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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON
New horizons in immunotherapy

Guest Editor
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CELL & GENE THERAPY INSIGHTS

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EDITORIAL

European research infrastructures join forces to provide innovative cancer research services across Europe—how can they support the cell and gene therapy developer?

Emanuela Oldoni, Patricia Carvajal-Vallejos, Florence Bietrix & David Morrow

Research infrastructures (RIs) are facilities that enable the research community to use their specific technology platforms, resources, and services to conduct research and foster innovation. Europe's RIs exist to solve systemic bottlenecks, pushing forward the frontiers of scientific disciplines, and enabling transformative technological development ranging from translational medicine to biobanking to clinical trials. In 2022, European wide RIs, including oncology experts and patient associations, came together to battle cancer by providing innovative cancer research services across Europe. CanSERV is a new EU-funded project under the Horizon Europe program that aims to provide a comprehensive portfolio of oncology-related research services to all scientists in EU member countries, associated countries and beyond. The CanSERV portfolio provides capabilities across the full breadth of cancer research and the translational pipeline, from basic biology research through to the delivery of late-phase clinical trials. Central to this project will be to facilitate cancer therapeutic developers with a set of robust, standardized, and validated assays for the development of novel Advanced Therapy Medicinal Products including cell and gene therapies, therapeutic vaccines, and RNA-based immunotherapies. Through this new initiative, the canSERV consortium will strive to meet the needs of academic and industry users from the EU and beyond to create a more effective, streamlined, and defragmented European oncology research infrastructure landscape.

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WHAT IS canSERV? HOW DOES IT WORK?

Cancer research requires concerted efforts if we want to accelerate the development and implementation of anticancer solutions. With this in mind, the EU-funded canSERV [1] project brings under the same umbrella existing oncology research infrastructures across Europe. canSERV is coordinated by BBMRI-ERIC [2] which is a European research infrastructure for biobanking that has brought together 19 research infrastructures (RIs) and their connected service providers all across Europe with a total EU-funding of €14.8 million to support the most promising cancer research. This includes world-class European life science RIs (BBMRI, EURO-BIOIMAGING, ELIXIR, EU-IBISBA, EuroPDX, EU-OPENSCREEN, INSTRUCT, EATRIS, INFRAFRONTIER, EMBRC, ECRIN, MIRRI, ARIE, CCE, EORTC and IARC) [2–17] that collectively covers all aspects along the development pipeline for oncology R&D, including providing users a guidance for navigating the entire translational value chain. These RIs provide to cancer research a range of services through transnational access (TNA) programs allowing researchers to access facilities, equipment, and resources that they may not have access to in their institution or at a national level. Scientists from academia and industry that want to benefit from the support of canSERV can submit proposals once calls open in the coming weeks, which will be evaluated based on their feasibility and scientific merit (independent peer review) following the process outlined in Figure 1.

Support for TNA to the canSERV services can be provided to researchers from public and private entities worldwide. Several limitations are applied to users from non-EU/Associated states as they can only represent 20% of the total budget dedicated by canSERV to TNA activities. As the aim of TNA activities is to facilitate access to unique services (Figure 2) and resources usually unavailable to most scientists, and to promote research with a cross-border dimension, selected projects must be conducted in a country different from that of the users' affiliation. Proposals can be submitted by a group of scientists through specific calls which are launched periodically. The first open call for canSERV will open in Summer 2023 and similar open calls are planned for the next two years as long as funds are available. Young scientists (PhD students, post-doctoral or early career researchers), scientists with limited prior experience in using facilities or resources through TNA, and Small &

FIGURE 1

How can you access free CanSERV services?

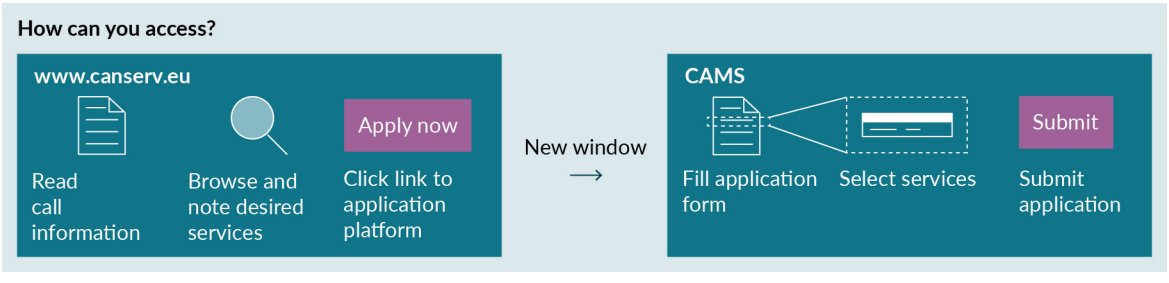

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FIGURE 2

Service catalogues available through canSERV.

Medium Enterprises (SME) are particularly welcome to apply for these services and support (Figure 1).

HOW DOES canSERV SUPPORT THE CELL & GENE THERAPY DEVELOPER?

Providing a wide range of state-of-the-art services that speed up each therapeutic and biological development process is core to the CanSERV mission (Figure 2). Central to this is the provision of robust, standardized, and validated assays for the development of novel Cell and Gene therapy products which have shown great promise in the fight against cancer. EATRIS, the European Research Infrastructure for Translational medicine [18–23] has a dedicated Advanced Therapy Medicinal Products (ATMP) platform which offers over fifty-one state-of-the-art European centers covering the entire ATMP production and development pipeline. The platform provides the most qualified and state-of-the-art technologies for the critical issues in this development area, such as specialized GMP facilities, imaging facilities for *in vivo* animal studies, availability of dedicated/ tailored animal models, clinical expertise, and access to patients for high prevalence and/or rare diseases, as well as to clinical facilities. The platform also includes a network of experts for regulatory affairs specialized in the ATMP field to ensure compliance with the preclinical and clinical development guidelines within Europe. Specific EATRIS ATMP resources available through CanSERV include services that facilitate the engineering of chimeric antigen receptor (CAR-T), CAR-NK, CAR-NKT, and dendritic cells for solid tumors and for highly immunosuppressive tumor microenvironments. In addition, EATRIS is also providing services that address cancer-specific viral vectors carrying therapeutic genes and/or mRNA sequences that block cancer growth and spreading. Services that evaluate and validate ATMP's efficacy on *in vitro* cancer cell (established or patient-derived) platforms are also accessible through the project

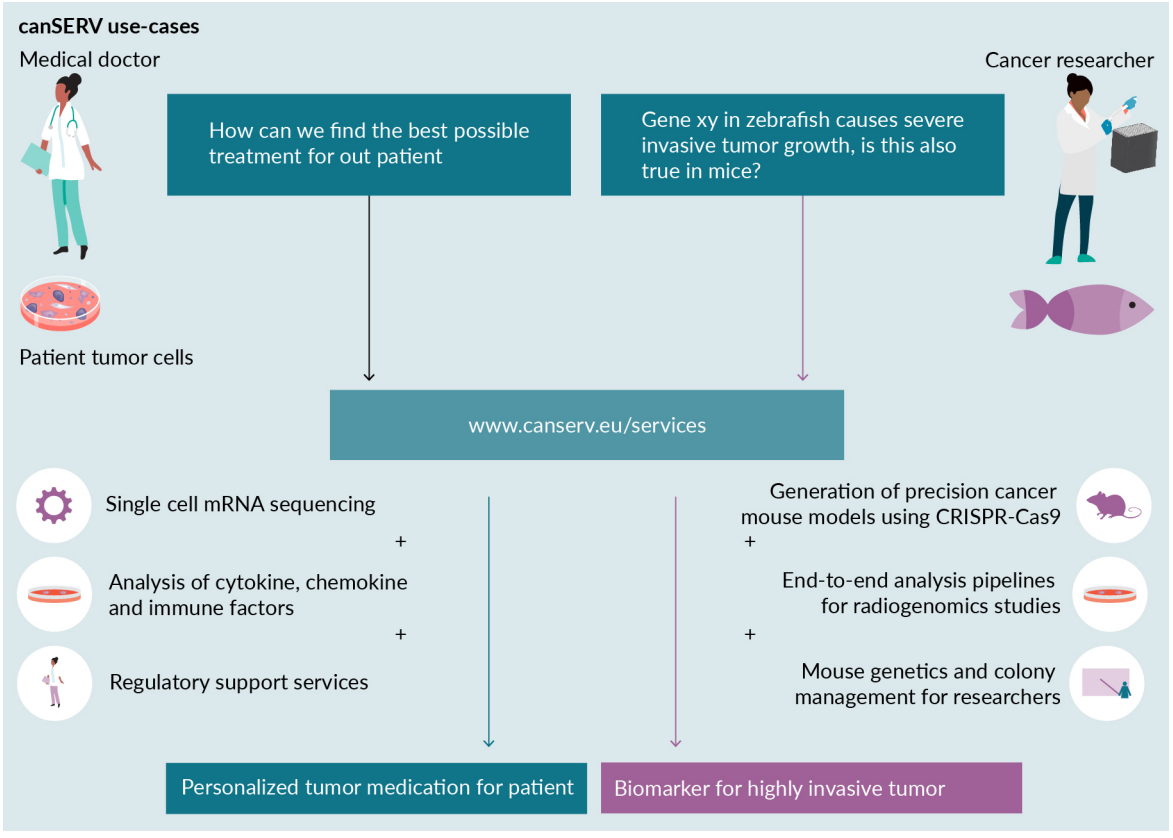
including support for the *in vivo* testing and validation of ATMPs in relevant, tried and tested animal models. Finally, the ATMP developer will have the ability to apply a broad range of free *in vitro* and *in vivo* immune monitoring and profiling technologies to investigate the associated immune response to these therapies, essential to their development towards the clinic (Figure 3).

Outside of the specific support to the cell and gene therapy developers listed above, EATRIS also brings the expertise and resources of twenty-two of its 150 institutions into the canSERV project to support different therapeutic areas in cancer research. Together, they will lead the process to provide high-quality services that will support the optimization of existing screening programs, the advancement of novel approaches for screening and early detection, identification of new biomarker sets, as well as contributing to the development of novel therapeutics based on molecular predictors. EATRIS and its partners will provide the necessary expertise and guidance on regulatory requirements and methodological standards for personalized medicine research in addition to support activities on innovation management to help accelerate adoption in the Personalized Oncology domain.

Finally, through the canSERV project, the European Network for Personalized Oncology (ENPO) aims to provide state-of-the-art guidance and support to accelerate the implementation of personalized oncology. The ENPO will establish the European Molecular Tumor Board Network (EMTBN), which aims to guide the establishment of MTB Standard Operating Procedures (consensus standards) and the set-up of a public registry of MTB recommendation outcomes. The ENPO and

FIGURE 3

CanSERV use case example.



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EMTBN will not be limited to the canSERV consortium partners but open to the wider community to be as inclusive and engaging as possible. Although participation in the networks is on a voluntary basis, some specific activities can be considered for cost reimbursement for those who will actively contribute. Registrations are now open for interested parties [24].

CONCLUSION

The mission of the canSERVs project and the RIs, patient organizations and Cancer societies involved, is to make cutting-edge and customized research services available to the cancer research community to enable innovative R&D projects and foster precision medicine for patients benefit across Europe (Figure 3).

By connecting, coordinating, and aligning existing oncology and complimentary RIs, canSERV can capitalize on the critical mass of experts and innovative services available including those that facilitate next generation ATMP development offered by canSERV and their extended network. This new concept of facilitating research will lay the foundation for a long-standing pan-European collaboration that can foster innovative research projects and bring precision medicine solutions to benefit the cancer patient community for years to come. canSERV represents an exciting opportunity to fill in the gaps in the developmental programs of novel cancer therapeutics. It is our hope that the cancer research community avails of this fantastic opportunity, and we wish you luck in the process!

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9. EATRIS. eatris.eu.
10. Infrafrontier. infrafrontier.eu.
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AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

NEW HORIZONS IN IMMUNOTHERAPY

SPOTLIGHT

EXPERT INSIGHT

Harnessing chimeric antigen receptor (CAR)-T cells as a potential treatment for Alzheimer's disease

Lauren Sarko & Krishanu Saha

Alzheimer's disease (AD) significantly burdens global healthcare systems given limited treatment options to delay or stop disease progression. Chimeric antigen receptor (CAR) T cell therapy, an immunotherapeutic approach that has produced remarkably effective responses in cancer, offers a potential avenue for the treatment of AD. Here, we discuss three significant challenges of adapting CAR-T cell therapy for AD: (i) identifying a suitable antigen target; (ii) limited permeability of the blood–brain barrier; and (iii) long-term persistence and durability of manufactured CAR-T cell products. Potential strategies to overcome these hurdles provide an attractive opportunity to revolutionize the treatment for AD and potentially other neurodegenerative disorders.

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Alzheimer's disease (AD) is a neurodegenerative disorder often characterized by the accumulation of extracellular plaque deposits of amyloid-β peptide (Aβ) as well as neurofibrillary tangles (NFTs) consisting of hyperphosphorylated tau [1]. Aging is the most common risk factor for AD. With increased life expectancy and an aging population, the World Health Organization (WHO) projects that individuals impacted by dementia, a primary cause of disability in elderly individuals, will dramatically increase from 55 million to over 135 million people by 2050 [2–4]. Despite significant recent progress in biologics and cell/gene therapy, only a handful of therapies targeting the brain have been approved, leading to an annual cost in the USA of ~$0.8 trillion [5]. Finding potential treatments that restore cognitive and physiological function as well as improve overall quality of

life for these individuals is crucial for those suffering and genetically predisposed for developing AD.

To date, there has been little to no success in the translatability of promising preclinical results into therapeutic interventions for AD due to the complex etiology involving genetic, environmental, and lifestyle factors. There are several cellular and molecular hypotheses for the cause of AD: the dysfunction of cholinergic function in neurons, the accumulation and deposition of oligomeric or fibrillar Aβ peptide, excessive or abnormal phosphorylation of tau protein, and more recently the accumulation of senescent cells impacting brain cognition [6–8]. While there is no clear consensus for what causes AD pathogenesis, there are various interventions aimed to help manage symptoms and improve quality of life. Presently, there are seven drugs approved by the US Food and Drug Administration (FDA). These include cholinesterase inhibitors to improve cognitive symptoms of AD (rivastigmine, galantamine, memantine, and donepezil) [9–12] as well as monoclonal antibodies that target Aβ and reduce plaque burden (aducanumab, lecanemab) [1,13,14]. These interventions, while helpful, are unable to reverse or stop AD progression. Emerging evidence has shown the development of an active immunotherapy may be the key to managing and protecting the brain from cognitive and behavioral deterioration [3,15,16].

The adaptive immune system plays a significant role in the pathophysiology and progression of AD [17]. In particular, T cells are dramatically altered in AD due to aging; however, the mechanisms behind these changes remain poorly understood. With aging there is an observed decrease of regulatory T cells (Tregs), limiting the ability of T cells to mitigate inflammation, allowing a pro-inflammatory environment to persist [18]. Due to this decrease in Tregs, there is an increase accumulation of senescent T cells, which are defective in their ability to proliferate and secrete inflammatory factors [19]. Furthermore, cytotoxic CD8 T cells have been shown to be impacted in AD with increased presence of exhaustion markers in cytotoxic CD8+ T cells within AD tissue [20]. Exhaustion of T cells is a state of dysfunction in which they lose their ability to attack their specific target due to chronic antigen stimulation and can arise from chronic neuroinflammation [21]. One possible hypothesis for AD therapy would be to restore the adaptive immune system balance in AD individuals by correcting the dysfunction of aged CD8+ T cells as well as increasing the number of functional Tregs present in the brain.

Cell therapy is one promising avenue to restore the disrupted immune balance observed in AD [22,23]. CAR-T immunotherapy, in particular, consists of T cells that have been redirected to attack a desired target antigen without any need for peptide presentation. To generate these cells, T cells are isolated from a patient and bioengineered *ex vivo* to incorporate a synthetic receptor. The receptor contains a single chain variable fragment (scFv) that binds to a target cell surface antigen. After engineering, the modified cells are injected back into the same patient (Figure 1). These therapies have shown remarkable clinical response with six currently approved FDA products for hematological malignancies and hundreds of clinical trials in progress [24,25]. As the field of CAR-T therapy continues to expand outside of cancer, there are still several challenges with adapting this therapy platform to target and eliminate antigens shown to be associated with AD. Here we discuss three of the main challenges we see impacting the translatability of manufactured CAR-T cell products as a potential therapy for AD and discuss possible solutions to these barriers.

IDENTIFYING A SUITABLE CHIMERIC ANTIGEN RECEPTOR TARGET

One of the challenges with adoptive CAR-T cell therapy for AD is identifying a suitable antigen target for the CAR. Antigens act as

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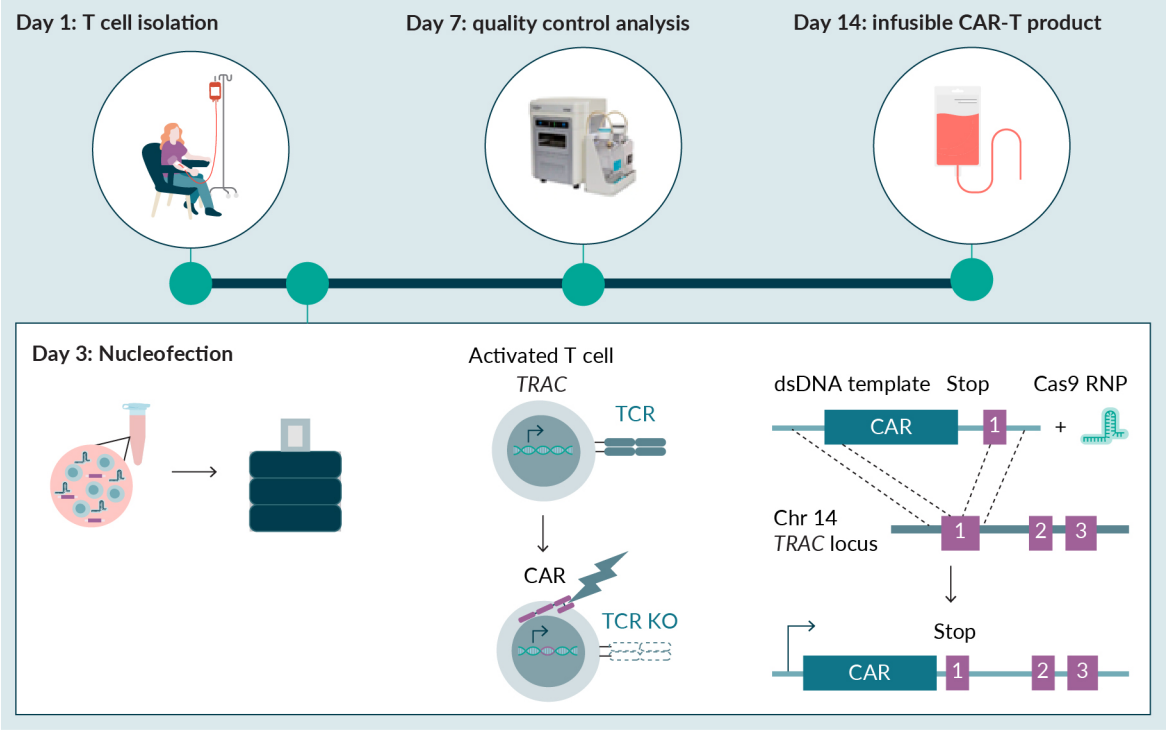
FIGURE 1

Chimeric antigen receptor (CAR) T cell manufacturing overview.



Shown here is a 2-week process for CRISPR mediated gene transfer of a CAR into primary human T cells. CAR strategies can be designed against antigens on dysfunctional cells in the AD brain.

a molecular fingerprint to a cell. When determining a desired antigen target for a CAR, there are several variables to consider such as coverage, specificity, and expression stability [26].

One key antigen of interest for AD CAR-T strategies is Aβ. The amyloid hypothesis is the most extensively studied concept of AD and proposes that deposition of the Aβ peptide in the brain is a central event in disease pathology. As of August 2023, there have been 34 AD immunotherapies targeting Aβ that have been investigated in clinical trials, with only two being approved by the FDA [27]. Of these immunotherapies in development there are a variety of therapeutic interventions including vaccines, monoclonal antibodies, polyclonal immunoglobulins aimed at reducing Aβ production/aggregation as well as promoting clearance targeting Aβ peptide fragments [28–31]. Aβ poses as an exciting potential CAR-T cell target as antibodies against Aβ have been shown to reduce amyloid plaque burden and mitigate cognitive decline in AD patients [14]. With the approval of monoclonal antibodies—aducanumab and the recent approval of lecanemeb—one could adapt the scFv fragment domains (VL and VH) of the approved antibodies to be a binder of Aβ in an anti-Aβ CAR. However, one factor that must be considered is that Aβ oligomers are soluble and may not be on the surface of cells. While soluble antigens may not trigger CAR signaling [32,33], newer engineering strategies to mechanically couple extracellular spacers with intracellular signaling domains may be able to activate signaling within T cell in Aβ-dependent manner that is therapeutic. These anti-Aβ CAR-T cells would not directly clear the amyloid plaques, but could stimulate an immune response to the plaque by recruiting neighboring cells like microglia. In contrast, CAR macrophages against Aβ [34] may be able to directly clear amyloid plaques.

Developing anti-Aβ CAR immune cell therapies could be revolutionary by programming the patient's immune system to eliminate harmful Aβ peptide.

Other emerging antigens of interest are on senescent cells. Senescent cells no longer proliferate and are often characterized by a permanent state of cell cycle arrest. These cells have been observed to play an important role in the overall onset and aggravation of diseases such as AD; where increased senescence has been seen in several cell types including astrocytes, microglia, and neurons, respectively [8,16]. Senescent cells are an attractive cell target for a neurodegenerative CAR-T cell therapy, as these cells have been shown to have increased expression in the brain and the removal of the cells has been shown to restore cognitive function in preclinical studies with senolytic drugs [35]. This data also suggests that cell senescence promotes Aβ and tau pathologies and that removing these cells could be a key strategy to prevent further neurodegeneration and disease progression [36–38]. Additionally, CAR-T therapies aimed to eliminate senescence have already shown to be successful in diseases such as liver and lung fibrosis to reverse senescence-associated pathologies, by targeting the urokinase plasminogen receptor (uPAR) and the natural killer group 2 member D ligands (NKG2D), which are markers on the surface of senescent cells [39,40].

Overall, further advances in identifying target antigens potentially with new technologies like single-cell RNA sequencing and special transcriptomics, are crucial for the development of CAR-T cell immunotherapy and a step forward to create an effective therapy for AD.

LIMITED PERMEABILITY OF THE BLOOD–BRAIN BARRIER IMPACTING CAR-T CELL EFFICACY

Enhancing CAR-T cells to efficiently bypass the blood–brain barrier (BBB) is another hurdle in developing efficacious treatments. Targeting the brain with therapeutic agents has been a key challenge in the treatment of AD. The BBB has evolved to restrict the transport of nearly all large molecules, including many therapeutic peptides, nucleic acids, antibodies, and growth factors (molecular weights larger than 400 Da) [41]. In neurodegeneration, the integrity of the BBB can be greatly impacted, ultimately affecting the transport of cytokines and other factors as well as the rate of cell trafficking to the brain parenchyma. While CAR-T cells have been shown previously to cross the BBB in the presence of brain inflammation, it is still unclear how trafficking of these cells could be altered in the AD environment [42].

During AD there is an increase in cytokines secreted by aged T cells in response to Aβ and tau protein [43]. The increase in cytokines leads to the vascularization of the BBB allowing it to become more permeable and increase trafficking of immune cells [42]. While this is beneficial in the context of CAR-T immunotherapy, the influx of additional aged T cells can contribute to neuroinflammation and AD pathology. Tregs, however, have been shown to restrain cognitive decline by fostering the recruitment of cells with anti-inflammatory/suppressive activity. These Tregs are in short supply in aged individuals [18]. To enhance the presence of Tregs in the AD brain, preclinical research has investigated the *ex vivo* expansion of Tregs in AD individuals and showed that reinfusion into animal models not only restored but also enhanced their immunosuppressive function, reduced the number of reactive glial cells, and alleviated amyloid plaque burden [44]. CAR-Tregs have been successfully generated [45], and these cell products could potentially cross the BBB and suppress neuroinflammation to establish an adaptative immune system balance in AD. Further research and clinical models are needed to understand how to safely and effectively traffic adequate CAR-T cells through the neurodegenerative BBB for therapeutic purposes.

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LONG-TERM PERSISTENCE & DURABILITY OF MANUFACTURED CAR-T CELL PRODUCTS

Increased persistence and durability of CAR-T cells within the neurodegenerative environment is crucial for durable efficacy. Like cancer, AD progresses over an extended period requiring long-term persistence and surveillance of T cells within the brain microenvironment. Limited persistence is a significant bottleneck in the field of CAR-T therapies due to T cell exhaustion [21]. To overcome this barrier, is it important to understand the neurodegenerative microenvironment in order to manufacture a CAR-T cell product that can ensure longevity and functionality over an extended period, even years post infusion.

One way to prolong persistence of CAR-T cells is to utilize naïve T cell populations such as stem cell memory T (TSCM) cells and central memory T (TCM) cells [21]. CAR-T cells derived from memory and naïve T cell subsets have shown greater potency and proliferation than those derived from effector memory T (TEM) cells in preclinical models [46]. To achieve desired T cell phenotypes, we can use non-viral manufacturing methods such as CRISPR-Cas9 gene editing (Figure 1), which has been shown to promote a more memory-like phenotype prior to antigen exposure [47]. The main hallmark of neuroinflammation in neurodegeneration is the presence of chronically-activated glial cells. In AD brains, disease-associated microglia (DAM) have been found [48]. DAMs are defined by the expression of genes, *APOE2* and *TREM2*, related to lipid metabolism and phagocytosis. Chronic DAM activation is hypothesized to contribute to the overall progression of AD, however, the overall role of these DAM cells is still unclear [49]. Developing a CAR-T cell that can augment the brain microenvironment by stabilizing glial cells—potentially modifying DAMs—may be able to generate a neuroprotective brain microenvironment, rather than a neurodegenerative one.

