL)=2
DNA ratio=1.5:2:0.6
(Rep/cap=helper: transgene GFP)
DNA:PEI MAX ratio=1:2

We evaluated two different transfection protocols, one of which uses HyCell TransFx-H and another that uses HyClone peak expression medium **(Figure 1)**. In a shake flask, Protocol 1 has been shown to produce high viral particle titers with several AAV serotypes. Protocol 2 starts with a higher cell density to achieve high AAV titer production.

Figure 2. Scalable rAAV production process.

Shake flasks | ReadyToProcess WAVE 25 | Xcellerex XDR-10 | Xcellerex XDR-50 | Xcellerex XDR-200

SCALABLE RAAV PRODUCTION PROCESS

Figure 2 illustrates a scalable rAAV production process with ReadyToProcess WAVE™ 25 and Xcellerex™ single-use bioreactors. This process allows scale-up of a HEK293T suspension cell culture from small-scale in shake flasks (20 mL) up to a 200 L culture in the single-use bioreactor.

Figure 3 reveals titer data obtained when completing three rAAV5 production runs in Xcellerex™ XDR-10 bioreactors using Protocol 1.

Figure 4 shows the resulting titers from rAAV9 production in ReadyToProcess WAVE 25 and Xcellerex XDR-200 with Protocol 2.

CONCLUSION

We have shown a scalable process from shake flasks to up to 200 L, resulting in industry-relevant titers for several AAV serotypes. Peak expression medium supports high cell density growth of HEK293 cells, but both media produced titers >1 × 10^{13} VG/L and >1 × 10^{14} VP/L.

Figure 3. Summary of three rAAV5 production runs in Xcellerex XDR-10 using HyCell TransFx-H medium.

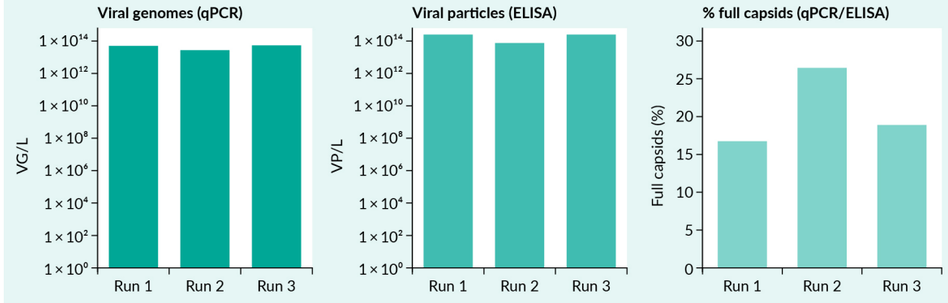
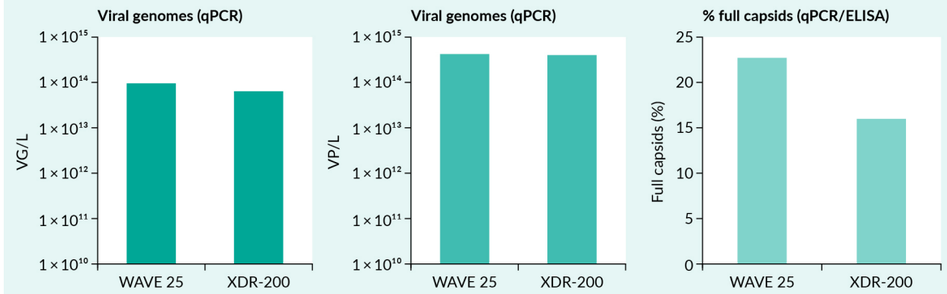


Figure 4. rAAV9 production in ReadyToProcess WAVE™ 25 and Xcellerex XDR-200 bioreactors with HyClone peak expression medium.



CELL & GENE THERAPY INSIGHTS

Cell & Gene Therapy Insights 2024; 10(2), 187; DOI: 10.18609/cgti.2024.027

In partnership with: cytiva

CELL & GENE THERAPY INSIGHTS

SCALABILITY

INTERVIEW

Advancing AAV production scalability: enhancing purity, productivity, and yield

Abi Pinchbeck, Commissioning Editor, *Cell & Gene Therapy Insights*, speaks to **Ashish Saksule**, Principal Scientist, Vector Core Lead, Vertex Pharmaceuticals, about ongoing efforts to improve vector productivity and titer, addressing issues with the separation of empty and full capsids, and increasing scalability in the downstream enrichment of AAV.

Cell & Gene Therapy Insights 2024; 10(2), 181–186

DOI: 10.18609/cgti.2024.026

Q What are you working on right now?

AS: I lead the vector core lab at Vertex Cell and Genetic Therapies (VCGT) overseeing end-to-end viral vector production and building in-house capabilities to support research and process development. My primary focus is on developing a scalable and universal platform for AAV, encompassing commonly used serotypes and engineered or modified AAV capsids.