Another method that can provide durability in CAR-T cell products is the development of newer CARs that have added functionality. For example, 'fourth generation' CARs can express the CAR and secrete additional factors to modify the local microenvironment. Such next-generation CARs could be engineered to resist the immune-hostile environment in the brain microenvironment to overcome immune exhaustion and evoke a long-lasting immunological response by secreting specific cytokines, chemokines, and proteins [50]. Long-term persistence ensures CAR-T cells can remain active and engaged in the ongoing fight against AD, therefore, minimizing the chance of relapse, progression, as well as reducing the frequency of T cell infusions. Continued research focusing on optimizing CAR-T cell design and developing strategies to enhance persistence are crucial to improve extended functionality within the complex neurodegenerative microenvironment.

TRANSLATIONAL INSIGHT

Chimeric antigen receptor (CAR) therapies have been revolutionary for the treatment of specific cancers, with promising preclinical results in several other diseases such as fibrosis, HIV, and pulmonary heart disease [39,51,52]. A CAR-T cell therapy to treat AD is currently in a preclinical phase of development, where ongoing research focuses on identifying suitable antigen targets on degenerating neurons and glial cells, determining the appropriate permeability of T cells through the BBB, and manipulating the neuroinflammatory environment within the brain.

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CELL & GENE THERAPY INSIGHTS

NEW HORIZONS IN IMMUNOTHERAPY

SPOTLIGHT

INTERVIEW

Developing universal AAV immuno-gene therapy for oncology

Traditional limits to the application of AAV-driven gene therapy are increasingly being eliminated. **David McCall,** Senior Editor, BioInsights, speaks to **Nicole Paulk,** CEO & Founder of Siren Biotechnology, about her team's work in creating both the world's first AAV-based immunotherapy in the oncology space, and the first universal AAV gene therapy product that can be used to treat multiple indications.

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Q What are you working on right now?

NP: We are attempting to make the world's first adeno-associated virus (AAV) gene therapy that can be used in the cancer setting. Normally, AAVs are only used as gene transfer agents in the Mendelian monogenic rare disease space. For the first time ever, we are bringing this modality into the oncology world.

With the same product, we are also making the world's first AAV gene therapy that is universal—that can be used to treat more than one disease. AAV gene therapies to date have been specific to a single indication, but we are trying to make an AAV that could be used to treat 20, 200, or even 2,000 different diseases, all from within a single gene therapy drug product.

Q How did you arrive at the concept of 'immuno-gene therapy'?

NP: The concept began within my lab at the University of California, San Francisco (UCSF), before Siren existed. We were thinking about what enabling technologies we could develop that would allow AAV gene therapies to act more broadly than the single disease context—in other words, at the platform level. We wanted to develop enabling technologies that would make any AAV gene therapy cheaper, easier, and faster to produce, so that we could treat more patients in more indications.

When we brainstormed the biggest roadblocks in the field that were holding us back, we kept returning to the fact that every AAV gene therapy is bespoke and personalized to a single indication. We wanted to change that. The concept of a universal payload that could be used to treat more than one disease led to us thinking broadly about what classes of payloads we could deliver outside of the Mendelian monogenic diseases space that would be capable of broader use to do things such as modulate cell signaling, cell behavior, cell interactions, and cell states. We had no initial intentions of targeting oncology. However, one of the classes of payloads that we became interested in was engineered cytokines because of how pleiotropic they are and after three or four years of work, we leaned into the applications in the oncology space using these payloads.

Q What are some of the key advantages of Siren's approach over other cancer immunotherapy modalities?

NP: AAV has never been used in any cancer setting in humans; it is a brand-new modality in this field. When we were making libraries of different payloads back in my lab at UCSF, we were thinking about the advantages that AAV could bring to a cancer setting. We got excited about melding the two worlds of AAV gene therapy and engineered cytokine immunotherapy because we thought we could address one of the big challenges that cytokine immunotherapies have: their half-life. The cytokines your body makes have short half-lives by design: every cell in your body is responsive to the effects of cytokines and they are exceptionally potent, so you don't want them active for too long.

The extensive efforts made to extend the half-life of therapeutic cytokines (e.g., by PEGylation and other technologies) have not being particularly successful. We thought that by vectorizing cytokines specifically within an AAV, we could create a huge advantage. AAV is unique compared to classic oncolytic viruses in that it isn't lytic, doesn't make copies of itself, or travel between cells. Therefore, you have this beautiful, continuous, and yet highly self-limiting expression of payload that lasts only as long as the host cell survives (in our case, a tumor cell). As soon as that tumor cell dies, you see an immediate end to the expression from the AAV. We saw this as a unique opportunity to leverage the strengths that AAV has in other settings and bring them into the oncology world. Now we can make continuous copies of the cytokine locally wherever we deliver the AAV, and when that host cell that the virus transduced dies, expression ceases immediately.

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"As soon as that tumor cell dies, you see an immediate end to the expression from the AAV. We saw this as a unique opportunity to leverage the strengths that AAV has in other settings and bring them into the oncology world."

We were not oncologists when we started this project from within my lab at UCSF; we were simply making libraries of AAVs and testing them in different 'use case' sandboxes. The combination of extreme technical knowledge about AAV with a naïveté about the oncology world allowed us to, in some sense, have the audacity to come into this new space. Oncology is a field with immense technical expertise and a long history, and bringing a new modality into a space in which you are not an expert is no small feat. From the safety of academia, we were willing to do so. Obviously, this was not done alone. We collaborated with world-leading experts in oncology at UCSF, particularly neuro-oncology since our lead candidate focuses on the brain. Practicing clinicians and research professors from the UCSF Brain Tumor Center worked closely with us as part of the Glioblastoma Precision Medicine Program we joined, providing an expert knowledge base for us to collaborate with.

Q Beyond your own work, where do you see signs of progress in AAV gene therapy in terms of addressing some of the longstanding barriers and issues in the field, and advancing into new application areas?

NP: One of the key bottlenecks for the field is manufacturing—producing and purifying AAV vectors. Every year we get better at this; and now we are starting to get to the point of our 'Genentech moment', referencing how monoclonal antibody manufacturing evolved so quickly following the field's entry into the commercial sphere. I believe that well within the next decade, we will be at the point where manufacturing huge quantities of AAV is a non-issue. As we improve on that front, it will help every AAV gene therapy company in terms of development times and capital needs, no matter what therapeutic area they are in.

It will no longer be necessary to go out and raise $250 million each financing round in order to bring in one new program—if we can make it so that a standard series B only needs to be $50 million instead of $250 million, that completely changes the game. This space is an exceptionally expensive one at the moment, so any reductions in capital needs allow more players to be at the table and will speed up their R&D pipelines.

Regarding other barriers to AAV like preexisting immunity, others have some promising approaches in development that I am really excited about. However, none are quite ready for prime time today, which is one of the reasons why Siren strategically picked the brain and the

eye to begin with: as they are immune-privileged tissues, we do not have to face the challenges surrounding preexisting immunity and 100% of our incident and prevalent populations will be eligible for our immuno-gene therapies. By the time we move into other indications, innovations in preexisting immunity will have caught up and be ready to implement.

Q As CEO of a new biotech in the space, how have you approached the challenges in securing financing in these relatively difficult times—do you have any tips for success in this regard?

NP: You have to just live, eat, and breathe fundraising. No matter the size or stage of the company, everyone is struggling to raise funds right now.

Particularly for early-stage companies, it is important to remember that venture dollars from classic biotech investors and big pharma partnerships are not the only source of funds. There are also techbio and tech investors to consider. You can always go beyond venture capital dollars, too. For example, at Siren, we are in the process of grant writing at the federal level (NIH and DOD grants), the state level (CIRM grants here in California), and from relevant disease foundations and societies. If you spun out of a university, reach back out to your university as they often have funds that can help support you. There are sometimes programs from within your state, particularly if you are in one of the big biotech hubs—major cities often have specific city-level programs. Get creative. Ask your vendors for highly deferred payment programs. Ask to get supplies you need in exchange for free advertising for that company. Buy used instruments when other companies fold. Share instruments with other startups in your building. Unabashedly ask every one of your vendors for a steep discount to keep you as a client. Keep your headcount low and stay lean and mean and laser-focused on de-risking your technical risk for your lead only. Meet regularly with your team to go over expenses, think through 'what is essential to stay alive', and drop everything else. Just hustle, get creative, and don't give up. If you survive this as a company, you will be absolutely unstoppable when the market rebounds. We have all seen the data; the biggest companies in history typically started during a downturn. Hustle like rent is due.

Q Looking to the future, what might be some important opportunities for further development and application of Siren's technology?

NP: We are starting with oncology because it is the simplest use case and the one with the largest markets and the clearest clinical trials in terms of endpoints and regulatory paths. There is no rule saying that the cytokines we are delivering can only be used in the oncology setting, though—they could be applied in autoimmunity settings, infectious diseases, and more. When we say that our assets are a platform, we mean it. Oncology might just be the tip of the iceberg.

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Q What are your key specific priorities for Siren over the next couple of years?

NP: We are laser-focused on getting to the clinic as fast as possible, since this is going to be a first-of-its-kind product to be tested in humans. We are working to get all of the investigational new drug (IND)-enabling experiments, process development, and manufacturing done, and our IND submitted as quickly as we can. All eyes are on the clinic right now.

BIOGRAPHY

DR NICOLE PAULK is the CEO, Founder and President of Siren Biotechnology, and the inventor of Siren's immuno-gene therapy platform technology. Previously, she was faculty in the UCSF Department of Biochemistry and Biophysics in San Francisco and left to lead Siren. Dr Paulk has a BSc in Medical Microbiology, a PhD in AAV Gene Therapy and Regenerative Medicine from OHSU, and completed her Postdoctoral Fellowship and Instructorship in Human Gene Therapy at Stanford University prior to starting her lab at UCSF. She is a pioneer in the development of next-generation AAV platforms for gene repair, gene transfer and gene editing for numerous rare diseases and cancer, directed evolution to evolve novel AAV capsids, and comparative multiomic approaches to interrogate translational AAV biology. Dr Paulk is a global KOL in gene therapy and consults extensively for big pharma, writes draft guidance for the FDA, and sits on the SABs for Sarepta, Astellas, Dyno Tx, CEVEC, Excision Bio, Whitelab Genomics, Johns Hopkins Gene Therapy Initiative, and several stealth newcos. She is regularly quoted in STAT, Endpoints, *The Wall Street Journal*, *The Economist*, *The Boston Globe*, Phacilitate, GEN, Biopharma Dive, Evaluate Vantage, *SF Business Times*, *Wired*, *MIT Tech Review* and, *Chemical & Engineering News* and is a regular guest on Biotech Clubhouse. Dr Paulk sits on the Scientific Editorial Boards of the journals *Nature Gene Therapy* and *Human Gene Therapy*, and is extensively involved in numerous committees and leadership positions within the American Society of Gene & Cell Therapy. Dr Paulk has invented numerous AAV gene therapy technologies that have been shared/licensed to dozens of gene therapy companies and nonprofits groups working in rare diseases.

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CELL & GENE THERAPY INSIGHTS

NEW HORIZONS IN IMMUNOTHERAPY

SPOTLIGHT

INTERVIEW

Does synthetic biology hold the key to unlocking solid tumors for cell and gene therapy?

While cellular immunotherapy holds promise in treating liquid and potentially solid tumors, considerable challenges prevent these therapies from being wholly effective. **David McCall,** Senior Editor at BioInsights, speaks with **Ming-Ru Wu,** Assistant Professor, Dana-Farber Cancer Institute and Harvard Medical School, about his work in employing synthetic biology principles, AI, high-throughput screening, and multiomics tools to design a new wave of immunotherapies with the potential to advance the field into the mainstream of cancer therapy.

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Q What are you working on right now?

MRW: I started my lab at the Dana-Farber Cancer Institute about 3 years ago. Before becoming a scientist, I was trained as a physician in Taiwan. Then I came to the USA for my PhD degree. I initially trained as an immunologist during my PhD, then further trained as a synthetic biologist during my postdoc. I have been fortunate to combine synthetic biology with immunotherapy since my postdoctoral work. In my lab, we continue to focus on

harnessing the tools and design principles of synthetic biology to overcome the outstanding challenges in cancer immunotherapy. That is our central goal.

Q What advantages are you seeing in employing the tools and design principles of synthetic biology, specifically in the cancer immunotherapy setting?

MRW: We focus on developing cell and gene therapy based approaches for cancer immunotherapy. For cell therapy, our primary focus is to leverage synthetic biology toolkits to overcome the outstanding challenges facing CAR-T cells. We aim to develop new sensors and devices for CAR-T cells, allowing them to become more intelligent, powerful, and controllable. For gene therapy, we are developing various forms of cancer-targeting gene circuits. Gene circuits usually contain a group of gene cassettes that can work together to perform a more complex decision-making process.

You can think of these cancer-targeting gene circuits as nanorobots. Upon delivery into the body, the nanorobots can enter normal and tumor cells. Nothing much will happen when they get into normal cells, but when they get into tumor cells, the nanorobots know they are within the enemy. They will then reprogram the tumor cells to produce all sorts of therapeutic proteins from within, thus localizing at the tumor site. Our gene circuits can program the tumor cell to produce any genetically encodable protein therapeutic. Instead of directly utilizing these gene circuits to trigger tumor cell death, we explicitly use gene circuits to educate the immune system to attack tumors.

This gene circuit therapy approach is relatively unique in the cancer immunotherapy space. Some synthetic biologists do it, but most focus on triggering killer genes. However, an immune response can be particularly useful in this context because an immune response usually has a bystander and memory effect. If you trigger killer genes, the bystander killing effect is not very strong, so you will need to have highly efficient circuit delivery to trigger robust therapeutic efficacy.

Because cancer is a highly aggressive enemy, even if you can deliver gene circuits to 50% of all tumor cells and eliminate them, the other 50% will become 100% in a few days. By contrast, an immune response is self-amplifying, so in a way, it can trigger the proper level of therapy according to how many tumor cells are present. Of course, another major feature of the immune response is that if we trigger it correctly, it can have memory capability and thus, can be triggered again in the future to prevent tumor relapse. Preventing tumor relapse is a crucial feature of immunotherapy.

Q As an immunologist, what is your take on the current state of play in terms of the migration of cellular immunotherapies into solid tumor indications?

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MRW: Conceptually, if we can treat hematologic malignancies, there is no apparent reason why we cannot treat solid tumors. But of course, there are significant challenges. For one thing, a solid tumor is a tumor mass that creates a high hydrostatic pressure, causing the blood vessels to collapse and limiting the immune cells' penetration. And even if there is sufficient immune cell infiltration, the tumor microenvironment is very immunosuppressive. This immunosuppressive microenvironment can render the army of immune effector cells much less potent.

> "The promise of allogeneic therapies lies in the potential to reduce [the] cost significantly."

Another challenge is that a successful therapy needs to wipe out almost all tumor cells. I mentioned that the immune response has memory, but immune memory can only start to form when you have wiped out almost everything. While wiping out all tumor cells, if T cells work too hard without proper rest, they will also go down the path of exhaustion.

But the good news is that basic research allows us to understand the bottlenecks and challenges more and more clearly. This capacity gives us more opportunity to design therapies that really target the weaknesses of our enemies.

Researchers are working hard to develop strategies to tackle each and every obstacle that I point out. There has not yet been a complete success with solid tumors, but we have seen partial success in solving some of these problems.

Q What for you are the current key challenges and potential promising approaches in the allogeneic cellular immunotherapy space?

MRW: Autologous therapy has shown superior efficacy in a few cancer types, but the cost is high. The promise of allogeneic therapies lies in the potential to reduce that cost significantly. Another promising aspect is that you can pick the best possible donor immune cells as the starting material rather than relying on the cells of very sick, frequently immunocompromised patients. We now understand that the starting material matters; if you pick the best, healthiest donor, then you are in a much better position from the start.

One challenge is that the host immune system can reject the allogeneic cells. This can be solved by removing the human leukocyte antigen (HLA) molecules on the donor cells—however, this may leave those cells prone to natural killer cell killing. It becomes quite tricky—you may need to put some non-classical HLA or minor histocompatibility antigens back to create a balance. The best way to reach that balance is still an open question.

Another challenge is that allogeneic cell therapy can cause graft-versus-host disease. This can be solved by removing the T cell receptors (TCRs) on the donor cells. However, TCRs also constantly interact with self-antigens, generating low but constitutive signals called tonic signaling. Tonic signaling is essential for various cellular processes, including T cell homeostasis

"If we can train an AI model that understands the design principles of promoters, we can ask the AI model to help us to explore the vast design space and identify the even better sequences out there."

and survival. So, if the TCR has been removed, would that engineered T cell remain as effective as a regular T cell? I think that is still an open question, too.

And of course, all the above manipulations involve multiple engineering steps on the cells *in vitro*, during which the T cells become less potent. We have yet to figure out all the technical things we need to do to maintain healthy allogeneic T cells so that they can perform as well as those in autologous therapy.

Q What is the collision of high content screening, spatial genomics and transcriptomic, and proteomics delivering to the field in practice today? Where and how can it be harnessed to drive further advances?

MRW: These collisions are synergistic. Now, we can use high-throughput screening to supplement the design-build-test-learn cycle, which helps us to understand how to design better genetic elements. High-throughput screening is extremely useful for us to build better circuits, or even simply to build better sensors. For example, we use synthetic promoters to target cancer cells, but it is not apparent what DNA sequences make good promoters. However, with high-throughput screening, we can design close to 100,000 promoters and measure the activity of each and every promoter with a single experiment. That in itself is already extremely useful. In addition, within the same experiment, we can curate a dataset containing information on 100,000 DNA sequences and their tumor specificity. This dataset allows us to train a deep neural network as an 'oracle' to help us further design and optimize promoter sequences.

In addition, we can do high-throughput perturbations, and measure what happens after the perturbations at the single-cell level. Combining high-throughput screening or perturbation approaches with multiomics analysis allows us to inform causality and identify actionable targets.

In a more general context, the collision of high-content screening, genomics, transcriptomics, and proteomics allows us to understand our enemy with unprecedented granularity. We may know exactly how tumors are orchestrated. For example, when I say 'immunosuppressive', it is not the entire tumor microenvironment that is suppressive. Sometimes, the suppressive zone is more specialized and specific. We can now understand that at a more granular level. This is very powerful.

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Q How about the application of AI and machine learning? Where are you seeing effective applications in practice there?

MRW: AI is the new electricity, and it is already broadly impacting our lives. I will use one project in the lab as an example to illustrate the role of AI in biomedical research. I mentioned that we could use a single experiment to measure the activity of 100,000 promoters. Usually, we can already identify outstanding promoters with this high-throughput measurement. But if you imagine a promoter having 100 base pairs, all the possible DNA sequences for this promoter can reach 4^{100} possible A-T/G-C combinations. Just by probability, there are likely even better promoters out there we have not designed. So we think, 'Can we take this one step further?' If we can train an AI model that understands the design principles of promoters, we can ask the AI model to help us to explore the vast design space and identify the even better sequences out there.

In addition, AI has been used for imaging diagnosis and prognosis predictions. These are also very exciting frontiers of AI in biomedical research.

Q Looking to the future, how do you weight up the prospects for cellular immunotherapy in the broader context of the immuno-oncology space as a whole?

MRW: I do believe cellular immunotherapy, whether it is adoptive cell therapies like CAR-T cells or using gene circuits to trigger an immune response *in vivo*, will become the mainstream of the new oncology space. Having said so, I believe the optimal therapy must combine the best from all fields.

After all, when we use adoptive cell therapy to treat a solid tumor mass, it is like peeling an onion. The therapy will start the peeling, but the 'onion' that is the tumor will still grow from within. Unless the peeling is faster than the growing and the tipping point is reached, the tumor will ultimately win. That is why I believe that the best therapy needs to combine the best of all fields—surgery, small molecule inhibitors, and cell and gene-based immunotherapy.

It will be a totally different scenario if we use a combination therapy that combines the best from all fields to treat a large tumor mass. The first effective step will likely be surgical removal. When you do so, you remove tens of hundreds of billions of tumor cells. This also means that the tumor microenvironment will be largely gone. After surgical removal, there may still be some residual tumor or micrometastasis which might not be visible to the physician. At this point, several methods could be important. Small molecule therapy can be very effective, especially the highly tumor-specific ones. Those small molecules can also penetrate the tumor mass more readily because they are small. They can further help us to clear the residual tumors. Then, we have immunotherapy taking over the battlefield. Now the suppressive microenvironment is much weaker. Immunotherapy can fully unleash its power. In addition, a good

immune response usually comes with various flavors—allowing us to tackle tumor heterogeneity. Then the immune memory will further protect patients from tumor relapse. Of course, this is the best-case scenario, but as long as we combine the best of all fields, achieving a curative response is possible.

Q Finally, can you sum up one or two key goals and priorities that you have for your own work over the foreseeable future?

MRW: One thing we focus on is to translate our cancer-targeting gene circuits into the clinic. Our cancer-targeting circuit therapy works well *in vitro* and *in vivo* in mouse models. But how do we ensure that it will work well in humans? There is a significant knowledge gap there, and overcoming that gap is still an open question for the field. There are always people who do not consider mouse model data to be reliable, but it remains the best research tool we have before doing a human trial. Figuring out how to tell when this data is reliable, and when it is not, is key. And even when we know the mouse model is not representative of human disease, can it still be used to inform action steps? We are constantly thinking about developing experimental or machine-learning-based approaches that allow us to bridge this gap.

BIOGRAPHY

MING-RU WU received his MD from Tzu Chi University in Taiwan. He obtained his PhD in Microbiology and Immunology from Dartmouth College in 2015, where he developed several natural killer cell receptor-based chimeric antigen receptor (CAR)-T cells, bispecific T cell engagers (BiTEs), and tumor-targeting nanoparticles. He conducted postdoctoral research at the Synthetic Biology Center at MIT, where he developed cancer-targeting gene circuits and high-throughput cell state-sensor engineering methods. He is currently an Assistant Professor at Dana-Farber Cancer Institute and Harvard Medical School. His lab is harnessing the power of synthetic biology, bioinformatics, and machine learning, to develop effective cell- and gene circuit-based cancer immunotherapies. Synthetic biology is a field that applies rigorous engineering paradigms from electric engineering and computer science (i.e., modularity, orthogonality, tunability, and composability) to genetic engineering. It aims to program living cells with novel functions, such as Boolean operations, analog computing, signal integration, and event recording, to enable sophisticated user-defined functions for sense-and-respond adaptive therapies—medicines that can change their behaviors in response to disease condition. Combining synthetic biology with bioinformatics and machine learning enables one to identify ideal targetable disease signatures, explore a very large circuit design space, and reliably predict circuit performance, for developing the next generation of cancer immunotherapy.

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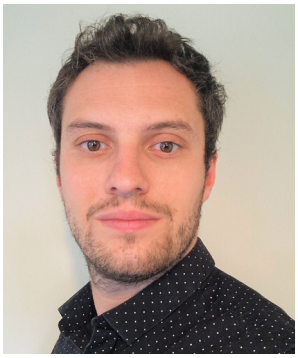
CELL & GENE THERAPY INSIGHTS

NEW HORIZONS IN IMMUNOTHERAPY

SPOTLIGHT

iNKT cells for allogeneic cell therapy

Eleni Chantzoura & Paul Ibbett
MiNK Therapeutics



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VIEWPOINT

"Allogeneic iNKT cells represent a game-changing option as they have a range of direct and indirect tumor killing mechanisms that can be augmented by CAR, TCR, or engager modalities."

Cell therapy has gained a lot of momentum for the treatment of hematological tumors, with six CAR (Chimeric Antigen Receptor) T cell therapies being recently approved by the Food and Drug Administration (FDA) (Table 1). However, while current treatments show phenomenal efficacy, they are autologous, meaning that cells from the patient are isolated, transduced with the CAR, and expanded *ex vivo* before being re-introduced to the patient. This tailor-made approach comes with a high cost as a new drug is made for each patient. Additionally, the lead time is prohibitory for many late-stage patients as it might take up to a month before the cells can be re-injected into the patient.

Consequently, it came as no surprise when in late 2022, the European Medicines Agency (EMA) became the world's first agency to grant marketing authorization for an allogeneic cell immunotherapy [1]. Ebvallo™ (tabelecleucel) harnesses T cells from healthy donors to treat patients over 2 years old with relapsed or refractory Epstein–Barr virus positive post-transplant lymphoproliferative disease (EBV+ PTLD) who have received at least one prior therapy. This is the answer of the field to the logistical challenges of autologous cell therapy described above.

Even though the use of T cells from healthy donors for 'off-the-shelf' cell therapy has shown promising results, it comes with its own challenges. To begin with, the T cell receptors (TCRs) from those cells can induce immune responses in the recipient leading to graft-versus-host disease (GvHD) that affects the efficacy of the treatment. Similarly, those allogeneic cells will eventually be recognized by the host's immune system leading to their rejection, which makes persistence one of the biggest questions facing allogeneic cell therapy. Finally, T cells do not easily infiltrate solid tumors, which represent the next big milestone for immuno-oncology [2].

Taking the above into consideration, scientists have started exploring different immune cell types for cell therapy, including natural killer (NK) cells, γδ T cells, or even macrophages [2]. Among those emerging cell therapy vehicles, invariant natural killer T cells (iNKTs) have unique properties making them naturally suitable for allogeneic applications. They are a very rare type of αβ T cells with an invariant TCR that recognizes the non-classical MHC molecule, CD1d, presenting cancer-associated glycolipids. In addition to their effector T cell function, they also demonstrate the rapid activation kinetics

TABLE 1

Summary of approved CAR-T therapies [9–11].

Brand name	Generic name	Company	Year of FDA approval	Target antigen	Indication	Pivotal trial	ORR/CRR
Abecma®	Idecabtagene vicleucel	BMS	2021	BCMA	r/r multiple myeloma	KarMMa	72%/28%
Breyanzi®	Lisocabtagene maraleucel	BMS	2021	CD19	r/r large B-cell lymphoma	TRANSCEND	73%/54%
Carvykti®	Ciltacabtagene autoleucel	Janssen	2022	BCMA	r/r multiple myeloma	CARTITUDE-1	98%/83%
Kymriah®	Tisagenlecleucel	Novartis	2017	CD19	r/r B-cell precursor ALL r/r large B-cell lymphoma	ELIANA JULIET	83%/63% 50%/32%
Tecartus®	Brexucabtagene autoleucel	Gilead	2020	CD19	r/r mantle cell lymphoma	ZUMA-2	87%/62%
Yescarta®	Axicabtagene ciloleucel	Gilead	2017	CD19	B-cell lymphoma, r/r follicular lymphoma	ZUMA-1 ZUMA-5	72%/51% 91%/60%

ALL: Acute lymphoblastic leukemia; BMS: Bristol-Myers Squibb; CRR: Complete response rate; ORR: Overall response rate; r/r: Relapsed/refractory.

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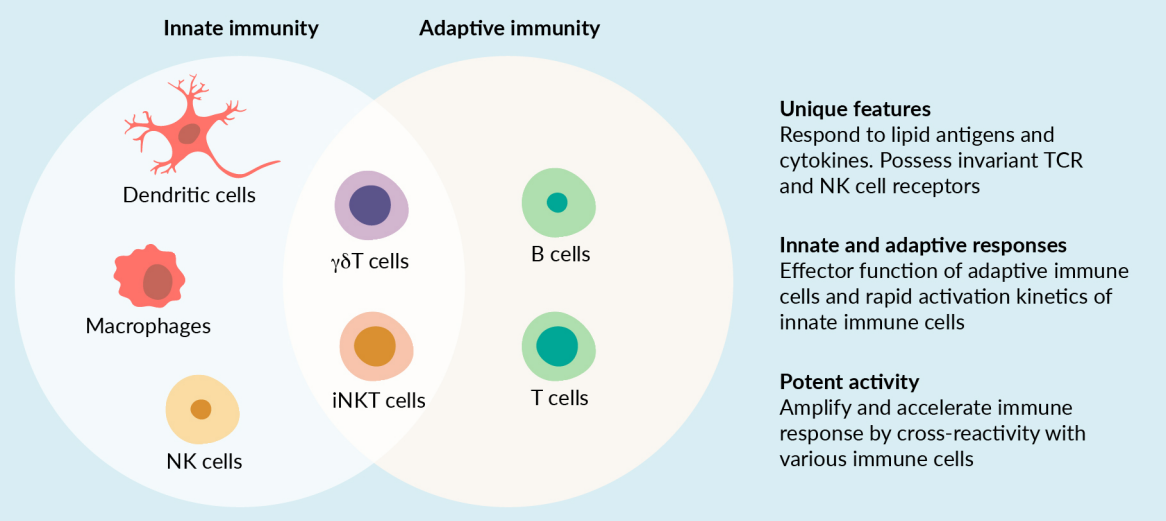
FIGURE 1

iNKT cells are distinct in the cell therapy landscape, combining both innate and adaptive immunity.



of innate immune cells (Figure 1), as they express many activatory NK receptors (e.g., NKG2D, DNAM) targeting stress ligands on tumor cells, while they largely lack the inhibitory receptors [3].

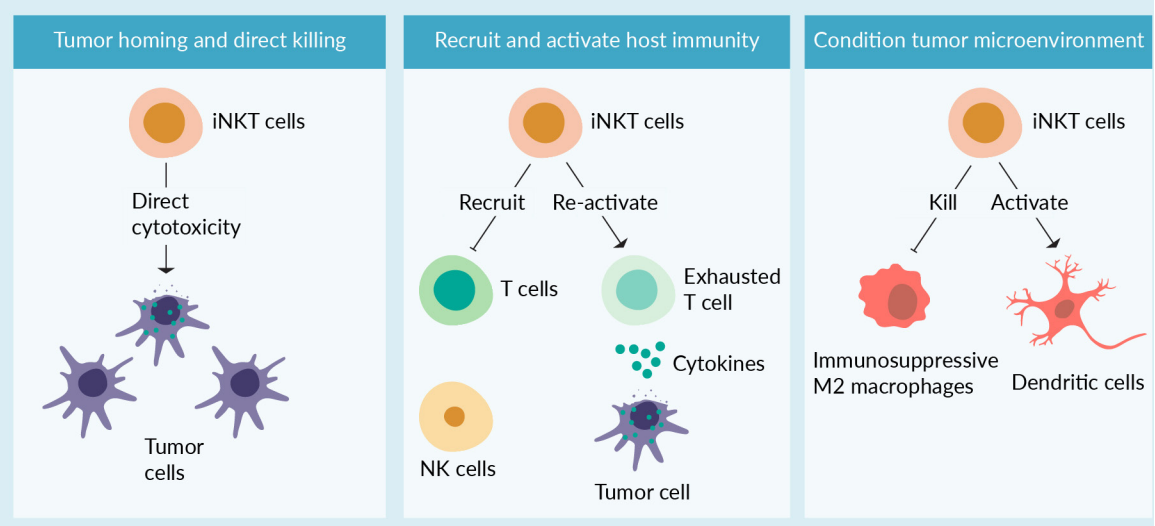
Beyond direct tumor lysis, iNKT cells can elicit a cytotoxic response by recruiting innate or adaptive immune cells and reshaping the tumor microenvironment (Figure 2). They can promote long-term cytotoxic $CD8^+$ T or NK cell responses whilst stimulating the maturation of dendritic cells (DCs) via cytokine stimulation. In parallel, they can counteract myeloid-derived suppressor cells (MDSCs) whilst inhibiting tumor-associated macrophages (TAMs) [3]. iNKT cells also have strong tissue-homing properties that can be critical in allowing the cell therapy to penetrate tumors.

Another advantage of iNKT cells over other cell therapies is their reduced risk of triggering GvHD, as their invariant TCR is common in all people and recognizes a non-polymorphic target [3]. Since one of the mechanisms of action of iNKT cells is the reactivation of the immune system, they can be administered without lymphodepletion, a short course of chemotherapy to kill host's immune cells before receiving adoptive cell therapy (ACT). It has been shown that lymphodepletion creates more space for the infused cells, prolongs their persistence, and modifies the hostile tumor microenvironment. However, lymphodepletion has substantial toxicity and in some cases, patients are not eligible for cell therapy trials as they may not survive lymphodepletion. For example, there is a higher risk of infections and certain lymphodepletion agents are associated with specific negative effects (for instance, fludarabine can cause fevers and neurotoxicity) [4]. Thus, avoiding lymphodepletion could lead to better efficacy and make this treatment available to more patients. Lymphodepletion may also be disadvantageous in the setting of allogeneic cell therapy as the host immune system is significantly damaged as a result, meaning it may not generate long-lasting host anti-tumor immune responses by the time the therapy is cleared.

Many human clinical trials have begun to exploit iNKT cells in blood and solid tumors in an autologous or allogeneic format (Table 2). In all these cases, treatment was well tolerated even at doses of 1 billion cells. Notably, allogeneic AgenT-797 iNKT cell administration showed clinical benefit in patients with heavily pre-treated solid tumor cancers, including

FIGURE 2

iNKT cells directly attack tumor cells, recruit host immunity, and reshape tumor microenvironment.



a partial response in 3L gastric cancer. Even though agenT-797 was administered without lymphodepletion, it was detectable in the periphery for ~8 weeks after administration and was well-tolerated either alone or in combination with anti-PD-1 therapy [5].

Following the promising clinical results of iNKT cells, the next step would be to increase potency by re-directing them towards the tumor. Based on preclinical studies, there are three different approaches to this end:

1. Introduction of a CAR: iNKT cells can be modified to express CAR molecules that mediate tumor killing whilst harnessing the host's immune system. Comparison of CD19-CAR iNKTs versus CAR-T cells in a syngeneic ACT model demonstrated that iNKT cells provide better tumor growth control partially by recruiting endogenous T and NK cells [6];

2. Introduction of a TCR: TCRs have the added advantage of targeting intracellular antigens. As iNKT cells are T cells, they can be modified with a TCR without the need for additional CD3 expression. Preclinical studies showed that TCR-iNKT cells have higher *in vivo* efficacy in comparison to CD8$^+$ T cells modified with the same TCR by simultaneously targeting the tumor and modulating suppressive myeloid populations [7];

3. Combination with engagers: one group developed covalent conjugates of soluble CD1d with photoreactive analogues of α-galactosylceramide, the first glycolipid identified to activate the iNKT TCR when presented on CD1d. They fused those conjugates to anti-HER2 scFvs, generating bispecific T cell engagers (BiTEs) with one arm binding to iNKT cells and the other to HER2 on tumor cells. Repeated administration of those conjugates either alone or in combination with an anti-CTLA-4 antibody in *in vivo* models led to tumor growth control via iNKT activation, maturation of DCs, and secondary activation of NK and T cells [8].

In summary, despite the successes of autologous CAR-T therapies in liquid cancers, a new paradigm is needed to address cost and access issues as we move towards the solid tumor space. Allogeneic iNKT cells represent a game-changing option as they have a range of direct and indirect tumor killing mechanisms

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TABLE 2

Summary of oncology clinical trials involving administration of iNKT cells [12–14].

Trial identifier	iNKT origin	Additional components	Tumor type	Trial phase	Evaluable responses
Motohashi *et al.* 2006	Autologous	None	Advanced/refractory NSCLC	Phase 1	SD (4/6)
UMIN000000722	Autologous	aGalCer-pulsed APCs	Refractory HNSCC	Phase 1	PR (3/8) SD (4/8)
UMIN000000852	Autologous	aGalCer-pulsed APCs, salvage surgery	Recurrent HNSCC with indication for salvage surgery	Phase 2	PR (5/10) SD (5/10)
NCT00631072	Autologous	+/- GM-CSF	Melanoma (stage IIIB-IV)	Phase 1	SD (3/9)
NCT03093688	Autologous	Autologous CD8 T cells	Advanced lung, gastric, pancreatic, HCC or CRC	Phase 1	Ongoing
NCT03175679	Autologous	IL-2, tegafur	Relapsed/advanced HCC	Phase 1	Ongoing
NCT04011033	Autologous	Cyclophosphamide, IL-2, TACE procedure	Relapsed/advanced HCC with indication for TACE	Phase 2	Ongoing
NCT04754100	Allogenic	None	Relapsed/refractory MM	Phase 1	Ongoing
NCT05108623	Allogenic	+/- approved ICIs	Relapsed/refractory solid tumors	Phase 1	Ongoing

CRC: Colorectal cancer; HCC: Hepatocellular carcinoma; HNSCC: Head and neck squamous cell carcinoma; ICI: Immune checkpoint inhibitors; MM: Multiple myeloma; NSCLC: Non-small cell lung cancer; PR: Partial responses; SD: Stable disease; TACE: Transarterial chemoembolization.

that can be augmented by CAR, TCR, or engager modalities. Their tissue-homing properties, lack of GvHD risk and requirement for lymphodepletion, and a maturing body of clinical safety and efficacy data makes them highly attractive as the basis of any cell therapy product.

BIOGRAPHIES

ELENI CHANTZOURA is the Director of Discovery in MiNK Therapeutics, a cell therapy company focusing on the discovery, development, and commercialization of iNKT cell-based therapies to advance cures for cancer patients. Eleni's team not only lead the discovery efforts in MiNK, but they have also been instrumental in implementing novel technologies to enhance iNKTs both for research and manufacturing processes.

PAUL IBBETT is Scientist II in MiNK Therapeutics, where he has been working on CAR and TCR discovery and iNKT cell engineering to enhance anti-tumor activity. Paul also leads the MiNK genomics team that utilizes a range of genomics tools including single cell RNAseq to enable a better understanding of iNKT-based cell therapy products.

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CELL & GENE THERAPY INSIGHTS

NEW HORIZONS IN IMMUNOTHERAPY

SPOTLIGHT

INTERVIEW

Increasing patient access to cellular immunotherapy through RNA gene writing

Current CAR-T cell therapies are beset by challenges relating to manufacturing and supply chain cost and complexity, as well as safety and efficacy, all of which limit the number of patients who can potentially benefit from them. **David McCall,** Senior Editor at BioInsights, speaks with the Chief Technology Officer of Cell Therapy at Tessera Therapeutics, **Madhusudan Peshwa,** PhD, about his work in addressing these challenges with an RNA gene writing platform combined with a lipid nanoparticle (LNP) delivery system for therapeutic application.

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Q What are you working on right now?

MP: Tessera Therapeutics is developing a genome engineering technology platform that we call Gene Writing™. We believe that this platform goes above and beyond existing technologies, and we are utilizing it in the context of developing potentially curative one-time treatments for a variety of different diseases. By writing in new genes, or by rewriting existing genetic code through making alterations at either a single nucleotide/base pair level or in larger stretches, we can effectively develop a functional cure for patients with a variety of unmet medical needs.

My specific role as Chief Technology Officer for Cell Therapies is to help drive our cellular immunotherapy programs from ideation all the way through to productization.

Q Can you tell us more about Tessera's Gene Writing platform and what differentiates it from other gene editing/cell engineering technologies?

MP: Nature has evolved an efficient way to write genes through mobile genetic elements where pieces of code are taken from one part of the genome and pasted into another part of the genome. The idea behind the creation of Tessera, which was founded by Flagship Pioneering, was to harness nature's greatest genomic architects and repurpose them for therapeutic applications. Tessera embarked upon an exercise to screen for mobile genetic elements across the kingdom of life, engineer them, and functionalize them to develop therapies.

These technologies come in two formats. They can either use DNA as a template (which we call DNA writers) or use RNA as a template (which we call RNA writers). We are most excited about an all-RNA system to engineer the genome because it gives us significant advantages in the context of not having to use plasmid DNA, not having to use a virus, and being able to multiplex multiple edits so you can pre-program a cell to exhibit a designed pattern of biological activity. It is also much more rapid to iterate these technologies through non-viral delivery approaches and RNA-based therapeutic approaches. This gives us a significant advantage in going from the bench to the bedside.

The fundamental premise behind this technology also differentiates it from other gene editing tools. When one thinks about genome editing, one typically thinks about nucleases, like transcription activator-like effector nucleases (TALENs), clustered regularly interspaced short palindromic repeats (CRISPRs), and others. The fundamental mechanism of action for these technologies is to act as molecular scissors. However, whenever you cut the DNA and you have a double-strand break, the cell tries to repair itself and oftentimes, your desired correction of that cut site competes with these DNA damage response pathways. This causes lower efficiencies of correction, along with insertions or deletions at that locus. This process introduces lower efficiencies of desired change and a larger likelihood of translocations, causing concerns about safety and toxicity.

Tessera's Gene Writing technology does not rely on making a double-strand DNA break. This allows for significant opportunity to have improved fitness and efficiency of the edits, giving us higher specificity and a lower likelihood of translocations. It is therefore a more robust platform that can be applied across multiple different types of cells and multiple different disease indications.

Q What are some of the key considerations and development steps related to Tessera's harnessing of a lipid nanoparticle (LNP) delivery system for application in the therapeutic areas and indications you are targeting?

MP: The traditional workhorses for delivery of *ex vivo* therapeutic applications have been viral vectors because that is where cell and gene therapy originated.

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Over the past few years, we have seen the advent of non-viral delivery technologies, especially when it comes to genome engineering applications. We may be on the cusp of the very first cell-based therapeutic engineered using electroporation, with CRISPR Therapeutics and Vertex Pharmaceuticals filing a biologics license application application for regulatory review and potential commercial approval in the very near future. So, when one thinks about non-viral approaches in the context of *ex vivo* applications, there are options out there.

"We have demonstrated the ability to deliver our RNA writers (as well as templates) to revert mutations to wild-type in a variety of animal models as well as *in vitro* models of various diseases."

Although we have six commercially approved chimeric antigen receptor (CAR-T) products on the market today, there are long waiting lists for them because of supply chain limitations, lack of availability of viral vectors, manufacturing slot limitations, scheduling complexities, and logistics and cold chain requirements. Today, only about 25% of the patients who are eligible to receive *ex vivo* CAR-Ts actually receive them. In addition, if a patient has a rapidly progressing disease, there are multiple challenges above and beyond the supply chain and logistics limitations, including the need for bridging therapies. All of this requires us to think about how we can disrupt that *ex vivo* CAR-T model and market.

What is emerging from that paradigm is a movement to create *in vivo* CAR-Ts. This is about combining gene editing tools with the right delivery vehicle. In that particular context, we take a lot of comfort from the fact that the LNPs have been validated for RNA interference (RNAi) delivery, with Alnylam Pharmaceuticals leading the way in that space in the context of delivery to the liver.

We are always learning from past enhancements, and engineering LNPs specifically for our purposes. Given that Tessera's RNA writer technology relies on an all-RNA system to write genes, it allows us to leverage past research with LNPs to perfect the delivery of our RNA molecules to various organs, cells, and tissues. The LNP system has largely evolved in the context of RNAi molecules to be effective delivery vehicles for targeting the liver. We have demonstrated the ability to deliver our RNA writers (as well as templates) to revert mutations to wild-type in a variety of animal models as well as *in vitro* models of various diseases. This includes phenylketonuria (PKU), where we achieved upwards of 40% *in vivo* liver rewriting efficiency at the *PAH* locus, the gene associated with PKU, in a non-human primate model. We know from past research that around 10% correction may potentially be sufficient to be clinically effective as a curative therapy. We are therefore really excited about the prospect of delivery to the liver for this particular indication.

By the same token, we have also been working on figuring out how to take our LNP delivery learnings and go beyond the liver to other organs and tissues. We have data on delivery to hematopoietic stem cells and T cells, which speaks to how and where this technology will continue to evolve and mature moving forward.

With any gene delivery system, the first tenet is to do no harm. The second tenet is to get specificity of the desired change that you are trying to make. The third aspect that we need to focus on is ensuring that we have a dynamic range because different diseases target different cells that may be present in different amounts. The abundance matters, the dose matters, and where the cells reside within the body or outside the body matters. It is about looking at the combination and convergence of all of these facets in order to optimize and tune our delivery platform for a specific therapeutic application.

Q What are the advantages of Tessera's approach in terms of application in cellular immunotherapy, specifically?

MP: The 'poster child' of cellular immunotherapy is CAR-T cells, with six products commercially approved in the United States, two additional ones in China, and many more in clinical trials. A predominant majority of these CAR-Ts target hematological malignancies, where the pathophysiology of the disease and access to the tumor is different than in solid cancers. There is also greater availability of antigens that can be targeted in hematological diseases than in solid cancers.

However, if you look at the entire unmet need from a cancer treatment perspective, hematological malignancies are the tip of the iceberg, making up 7–10% of all cancer indications, whereas 90% is made up of solid cancers. The big challenge with hematological diseases is about driving access to more patients, which is difficult due to the supply chain and manufacturing obstacles we discussed previously. Approximately 80% of patients who could benefit from CAR-Ts do not have the ability to access.

The other facet is that when you take cells out of the patient, put them on plastic, cryopreserve, thaw, and manipulate them, you essentially decrease their immunological fitness. By the time you come up with your final drug product, the immunological fitness of the cells is significantly reduced from what the T cell's immunology and basic biology is *in vivo*.

If there were approaches where you could actually create CAR-Ts *in vivo* without the complexity of *ex vivo* manufacturing challenges, you would not only have improved patient access, but you would have immunologically more fit cells that would improve the therapeutic index going forward.

If we extend beyond hematological malignancies to solid cancers, one of the big challenges other than the fact that antigen availability may be more heterogeneous or not as well defined, is that these solid cancers reside in a very complex microenvironment. CAR-T cells have to enter that microenvironment, penetrating inside to find the tumor cells, which may have differential levels of expression of the antigen. In that microenvironment, there is also a significant immunosuppressive regimen, which is what the tumor uses to disguise itself from being recognized by immune cells. CAR-T cells therefore require a broad diversity of affinity and specificity in order to recognize diverse levels of antigen expression, to home and penetrate, to find the tumor cells within this solid packed tissue, and to exhibit anti-tumoral function while not being subject to the immunosuppressive environment.

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"As we think about the future, we see many opportunities to continue to invest in the platform and build robust outcome and characterization measures, which will allow us to look beyond both hematological malignancies and solid tumors."

In order to design such a T cell with multiple biological functions, you must have the ability to multiplex and modify different genes concurrently in a synergistic manner. It is very challenging to modify three, four, five, six genes using viral vectors or using gene editing tools in the conventional sense where you are introducing double-stranded breaks. In the context of using an all-RNA system without employing viral vectors, it is potentially much easier for us to multiplex, and we hypothesize that we can do this. We have shown the ability to very efficiently add and edit genes at the same time using an all-RNA system inside primary T cells to generate CAR-T cells. This ability to multiplex, where you are combining the ability to write different CARs and knockdown different genes, or introduce gain of function to different genes, gives us the ability to pre-program a T cell to handle more complex disease environments such as solid cancers.

Even beyond solid cancers, there is excitement about applying T cells to other disease areas. As we think about the future, we see many opportunities to continue to invest in the platform and build robust outcome and characterization measures, which will allow us to look beyond both hematological malignancies and solid tumors.

Q What priorities do you have for your work and for Tessera over the next few years?

MP: We believe that our platform is disruptive and allows for the potential to create clinically meaningful therapies with positive benefit/risk and potentially curative from a clinical perspective. As with any new technology, our goal is to be very deliberate in our approach. We want to generate appropriate scientific data in a variety of *in vitro* and preclinical animal models, and to drive the platform into many different therapeutic areas where we think there are significant unmet needs. That is what we will be focused on in the near future.

Looking further ahead, we are building a product portfolio and look forward to progressing to human clinical trials, either independently or through potential partnerships. Developing therapeutics takes a village, and we must have the right set of stakeholders and partners as we go forward to ensure we are bringing these potentially transformative therapies to the greatest possible number of patients in need.

BIOGRAPHY

MADHUSUDAN V PESHWA serves as Chief Technology Officer, Cell Therapy, for Tessera Therapeutics and is responsible for developing the strategy and executing the operating plan encompassing the design, development and manufacture of Tessera's proprietary mobile gene element engineered cell therapy product portfolio. Dr Peshwa is a member of the College of Fellows at the American Institute for Medical and Biological Engineering (AIMBE). Prior to joining Tessera, Dr Peshwa was CTO at Mana Therapeutics, an immunotherapy company focused on the development of allogeneic, multi-tumor-antigen-targeted, non-engineered, T cell immunotherapies with additional oversight of Quality Assurance and Quality Control functions. Previously, Dr Peshwa was CTO and Global Head of R&D for the Cell and Gene Therapies business at GE Healthcare (GEHC). Prior to these roles, Dr Peshwa held various executive positions at MaxCyte, Inc., NewNeural LLC, and Dendreon Corporation. In addition to his broad industry experience, Dr Peshwa has served as Principal Investigator/Co-Investigator on multiple grant-funded research studies, is an inventor of seven issued US patents in the field of cell therapy, and has served in various consultative, advisory, and board capacities to industry, government, not-for-profit, and financial organizations. Dr Peshwa earned his PhD in Chemical Engineering from the University of Minnesota and his BTech in Chemical Engineering from the Indian Institute of Technology in Kanpur, India.

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CELL & GENE THERAPY INSIGHTS

NEW HORIZONS IN IMMUNOTHERAPY

SPOTLIGHT

INTERVIEW

Rearming the immune system with personalized allogeneic CAR-T cell therapy

Off-the-shelf cellular immunotherapies have historically been expensive to produce and carry safety risks such as graft-versus-host disease (GvHD). **David McCall,** Senior Editor at BioInsights, speaks with **Olivier Negre,** Chief Scientific Officer at Smart Immune, about overcoming these and other barriers through the combination of allogeneic cell therapy and personalized medicine.

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Q What are you working on right now?

ON: My research department at Smart Immune is working to transform outcomes for immuno-compromised patients with life-threatening diseases such as high-risk blood cancers and primary immunodeficiencies like severe combined immunodeficiency (SCID). Smart Immune is developing a T cell progenitor platform to re-arm patients' immune systems. With our *ex vivo* lymphoid niche technology, we can differentiate large numbers of allogeneic T cell progenitors from hematopoietic stem cells in only 1 week, rather than the 1 year it takes inside the body. Clinical trials are already in progress to accelerate

the T cell reconstitution in SCID and leukemic patients after allogeneic hematopoietic stem cell transplant (allo HSCT). In parallel, we are developing a new generation of allogeneic CAR-T cells to treat liquid malignancies, and we would also like to treat solid tumors in the long run.

Q Tell us more about the Smart Immune therapeutic platform—what differentiates it?

ON: ProTcell has the potential to combine the advantage of an off-the-shelf product, produced at a large scale, immediately available, and affordable, with the characteristics of personalized medicine, targeted and well-tolerated by the patient. Through a proprietary process, Smart Immune produces large numbers of clinical-grade T cell progenitors that travel directly to the thymus of the patient in order to complete their differentiation and education. They are educated by each individual patient which means that they can become tolerized to each patient. The thymus plays a critical role to avoid generating T cells that target self-antigens. By educating allogeneic T cell progenitors, it can prevent GvHD.

This process enables the use of allogeneic cells of good quality, which have not been exposed to chemotherapies, for instance. Those cells, in fact, can be chosen based on human leukocyte antigens (HLA) in order to optimize the match between the donor and the patient and help avoid cell rejection. We plan to develop a biobank of donor cells to drive our development programs.

Q More specifically, what are some of the features of the Smart Immune approach that offer encouragement that it can address some of the barriers that have hindered other allogeneic cellular immunotherapy approaches, such as persistence and durability of response?

ON: Allogeneic therapies using mature T cells as a starting material necessitate genetic modification and complex manufacturing processes in order to avoid rejection and graft-versus-host disease. Intensive modification of the cells increases the cost of manufacturing and decreases the fitness of the cells—something we are currently seeing with allogeneic CAR-T cells, for example. Overall, allogeneic products made from mature T cells are vulnerable to attack by other immune systems and have a reduced lifespan. We would like to tackle those issues through our approach and produce allogeneic T cells with better fitness and longer persistence while capitalizing on the potential to be produced off-the-shelf at a reduced cost per dose.

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Q Many in the field are now pivoting toward *in vivo* engineered cell therapy approaches—what is your view on this trend?

> "We would like to … produce allogeneic T cells with better fitness and longer persistence while capitalizing on the potential to be produced off-the-shelf at a reduced cost per dose."