Although modified AAV capsids look similar to the parental serotype of AAV, they can behave very differently during production and purification. There are many challenges in developing processes for new viral vectors, requiring novel innovations and technologies to solve.

Most of my work is focused on the optimization and development of robust purification platforms for viral vectors to efficiently produce higher yield and quality vectors. Recently, I have started transitioning towards a new position in cell therapy, leveraging the potential of stem cells including hematopoietic and pluripotent stem cells. This transition reflects the strategic move to contribute towards accelerating process development in the field of regenerative medicine with the therapeutic application of stem cells.

Q What are the key current challenges and pressure points relating to the scalability of recombinant AAV (rAAV) vector processes?

AS: AAV is a powerful vector technology most used in gene therapy clinical trials. However, achieving scalable packaging and production of rAAV remains challenging across all stages of development. Starting with the preclinical research phase, one key challenge is selecting the right AAV serotype or engineered version of AAV. The selection and development of scalable platforms across these variants of AAV can be challenging. Early developmental efforts into designing a universal platform technology across different AAVs can be beneficial.

Another critical issue is that the processes are not linearly scalable. Scientists often conduct optimization at a smaller volume and consider it to be proportionally linear when scaling up, but due to differences in scale-down and scale-up model devices, processes often cannot be scaled linearly, posing a major challenge during process development. Furthermore, the lack of orthogonal and real-time analytical techniques is a further challenge while developing and scaling up processes. As an industry, we still lack standardized analytical alignment, though there has been significant progress in recent years in providing viral vector-specific characterization tools, in addition to helpful specific guidelines from regulatory authorities.

Another challenge surrounds costs and resources. The scaling up of rAAV vector requires significant resources, including infrastructure, equipment, and skilled personnel. Cost–effectiveness and resource optimization are key considerations in scaling up production to ensure the affordability and accessibility of gene therapy treatments for everyone.

Q How does this compare to experiences with lentiviral processes?

AS: I began working with lentivirus at the beginning of my career, and I find it to be a more challenging vector than others. Lentiviral and AAV processes share some similarities, such as upstream transfection protocols and filtration techniques, but they exhibit notable differences in terms of harvesting, lysis mechanisms, structural characteristics, stability, processing time, and overall process yields.

Lentiviruses can integrate their genetic material into the host cell's DNA, and upon activation, they produce new viral particles that are released through budding and are automatically

"...rather than thinking about increasing productivity, we need to think about increasing the therapeutic index of the drug..."

released upon their production without the requirement for an additional lysis method. For AAV, an external chemical or physical lysis method is required, and the clarification will look different based on differences in the lysis method. Chemical or detergent-based lysis introduces additional challenges to the clarification step.

Enveloped viruses like lentivirus exhibit significant challenges around stability during processing, storage, and freeze–thaw cycles compared to non-enveloped viruses like AAV. We have observed significant vector titer losses of >50% of lentivirus if left overnight or through multiple freeze–thaw cycles. In contrast, AAV can be left for a few days and can tolerate multiple freeze–thaw cycles without significant losses in vector titer.

Lentivirus and AAV processes employ similar filtration technology for the purification and concentration of viral particles. However, the specific filtration requirements are different based on their size differences. Lentivirus has a larger size at approximately120 nm, while AAV is one of the smallest parvoviruses at around 20 nm. The sterile filtration of lentivirus poses unique challenges due to its size and the molecular weight cutoff of standard sterile filtration. Thus, there is a need for sterile filtration optimization, larger or multilayer sterile filters, and rigorous testing and quality control to ensure the removal of microbial contaminants while retaining lentiviral activity and titer. Overall, understanding these distinctions is essential for optimizing production processes for both lentivirus and AAV and ensuring the successful application of viral vectors in gene therapy.

Q Where might the required improvements in productivity/yield/titer come from? What promising technological innovations are you seeing within the space?

AS: The improvement of productivity and titer is the million-dollar issue that everyone in the industry is working to answer. These improvements are critical for advancing medical treatment and making viral vector-based therapies affordable and easily available to all patients in need.

First, rather than thinking about increasing productivity, we need to think about increasing the therapeutic index of the drug, thus reducing the dosage level while maintaining efficacy. This can be done in a few ways that utilize vector engineering and design. One method is developing vectors with enhanced selectivity. The greater the selectivity for the intended target or tissue, the less off-target effects and viral toxicity. Incorporating tissue-specific promoters and regulatory elements can be helpful here. Another approach is optimized administration and combination therapies. This includes the development of synthetic vectors, engineered capsids, and hybrid vector systems with optimized properties for therapeutic applications.

"Having real-time monitoring and automation within workflows can improve process consistency, reducing human error and thus increasing throughput."

To increase yields and titers, there are many ongoing efforts in upstream processing to optimize bioprocessing parameters such as culture conditions, including transfection optimization and cell line engineering to maximize productivity and yield. Utilizing novel cell culture systems such as perfusion bioreactors for suspension systems or microcarrier culture systems for adherent platforms can help enhance cell growth viability and viral vector productivity.

There is also ongoing innovation that can be incorporated into the purification process itself. Many processes currently used are traditional methods taken from the monoclonal antibody and protein industry and were not developed for use with viral vectors. These lack effectiveness considering viral size and the complexity surrounding the viral membrane and capsids. Advancing purification processes through the development of novel chromatography formats such as membrane absorbers and monolith devices will help to achieve better purification of viral vectors, particularly AAV. Having a universal affinity-based purification method and a scalable ion exchange chromatography system can help streamline the downstream processes to generate high purity and recovery of viruses.

Finally, integrating automation and robotics could enable increases in yield and quality. Having real-time monitoring and automation within workflows can improve process consistency, reducing human error and thus increasing throughput. Automated systems can also enable continuous operation and precise control over the production parameters, thus reducing batch-to-batch variability and ensuring resources are utilized effectively.

Q How are current solutions helping to address the challenge of empty/full capsid separation?

AS: Within AAV processing, the separation of empty and full capsids is a critical topic. We first need to attempt to address this from the upstream point of view. Increasing the efficiency of packaging will reduce the co-production of empty particles and increase the efficiency of producing full genome-containing particles. To further address this issue during purification, we need to introduce more real-time analytics to help identify the differences between the empty, partial, and full AAV particles so that effective purification techniques can be applied. For example, the isoelectric point difference between empty and full particles can be used to enable a baseline separation. However, this difference is very small, so we require novel tools to distinguish these so they can be effectively separated during the chromatography step.

For purification, many processes still utilize traditional methods such as ultracentrifugation. This technique can provide a high degree of separation and has been used for many early

academic or small-scale-based purification processes, however, it is not scalable. We need to develop more scalable processes such as ion exchange chromatography or a similar combination of chromatographic tools to separate empty and full particles utilizing baseline separation.

Q How can we achieve increased scalability in the downstream purification and enrichment of AAVs?

AS: There are multiple approaches to achieving downstream scalability. First, implementing process intensification strategies such as the use of high-capacity chromatography resins, continuous processing systems, and multi-column chromatography setup, can lead to higher throughput and increased scalability. Continuous chromatography systems can enable uninterrupted operation and higher productivity compared to traditional batch processing methods.

Another approach is technology integration. Using a combination of filter and chromatography in a single step or product can be beneficial to reduce the number of unit operations and product loss over multiple steps. One example from a vendor-specific application is the use of clarification with chromatography in a single unit operation. An example of this is Harvest RC chromatographic clarification, which utilizes filtration and ion exchange chromatographic purification in the same step. In our tests, this product has been shown to increase productivity through AAV clarification and reduce the need for an additional chromatographic purification step.

Another option is utilizing high throughput screening and optimization, which can enable the rapid evaluation of various purification conditions and parameters to identify the most efficient and scalable processes. Automated screening platforms can streamline the evaluation processes, thus allowing the rapid identification of critical process parameters. Then, the utilization of design of experiment and quality by design approaches can allow the systematic evaluation and refinement of these process parameters.

Q What are your key priorities, both for yourself and for Vertex Pharma as a whole, over the next 12–24 months?

AS: Currently, I am focusing on expanding my knowledge of scale-up and process optimization for viral vectors, in addition to looking at reducing the cost of goods to ensure these treatments are available at an affordable level to all patients.

Developing and training the next generation of skilled scientists in the field is also one of my key priorities. As I move to new leadership roles, I want to help other scientists develop viral vector-specific skills to help overcome the current shortage of skilled workers.

Finally, I am currently transitioning into the stem cell and regenerative medicine industry, focusing on establishing the cell therapy core lab at Vertex Pharma. These labs will help foster innovation and accelerate process development efforts for cell therapy at Vertex. I am looking

forward to this next chapter of my career so that I can contribute to scientific progress and learn as much as I can.

BIOGRAPHY

ASHISH SAKSULE is an accomplished cell and gene therapy process development expert, specializing in the bioprocessing of viral vectors. With over 10 years of experience, he has worked across various domains, including academic research, clinical stage vaccine development, and process optimization for cell and gene therapies. His expertise spans both upstream and downstream processing for viral vectors, including lentiviruses, AAV, live viruses, and proteins.