ON: The *in vivo* approach is interesting, but *in vivo* engineered cell therapy requires more time and more development to ensure optimal targeting and maximal efficacy in patients. It might reduce the cost and the manufacturing time of the product in comparison to *ex vivo* manufacturing, but it will make quality control of the final product more challenging, of course.

Also, the quality of the cells modified *in vivo* to generate therapeutic cells may be poor if the patient has been exposed to intense chemotherapies and/or radiotherapies. Allogeneic T cells or allogeneic progenitors from healthy donors may provide starting material of better quality.

Q Which emerging technologies are demonstrating the greatest utility in providing translatable R&D insights for the field?

ON: I think single-cell analysis tools such as proteomics, transcriptomics, and epigenetics analysis provide a better understanding of the cell products, which is key. The ability to perform complex cell differentiation, genetic, and epigenetic modification paves the way to more efficient and more specific immunotherapies for oncology, as well as for other indications like autoimmune diseases.

Q Where specifically are you seeing the practical application of AI and machine learning tools starting to pay dividends for the cellular cancer immunotherapy space in particular? And what will be some key next steps in this regard?

ON: Artificial intelligence and machine learning can be very helpful to us for donor selection, allowing us to maximize the compatibility of the allogeneic therapy and to reach as many patients as possible. AI is also instrumental in the molecular design of new ligands, antibodies, or chimeric antigen receptors.

Q The voluntary market withdrawals of Zynteglo® and Strimvelis® remain important reference points for those who suggest the European market access model for innovative biologics is broken—what can companies like Smart Immune bringing a new generation of cellular immunotherapies to patients do to prepare for a successful future beyond MAA approval?

ON: Gene therapy products like Zynteglo have demonstrated strong efficacy in clinical trials, as acknowledged by European Medicines Agency (EMA) approvals. However, the autologous nature of such approaches makes them logistically complex and costly. Allogeneic approaches may help to solve both issues, ensuring these products are more accessible for patients by allowing for them to be immediately available (since they are off-the-shelf), and less expensive (since it is possible to provide enough drug products for several patients with one batch).

Q Finally, can you pick out one or two key goals or priorities for Smart Immune over the coming 12–24 months?

ON: For the next 1 or 2 years, we would like to obtain clinical results with leukemia and SCID patients with our thymus-empowered ProTcell therapy and develop a new generation of long-lasting allogeneic CAR-T cells. We would also like to be able to evaluate them in clinics to improve the outcome of patients with blood cancers as well as with solid tumors.

Smart Immune was built on the complementary skill sets of the three cofounders: Marina Cavazzana has a tremendous amount of clinical experience; Isabelle André has deep knowledge of the lymphoid cells; and Karine Rossignol is driving the whole team towards our company goals.

BIOGRAPHY

OLIVIER NEGRE has a BEng in Biotechnology from ENSTBB and a PhD in Cell and Molecular Biology from Paris Diderot university. With over 20 years of experience in biotherapies both in France and the USA, including recombinant protein production (BioProtein Technologies) and gene therapy (bluebird bio), he is currently a board member of the French Society of Gene and Cell Therapy, co-president of the Gene & Cell Therapy Institute, co-founder of Biotherapy Partners, and serves as Chief Scientific Officer at Smart Immune.

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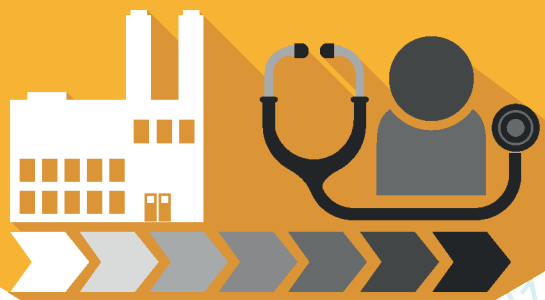
CELL & GENE THERAPY INSIGHTS

DIGITIZING THE CELL AND GENE THERAPY SUPPLY CHAIN



CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN CHANNEL:
Digitizing the cell and gene therapy supply chain

August 2023
Volume 9, Issue 7

COMMENTARY

Cell and gene therapy commercial off-the-shelf software components

Parris Farr

CELL & GENE THERAPY INSIGHTS

DIGITIZING THE CELL & GENE THERAPY SUPPLY CHAIN

CHANNEL CONTENT

COMMENTARY

Cell and gene therapy commercial off-the-shelf software components

Parris Farr

This article aims to provide readers with an understanding of how the use of commercial off-the-shelf software (COTS) makes cGMP cell and gene therapy manufacturing easier, safer, reliable, scalable, compliant, and cost-effective from early research and development (R&D) through commercial production. Several biotech and pharma COTS products that support GxP manufacturing and list common functionalities will be described. This is by no means an exhaustive list of available products. This article will provide a high level of understanding of the COTS landscape and the need for good research and planning, as well as the importance of taking an interdisciplinary approach in cGMP cell and gene therapy manufacturing. As can be seen from the variety of expertise and skills required throughout the manufacturing process, we cannot operate safely and efficiently in siloed teams as we work through the initial developmental biology, then materials engineering, and finally cell and gene therapy manufacturing.

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ASSUMPTIONS

The reader:

- Has a working understanding of what cell and gene therapies are, how GxP manufacturing, processes, and practices work at a high level, and data, warehousing, supply chain and logistics general practices, risks, and issues;
- Knows enough about technology to understand that software in a GxP environment very often integrates with

other software and equipment and rarely stands alone;

- Needs a better understanding of the capabilities and features of some of the technology available to them so they can make better decisions and improve manufacturing outcomes.

GENERAL NOTES

- There is no single, clear definition for GxP COTS software. Each product is comprised of various components or modules that often overlap with similar components in other COTS products. The spread and depth of these modules differ widely between products and are highly dependent on how they are implemented and configured;

- Almost all COTS products come with built-in workflows and summary data management functionality. These functionalities likely have significant differences from one product to another. Be certain that you understand the differences between similar functionalities when comparing products;

- Pay close attention to what you really need versus what is nice to have. Every feature adds complexity. Choose software that is easy to use and supports configuration and process changes that don't require excessive IT or vendor support. That being said, think carefully about the IT resources you will need to properly own and manage the software. This is especially true of smaller companies and start-ups that don't have an IT team to support them;

- Work with the vendor to get a clear picture of the support options they offer and how much they cost, as well as various skills and roughly how many humans you will need in house to maintain the functionality you need. Software patches, hot fixes, and upgrades happen regularly and must be validated;

- Although COTs products offer specific functionality that in theory could be used out of the box, customizations are often necessary to meet your particular requirements. Once customization is complete, configuration is required. Configuration complexity is frequently underestimated and iterative. The more you learn about the software through demos, videos, vendor documentation, and so on, the less rework you will have when you work on configurations;

- Total cost of ownership is often conjured up at a high level and isn't always carefully vetted from the bottom up by people who know the technology landscape, understand who is available to do the work, whether the necessary skill sets reside in house, peripherals (printers, scanners, tablets, and the like, documentation and validation efforts, ongoing maintenance, and support and more. Software that offers a wide selection of modules and various options like ERP, MES, or LIMS systems is especially prone to budget overruns and missing delivery timelines;

- GxP COTS products are commonly integrated with other COTS products and lab equipment, which have their own GxP software. Integrations can be quite complex and often take more time, money, and staff than anticipated. Think about possible future integrations when considering a COTS product and investigate whether those products are compatible before you purchase;

- GXP COTS products all include the following features, so I didn't include them in the feature lists for each product:

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- User authentication and authorization;
- Role Based Access (RABC) configuration and basic levels of security;
- Data encryption in transit and at rest;
- Audit trails;
- CFR 21 Part 11D compliance and e-signatures;
- Validation of digital products.

- The list of software is meant to give you an idea of some of the critical components required to manufacture cell and gene therapies and how the industry is solving the challenges we face today. It is not intended to be an all-inclusive list;
- Some of the software described below may be either stand-alone or included as modules in large platforms;
- Categories of COTS software, Electronic Notebook (ELN), or Lab Information Management System (LIMS) for example, are simply categories of software products. One cannot assume a clear, definitive, all-encompassing definition that fits every product in a category because each product differs from the next. When referring to any of these categories, the intent is a general reference to the category of products;
- Always assume that the software required for GxP manufacturing must be validated.
- Because some of the acronyms and terms may not be familiar, we have added a glossary and references as **Supplementary Material**. Please refer to this material when looking for definitions or content source material.

COMMON GxP COTS IMPLEMENTATION, CONFIGURATION

- Cell Orchestration Platforms
- Document Management Systems (DMS)
- Electronic Batch Records (EBR)
- Electronic Lab Notebook (ELN)
- Enterprise Resource Platforms (ERP)
- Environmental Monitoring (EMS)
- Laboratory Information Management (LIMS)
- Manufacturing Execution Systems (MES)
- Quality Management Systems (QMS)
- Supervisory Control and Data Acquisition Systems (SCADA)

CELL ORCHESTRATION PLATFORMS

Cell orchestrations platforms document everything that happens to biological tissue from vein to vein as it moves from location to location and is processed into a therapy along the way. Robust tracking product capabilities are required to comply with regulatory requirements. Cell orchestration platforms are commonly integrated with internal systems including ERP, LIMS, MES, etc.

Available GxP functionality includes some or all of the following, depending upon vendor capabilities and the team's requirements:

- Allows real-time visibility across different patient batches and products;
- Authorization workflows and e-signatures;
- Capacity planning and forecasting;

- Condition management and deviation reporting;
- Defines and manages the best routes to transport patient specific materials in real-time;
- Enables algorithmic equipment and clean room allocation;
- Facilitates conflict resolution and feedback and ability to visualize and manage variability impact;
- Facilitates dynamic slot allocation;
- Facilitates viewing, generating, and uploading COI/COC certificates;
- Improves adherence to schedules;
- Internal and external exception handling;
- Label generation, upload, and print capability;
- Manufacturing efficiency and asset utilization optimization;
- Manufacturing facility allocation;
- Master data management (MDM);
- Multiproduct management;
- Order and task management;
- Patient order confirmation workflow;
- Predictive patient delivery;
- Provides audit and application logging and monitoring;
- QA review scheduling;
- QC release scheduling;
- Reschedule patients according to resource availability with insight into potential bottlenecks;
- Shipping and logistics management;
- Slot change and cancellation management:
  - Supply chain partner portals allow users to see order data, label printing, key milestones capture, goods receipts, biospecimen disposition, product shipment details, and *ad hoc* events.

DOCUMENT MANAGEMENT SYSTEMS

Document management systems (DMS) are the single source of truth for everything that happens in the manufacturing process. They are critical to compliance and quality requirements across GxP organizations. They may be standalone or a component of an Enterprise Content Management system and DMS are commonly integrated with workflow and content management systems.

Available GxP functionality includes some or all of the following, depending upon vendor capabilities and the team's requirements:

- Annotation and Stamps;
- Built in manual, rules based, and/or dynamic workflows;
- Data validation configuration;
- Document distribution via electronic links or attachments;
- Document search and retrieval with a unique document identifier, partial search terms, a Boolean expression containing keywords or example phrases;
- Hard copy reproduction;

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- Image capture primarily from paper documents;
- Metadata extraction and storage, for example author's name, date created and modified, time stamps, etc.;
- Proofreading, peer and/or public review, authorizations and approvals, printing, and publishing capabilities;
- Real time collaboration;
- Roll-back, to activate a prior version in case of an error or premature release;
- Store and manage electronic documents;
- Unique document identifiers and classification through metadata or word indexes;
- Versioning and check in/check out.

ELECTRONIC BATCH RECORDS

Electronic batch records (EBR) software electronically executes and stores manufacturing recipes, documents, and processes according to regulatory and compliance requirements. A batch record is a quality-controlled document that collects all the data and information such as materials, equipment, people, data, labels, and events during the production of a regulated product. This records everything that happens to the tissue during the manufacturing process such as:

- Who did what, and when?
- Which materials were used?
- Which procedures were followed and were the results within specifications?

They ensure error-free and guided execution of the entire production process and right-first-time manufacturing. EBR replace paper-based batch records with electronic documents, thereby eliminating time-consuming and error-prone manual comparisons and approval procedures. They significantly improve processes and product quality.

Different versions of EBR products are available for different phases of a product lifecycle. When working in research and development (R&D), creating standard processes, builds of materials (BOMs), kitting, labeling, packaging etc. is a blank slate. The team works iteratively to discover how to efficiently manufacture their new product. In order to do that, they need highly flexible software that complies with GLP practices. They can refine their efforts without having to adhere to the unyielding GMP compliance requirements that apply to commercial products. When the product is ready for commercialization, regulatory bodies require GMP compliant software that includes QC testing and doesn't allow for process, BOM, kitting or labeling, packaging, or any other deviation. Carefully consider and balance your requirements for a highly flexible EBR versus more rigid requirements that regulatory and compliance guidelines suggest, especially when manufacturing small batch sizes with highly variable processes. EBR functionality is typically included in MES.

Available GxP functionality includes some or all of the following, depending upon vendor capabilities and the team's requirements:

- Automatically runs plausibility and completeness checks;
- Digital execution and documentation;
- Efficient electronic paperless batch documentation;
- Electronically executes manufacturing recipes and documents processes;
- Electronically maps and records master batch records as graphical structures;

- Paperless batch documentation, routing, and approval;
- Real-time data capture.

ELECTRONIC LAB NOTEBOOK

Electronic lab notebook (ELN) streamlines lab activities and supports collaboration between lab members. Users have concurrent access to relevant information with no risk of creating conflicting document versions. Researchers can access their records remotely, which is especially useful when physical access to facilities is limited.

They remove the need to transport physical laboratory notebooks between locations and reduce the risk of cross-contamination and data loss. There is no need to print documents or fill out forms manually. Data is secure and data integrity is significantly higher. They track materials, automate tasks like order processing, shipping, and other tasks related to stock control, and support best research practices. Overall operational efficiency and adherence to regulatory and compliance requirements improve measurably.

Available GxP functionality includes some or all of the following, depending on vendor capabilities and the team's requirements:

- Accurate routine experimental information capture;
- Assign tasks to team member;
- Data annotation and linking to relevant experiment capability;
- Data sharing and export;
- Dataset and report gathering, visualization, and comparisons;
- Draw chemical compounds, reactions, and query molecules easily and integrate into lab documents and reports;
- Encrypted data transfers;
- Inventory management capability;
- Materials tracking;
- Optimized search capability to templates tailored to each facility's needs;
- Pre-populated protocols, standard operating procedure templates and automation rules;
- Research tracking;
- Secure documents sharing capability via email or file-sharing platforms;
- Secure records storage in one location;
- Stock control task automation;
- Workflow automation.

ENTERPRISE RESOURCE PLATFORMS

Enterprise resource platforms (ERP) are suites of integrated applications that function together as a business process management software solution. They integrate with different computer systems across many departments in an organization together to manage various functions across the enterprise. Because of the capacity of ERP systems to address both information needs as well as regulatory requirements of all departments and functions across a company, they have become an important reference solution for large pharmaceutical companies.

Without an ERP application, each department would have its system optimized for its specific tasks. With ERP software, each department still has its system, but all of the systems can be accessed through one application with one interface.

When properly implemented, ERPs eliminate similar and/or duplicate systems

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and incompatible technology, tie together business processes from multiple sources, and enable the flow of data between them. This eliminates data duplication, enables huge improvements to data integrity, and boosts security. They provide an integrated and continuously updated view of core business processes using common databases maintained by a database management system.

ERP software offers a wide variety of benefits including but not limited to:

- Eliminating data duplication and paper records and critical data stored in spreadsheets;
- Enabling global integration by eliminating barriers of currency exchange rates, language, and culture;
- Facilitating information flow between all business functions and managing connections to internal and external stakeholders;
- Gaining operational efficiencies;
- Improving quality;
- Integrating planning across departments;
- Managing validation and regulatory compliance;
- Minimizing production delays;
- Simplifying demand forecasting;
- Standardizing and automating processes across departments throughout the enterprise;
- Streamlining inventory management.

Available GxP functionality includes some or all of the following, depending on vendor capabilities and the team's requirements:

- Customer Relationship Management (CRM);
- Financial Accounting and Controlling (FICO);
- Financial Supply Chain Management (FSCM);
- Human Resource Management (HRM), also known as Human Resource (HR);
- Manufacturing;
- Materials Management (MM);
- Order Processing;
- Plant Maintenance (PM);
- Production Planning (PP);
- Project System (PS);
- Quality Management (QM);
- Sales and Distribution (SD);
- Supplier Relationship Management (SRM);
- Supply chain management.

ENVIRONMENTAL MONITORING SYSTEMS

Environmental monitoring systems (EMS) allow teams to monitor and control viable, non-viable, and environmental contamination. They are location based to support environmental workflows and include temperature monitoring, particle counts, and microbial testing to check the cleanliness of laboratories, cleanrooms, clean areas, warehouses, and storage rooms. They also support utilities monitoring and gowning qualifications. Customized reporting allows laboratory technicians to preempt refrigerator or freezer failure and take actions to protect cell

cultures and priceless, irreplaceable research samples. EMS software is audit proof, scalable, and secure.

The environment in which the product is manufactured and stored is critical in order to ensure that the product remains viable and safe through the manufacturing, storage, and logistics processes. Failure to adhere to could mean loss of the product and time to market complications.

Available GxP functionality includes some or all of the following, depending upon vendor capabilities and the team's requirements:

- Active air monitoring systems;
- Ad hoc sample generation;
- Ad hoc analysis in case of excursions;
- Air monitoring media;
- Automated processes, analytics, and standard and customized reporting;
- Configurable, role-based alarming;
- Microbial sampling;
- Particle counts;
- Passive air monitoring;
- Regulatory and compliance;
- Remote warning and alarm notifications;
- Scheduling;
- Surface and personnel monitoring;
- Temperature mapping.

LABORATORY INFORMATION MANAGEMENT SYSTEM

Laboratory Information Management System (LIMS) software is a platform built around a centralized database which is made up of a massive amount of sample data. LIMS solutions digitally record, track and store sample metadata, results and instruments from the time it enters a lab until processing is complete. Samples can easily be audited from creation, to release, usage, and disposal. They eliminate manual data entry, reducing the opportunity for human error, which supports the lab's efforts to remain compliant with regulatory regulations and industry best practices. They are secure, automated, and scalable.

In the past, LIMS were not often integrated with other systems or equipment, which limited direct data capture and metadata. As the need for gathering huge amounts of data from a variety of sources expands, LIMS integration capabilities are improving. LIMS are now commonly integrated with lab equipment, ELN, and QMS, and other COTS software.

It is worth noting that LIMS tools tend not to handle quality management very well. It is good practice to consider finding a QMS solution that integrates well with the existing LIMS software to improve compliance and drive efficiency from lab bench to product release.

Available GxP functionality includes some or all of the following, depending on vendor capabilities and the team's requirements:

- Allows analysis to be conducted in the context of the entire product, not just a single batch;
- Automates and streamlines workflows, processes, and reporting;
- Centralizes access and storage of quality control (QC) information;
- Checks quality of a batch manufacture and/or water throughout a distribution system with reliable traceability for each sample;
- Creates internal and client reporting;

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- Documents, tracks, and manages inventory relating to controls and samples;
- Enables data entry by way of barcode scanning and manual entry, data upload directly from lab equipment and workflow automation;
- Identifies errors from testing or experimental runs and flag them;
- Initiates downstream data analysis workflows;
- Manages lots and releases, as well as sample and assay data;
- Monitors batch usage and performance;
- Offers visibility into sample data;
- Performs instrument run monitoring;
- Prevents the use of expired lots;
- Prioritizes and performs batch runs;
- Provides flexible architecture, and data exchange interfaces;
- Provides sample inventory configuration with data trending;
- Removes the lab's reliance on manual documentation and maintains data integrity;
- Standardizes and automatically prevents poor-quality samples from processing;
- Supports comprehensive case-centric clinical data;
- Supports regulatory and compliance tasks;
- Tracks data from sequencing runs over time and across experiments;
- Tracks reagents and lots, as well as distribution of lots among lab members.

MANUFACTURING EXECUTION SYSTEMS

Manufacturing execution systems (MES) allow users to gain insights into manufacturing operations, analyze potential actions to improve performance, maximize efficiency, and cut cost by connecting, monitoring, and controlling complex manufacturing systems and data flows on the factory floor from order release through product delivery. They reduce error rates and manufacturing costs, shorten time-to-market, and increase manufacturing efficiency to enable workers to collect real-time data at the moment they need it. Until recently, MES have generally been best suited to large scale production environments.

Full blown MES software is more practical in a big pharma commercial setting than in a small start-up or early R&D process development, clinical trial manufacturing, and scale up setting. They are very complex and expensive, and they require significant maintenance and a team to support them. Smaller, lighter MES options are coming onto the market that are better suited for CGT manufacturing.

Available GxP functionality includes some or all of the following, depending upon vendor capabilities and the team's requirements:

- Approval management;
- Automatic document version management;
- Controls, monitors, and documents manufacturing processes digitally in real time;
- Detailed sequencing;
- Dispatching production unit;
- Document control;
- Electronic approval management;

- Enforces process requirements;
- Labor management;
- Maintenance management;
- Performance analysis;
- Process management;
- Product tracking and genealogy;
- Production analysis;
- Quality management;
- Real-time data collection;
- Resource allocation and status.

QUALITY MANAGEMENT SYSTEMS

Quality management systems (QMS) are comprehensive digital systems that house complete records of business processes and are a central tool for customer feedback, issues, policies, suppliers, documents, risks, incidents, training records, equipment, audits, and inspections. This enables the system to provide real time custom task lists for each user. They can automatically pull in data from an ERP or mobile applications and can send notification such as review requests, change updates and alerts.

QMS software is usually made up of several different modules that enable quality activities from document management to corrective and preventative actions. They are commonly integrated with LIMS systems, which can help improve compliance and drive efficiency from lab bench to product release.

The benefits and advantages of using QMS software include:

- Accurate risk forecasting;
- Automated workflows improve communication flow;
- Empowered quality, risk, audit, and operations teams;
- Enhanced process management;
- Enriched products and services;
- Heightened productivity;
- Improved quality metrics;
- Increased customer satisfaction and retention;
- Real time incident response;
- Reduction in errors;
- Simplified ISO standard certification.

Available GxP functionality includes some or all of the following, depending upon vendor capabilities and the team's requirements:

- Quality assurance;
- Quality control;
- Quality control planning;
- Quality improvement.

SUPERVISORY CONTROL & DATA ACQUISITION

Supervisory Control and Data Acquisition (SCADA) systems include software and hardware elements that enable industrial organizations to control industrial processes locally and/or at remotely, monitor, gather, and process data in real time, directly interact with devices such as sensors, and record events as data.

They incorporate microcomputers that communicate with an array of objects including but not limited to factory machines, human-machine interface (HMI), sensors, and end devices, then route the information from

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those objects to computers with SCADA software installed. They increase productivity and maintain efficiency, process data to facilitate smarter decisions and communicate system issues to help mitigate downtime.

Available GxP functionality includes some or all of the following, depending on vendor capabilities and the team's requirements:

- Creates formulas used for calculating values and input channel status, and calculating command values;
- Control;
- Data acquisition and transmission;
- Data collection, storage, and retrieval;
- Electrical communication;
- Human-machine interface (HMI);
- Monitoring;
- Report generation.

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CELL & GENE THERAPY INSIGHTS

LATEST ARTICLES:

Driving the expansion of mRNA into the therapeutic sphere

Alejandro Becerra, Thermo Fisher Scienitific, **Andreas Kuhn**, BioNTech, and **Metin Kurtoglu**, Cartesian Therapeutics

The advanced therapies industry is heavily engaged in capitalizing upon the success of mRNA-based COVID-19 vaccines. Novel therapeutic applications in major disease areas, including oncology, continue to show promise in preclinical and early clinical studies, yet challenges remain. *Cell & Gene Therapy Insights* brought together a panel of industry experts to discuss the expanding reach of mRNA technology, exploring how and where it will impact the advanced therapies space moving forward.

Here are some of the highlights…

Cell & Gene Therapy Insights 2023; 9(6), 783. DOI: 10.18609/cgti.2023.098

What are some of the major challenges that face the field as it migrates from infectious disease vaccines into therapeutic drug applications?

"Using mRNA to vaccinate against infectious diseases works really well. The mRNA itself is very immunogenic and the body will immediately react to it. However, when you go after a disease that needs a long-term therapeutic effect, it will be challenging to produce the right type of RNA in a formulation that results in sustained therapeutic activity."

—Mertin Kurtoglu

"From the development perspective, the purity of the mRNA is critical, and closely associated with purity are the analytical challenges. A purification process is only going to be as robust as the analytics that are available to develop it. It will be critical to establish better methods in order to characterize the product-related impurities."

—Alejandro Becarra

"With vaccines, only relatively small amounts of protein are needed in order to obtain a huge amplification by the immune system. On the other hand, using mRNA for the expression of functional protein requires several orders of magnitude higher expression of that protein. Therefore, looking into improved expression of the mRNA is key—for example, through improved sequence design."

—Andreas Kuhn

Looking at mRNA therapeutic manufacturing, what are the main limitations with the current processing tools and technologies?

"The design of the mRNA is the biggest challenge in mRNA manufacturing. How much mRNA is needed to make enough protein in order to achieve the therapeutic function? The answer is that the amount of mRNA required depends greatly on the design of the mRNA. If you can design an mRNA where you only need a microgram to give the desired therapeutic effect, then manufacturing is no longer going to be a challenge. The second challenge relates to the delivery system: whether you are using a LNP or a cell, the limitation and bottleneck right now is in scaling up."

—Mertin Kurtoglu

What will be the key technological and platform developments and innovations required to address mRNA downstream processing challenges?

"There are ongoing efforts to improve the purification toolkit for the mRNA field. More specifically, when we are looking at eliminating double-stranded RNA (dsRNA) from the final product, current efforts focus both on the *in vitro* transcription (IVT) reaction and the downstream process."

—Alejandro Becarra

"There is a lot of existing knowledge on purifying biological molecules that can be applied to mRNA, including on the analytics side. We will need improved analytical techniques to better understand what the molecule is that we have in hand."