Ashish holds a Biotechnology degree from Harvard University and a Chemical Engineering degree from Michigan Technological University. Currently, he serves as a Principal Scientist and Core Lead at Vertex Pharmaceuticals, where he oversees the Vector Core facility and the Cell Therapy Core facility. Prior to this, Ashish contributed to the Rare Disease Gene Therapy program at Takeda and worked at MilliporeSigma on virus and gene therapy process development for AAV and LV. His passion lies in developing cutting-edge bioprocessing tools for cell and gene therapy, with the goal of making these life-changing treatments more accessible and affordable for all patients.

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AUTHORSHIP & CONFLICT OF INTEREST

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Volume 10, Issue 2

CELL & GENE THERAPY INSIGHTS

LATEST ARTICLES

INTERVIEW

Gene therapy is having a monumental year: how can the industry keep up with supply?

Lauren Coyle, Commissioning Editor, *Cell & Gene Therapy Insights*, talks with **Suman Subramanian**, Head of Commercial Operations, Catalent Cell and Gene Therapy, to discuss the major achievements in the gene therapy market in 2023, with a focus on reliable manufacturing processes and strategies to enhance predictability while reducing waste in gene therapy manufacturing.

Cell & Gene Therapy Insights 2024; 10(2), 259–265

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Q Can you discuss the key milestones achieved in the gene therapy market in 2023 and how these advancements have impacted the therapeutic landscape?

SS: Gene therapy is experiencing unprecedented growth, surpassing expectations, especially in the post-pandemic era. The past 2 years have served as a testament to this growth, marked by a significant number of approvals. In 2023, notable approvals included that of ELEVIDYS for Sarepta and ROCTAVIAN™ for BioMarin, each representing distinct drugs that have the potential to shape the industry. ELEVIDYS addresses muscular dystrophies, catering to a substantial patient population with a high demand for the product. ROCTAVIAN targets hemophilia A, making it equally significant in its therapeutic impact.

These approvals are shaping the future trajectory of gene therapy. Current estimates indicate that the market continues to grow at a double-digit rate, steadily approaching the

US$20–30 billion mark. The gene therapy space is indeed an exciting and dynamic field to be a part of at this point in time.

Q What supply chain strategies have proven effective in addressing accelerated timelines associated with gene therapy development?

SS: One of the most common inquiries we receive from new clients revolves around accelerating timelines when engaging with a CDMO. The biggest factor that impacts timelines in the gene therapy space, given its time sensitivity, is material procurement and analytical work. Post-pandemic, the focus on materials and supply chain has intensified due to significant lead times and challenges in procuring products.

Most CDMOs and innovator companies have increasingly adopted the strategy of standardized materials and specifications. Although the gene therapy market is not entirely commoditized, certain elements can be standardized. Moving towards standardized materials and specifications is crucial. This approach allows for different fulfillment strategies such as maintaining stock for certain materials. By maintaining a certain safety stock and swiftly pulling materials when needed, we can substantially reduce the material requirements' impact on the overall timeline, thus reducing the overall program risk.

Another important challenge in the gene therapy supply chain is material movements within the facilities. Establishing and implementing more traditional methods, such as trying to establish a supermarket approach for 'just-in-time' materials, is essential. In the bioprocessing industry, certain materials require just-in-time delivery, limiting their storage time at ambient temperatures. Having certain tools in place to precisely track when the materials are needed is critical. These strategies are proving to be efficient and will likely continue to play a significant role as the gene therapy space expands.

Q Focusing on the challenges in developing reliable and scalable manufacturing processes for gene therapies, what strategies or innovations can be implemented to ensure a robust manufacturing pipeline?

SS: The most important aspect is addressing standardization and the adoption of a platform process. Regulatory agencies, including the US FDA, have already indicated the importance of innovators, manufacturers, and CDMOs embracing a platform process. This approach provides a more predictable and streamlined manufacturing environment. Currently, platform processes have not reached the peak of where they need to be, and there is significant interest from clients moving towards the platform process.

The reasoning behind a platform process lies not with the amount of DoEs attempting to achieve the end goal, but with trying to perfect an improvement in the overall process. The focus is on directing energy into certain critical DoEs, based on experience and history, that have proven to be the most effective.

Another important consideration is equipment. Avoiding the creation of multitudes of equipment trains that complicate the process is crucial. A predefined equipment train will allow for easy integration into the platform. Equally important is not tying the equipment

"Current estimates indicate that the [gene therapy] market continues to grow at a double-digit rate, steadily approaching the US$20–30 billion mark. The gene therapy space is indeed an exciting and dynamic field to be a part of at this point in time."

train to a specific scale, which has remained a challenge in the industry. Multiple equipment trains with different scales can be offered, giving clients flexibility, but also remaining within the confines of being a platform process.

Lastly, enabling partnerships within the industry remains indispensable. Taking a product from a gene to the clinic largely relies on multiple partner collaborations, each contributing unique expertise, both in-house and external. Understanding how to work with partners and establish relationships to predefine your service level expectations, certain test timeframes during production, and establishing information upfront with clients is paramount.

Q Can you elaborate on the specific challenges in AAV development and manufacturing, and how the industry is working to overcome them?

SS: When looking at over 80% of the molecules pipeline, most clients within this space are small emerging biotech companies and this encompasses the demographic of our clients. Several of these challenges are coming up from an academic or a lab setting where there is not a lot of data to prove how these viral vectors can be scaled. Although there is a wealth of hypotheses and experiences behind the behavior of certain viral vectors and other biopharmaceuticals, inconsistencies and limited data remain.

There remains a lack of robustness in the process of transitioning molecules from the clinic to late-stage manufacturing, with GMP readiness being a consistent challenge. The documentation and adaptation of these processes hold the potential to move towards automation in the future.

While bioprocessing, specifically gene therapy, is not a fully automated process yet, standardization and moving toward a platform may enable some automation. Initiatives such as adapting to paperless technology and implementing e-batch records will enable advancements to overcome these challenges.

Q Integrated solutions from raw materials through commercial supply are crucial for gene therapy development. How can companies effectively plan early for a commercial-ready process, and what role does collaboration with experienced partners play in achieving this goal?

SS: Gene therapy manufacturing can be inherently complex, comprising of various processes, materials, and technologies. Clients want an end-to-end provider capable of guiding their gene or sequence from inception to the clinic. Companies that can provide the end-to-end

> "Having partners with extensive regulatory landscape experience and previous success in taking molecules through the regulatory approval cycle is indispensable."

supply plan, or as we call it, an integrated supply plan, play a pivotal role. It entails seeking the starting material and strategizing from day one to the endpoint, such as the clinical setting.

Clients are actively looking for partners who simplify the supply chain and who can assemble various pieces of the process together, while also taking accountability for bringing the product to the clinic. This approach simplifies the program governance, eliminating the challenges of dealing with multiple partners as well as simplifying structures, commercial models, and coordinating dates. It de-risks the entire supply chain and also reduces the complexity.

Q Further to that, how can a partner with commercial manufacturing experience contribute to the success of gene therapy development, both in terms of solidifying processes and navigating the regulatory pathways?

SS: The field is continually evolving with an emphasis on the regulatory landscape. Having the right partner who comprehensively understands the regulatory agencies' requirements, possesses familiarity with global expansion, and holds experience and state-of-the-art facilities is key. This is partly due to regulatory agencies and their guidelines undergoing frequent changes.

Experience in dealing with the FDA and international agencies, what they are seeking, understanding the audit requirements for facilities, and adapting specificities are all important considerations. Clients often overlook these aspects earlier in the process, leading to a bottleneck as they progress through filing, IND submission, and the later stages of clinical phases.

Having partners with extensive regulatory landscape experience and previous success in taking molecules through the regulatory approval cycle is indispensable. Establishing protocols with companies such as Catalent, who can bring valuable experience, is a critical aspect of ensuring success in gene therapy development.

Q Given the importance of supply chain management and delivering gene therapies to clinical trial locations, what specific needs and challenges should be considered and how can they be effectively addressed?

SS: Traditional methods of managing the supply chain and its various aspects remain in place but one key aspect that has an increasing importance is the focus on business continuity planning. Dual or multi-sourcing of critical materials is vital, not only from a commercial competitiveness in pricing standpoint but also to ensure the security of the supply chain.

Several emerging excipients and consumables are becoming new considerations in finding the right suppliers, for example suppliers with an existing Drug Master File, and securing

long-term partnerships are essential. Lessons learned from COVID-19 emphasized the importance of securing partnerships and establishing long-term contracts to help enable the right level of discussions with partners, ensuring service level expectations and lead times are met.

Another important consideration is storage capacity, as most gene therapy products are single-use and can take up significant space and consume substantial amounts of product for processes. Enabling strategic storage capabilities, both within and outside networks, allows swift product movement. Consideration also needs to be taken when looking at partners to help expand support for the patient and product needs.

Q How is the space evolving in terms of the ability to leverage platforms, for instance, in platform assay development? What are some of the keys to capitalizing on the benefits of this?

SS: The first considerations when discussing a platform are cost-effectiveness and reduced timelines due to the plug-and-play nature of a platform. Each time a platform is used, the redundancy and development are minimized, specifically in terms of analytical methods and associated documents.

In certain cases, it is essentially a plug-and-play scenario, resulting in a significant amount of time and rework being saved. This, in turn, has cost benefits passed on to clients, further reducing timeline risks due to the ability to execute specific processes with a specific platform, multiple times across several years.

On the analytics side, adopting platform assays involves adapting the client's gene of interest or other specific needs into an existing platform. This approach avoids the need to redevelop an entire assay, saving weeks or even months. Suitability assessments can be conducted to better understand the suitability of the platform within weeks of engaging the client.