—Andreas Kuhn

What are the key areas for improvement in the analytical toolkit?

"One of the challenges at this moment is the diversity of methods used to analyze the same parameter. One example is measuring RNA integrity, which indicates the amount of full-length RNA versus the amount of degradation products or truncated transcripts. Analysis of RNA integrity can be performed by using a large variety of techniques and you can question how the results of these different techniques correspond to each other. Harmonization and standardization of analytics are very important moving forward."

—Andreas Kuhn

Watch the webinar here

Read the full transcript here

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Navigating evolving regulatory CMC guidance in the AAV gene therapy field

Michael Brewer, Thermo Fisher Scientific, **Alexis Cockroft,** Lex Regulatory Ltd, **Christina Fuentes,** Dark Horse Consulting Group, **Christine Le Bec,** Sensorion Pharma, **Yan Zhi,** CSL Behring

Analytical tool innovation is delivering a new depth of understanding of AAV vectors and the impact of elements, such as full-empty capsid ratio. But with this new knowledge comes uncertainty, as regulators and developers alike struggle to keep pace with the speed of progress in what is a still nascent and relatively unstandardized field. *Cell & Gene Therapy Insights* brought together a panel of regulatory, analytical, and process experts to examine some of the key developments in the CMC landscape for AAV-based gene therapy. The panel dissected current and future regulatory guidance, offering advice to those seeking to avoid the setbacks that have recently hindered products in late-stage development. Here are some of the highlights…

The US FDA has been clear in relaying the importance of full/empty/partially-full capsid ratio to the safety and efficacy of the final drug product—can you expand on the tools and methods that stand out for you in terms of maximizing the quality of the final vector product in this specific area?

"There has been an evolution towards dPCR and array-based digital PCR platforms, which have improved accuracy. This is particularly critical for counting vectors.

The method chosen for the quantitation of viral capsid and correlating the ratio of full/empty is also critical. People frequently use ELISAs for this purpose, but there have been developments in analytical ultra performance liquid chromatography (UPLC) methods that can distinguish between empty, full, and partially-full capsids. This method can even determine a percentage of overly-full capsids that have fragments of host cell DNA incorporated into the vector."

Michael Brewer, Thermo Fisher Scientific

"People are mostly trying to enrich capsids at the beginning of the process. Many are working more on vector design and using two plasmids instead of three to enrich capsids and achieve a high yield. It is difficult to achieve 100% full particles during purification - you can achieve 90–95% if you pre-refine with cesium chloride gradients, although this type of process can pose challenges for clinical applications. For clinical applications, ion chromatography approaches such as anion exchange chromatography (IEX) are the most commonly used methods.

We must bear in mind the definitions of full and empty particles. We must fully characterize these and know what is inside. Next-generation sequencing (NGS) is being used to understand the quantity and the size of the host cell DNA, the plasmid DNA, and the capsid itself."

Christine Le Bec, Sensorion Pharma

How would you go about determining partially-full capsid percentage as a critical quality attribute (CQA), and then establishing a release assay and release specifications?

"Everyone knows an empty particle is an impurity – there is no question about that. However, if you have data to show the intermediate species actually has a biological effect, then the conversation changes: it is no longer an impurity, it's just a different form of your product. This might open the door to consider full and partially-full particles together."

Yan Zhi, CSL Behring

"Setting the acceptance criteria for partially-full capsid population is really dependent on the capabilities of the analytical methods you are using to differentiate or discriminate between populations."

Michael Brewer, Thermo Fisher Scientific

What would you say are the 'must-do's' in terms of early product development?

"I strongly advise an integrated development plan from an early stage and applying quality by design (QbD) principles. A quality target product profile should be created early and CQAs should be considered. An analytical development plan is also needed early on.

When considering what is needed for a clinical trial application…

- As a minimum, one should know both the FDA and the EMA guidances for clinical trial applications off-by-heart
- As early as is feasible, critique your vector design, and question every single component for its benefit and safety profile
- You need at least one batch manufactured in accordance with your proposed manufacturing process for the clinical trial
- Design your production so that you can have an initial batch assigned to stability, in order to have stability data, and indicate which (potential) CQAs will be tested. Once you have established a stability profile, you can propose a meaningful, practicable shelf-life
- Ensure you have completed compatibility studies in addition to a potency assay. Qualify the assay that will be used for dose determination, otherwise you risk wasting clinical data if those results are not robust
- Process-related impurities, such as any raw materials being used that could have a potential toxicological or pharmacological action, should be risk assessed
- Qualify your starting materials and consider whether each material you are using is the best for the job."

Alexis Cockroft, Lex Regulatory Ltd

"Take retains, especially if you are starting this work early. You may not have all your methods developed yet, so having those retains will be really important, particularly once you have your dose determining assay.

In addition, the earlier you can make the transition to a representative manufacturing process, the more of your preclinical data you can leverage later.

Finally, you need to save your preclinical lots because ideally, you will test your titer assay on those retains. Once you have your qualified method in place, you can again leverage the data to determine your starting dose range for clinical use."

Christina Fuentes, Dark Horse Consulting Group

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CELL & GENE THERAPY INSIGHTS

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CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Addressing regulatory guidance for HEK293 cells & AAV-based therapeutics manufacturing

Mike Brewer

Testing and characterization in cell and gene therapy manufacturing is critical for AAV-based therapeutics. Regulatory guidance affecting those using HEK293 cells or AAV-based therapeutics is evolving over time, especially pertaining to quantitating residual host cell DNA and analyzing its size via E1A fragments, quantitating residual plasmid DNA (pDNA), and detecting the presence of the E1A oncogene. Integrated solutions leveraging real-time PCR or dPCR technologies are necessary to meet regulatory needs.

This article will cover some of the latest regulations around residual DNA amounts in the product, as well as quantitating host cell and pDNA, and the presence and size of the E1A oncogene. Integrated dPCR and qPCR assay solutions will also be introduced.

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RESIDUAL HOST CELL DNA TESTING GUIDANCE

Regulatory guidance for residual host cell DNA testing has existed since the beginning of the recombinant DNA biopharma revolution starting in the early 1980s. That guidance has evolved over time maintain pace with technological advances. For example, the most recent chemistry, manufacturing and control (CMC) guidance for human gene therapy investigational new drug (INDs) involves taking steps to minimize the biological activity of any residual DNA associated with a viral preparation. This can be accomplished by reducing the size of the DNA to below the size of a functional gene and decreasing the amount of residual DNA. The recommendation is that the amount of residual DNA is limited to 10 ng per dose and the DNA size to below ~200 base pairs (bp).

It is recommended that sponsors carefully consider the characteristics of cell lines used in the manufacture of viral vectors that may impact the safety of the final product, including the presence of tumorigenic sequences. The Human Gene Therapy for Neurodegenerative Disease FDA Guidance for Industry

advises limiting residual host cell DNA levels. Historically, guidance for industry was first developed for cell culture-based vaccine manufacturing in February 2010. This mentioned the potential risk of residual host cell DNA from certain host cell lines and provided guidance on decreasing its biological activity by size reduction.

Testing for residual DNA in AAV manufacturing occurs at the end of purification. Optimizing the manufacturing process to reduce non-vector DNA contamination in the final product is recommended.

A SOLUTION FOR MEASURING & QUANTITATING RESIDUAL HOST CELL DNA

The Applied Biosystems™ resDNASEQ™ Residual DNA Quantitation System includes optimized sample preparation and assays specific to the host cell DNA. The system is highly sensitive with an overall method limit of quantitation (LOQ) of as low as 1.5 pg/mL of test sample for mammalian DNA, and 15 pg/mL for bacterial or yeast cell culture processes. Generally, the workflow leads to a rapid result in <5 h, giving highly consistent results, especially when using the automated sample preparation options. There is typically an extremely low failure rate due to percentage coefficient of variation (CV) in the triplicate samples. This system leverages a worldwide support network, technical validation support, as well as hands-on training by expert Field Application Specialists.

The resDNASEQ Quantitative HEK293 DNA Kit is an assay specifically for HEK293 cell line-based manufacturing processes. This quantitative PCR (qPCR)-based system includes precisely quantitated, highly purified genomic DNA from an established Thermo Fisher Scientific HEK293 cell line as a standard control. The kit has a high adoption rate, with 50% of the top 100 large pharma companies worldwide relying on the reliable performance of resDNASEQ. The quantitation of the host cell DNA with this assay is independent of the DNA size. The resDNASEQ system has also been shown to give reliable results across multiple stages in the gene therapy manufacturing process.

QUANTITATING FRAGMENT LENGTHS

Guidance on specific gene testing for HEK293 cells states that in addition to controlling the host cell DNA content and size, the level of relevant transforming sequences should also be controlled. In this case, products made in 293 cells should be tested for specific genes in 293. A resDNASEQ Quantitative E1A DNA Fragment Length Kit has been developed for gene therapy and cell-based vaccine manufacturers who use HEK293 and need to quantitate the fragment lengths of residual DNA to adhere to new regulatory guidelines for the clearance of residual DNA fragments >200 bp, and to identify the oncogenic potential. It has a 3-in-1 assay design to allow the differentiation of three different fragment lengths (86 bp, 200 bp, and 478 bp). The assay has ultra-high sensitivity, being able to accurately quantitate down to 30 copies of the target in the qPCR reaction. The results shown in Figure 1 demonstrate linearity and high efficiency to enable quantitative results across a broad range of DNA concentrations. The resDNASEQ™ E1A DNA Fragment Length Kit is a comprehensive product solution with the same rapid testing and sample prep chemistry as the resDNASEQ™ Residual DNA Quantitation System.

Measuring E1A fragments is an excellent approach for assessing the effectiveness of DNA size reduction steps, and also ensuring the oncogenic E1A gene has reduced to a size that is ≤200 bp E1A is an oncogene that is transformed into HEK293 cells. It is essential for the transcription of other viral genes, which are responsible for viral DNA synthesis and play roles in modulating the expression of host genes. Adeno-associated

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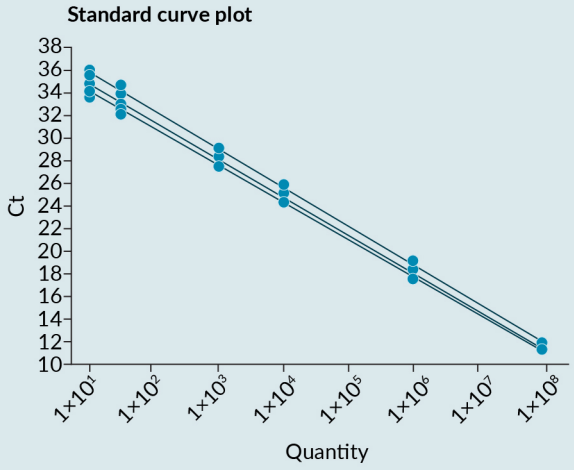
FIGURE 1

Standard curve performance of the resDNASEQ Quantitative E1A DNA Fragment Length Kit.



	Short	Medium	Long
Fragment size (bp)	86–200	200–476	>476
Slope	–3.284	–3.363	–3.433
R^2	0.999	0.999	0.999
Efficiency (%)	101	98	95

Ct: Cycle threshold: bp: Base pairs.

virus (AAV) is actively used as a gene therapy vehicle to transport modified genes into the cells. Typically, recombinant AAV is manufactured in HEK293 cells, and HEK293 is also a frequently used cell line in the production of cell-based vaccines. Manufacturers want to ensure efficiency in the DNA size reduction step (i.e., benzonase) and ensure clearance of the E1A gene in the final product.

PLASMID VECTOR QUANTITATION: KANAMYCIN RESISTANCE GENE KIT

Typically, multiple plasmids are co-transfected into the cells as part of the manufacturing process to induce the production of recombinant AAV. For gene therapy/vaccine manufacturers who are currently using plasmids in their workflow, and for plasmid manufacturers themselves, Thermo Fisher provides a robust, easy-to-use, highly sensitive multiplex qPCR assay to measure residual pDNA by targeting all common alleles of kanamycin resistance genes. This enables measurements of the presence and the removal of the pDNA during purification. It can also be used by plasmid manufacturers to quantitate pDNA through purification and in the final purified form. As a comprehensive solution, the product has high sensitivity and targets the common alleles in plasmids commonly used in the AAV workflow. The kit enables accurate quantitation as low as 30 copies in a test sample.

Amplification plots were generated using serial dilutions of pDNA standard (ranging from 15 copies to 300,000 copies provided in the kit), as shown in Figure 2. The broad linear range allows the testing of a wide range of Kanamycin-resistant pDNA samples.

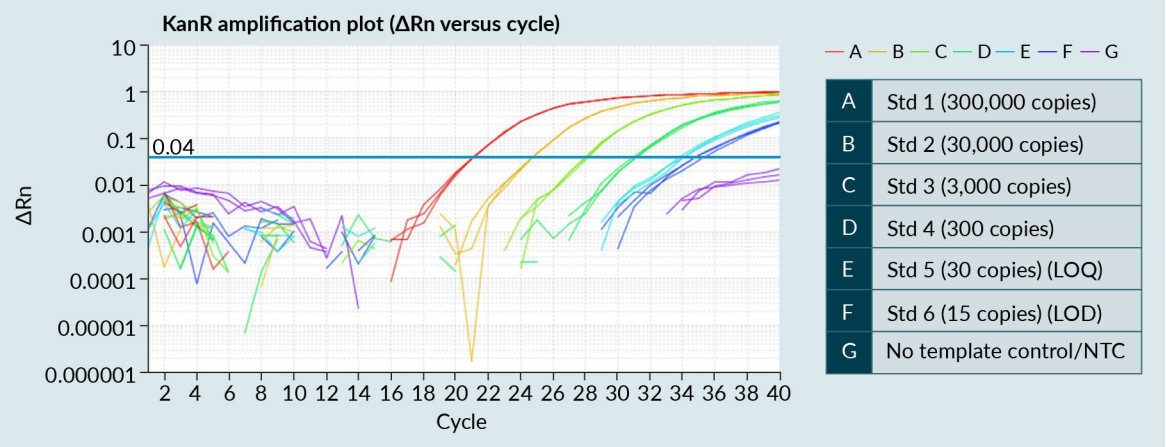
Utility is shown throughout the manufacturing process and in the manufacturing of plasmids. The standard curve performance is very linear, demonstrating that the Kanamycin Resistance Gene Kit is capable of enabling quantitation across a broad range.

AAV VIRAL PARTICLE QUANTITATION

At the 70th Cellular Tissue and Gene Therapy Advisory Committee meeting in September 2021, additional guidance surrounding the risk of hepatoxicity observed in clinical trials with high doses of AAV vectors was discussed. The fact that many AAV products contain

FIGURE 2

Amplification plots showing the sensitivity and dynamic range of the Kanamycin Resistance Gene Kit.



Std: Standard.

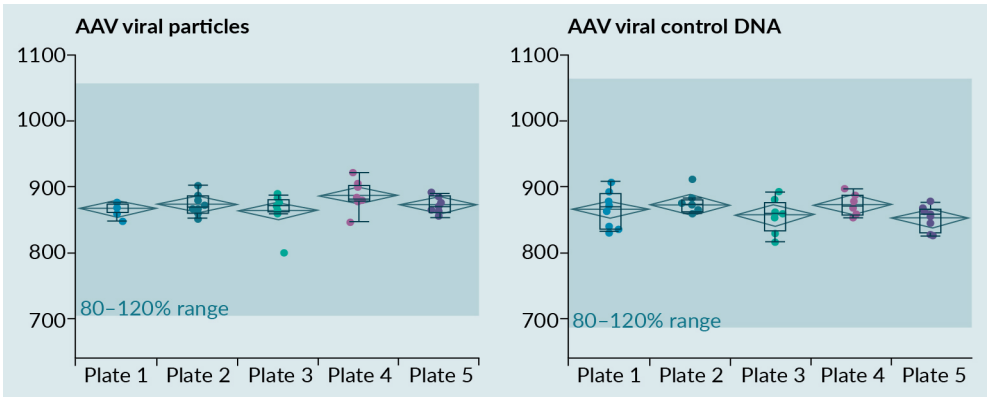
significant amounts of empty capsids was noted. It was concluded that assays for empty capsids need better standardization, and more effort is needed to comprehensively characterize empty capsids and other byproducts of AAV manufacturing.

However, before specific guidance on acceptable levels of empty capsids can be provided, there needs to be a better understanding of the manufacturing process. To help address this issue, Thermo Fisher has a dPCR assay that provides a sensitive and accurate quantitation of AAV genomes. dPCR is a method of quantifying nucleic acid targets without standard curves by dividing the bulk reaction into thousands of smaller, independent reactions. Individual molecules are amplified in each microchamber, and positive and negative microchambers are simply counted.

Results of the readout of the dPCR assay for counting viral genomes of AAV are shown in Figure 3. This assay targets the ITR2 sequence of AAV viral particles, as well as a DNA control. The Absolute Q dPCR instrument demonstrates consistent quantification

FIGURE 3

Demonstrating consistent AAV quantification across plates.



AAV: Adeno-associated virus.

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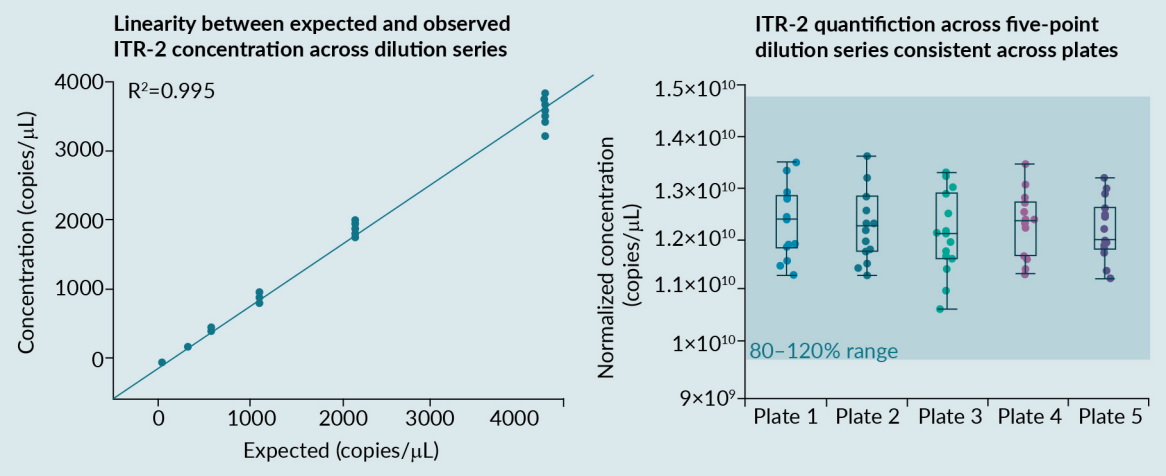
FIGURE 4

Linearity between expected and observed ITR-2 concentration across dilution series.



ITR: Inverted terminal repeats.

of ITR2 sequence using both AAV particles and AAV DNA control.

Figure 4 shows the linearity of the dPCR solution across a five-point AAV dilution series. The accuracy and consistency of the data are high, even at lower concentrations of the AAV targets (<1,000 copies/test sample).

CELL CULTURE IMPURITIES: qPCR TESTING FOR MYCOPLASMA

Mycoplasma testing guidance from FDA states that vector safety testing should include microbiological testing, such as sterility, mycoplasma, endotoxin, and adventitious agent testing, in order to ensure that the chimeric antigen receptor(CAR-T) cell drug product is not compromised. Mycoplasma testing is required at cell culture harvest for virus used in gene therapy and for transduction of T cells in cell therapy. In the manufacturing process of recombinant AAV, mycoplasma testing is typically done at the stage of bulk harvest from the bioreactor producing the recombinant virus. Downstream, residual DNA impurities such as plasmid host cell DNA and E1A are measured.

The MycoSEQ Mycoplasma Detection System is an integrated sample preparation and qPCR assay for the detection of mycoplasmas that is accepted by regulators across multiple therapeutic modalities, including recombinant proteins, monoclonal antibodies, vaccines, and viral vectors. It can also be used in cell-based therapy manufacturing as a release test.

This assay was designed to exceed the guidelines as a nucleic acid amplification technique (NAT)-based alternative method for lot-release testing. It includes an optimized sample preparation developed for a protocol starting with 10+ mL of the starting test sample to enable an analysis of 1 mL of test sample equivalent in a qPCR reaction. The system and instrument have an integrated software platform that enables full compliance with 21 CFR Part 11 electronic records expectations. Instrument Installation Qualification and Operational Qualification services are offered, with an extensive network of field application scientists offering instrument training and providing guidance on method qualification, implementation, and validation.

The method provides highly confident results, uses objective multi-parameter analysis, and incorporates a proprietary and patented discriminatory positive control. It enables the differentiation of real mycoplasma targets and accidental cross-contamination

with a positive extraction control. It is also highly sensitive and consistent across the various mycoplasma species: the assay is designed to support the detection of over 140 species and is sensitive down to 1–3 genome copies per qPCR reaction. It has also been demonstrated to be specific to mycoplasma species and does not detect microorganisms or non-mycoplasma microorganisms that are related genetically to mycoplasma.

Following validation, regulatory filing, and review, end users have received regulatory acceptance to use MycoSEQ across a variety of therapeutic modalities including cell culture, cell therapy, and tissue therapies. This complete sample-to-answer solution typically uses the cartridge-based magnetic bead processing system the AutoMate Express. Following extraction of the DNA from the samples, the MycoSEQ qPCR assay is employed, typically running on the Applied Biosystems™ QuantStudio 5 or 7500 Fast Real-Time PCR instruments, with analysis on the fit-for-purpose, application-specific AccuSEQ software.

The Ct value obtained with this assay is a measure of the amount of DNA present at the beginning of the qPCR reaction. Ct data can be compared from experiment to experiment and lab to lab, and is shown to be consistent across studies.

SUMMARY

The constantly evolving regulatory environment for HEK293 cells and AAV-based therapeutics manufacturing necessitates integrated solutions to quantitate residual host cell DNA, fragment lengths, residual pDNA, AAV genomes, and cell culture impurities. Thermo Fisher Scientific has established a multitude of real-time qPCR and dPCR assays in order to enable the manufacture of HEK293 and AAV-based therapeutics whilst meeting all regulatory requirements. Each of these assays has been validated by multiple end users globally.

Q&A

Mike Brewer

Q I've heard that sample preparation is not needed for analysis with dPCR. Can you provide more details of that?

MB: This is a somewhat nuanced issue. In cases where the concentration of analyte is significantly high, where the sample can be diluted prior to analysis by dPCR, there is no sample prep needed. That is also the case for qPCR. As you move deeper into a purification process where the amounts of target DNA are reduced and the amount of protein or virus and excipients in the sample are much higher, it is often necessary to use a sample prep, whether using qPCR or dPCR.

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Q I noticed that the regulatory specifications on host cell DNA levels are in nanograms of DNA per dose. Can dPCR, which reports in copies of DNA in a reaction, be used for host cell DNA testing?

MB: It is often the case that when you are looking to find the right tool for a job, there is a good tool and a best tool. dPCR counts copies of the target, whilst qPCR measures the amount of DNA in a test sample through the comparison of qPCR of the DNA in the test sample to a standard curve generated by qPCR analysis of known amounts of the standard DNA. Typically, qPCR reports in nanograms, picograms, or femtograms of DNA, aligned with the regulatory guidance. Regulatory guidance and the expectations on limits that are acceptable per therapeutic dose are in nanograms.

When using dPCR, a well-designed study must be performed to be able to accurately correlate the number of copies in the digital readout to the mass of the DNA in the test sample. That can be a challenging experiment to carry out, especially because the size of the DNA can make a big difference.

BIOGRAPHY

MICHAEL BREWER is the Director, Global Principal Consultant, Regulatory for the BioProduction Group (BPG) at Thermo Fisher Scientific. In this role, Michael is responsible for providing global support to BioProduction customers and serving as the regulatory thought leader and expert across all technology areas within BPD. Prior to moving to this role, he led the Pharma Analytics business, a team responsible for development and commercialization of testing applications for microbiology, analytical sciences and quality control. The products are fully integrated solutions for glycan profiling, bacterial and fungal identification, mycoplasma and viral detection and host cell DNA and protein quantitation. Michael has over 30 years of experience in the biopharma industry, including, Scios, Synergen and Amgen in a variety of roles including discovery research, analytical sciences and quality control. Prior to joining Thermo Fisher Scientific, he led a group at Amgen that developed qualified, validated and implemented molecular methods for host cell DNA quantitation, contaminant (mycoplasma, virus and bacteria) detection, contaminant identification, strain typing and genotypic verification of production cell lines. Additionally, his group supported regulatory submissions including IND, NDA, and CMC updates, Regulatory inspections, NC/CAPA investigations, contamination investigations and remediation and developed regulatory strategy for implementation of new methods.

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ThermoFisher SCIENTIFIC

AUTHORSHIP & CONFLICT OF INTEREST

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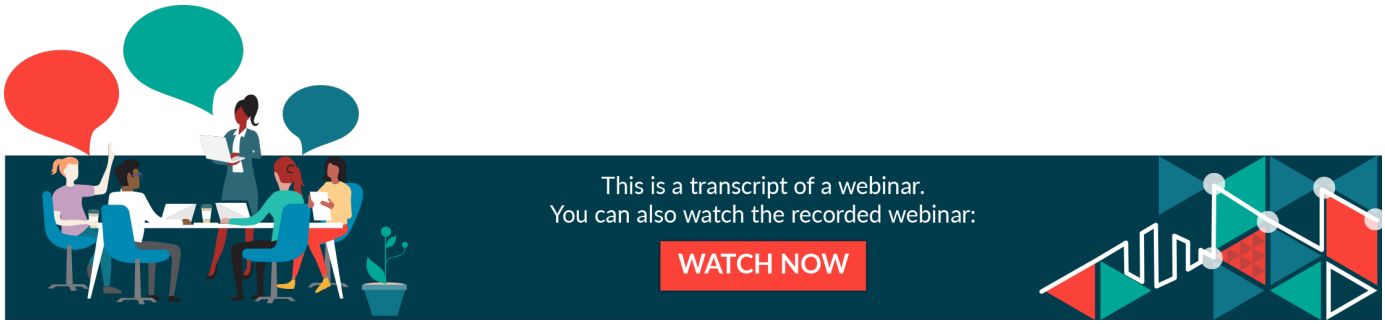

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The way forward in residual DNA quantitation.