Another significant aspect is the predefined and templated report summaries of how the assays and platforms perform. This not only helps to shorten the cycle of the work but also reduces the overall timeline and associated costs.

Q 'Right First Time' is a key aspect of the Catalent approach. Can you pick out some specific areas where this is particularly important?

SS: The concept of 'Right First Time' is not new to Catalent and has always existed in the industry, but it is evolving in various spheres of life sciences. In today's landscape, with more focus on platforms and standard processes, the expectation is to achieve accuracy the first time. Speed is of the essence to the market and not achieving 'Right First Time' could mean losing the race to the market.

The most important complexity is the tech transfer of a process. Whether it is transferring a process within a CDMO's network of facilities or from a client, it is a critical phase. Implementing an efficient protocol with defined stage gates for decision-making along with risk identification significantly reduces the risk and increases the chance of getting it 'Right First Time'.

Effectively measuring key performance indicators and metrics during the manufacturing process plays a vital role. Most manufacturing processes, whether platform or non-platform,

"Implementing an efficient protocol with defined stage gates for decision-making along with risk identification significantly reduces the risk and increases the chance of getting it 'Right First Time'."

can have predefined success criteria of what is expected, ensuring the right schedule, people, and talent at the right steps of the process. Regular stage gates, checkpoints, and communications greatly enhance the chance of success. Conversely, getting it wrong the first time can result in a ripple or cascading effect of losing a slot in the schedule that was meant for another program or another batch.

Q Finally, the focus on simplification of the manufacturing process includes minimizing unnecessary tweaks and reducing the number of supplier notes. Can you discuss some examples of successful simplification strategies in gene therapy manufacturing, and how they have positively impacted efficiency and reliability?

SS: Simplification always leads to more predictability, leading to less waste in the overall process and a significant improvement in the COG. COG is by far the single most important aspect looked at to scale programs, especially for rare diseases where batch yield may be limited.

Our continuous improvement teams play a pivotal role in identifying opportunities to simplify processes, eliminate waste, and cut redundancies. This focus on simplification not only has a measurable impact on the COG but also addresses areas where deviations in the process may occur.

Efficient application and enforcement of corrective and preventative actions are essential for maintaining the facility. Given the specificity of the area, historical data can help to identify potential deviations, ultimately reducing timelines, since the ability to manufacture correctly and release the product to patients is of utmost importance.

Manufacturing, along with effective documentation and reduced deviations all allow for the release of the product or disposition. Further, having trained operators within the organization to comprehend the nuances reduces delays and the associated risks of these complex operational aspects.

Predictable costs and lower contingencies are key considerations. Understanding the platform process, identifying waste, and utilizing tools such as enterprise resource planning and planning tools, contribute to predictable costs. This approach allows for an accurate estimate of program costs and minimal contingencies, ensuring that clients don't bear unnecessary costs later in the process.

BIOGRAPHY

SUMAN SUBRAMANIAN joined Catalent Cell & Gene Therapy Commercial Operations in 2022. In his role, he is responsible for integrating commercial strategy and fostering partnerships across the organization. Suman leads the BU commercial operations council/partnership to deliver best-in-class commercial performance. Before joining Catalent, he spent 6 years in various roles at West Pharmaceutical Services. During his time

there, he was tasked with integrating the world's largest API manufacturing facility into an existing supply network with over 3,600 supply nodes in Kalamazoo, MI and Puerto Rico.

Subramanian obtained a MSc in Pharmaceutical Systems Management from Rutgers, and Bioinformatics from the New Jersey Institute of Technology. In addition, he is fluent in speaking six languages and proficient in writing in them.

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AUTHORSHIP & CONFLICT OF INTEREST

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We hope you enjoyed reading this interview.
You can also listen to the recorded podcast here:

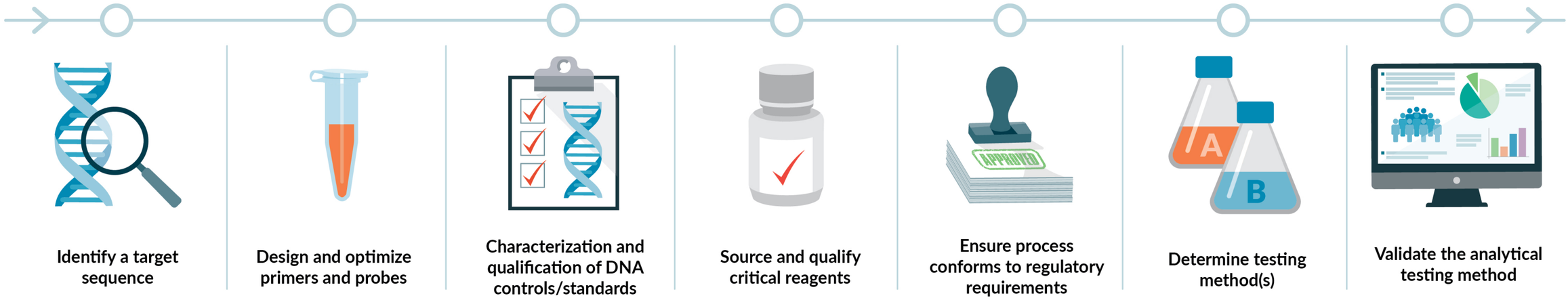
LISTEN NOW

What goes into developing an in-house method for quantitation of residual host cell DNA?

Ilaria Scarfone, Field Application Scientist, Pharma Analytics, Thermo Fisher Scientific

Robust product characterization and quality testing of cell and gene therapies ensures the safety, efficacy, purity, quality, and potency of the final therapeutic product. A key consideration for the process and product characterization process is deciding between in-house development of a test method, or adopting a commercial kit for quantitating residual host cell DNA in the manufacturing workflow. This poster explores the steps and challenges that go into developing an in-house residual testing assay.

Developing sensitive and robust residual DNA analytical assays requires a significant investment of both time and resources. For example, the first step is developing a sensitive and specific quantitation assay and pairing that with a sample preparation method that can enable efficient recovery of trace levels of DNA from complex matrices. Once that is accomplished, the next step in development involves the laborious task of developing and completing the documents that are required for a new analytical method suitable for use in cGMP applications, including standard operating procedures for the test method, critical reagent preparation and qualification, equipment operation and maintenance, method development reports, and validation protocols and reports. In addition, a key requirement is developing a robust protocol for purification, quantitation, qualification, stability assessment, and storage of quantitative standards that ensure consistent, accurate performance from lot to lot. The steps required to develop an in-house essay are illustrated in **Figure 1.** Each step demands distinct considerations.



1. The first steps involve identifying a target sequence so that specific primers and probes can be designed and optimized. Identifying the right target sequence necessitates the sourcing of internal expertise. If recruitment of the requisite in-house expertise is required, then the development process will be both lengthy and costly.

2. Next, characterization and qualification of quantitative DNA controls/standards need to be performed. This step can be resource-intensive because, to avoid cross-contamination, the purification of DNA standards must be carried out in a separate lab from the lab where routine testing occurs. Contamination from the DNA standards could interfere with routine testing and result in inaccurate data.

3. Critical reagents such as qPCR Master Mixes should then be sourced, evaluated, selected, and qualified. A sample preparation method that is effective for multiple test sample matrices and product concentrations must also be selected or developed. Even after development, every new lot of critical reagent needs to be qualified.

4. Subsequently, the overall method performance across the process must conform to regulatory requirements. If the developed assay proves to be insufficient in meeting regulatory guidelines, this can lead to increased costs and often more importantly, timelines to commercialize the drug product.

5. The same challenge applies to the final steps, which are to determine and validate analytical testing methods according to specific regulatory guidance. Host cell DNA should be validated as per the ICH Q2 (R1) guidance for analytical procedures. Establishment of validation protocols, execution of that validation, and generation of a report can be time consuming.

Commercial, fully integrated kits for residual host cell DNA testing, like the Applied Biosystems™ resDNASEQ™ Residual DNA Quantitation Kit, offer a solution to the challenges involved in developing residual DNA analytical assays. This commercial solution can dramatically reduce implementation timelines, essentially eliminating the time required for method development. This allows labs to quickly move to process specific method qualification and following that, generation of valid results.

Watch the webinar here

Read the full article here

CELL & GENE THERAPY INSIGHTS

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In partnership with: ThermoFisher SCIENTIFIC

FASTFACTS

Dissolvable microcarriers: a modular, high-yield solution for seed train through production