A fully integrated, easy-to-use solution to help you meet regulatory guidance

Applied Biosystems™ resDNASEQ™ kits are quantitative PCR-based assays designed to enable accurate quantitation of residual host cell DNA and residual plasmid DNA. This is crucial in demonstrating the removal of host cell and process-based plasmid impurities during the purification of biopharmaceutical products—a global regulatory requirement.

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CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Overcoming upstream & downstream process barriers for large-scale AAV production

Betty Woo & Jonathan Zmuda

Given the broad treatment potential and demonstrated efficacy of recently approved viral vector-based gene therapies, there is an established need for reproducible and consistent manufacturing solutions that yield high titer, high quality viral particles for clinical applications. It is critical to implement a robust manufacturing process that addresses the upstream and downstream production challenges of obtaining sufficient titers and purity for *in vivo* applications, whilst also meeting safety and regulatory requirements for clinical use.

This article discusses key barriers to addressing clinical needs and market supply of gene therapies. We will explore the implementation of solutions to overcome these issues, thereby optimizing AAV manufacturing processes and accelerating the development of safe and effective gene therapies.

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Industry experts and patient advocacy groups alike are excited and optimistic over recent regulatory approvals for cell and gene therapy products. Furthermore, the number of marketing authorizations in the field is expected to grow, including in exciting new areas such as CRISPR-based therapies targeting hematologic diseases. This article focuses on a key technology area driving the commercial maturation and industrialization of the field: adeno-associated virus (AAV).

The quality of AAV vector-based gene therapies has advanced in recent times in large part due to the development of fit-for purpose solutions, which can meet regulatory chemistry, manufacturing, and control requirements for safe and effective products. However, more general solutions, including plastics, basal media, general lab equipment, and analytical assays and instrumentation, are equally important to manufacturing success. It is critical for solution providers to continue exploring how these more general-purpose products may be improved to accelerate the commercialization of cell and gene therapies without adding to the undue cost or complexity that

is sometimes associated with more bespoke solutions—for example, in providing support to help meet regulatory requirements, or providing tools that meet raw material requirements for manufacturing processes. Supply chain is a further area where supportive capabilities can help manage capacity and demand in what remains a highly dynamic environment following the COVID-19 pandemic.

Thermo Fisher has focused on providing cell and gene therapy developers with a comprehensive portfolio of products, and contract development and manufacturing organization/contract research organization services, backed by regulatory support and a robust global supply chain network. Innovative platforms comprising cGMP manufactured reagents streamline tech transfer and progression of therapies to the clinic.

Here, we look at some of the key advancements in upstream and downstream GMP AAV manufacture and explain how this combination of process innovation and technical support can help accelerate speed-to-clinic.

A SCALABLE UPSTREAM PRODUCTION AND DOWNSTREAM PURIFICATION PROCESS FOR AAV

The Gibco™ AAV-MAX Helper-Free AAV Production System has a simplified workflow that enables high-titer AAV production at scales ranging from milliliters to thousands of liters. The components comprising the AAV-MAX System are manufactured free of animal or human derived components. The system is available in both research use only (RUO) and GMP manufacturing grades (CTS), allowing for a seamless transition from research through clinical and commercial product development.

Figure 1 shows the core components of the AAV-MAX System, at the foundation of which is the Gibco Viral Production Cells (VPC) 2.0 cell line—a documented 293F-derived clonal cell line adapted for high-density growth and suspension in Gibco viral production medium. To transfect the cells with high efficiency and at high densities, the AAV-MAX System utilizes the AAV-MAX Transfection Reagent and Transfection Booster. This is paired with the Viral-Plex™ Complexation Buffer, which is a chemically defined, protein- and animal origin-free complexation medium. The AAV-MAX Enhancer allows for two-to-five-fold improvements in viral titer. Finally, following viral vector production, the resultant AAV vector product is harvested from the production cells utilizing the polysorbate 20-based AAV-MAX Lysis Buffer.

CASE STUDY: PURIFICATION OF AN AAV6 ANTI-CD19CAR CONSTRUCT AT 50L BIOREACTOR SCALE

The aim of this study was to demonstrate consistent AAV productivity across a broad range of volumetric scales, from 125 mL to 5 L shake flasks, through 3 L and 15 L HyPerforma™ glass stirred-tank bioreactors, to the 50 L DynaDrive™ single-use bioreactor. Comparability of cell growth parameters, AAV titers, and metabolite profiles were assessed across all scales.

The AAV-MAX System offers streamlined protocols for convenient and reproducible scale-up. Figure 2 shows a typical production process.

In this example, step one, which commences at four days prior to transfection, starts with the inoculation of cells at approximately 0.6 million cells/mL in typical shake flask cultures. At step two (one day prior to transfection) the bioreactor is prepared and the cells are inoculated.

At this stage of the process, there is great flexibility available in terms of how to proceed with the seed train. In this particular example, dilution of the cells into the bioreactor is at 1.5 million cells/mL, which typically results in an approximate doubling of the cells overnight. Therefore, target transfection density of 3 million cell/mL (or

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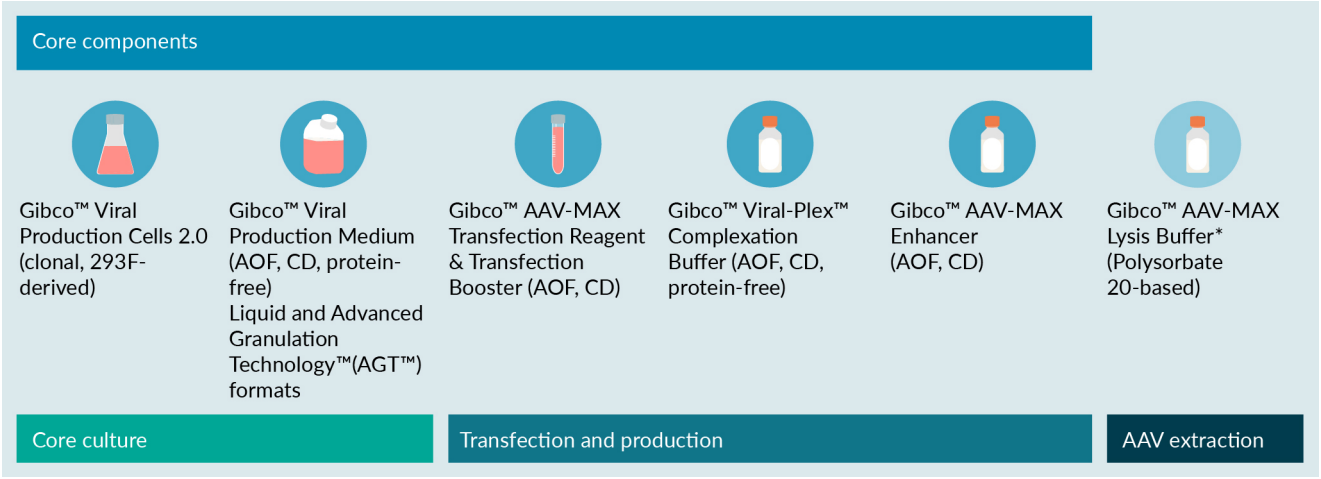
FIGURE 1

Components and workflow of the Gibco AAV-MAX Helper-Free AAV Production System

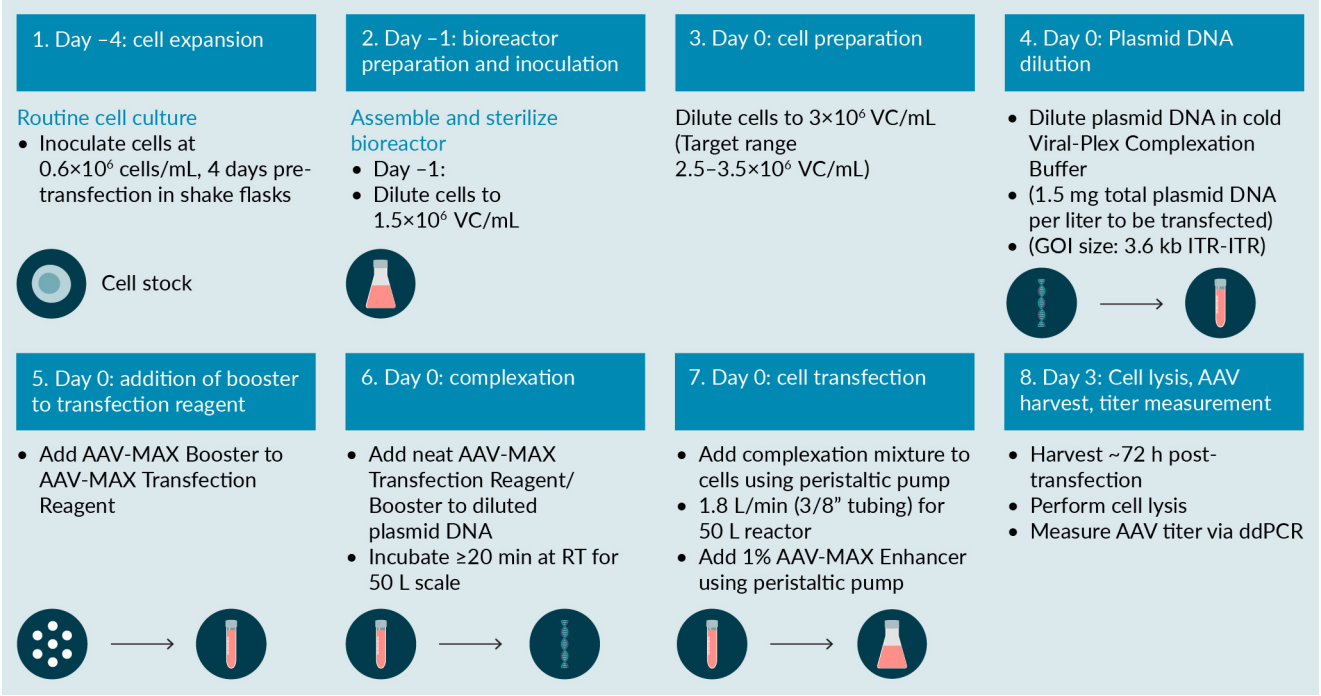


AOF: Animal origin–free; CD: Chemically defined.
*CTS AAV-MAX Lysis Buffer estimated availability in Q3 of 2023.

within a range of 2.5–3.5 million cells/mL) is achieved by step three (day zero). However, the cells could be seeded at a higher density one day prior to transfection, resulting in a higher cell number, which can then be reduced with fresh media to the desired range at the time of transfection. Equally, one might choose to seed the cells

FIGURE 2

Scalable bioreactor production protocol for convenient and reproducible scale-up.



AAV: Adeno-associated virus; GOI: Gene of interest.

at a lower density (e.g., 0.5 million cells/mL) three days prior to transfection, and to then grow them directly up to the target density at transfection. The robustness of both the VPC 2.0 cell line and the Viral production Medium offers a substantial degree of flexibility in this regard.

Step four is the dilution of plasmid DNA into the cold Viral-Plex Complexation Buffer. The AAV-MAX System utilizes a relatively low amount of Plasmid DNA (1.5 mg per liter of culture, which equates to approximately 0.5 mg/million cells to be transfected). At step five, the AAV-MAX Transfection Booster is added to the AAV-MAX Transfection Reagent in a 5 L Aegis™ bioprocessing bag. Step six is simply a case of adding the combined AAV-MAX Transfection Reagent/Booster solution to the diluted plasmid DNA, agitating to mix the contents, and then incubating for ≥20 minutes at room temperature for a 50 L scale production.

At step seven, the complexation mixture is added to the cells using a peristaltic pump at a rate of approximately 1.8 L/min, followed by the addition of 1% AAV-MAX Enhancer utilizing the same peristaltic pump. Finally, in step eight (on day three post-transfection) cells are lysed with the AAV-MAX Lysis Buffer to liberate the AAV, which is then collected and measured for titer.

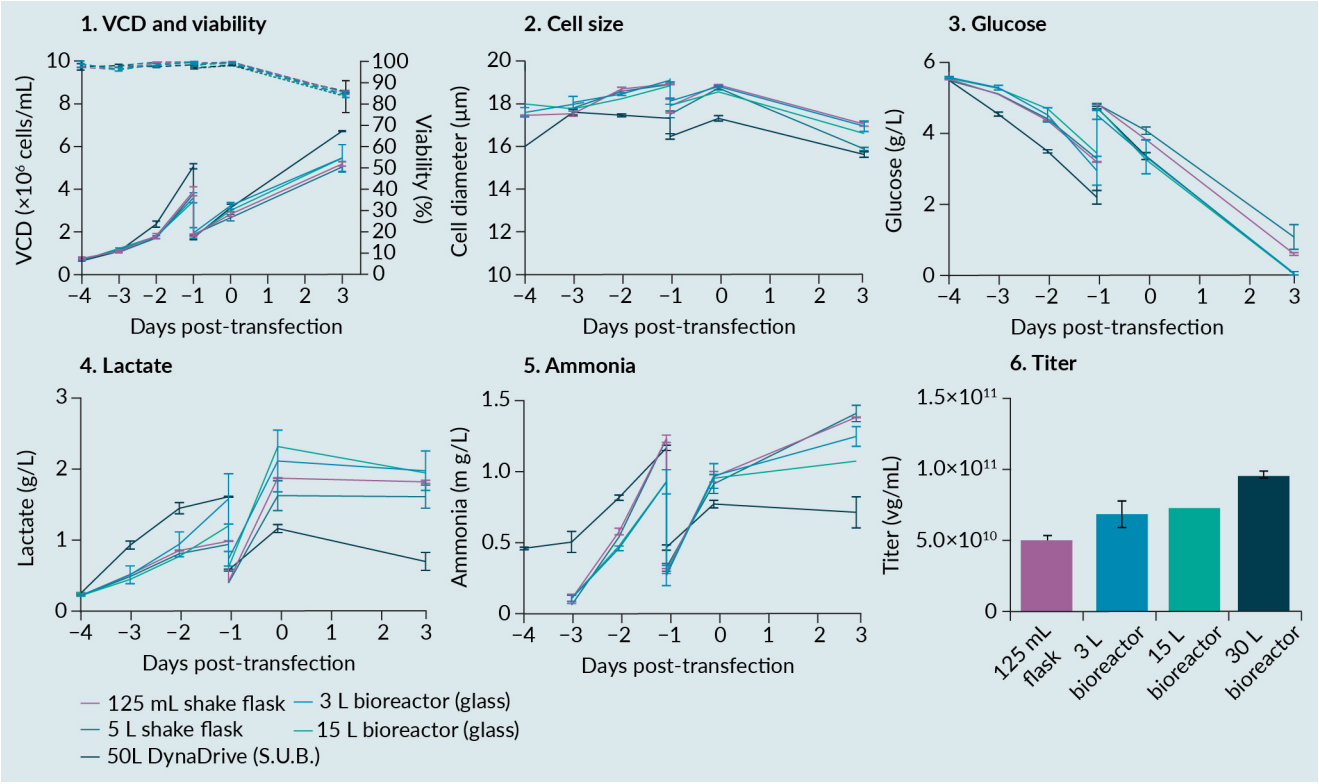
Figure 3 shows the viable cell density and viability across all production scales tested, ranging from a 125 mL shake flask through to the 50 L DynaDrive single-use bioreactor.

Graph one show the viable cell density and viability plots for both the seed train at the time of transfection (day zero) and at harvest (three days post-transfection). Results are comparable across the different scales, with 82–83% viability achieved at time of harvest.

Turning to graph three (glucose), as previously noted, the cells grew slightly faster in the 50 L DynaDrive bioreactor compared to

FIGURE 3

Consistency of production of AAV6-anti-CD19CAR across scales and platforms.



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the other conditions tested, which is demonstrated here by an accelerated glucose consumption rate. (Note: Because different cell counters were utilized to measure cell density, viability, and size at the 50 L scale versus all of the smaller scales, it cannot be ruled out that differences observed in glucose consumption and lactate production were the result of slight differences in actual seeding densities at the time of reactor inoculation. Similarly, it is possible that the perceived differences in cell size may also be a result of cell counter variability). Following transfection at day zero, however, the glucose utilization profiles are consistent across all scales, nearing exhaustion at the time of harvest.

Cell size (graph two) also trends comparably across all scales. So do the lactate and ammonia profiles (graphs four and five), which show very low levels, and consistent profiles across the entire production run regardless of scale. Finally, regarding overall titers, graph six shows a consistent trend in that the bioreactors provide at least comparable if not higher VG/mL compared to the shake flask controls. This suggests that the control of the bioprocess parameters enabled by the stirred-tank bioreactors may have a favorable impact on titer (VG/mL), with the expectation that additional optimization may increase titers further compared to the small-scale shake flask controls.

The next part of the study aimed to demonstrate that the AAV produced in the AAV-MAX System at the 50 L scale could be easily purified, leading to biologically active virus (Figure 4).

In the first step, cultures were lysed utilizing the AAV-MAX Lysis Buffer, followed by nucleic acid digestion by the Pierce Universal Nuclease. Primary clarification took place through a triple depth filter train. The filtered material was then frozen and shipped to another Thermo Fisher site for final downstream purification. At this point, post-thaw, the supernatant was reclarified utilizing the C0SP and the Saropore 2 XLG filters from the primary depth filtration train. The reclarified material was then concentrated by tangential flow filtration (TFF) before being loaded onto the POROS™ AAVX Column. Finally, an anion exchange purification step for enrichment of full capsids was carried out utilizing the POROS GoPure™ HQ Column.

Following concentration of the crude supernatant that passed through the triple depth filtration train, the concentrated material was loaded onto the POROS AAVX Affinity Column. The chromatogram in Figure 5 demonstrates a sharp elution peak with

FIGURE 4

Process overview of the purification of AAV6-anti-CD19CAR at 50 L scale.

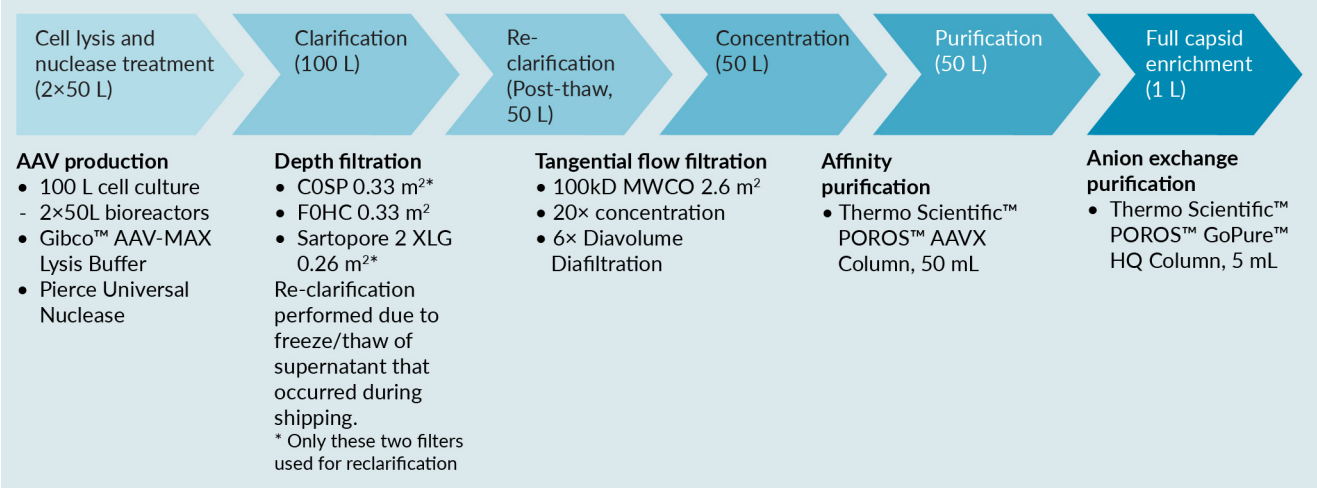
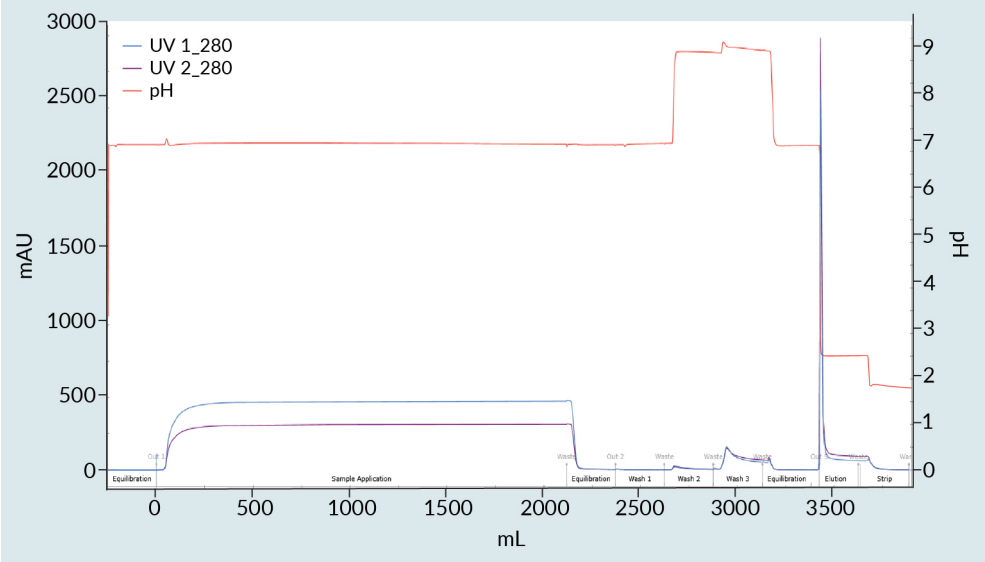


FIGURE 5

Affinity purification results.



a higher A260 than A280 ratio. This provided the first indication of a high percentage of full AAV capsids being present in the preparation.

The eluted AAV material from the AAVX affinity resin was then further purified in the POROS GoPure HQ Column to enrich for full capsids (Figure 6). To the left of the main peak, one can see the empty and partially full capsids, which are indicated by a higher A280 ratio compared to A260 ratio. In the main peak, this ratio has reversed, with A260 now greater than A280, indicating further enrichment of full capsids within the preparation.

Figure 7 shows the overall step yields and percent full capsid data across the 50 L downstream purification process.

FIGURE 6

Affinity purification results.

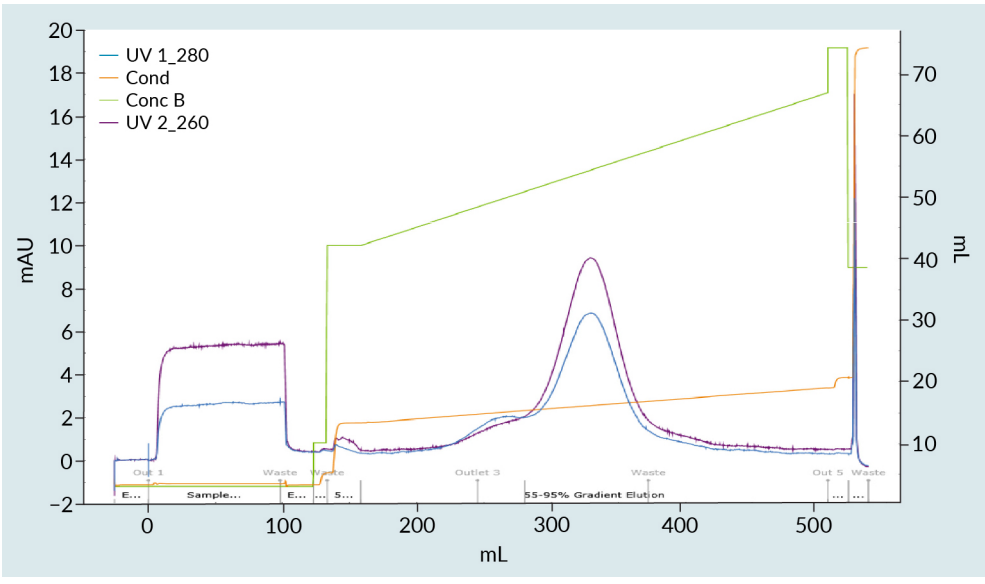


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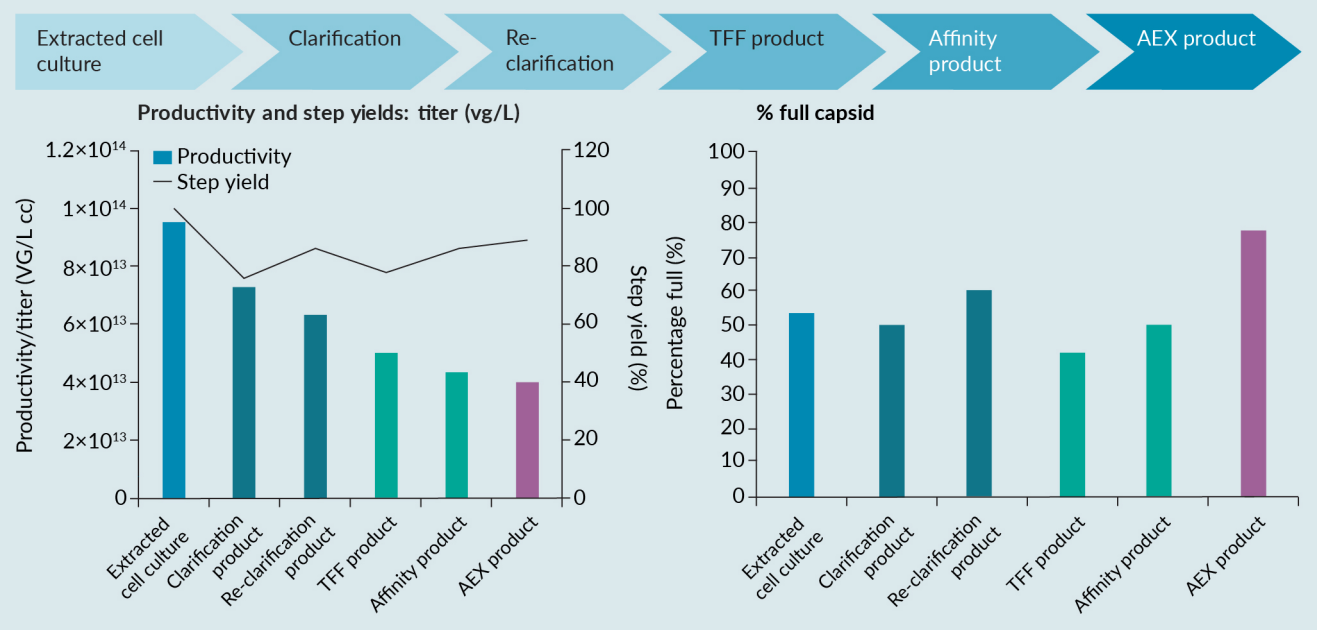
FIGURE 7

Step yields and percent full capsids.