Tom Bongiorno, Field Application Scientist, Corning Incorporated

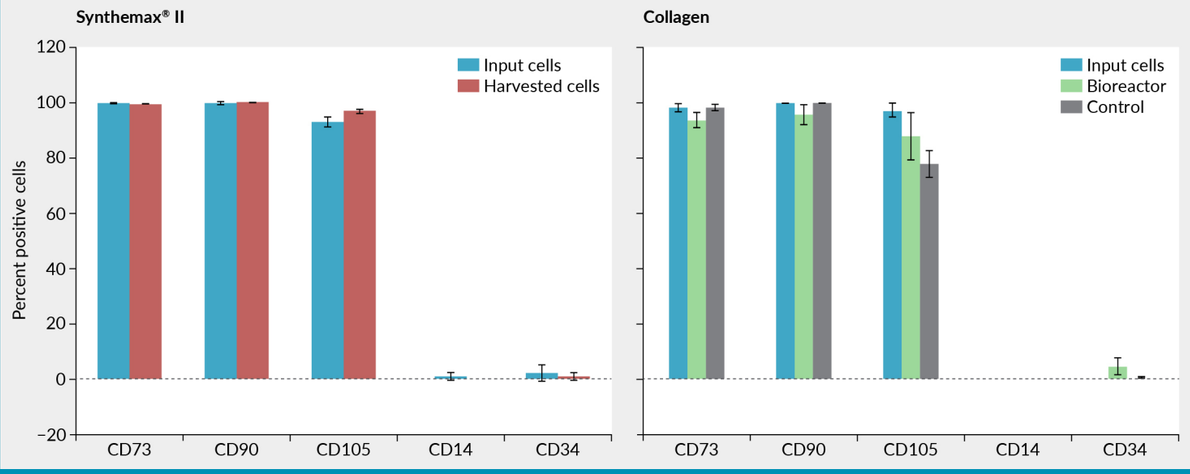
Dissolvable microcarriers offer an attractive solution for adherent cell culture. Here, we describe the advantages of microcarriers for adherent cell scale-up and provide data on the performance of dissolvable microcarriers for mesenchymal stem cell expansion.

Scaling up stem cell culture using traditional planar or multi-layer surfaces consumes large amounts of operating and incubator space. Microcarriers offer reduced space requirements and excellent scalability but can be challenging to harvest efficiently in a large-scale process. Dissolvable microcarriers offer a new approach, with drastically improved cell harvest recovery and reduced labor requirements. Paired with surface chemistry specifically designed for stem cell attachment and expansion, dissolvable microcarriers provide a novel scale-up solution for cell and gene therapy workflows.

WHY CHOOSE MICROCARRIERS?

Microcarriers, whether plastic or dissolvable, offer excellent modularity and are compatible with various upstream platforms, including cells from planar culture or direct from thaw. Additionally, microcarriers scale well, offering a solution for seed train through production. Disposable spinner flasks can be paired with microcarriers for volumes up to 3 L. Bioreactors, including traditional stirred-tank and novel vertical wheel bioreactors, can be paired with microcarriers for volumes in excess of 2,000 L.

Figure 1. Surface marker expression of input and harvested cells expanded on Synthemax II and collagen dissolvable microcarriers.



WHY CHOOSE DISSOLVABLE MICROCARRIERS?

Corning® Vanish™ dissolvable microcarriers offer several important advantages over plastic microcarriers:

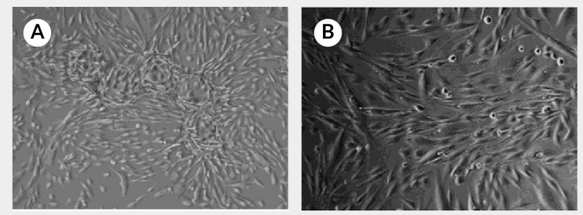
- Up to 2× cell recovery versus polystyrene microcarriers (>90% versus 60%)
- Bead-to-bead cell transfer enables homogeneous expansion and growth
- Proven surface chemistry, optimized for common cell types used in cell and gene therapies
- Animal component-free offerings
- Plastic-free microcarrier core, isolated from citrus peels

CASE STUDY: MESENCHYMAL STEM CELL EXPANSION ON DISSOLVABLE MICROCARRIERS

Mesenchymal stem cells (MSCs) expand successfully on the Corning® Vanish™ dissolvable microcarriers, including Synthemax II- and collagen-coated versions. Throughout the expansion phase, viability is maintained at 90% or greater.

With any adherent cell culture platform, it is particularly important to characterize the cells after removal from the surface. MSCs on both Synthemax II- and collagen-coated dissolvable microcarriers displayed characteristic surface marker expression (positive for CD73, CD90, and CD105, and negative for CD14 and CD34) **(Figure 1)**. The cells also demonstrated a capacity for tri-lineage differentiation.

Figure 2. MSC morphology after (A) migration from intact microcarriers to a well plate (no dissolution), or (B) replating after microcarrier dissolution.



The cells were karyotypically normal, and normal MSC morphology was observed following either migration from the intact microcarriers to a well plate **(Figure 2A)** or microcarrier dissolution **(Figure 2B)**. Cell expansion on the dissolvable microcarriers has also been demonstrated with DF-1 cells for vaccine production applications.

CONCLUSION

Corning Vanish dissolvable microcarriers offer a modular solution for seed train through production, enabling adherent cells to grow on the same platform throughout. The microcarriers dissolve in minutes, improving cell harvest efficiency and reducing the associated labor and material costs. The dissolvable microcarriers are derived from fit-to-purpose materials, including a plant-derived core that offers a favorable by-product profile following dissolution.

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