The left-hand graph demonstrates the productivity in step yields from the extracted cell culture through to the polished anion exchange product. The right-hand graph depicts the percent full capsids across the entire downstream process. As expected, no improvement in percent full capsids was observed before the anion exchange purification step. Following that step, the final product consisted of approximately 75% full capsids. It was particularly encouraging to note the high starting point at the extracted cell culture step of approximately 50% full capsids.

The final stage of the study involved demonstration of biological activity of the AAV6-anti-CD19CAR resulting from the 50 L process (Figure 8). This involved the transduction of primary T cells and observing functionality using a Nalm6 cell killing assay. The graph to the right shows percent cytotoxicity. The blue line represents the control T cells, which achieved approximately 10% cytotoxicity of target cells. However, once the T cells were transduced with the AAV6 anti-CD19 CAR, this percent cytotoxicity of the target cells rose to 82–83%, indicating efficient killing of the target cells by the AAV6-transduced T cells.

CONCLUSION

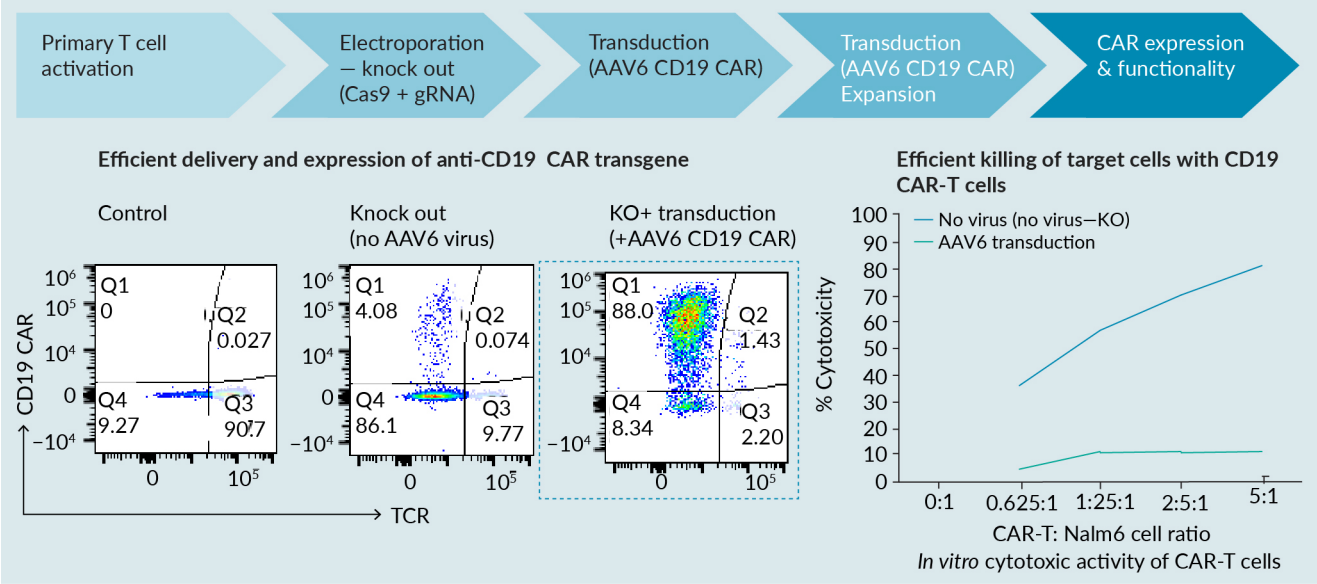
In summary, the Gibco CTS AAV-MAX System facilitates a seamless transition from research-scale to clinical and commercially relevant AAV production volumes. Scalability of the AAV-MAX System from 30 mL shake flasks up to the 50 L HyPerforma DynaDrive single-use bioreactor was achieved, demonstrating the ability to translate this upstream production system to large scale commercially relevant production volumes. Significantly, AAV vectors produced using the AAV-MAX System were compatible with downstream purification technologies. Data presented here showed the ease of purification at the 50 L scale using the POROS AAVX affinity resin and the resulting enriched capsid preparation following use of the GoPure HQ Anion Exchange resin.

With the addition of Thermo Fisher's array of AAV analytical test kits and analytical

instrumentation, as well as the Gibco Cell Therapy System reagents, the AAV-MAX System can help to de-risk viral vector manufacturing by enabling a seamless transition from research, to clinical, to commercial production of AAV.

FIGURE 8

Functionality study results: potency of CAR-T cells produced using AAV6 anti-CD19 CAR.



Q&A

Jonathan Zmuda

Q What aspects of the protocol are most important for achieving optimal and consistent titers?

JZ: You have to look at this system as a whole, but if I had to put an emphasis on two areas, one would be your cell health and growth parameters, which are

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foundational to everything that happens downstream from there, and the second would be the complexation reaction.

Regarding cell health and growth parameters, what we have seen through our AAV-MAX System is that our cell growth kinetics can be very consistent from very small scales, up to thousands of liter scales—in fact, we have grown the VPC 2.0 cell line all the way up to 3.000 L in single-use bioreactors. We also see highly comparable viable cell densities at every scale. My first suggestion would be that before you ever transfect your cells in a stirred-tank bioreactor, make sure that your growth kinetics match (essentially, identically) those in your shake flask cultures where you did all of your early optimization work. You should be able to attain a highly comparable, if not identical, profile. If you are going into your first transfection in a single-use stirred tank bioreactors and you are having cell health or viability issues, or seeing differences with your shake flask controls, it is essentially a recipe for achieving lower titers at the end of your run.

Regarding the complexation reaction: every transfection reagent is a little bit different, all of them have their different nuances. It is therefore key to follow the protocols very closely in terms of time and temperature, plasmid DNA amounts, transfection reagent concentrations, as well as mixing and addition to the bioreactor. With the AAV-MAX System, we see very robust complexation using fairly simple protocols. Your plasmid DNA is first diluted into your buffer, which is very stable over time. You then add your neat transfection reagent when you are ready to perform your complexation, mix appropriately (depending on your choice of complexation vessel) and allow that to incubate for 20 minutes up to an hour, and then pump it into the bioreactors. We have added the complex either by gravity feed or pumping through different heights of the bioreactor and seen very consistent and robust results. But it is certainly very important that you maintain consistency in that complexation reaction. I think that if you really nail down these two parameters, you are going to have good, consistent runs time and time again.

Q Do you feel that production runs greater than 1,000 L are possible using the triple transfection method?

JZ: Yes, absolutely. Part of the reason why we did the recent 1,000 L production run [1] was to better understand where the pain points are in the complexation process, and what may need to be done differently going forward. We were very pleasantly surprised to see that our very first run was highly effective. We were able to attain titers that were comparable or greater than those in our shake flasks controls. We definitely believe that should be readily scalable up to 2,000 L and beyond.

As mentioned earlier, we have grown our VPC 2.0 cell line all the way up to 3,000 L with very consistent growth kinetics compared to our controls, so based on what we've seen so far, we do feel that the transient transfection at very large scales should be possible.

Q Where have you seen the greatest challenges in scaling from early discovery formats to commercial-scale protocols?

JZ: At the risk of repeating myself, it really is about making sure that you are matching your conditions from your bench-scale to your bioreactors. Cell health is priority number one. If your cells are not growing at least as well as your small-scale controls, the cells will not transfect or produce as well as expected. Always make sure this parameter is locked down before proceeding further. Generally speaking, VPC 2.0 cells adapt very well into the high-density suspension protocols and are highly robust across scales. Furthermore, the trends that we have seen, where typically our VG/mL results tend to trend higher in the stirred-tank bioreactors than in the control shake flasks, indicates to us that there is potentially room for even greater improvement there. I know a lot of folks are utilizing instrumentation like the Ambr® 250 system, etc. to do medium-throughput process development. I think that is one place where you really want to look and make sure that, for your particular AAV vector, you are optimizing the parameters that are going to get you the best viral titers and product quality.

REFERENCE

1. Scalable production of AAV from shake flasks to 1,000 L single use bioreactors using the Gibco™ CTS™ AAV-MAX Production System. Cell and Gene Therapy Insights.

BIOGRAPHIES

BETTY WOO currently serves as Vice President of Cell, Gene, and Advanced Therapies, spanning biosciences, bioproduction, and laboratory products businesses across Thermo Fisher Scientific, leveraging 'total company' to address the needs of developers and manufacturers of cell and gene therapies. The business focuses on fit-for-purpose product solutions that in partnership with our customers, is increasing the accessibility of these life-changing cell and gene therapies to patients.

JONATHAN ZMUDA is a Director of Cell Biology R&D within the Biosciences Division of Thermo Fisher Scientific located in Frederick, MD (USA). Within Cell Biology, Jon leads various teams that focus on developing new technologies for protein expression, viral vector production, transfection and classical cell culture. Dr Zmuda received his PhD in Cell Biology from the University of Maryland, College Park and his undergraduate degree from Dickinson College in Carlisle, PA.

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AUTHORSHIP & CONFLICT OF INTEREST

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ThermoFisher SCIENTIFIC

AAV-MAX HELPER-FREE AAV PRODUCTION SYSTEM

STREAMLINE AAV PRODUCTION WITH A COST-EFFECTIVE, SCALABLE SYSTEM

Cost-effective, scalable adeno-associated virus (AAV) vector production is critical to meet commercial demand—and smooth scale-up to clinical production is essential. We created the Gibco™ AAV-MAX Helper-Free AAV Production System* to help reduce production costs and streamline your transition from research to clinical scale.

Make connections with an established partner.
Go to **thermofisher.com/aavmax**

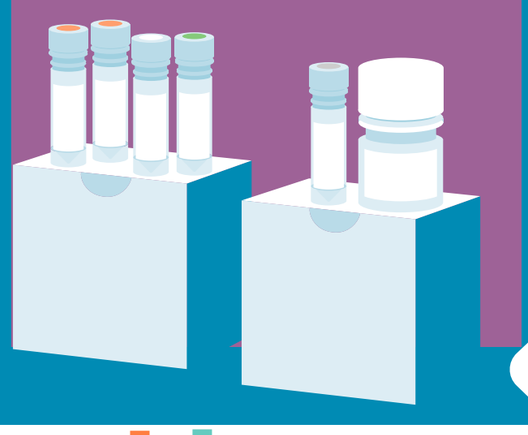
* cGMP will be available with the Gibco™ Cell Therapy Systems™ (CTS™) AAV-MAX Production System.
The content provided herein may relate to products that have not been officially released and is subject to change without notice.

RESIDUAL DNA TESTING:
Homebrew vs off-the-shelf solutions

To verify product quality, the amount of residual DNA in a drug's final dosage form must meet guidelines established by multiple regulatory agencies. Each regulatory agency has specific guidelines for acceptable limits depending on the product and therapeutic modality.

Companies that produce biopharmaceutical products must verify the quantity of residual DNA impurities throughout the purification process, and in the final drug product.

Commercial kits for residual DNA testing can shorten process development time and reduce in-house staffing, overheads, and expertise requirements. This lowers risk and results in a cost-effective solution overall.



STEPS TO SETTING UP YOUR OWN RESIDUAL DNA TESTING SOLUTION

CREATE A QUALIFIED METHOD FOR TESTING A MANUFACTURED BIOLOGIC FOR RELEASE

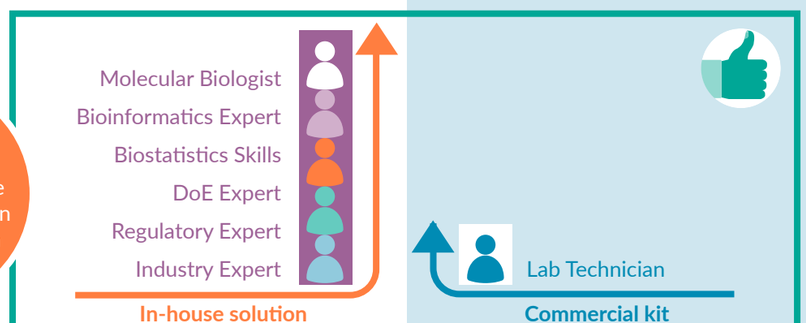
Do you have the right in-house expertise?

- **Identify target sequence** — Bioinformatics expert or Molecular Biologist with bioinformatics expertise
- **Design primers & probe** — Molecular Biologist
- **Optimize concentration of primers & probes** — Molecular Biologist with Design of Experiments (DoE) & biostatistics skills
  - Statistical software can help to design a shorter experimental plan
- **Characterization & qualification of DNA controls/standard** — Molecular Biologist and regulatory expert
  - Have you got a reliable source for your DNA controls?
- **Source and qualify reagents** — Molecular Biologist and regulatory expert
  - Source reagents
  - Qualification of reagents
  - Do you have a procedure for qualifying critical reagents?
  - What process is in place for dealing with delays from raw materials suppliers?
  - Have you defined a process to QC raw materials?
  - cGMP reagents can take up to a week to produce
- **Ensure process conforms to regulatory requirements** — Regulatory expert
  - Do you have the in-house expertise to un-derstand regulatory requirements?
- **Create test method** — Molecular Biologist with technical writing skills
  - Draft
  - Review
  - For commercial product: update method for own site
- **Qualification/validation of the analytical method** — Molecular Biologist, regulatory expert (knowledge of FDA and other regulatory agency requirements), industry expert
  - Draft qualification method
  - Review
  - Qualification & execution
  - Report
  - Draft validation method
  - Execution of the validation
  - Validation report

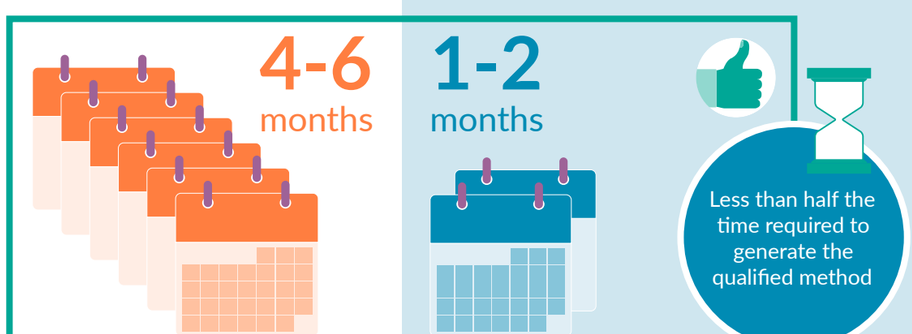
At this point, you have a qualified method that can be used to test a manufactured biologic for release

CREATING THE QUALIFIED METHOD IS ONLY THE FIRST HURDLE...

Staffing requirements and overheads are much greater for an in-house solution



Do you have the required expertise in-house?



Have you factored in the in-house time to create a qualified method?

METHOD MAINTENANCE IN-HOUSE

The in-house method requires routine testing and health evaluation

- Method health evaluation
- Inventory management
  Maintain an inventory of critical primers, probes and DNA controls
- Dedicated team to support the method in-house
- Re-qualification of each new lot of primer/probe mixes

TROUBLE-SHOOTING

Do you have the resources in-house to troubleshoot the issue when something goes wrong with the test method?

- Have you got a process in-house to identify the issue when the assay starts to fail?
- Do you have the in-house resources to troubleshoot the issue?
- Can you afford the time to get back on-line? It can take a week or more to check all parts of the process and get back online.

It can take a week or longer to get back online.

RISK MITIGATION

Are you able to afford a failed assay to delay batch release?

- Have you factored in delays for reagents failing qualification?
- Do you have a process in place to deal with delays and issues with your raw materials?

REGULATORY ISSUES

Do you have a dedicated person who can respond to questions coming from regulatory authorities?

- Do you have someone in-house to can answer regulatory questions regarding assay design and how it was validated?

STEPS TO SETTING UP A COMMERCIAL RESIDUAL DNA TESTING SOLUTION

The Applied Biosystems™ resDNASEQ™ Quantitative DNA Kits utilize a quantitative PCR (qPCR)-based system to detect plasmid DNA and host cell DNA from various cell types commonly employed in the development of gene therapies, cell-based vaccines, and similar biotherapeutics.

With the resDNASEQ system, you can achieve both sensitive and specific quantitation, ensuring a high level of confidence in the data obtained from a diverse range of sample types. This includes in-process samples with different sample matrices, as well as purified final products

The overall performance of the resDNASEQ method, including sensitivity, accuracy, precision, range, and linearity meets or exceeds the example described in USP <509>.

The resDNASEQ system offers a sample to result solution.

No requirement for assay component procurement

No requirement to qualify critical reagents

- **Qualification/validation of the analytical method** — Lab technician
  - Adapt qualification method (provided)
  - Review
  - Qualification & execution
  - Report
  - Draft validation method
  - Execution of the validation
  - Validation report

At this point, you have a qualified method that can be used to test a manufactured biologic for release

Support services include access to a team of experts including molecular biologists and regulatory experts meaning that you can run the process in-house with a Lab Technician. Thus reducing staffing and overheads costs.

When you use a commercial kit, you are covered from sample to result and everything in between. These risks are not your issue.

Access to a team of Regulatory Experts comes as part of the commercial kit.

"For those convinced that creating their own assays saves money, it's time to consider the hidden expenses. Troubleshooting DIY assays can become a time-consuming and costly endeavor. Commercial DNA quantitation kits offer a reliable, ready-to-use solution, ensuring accurate results without the unexpected financial detours of assay troubleshooting."

– James Baus

BENEFITS OF THE THERMO FISHER COMMERCIAL KIT

Reduced staffing and overheads in-house	Dedicated team of experts	Access to a team of experts including regulatory specialists
Lower risk	Support from sample to result	No unexpected delays or costs
Eliminated time needed to devekop assay in-house	Cost effective	Method creation and maintenance is handled for you

ThermoFisher SCIENTIFIC

CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

How can the gene therapy industry drive down the cost of goods to better serve patients?

Emmanuelle Cameau

The cost per dose of viral vector therapeutics is a challenge for the widespread accessibility of gene therapy products. Manufacturers and solution providers can work together to ultimately make these drugs more cost-efficient to manufacture and ultimately reduce cost per dose. This article will discuss how tools such as process intensification (PI) and cost modeling can help build an understanding of the main cost drivers and the impact process optimization can have on the cost per dose.

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GENE THERAPY COST PER DOSE

Over the past year, six new genomic medicine products have been approved, four of which are adeno-associated virus (AAV) or adenovirus (AV)-based gene therapies. The number of cell and gene therapy clinical trials is globally increasing with 58% of trials treating potential prevalent disorders. There is a need for healthcare systems and regulators to keep up with the pace of these therapies and work on better reimbursement strategies for improved patient access.

Viral vector-based gene therapies bring vast possibilities for treatments, but high cost per dose. The cost is often justified compared to lifetime treatment, although current healthcare systems are not able to provide these therapies to all those who need them. Cost per dose is influenced by R&D investment, manufacturing costs, lack of process maturity and adapted technologies, and analytical validation. Manufacturing costs show a 1,000-fold difference depending on the dose required, and studies have shown that only 2% of manufactured product actually goes into the patient [1].

To reduce the cost per dose to make these therapies more accessible, the industry is evolving quickly by innovating the way we produce them. Solution and technology providers have a key role to play in innovation, and drug developers are seeking higher performing processes. Collaboration between manufacturers and suppliers is key, as is ensuring communication with regulatory experts throughout the process.

To optimize the cost of manufacturing on the upstream side, cell line optimization, process optimization, plasmid optimization, and the use of stable cell lines should be considered. Process optimization and striving for better recoveries are necessary on the downstream side, although upstream optimization is the real cost-driving lever that will make these therapies more accessible. Key opinion leaders in the field agree that a decrease of 10× to 100× the actual manufacturing cost is needed to do this.

INCREASING UPSTREAM PRODUCTIVITY

The direct benefits of increased upstream productivity include obtaining more doses per batch and requiring fewer batches, smaller bioreactors, and fewer skids. This translates into more patients treated, reduced cost per dose, fewer materials, less labor, a lower consumable cost or capital expenditure, and potentially reduced footprint.

While developing an upstream transient process, the transfection reagent is key. In collaboration with inVitria and in the iCELLis™ Nano bioreactor from Cytiva for adherent cells, lentivirus production using FectoVIR AAV, which is traditionally marketed as a suspension transfection reagent, was shown to give a productivity improvement over traditional PEIpro. By screening several transfection reagents in the early stage, a process can be made more cost-efficient.

High upstream process efficiency means a lower cost of goods sold. Another example of an emerging tool in the market designed to drive down upstream costs is from Virica Biotech, which makes viral sensitizers (VSEs) that reduce the number of batch cycles required to achieve target yields. A 50% increase in upstream yield has been shown to decrease upstream manufacturing costs by 33% [2].

Another method of increasing upstream productivity is to optimize pDNA use. In performing AAV cost modeling, the importance of optimizing plasmid DNA (pDNA) use for all platforms is highlighted (Figure 1). Benchmark process modeling reveals the significant impact of labor and upstream consumables. GMP-grade pDNA and transfection reagents are a common cost driver in all processes, but are 50% less important in the iCELLis bioreactor batch process. Upstream consumables also contribute the most to multitray (MT) process costs.

Upstream decisions impact the downstream. For example, increasing productivity can lead to more contaminants, process-related impurities, and product-related impurities. This can increase the number of downstream processing steps needed, and requires considerations regarding capacity and sizing for both chromatography supports and filters. Optimizing the downstream alongside the upstream allows for optimizing the process and overall cost-per-dose. This approach requires good communication between teams working on the entire process.

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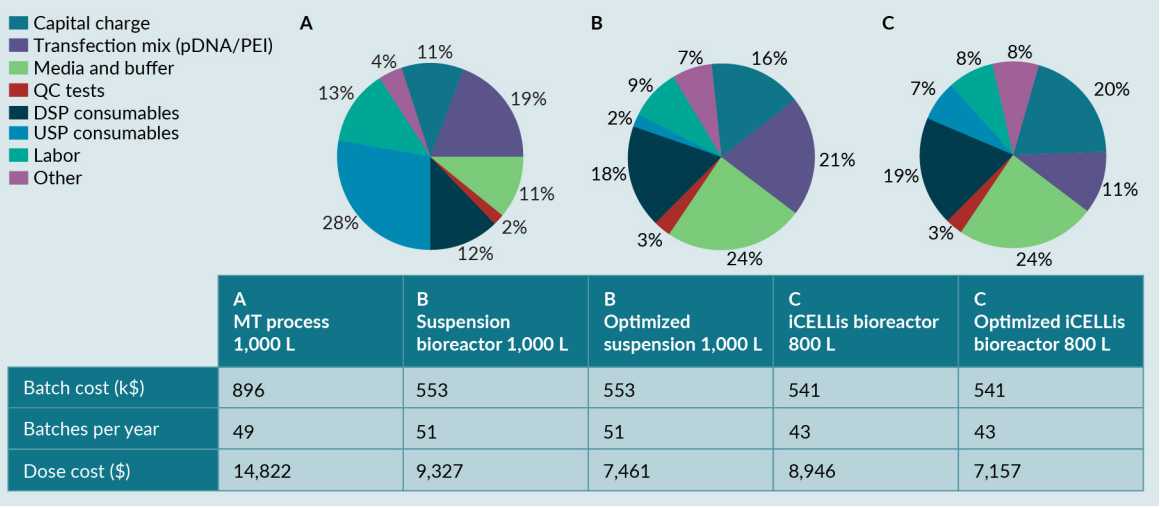
FIGURE 1

Cost modeling exercise comparing MT and bioreactors for AAV manufacturing.



	A MT process 1,000 L	B Suspension bioreactor 1,000 L	B Optimized suspension 1,000 L	C iCELLis bioreactor 800 L	C Optimized iCELLis bioreactor 800 L
Batch cost (k$)	896	553	553	541	541
Batches per year	49	51	51	43	43
Dose cost ($)	14,822	9,327	7,461	8,946	7,157

DSP: Downstream processing; MT: Multitray; QC: Quality control; USP: Upstream processing.

PROCESS INTENSIFICATION AS A TOOL TO REDUCE COST

Another way to reduce cost is through the use of process intensification. Process intensification looks to reduce areas of waste across the whole process, with drivers of reducing production cost, footprint, and time to market while enhancing manufacturing flexibility, scalability, and ease of use.

Case study: the development & scale-up of a HEK293 helper-dependent adenovirus process using the iCELLis bioreactor platform

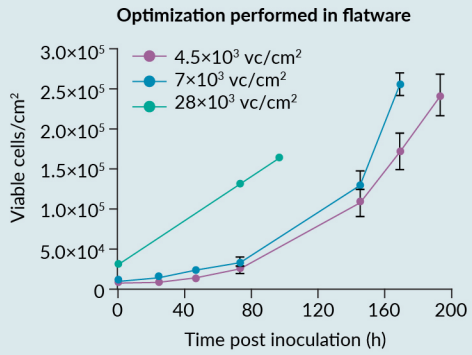
One example of process intensification was published by Cytiva in collaboration with Ensoma. The development and scale-up of a helper-dependent adenovirus (HDAd) process using the iCELLis bioreactor platform was performed. The goal was to move from a roller bottle process to an iCELLis 500+ process. Many aspects of the process were optimized, including the seed train. The team screened different seed train densities and identified a seed train cell density that reduced the burden of the seed train generation (Figure 2).

Process intensification of the lysis step was also completed. The lysis step of the process was initially freeze-thaw, which lacks scalability. The process was modified to ensure manufacturability and reduce the step duration. Through a screening of lysis buffers, the best-performing buffer in terms of yield was identified. An alternative surfactant being tested was eliminated as the resultant solution was too viscous. Maximum virus recovery time was achieved after a 2 h lysis in the iCELLis Nano bioreactor. The reduced lysis time enables a same-day harvest clarification process.

The final part of process intensification was to implement a rinse step, which was found to improve virus recovery. A negligible amount of virus was present in the supernatant prior

FIGURE 2

Seeding density screening to reduce seed train burden.



Seeding density (vc/cm²)	4,500	7,000	28,000
Growth post-inoculation (days)	8	7	4
CellSTACK®10 layers required to inoculate 333 m² iCELLis 500+ bioreactor	14	22	86

- Lower seeding density and extended growth duration screened in flatware
- Reduced seed density to 4.5×10^3 cell/cm² to minimize required number of N-1 passage vessels
- Target infection density increased to 2 to 3×10^5 cell/cm² in the iCELLis nano bioreactor

to lysis. After the cell lysis, the bioreactor was drained, a rinse step with buffer was performed, and then both the drain and the rinse were pooled and depth-filtered. A rinse at 330 m² scale recovered 20% of the virus.

ELEVECTA™ STABLE PRODUCER CELL LINE

A potential way to increase overall productivity and decrease manufacturing costs of gene therapies is to work on stable cell lines. The ELEVECTA stable producer cell line is a transfection-free and inducible AAV producer cell line that does not require a helper virus. It is flexible, giving the choice of either CAP™ or HEK293 cell lines. It offers minimal batch-to-batch variability and allows simple scale-up for large-scale viral vector manufacturing.

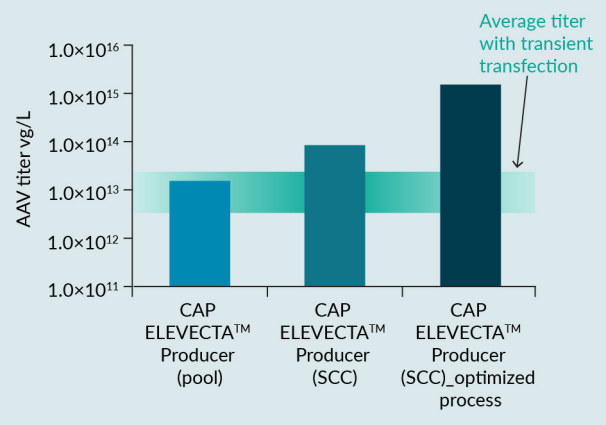
With the ELEVECTA stable producer cell line, titers can be increased significantly from the pool (Figure 3). Screening and process optimization led to titers of $>1\times10^{15}$ vg/L.

An example of process intensification that can be performed with these stable cell lines is the use of perfusion mode (Figure 4).

The cells were induced at the end of the exponential phase before several days of production. Increased production time led to a 10× higher cell-specific yield than with transient transfection.

FIGURE 3

Process optimization of CAP ELEVECTA cell line.



CHROMATOGRAPHY MECHANISTIC MODELING

Chromatography mechanistic modeling is a process intensification tool that can help to decrease the overall cost of therapies. This modeling uses software to create digital twins of the chromatography process. Mechanistic models use computer simulations based on known physiochemical phenomena involved

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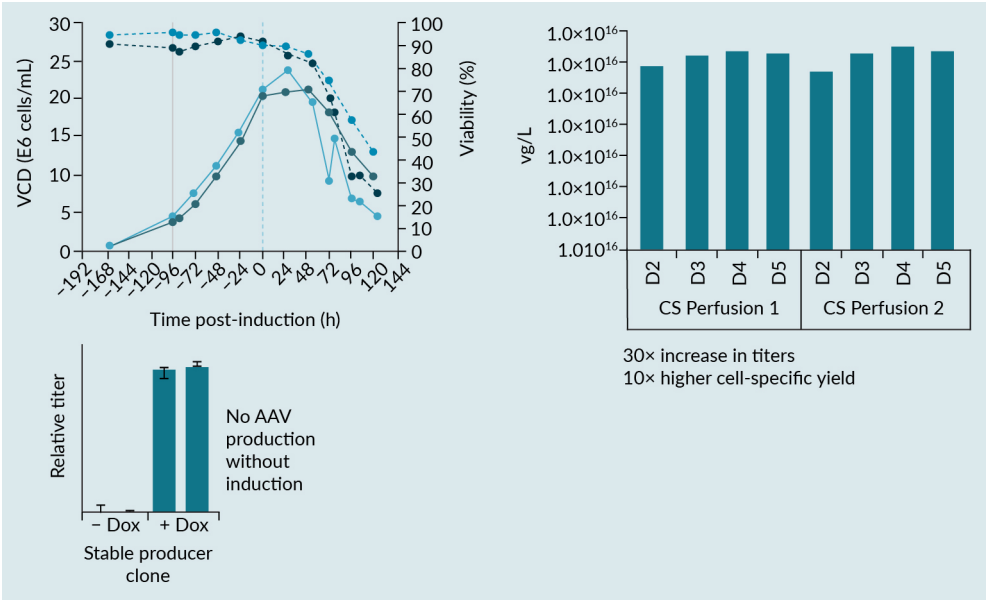
FIGURE 4

AAV production in perfusion mode with CAP ELEVECTA: a proof of concept at small scale compared with a batch process.



in chromatography. This enables thousands of purification options to be tested in a few hours, allowing for *in silico* process optimization.

In Figure 5, *in silico* process optimization of AAV manufacture was completed with the objectives of optimizing yield and purity of full capsid while maintaining a short experiment runtime. The optimized parameter here was the concentration of $MgCl_2$. A three-step elution process was established where empty capsids (Ve) elute at 5% B, viral genomes (Vg) at 10%, and impurities (Vi) at 24% with 7 mM $MgCl_2$.

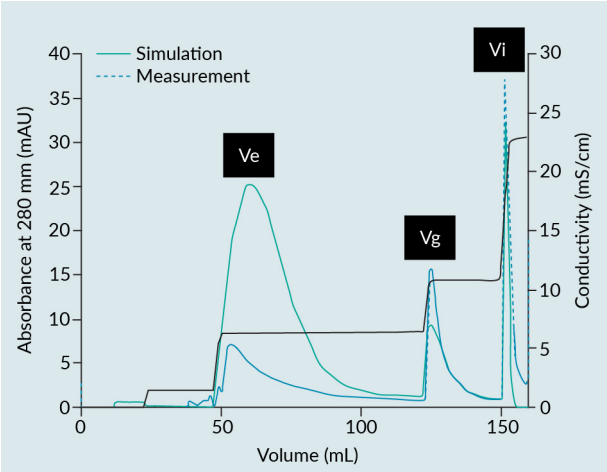
This allowed for the successful separation of all three AAV variants. The feed composition of the validation experiment differed in Ve content, which led to deviations in the peak shape during the first elution step. A good agreement was found between the Vg elution peak in the second step and the Vi in the third step. Overall, the process development goal was achieved, and at the same time, a profound understanding of the process was created. Process development was sped up using GoSilico™ Chromatography Modeling Software.

SUMMARY

Process intensification can be used as a tool to decrease costs, through a combination of

FIGURE 5

***In silico* process optimization of AAV.**



smart bioprocessing and the use of digital tools and process integration. Key concepts to keep in mind are adaptability and predictability. To decrease the cost of viral vector-based gene therapies, we need to develop new manufacturing and characterization tools and find ways to increase upstream productivity by 10× to 100×.

Process development and process intensification must be considered at the earliest stages of drug development in order to reduce R&D costs and the risks associated with developing new processes. We need to rethink the model for how these therapies are invoiced to patients to increase access of gene therapies, especially for ultra-rare diseases.

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Q&A

Abi Pinchbeck, Assistant Editor, BioInsights, speaks to **Emmanuelle Cameau,** Strategic Technology Partnership Leader, Genomic Medicines, Cytiva

Q What is the capacity of the iCELLis bioreactor?

EC: The iCELLis bioreactor goes from bench scale at 0.53 to 4 m^2 and large scale at 66 to 500 m^2.

Q Is the viral capsid gene already integrated into the ELEVECTA producer cell line?

EC: Producer cell line development starts with an ELEVECTA alpha cell line that has the rep and helper genes. You provide the capsid and the gene of interest (GOI) ; these

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are then incorporated into the alpha cell line. The producer cell line we provide to you will include all necessary genetic information for viral vector production.

Q Is the ELEVECTA producer cell line suitable for GMP manufacturing?

EC: Yes, we can deliver your cell line for GMP manufacturing.

Q What about the role of downstream in the optimization of your process?

EC: It is important that whatever is done on the upstream is accounted for in terms of its impact on the downstream. On the downstream side, many things can be changed, for example, using *in silico* software.

As industry providers, we support you to either achieve faster processing or a reduced number of steps while also maintaining the same level of purity and quality of the ultimate desired product. There is a lot of work being done on the analytics side such as the development of better analytic tools to be able to identify what we are purifying and the ultimate product characteristic we want.

Q How long will it be before affordable doses can be brought to the market given the speed at which technology is accelerating?

EC: This is a difficult question to answer, but I do think that the industry is starting to move forward in the right direction. Many people in the field are becoming conscious of this. Part of my work is to continue collaborating with the people who develop the therapies in the early stages so that they are mindful of the impact of whatever they do on the process development side on a larger scale. Processes need to be scalable as early as possible.

I am continuing to work with our customers and the people who are driving the science at the start to ensure that whatever they develop uses scalable technologies and smart bioprocessing to accelerate development. Time is often lost simply because when the GMP and scale-up stages are reached, the solutions that have been identified are not fit for purpose.

Q Could the tips you presented also be applied to lentivirus?

EC: Yes. Lentivirus is often less problematic because the expected doses and the total productivity are lower than that for adeno-associated virus (AAV). The largest portion of lentivirus produced are for *in vitro* use, so the quality requirements are lower than those for *in vivo* AAV. We do see some *in vivo* lentiviral therapies emerging, and all the principles I have talked about do apply to lentivirus.

Lentivirus is a very sensitive vector, so process time reduction and making sure you mitigate the risk of losing viable vectors all along the process are key.

BIOGRAPHY

EMMANUELLE CAMEAU has more than 15 years of experience in biotechnology process development and GMP production. Highly skilled in the field of cell culture applications, Emmanuelle joined Pall Biotech (now part of Cytiva) almost 11 years ago—first as a Bioprocess Specialist (BPS). She then transitioned to the Bioreactor Applications team, where she was Principal Bioreactor Specialist for 5 years. She then moved to the newly formed Gene Therapy Business unit where she has been Strategic Technology Partnership Leader for the past 1.5 years. Previously, Emmanuelle worked as Biotech Process Sciences Upstream Process development engineer at Merck Serono. She received her Biotechnology Engineer diploma from the former Ecole Supérieure d'Ingénieurs de Luminy (now Polytech Marseille). Emmanuelle is a keen horse rider and enjoys spending time with her husband and two daughters.

AFFILIATION

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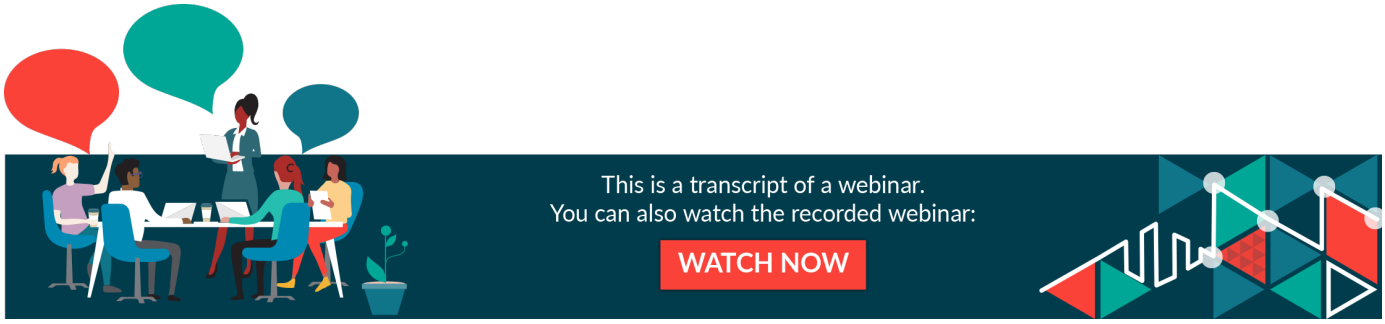


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You're facing challenges that never existed before, requiring new solutions. At Cytiva, we help accelerate the path from milestone to market with products that innovate with you. All while reducing risk and increasing accuracy. Together, we can accelerate brave science.
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CY19323-16Aug21-AD

FASTFACTS

Developing oligonucleotide therapeutics with confidence

Lucy Cook, Global Marketing Manager for Nucleic Acid Therapeutics, Cytiva

Investment is fueling the rapid growth of oligonucleotide therapeutics, igniting urgency in companies to secure stable supplies of oligonucleotide active pharmaceutical ingredients (API) in preparation for drug approval. This poster will highlight key considerations for therapeutic developers entering this market.

DIFFERENT TYPES OF NUCLEIC ACIDS

Nucleic acid therapeutics can achieve long-lasting clinical efficacy via gene inhibition, addition, replacement, or editing. Three types of nucleic acids used for therapeutic purposes: DNA plasmids (pDNA), mRNA, and oligonucleotides. This poster will focus on noncoding single-stranded oligonucleotides.

OLIGONUCLEOTIDE THERAPEUTICS

Oligonucleotides have an established market, with over 15 therapies launched commercially and over 1,200 in the clinical pipeline. Oligonucleotide therapies usually have a regulatory mode of action, interfering with or complementing sequences to alter protein expression. A vast majority of oligonucleotide therapeutics use interfering RNA (RNAi) for this purpose.

Most oligonucleotide therapeutics have been used for rare diseases such as spinal muscular atrophy though as shown in **Figure 1**, they are beginning to move into larger patient indications.

EARLY THERAPEUTIC DEVELOPMENT CHALLENGES AND CONSIDERATIONS

Table 1 shows the necessary considerations for the early stages of process development to harness the potential of an RNAi or another oligonucleotide therapeutic.

SCALING UP MANUFACTURING

Scaling up is a big milestone in oligonucleotide development. Seamless scale-up is essential for patient safety and for maximizing the potential of a new drug when it goes to market. Cytiva's scale-up strategy provides a good example of successfully scaling up manufacturing. Oligonucleotide synthesizers are designed to be part of a scalable platform fitting into a larger oligonucleotide production workflow with other scale-appropriate downstream purification and filtration equipment, shown in **Figure 2**. As a result, there is a solution for each step of the oligo production process, allowing the user to get high product yields at the desired scale.

Table 1. Addressing industry challenges.

Challenge	Considerations
Scalability and flexibility, which can be complex especially when working with novel processes	Being able to tap into the depth of prior knowledge and process experience can make a difference for in-house R&D and scale-up. Strong technology providers understand specific needs and can help provide flexible solutions. At large scale, solutions may include modular facilities, workflow configuration, and staff training
Deciding between insource or outsource	By outsourcing, you can take advantage of a CDMO's existing facilities (thereby gaining efficiency), but risk losing control of your process. Insourcing enables you to maintain control, build in-house experience, and save money on process development. This will require a team that can synthesize oligonucleotides, has expertise in chromatography and purification, and has strong analytical methods
Security of supply and budget strain due to unpredictable supply chains	Increasing manufacturing investment and building capacity is necessary to meet the growing demand of biotechnology solutions
Complex regulatory concerns	Take care to look at differences that may exist between regulatory bodies. These will need to be taken into account when testing your drug
Sustainability	Acetonitrile, which is used in synthesis, can be recovered and reused, or replaced with less hazardous solvents such as acetone. Using continuous diafiltration in the isolation step reduces water consumption by 30% compared to discontinuous diafiltration. Using anion exchange chromatography for purification can reduce hazardous waste burden

Figure 1. An overview of oligonucleotide therapeutic indications.

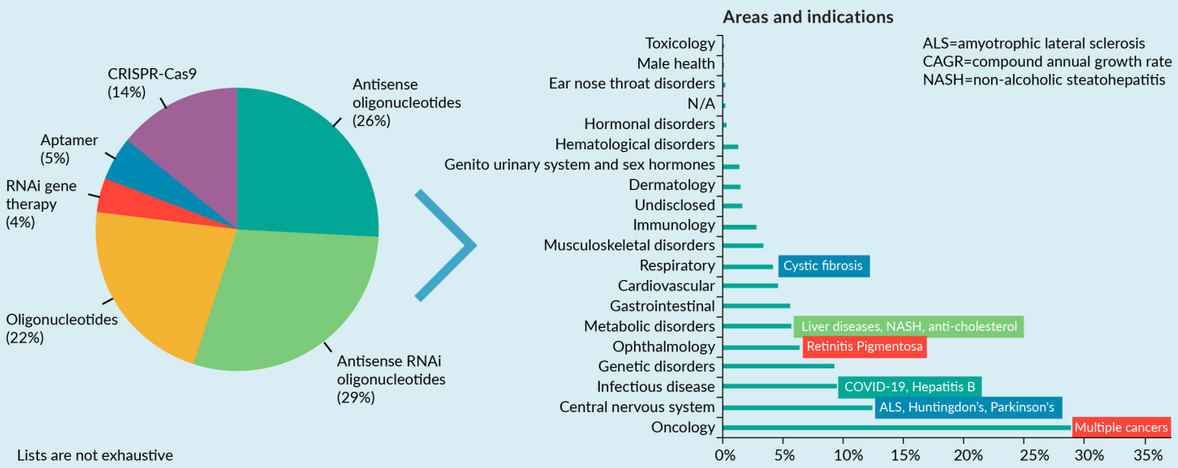
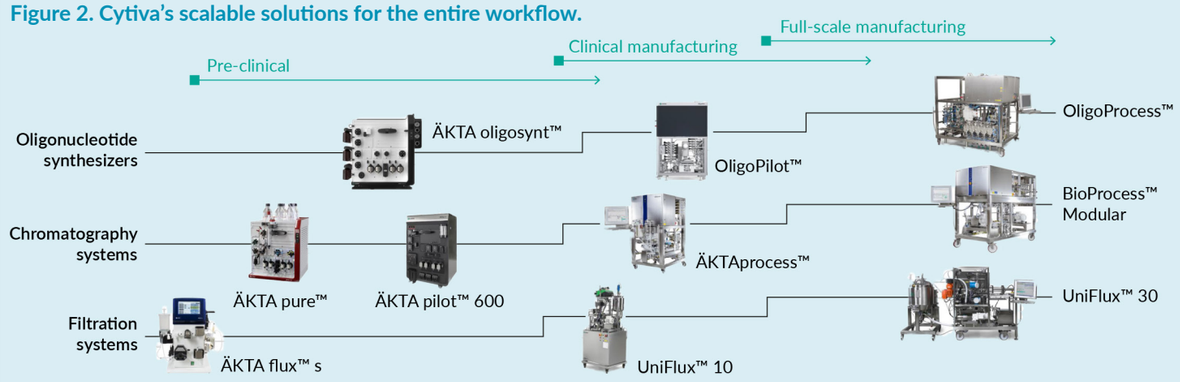


Figure 2. Cytiva's scalable solutions for the entire workflow.



CELL & GENE THERAPY INSIGHT

Cell and Gene Therapy Insights 2023; 9(7), 897; DOI: 10.18609/cgti.2023.114

In partnership with: cytiva

CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Future-proof your AAV process with a complete producer cell line

Dovile Gruzdyte

Stable producer cell lines helped to make mAb therapies the powerhouses they are today. To deliver on the promise of AAV-based gene therapy, we need similar technology. The challenge is that multiple genetic elements—rep, helper, capsid, and gene of interest—must be present. Ideally, everything needed to produce the required rAAV would be stably integrated into a single cell line. This article will describe an all-inclusive cell line platform that can be customized for a specific gene of interest and capsid.

Cell & Gene Therapy Insights 2023; 9(7), 851–858

DOI: 10.18609/cgti.2023.106

Despite a lot of dialog, cell and gene therapy (CGT) is still an emerging field. Since the first approval of a modern cell therapy in 2017, CGTs make up just 7% of all FDA-approved biologics and have a very low commercialization success rate when compared to their small molecule and other biologics counterparts.

Given the low commercialization success rate, the financial risk for a therapy developer is immense. Entering the world of prevalent diseases and increasing patient populations might be one way to reduce that risk, and we expect to see a growing number of CGTs being commercialized in the near future for large patient populations. This brings new challenges, notably the ability to make enough material to cover clinical trials and beyond.

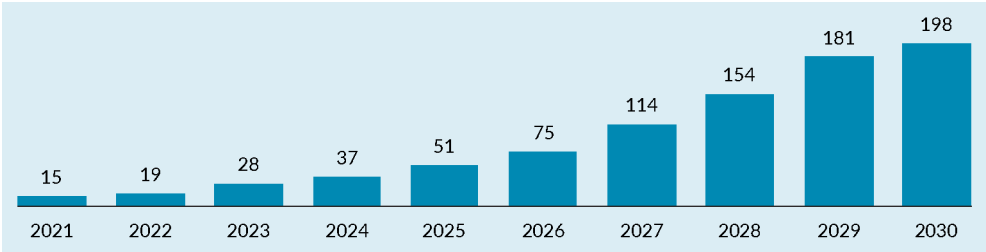
As shown in Figure 1, it was estimated that 19,000 patients would need viral vector material for commercial administration in 2022, and by 2024 that number is predicted to double. Current manufacturing methods and infrastructure are already at capacity and will need to evolve rapidly to keep up with demand.

CURRENT AAV MANUFACTURING METHODS

Selecting a production method as early as possible during development can be a defining moment for a viral vector therapy. If production methods and processes aren't fit for scaling, then the entire project can be put at risk due to the additional time and expense incurred.

FIGURE 1

Global commercial demand for viral vectors and plasmid DNA (in thousands of patients) 2021–2030 [1].



There are four distinct production methods of adeno-associated virus (AAV) that are most widely adopted (Figure 2). Classic triple transfection is the current state. These transient transfection systems allow the greatest flexibility as they don't require prior cell line generation and are therefore commonly used in the early stages of development to rapidly optimize and test lead candidates. However, the scalability of transient transfection processes is limited.

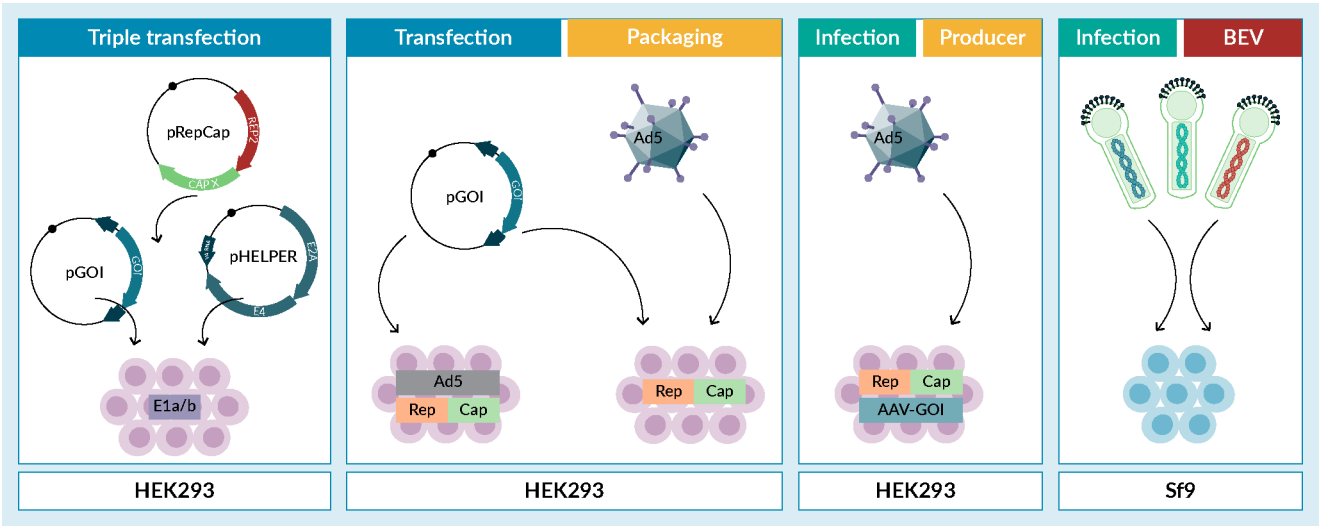
To improve the scalability, packaging or producer cell lines were developed. Scalability is improved due to fewer components needing to be integrated; however, the transfection step and infection step are still required.

An alternative production platform, the baculovirus expression vector system, uses Sf9 insect cells, making it very scalable and cost-effective. However, insect cell systems produce viral vectors with low infectivity due to non-mammalian post-translational modifications, leading to a requirement for higher doses.

All of these systems require manual steps that lead to batch-to-batch variations. It is, therefore, paramount that operators are intimately familiar with the process to maintain as much consistency as possible. When we think about where AAV is manufactured, around 70% is manufactured in contract development and manufacturing

FIGURE 2

AAV production methods.



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organizations. If the production method is highly variable, involves many manual tasks, and is prone to error, it will be harder to carry out tech transfer, resulting in lower performance and variability in productivity of each batch. This makes it hard to plan batches accurately for clinical studies and beyond, and can cause significant delay.

A TRUE PRODUCER CELL LINE FOR AAV PRODUCTION

The scale, accessibility, and cost of mAbs only became manageable when producer cell lines became available. Cytiva now offers the highly similar ELEVECTA™ cell line for AAV production.

All components for AAV production are stably integrated into the genome of the customer's ELEVECTA producer cell line for continuous manufacturing. The cells require no transfection of plasmids or infection with a helper virus at the manufacturing stage, just the addition of an inducer agent. This simple production process allows for minimal batch-to-batch variability, saving valuable time in tech transfer and producing high-quality material.

The ELEVECTA producer cell line is by stably integrating the tailor-made AAV vector components, including the serotype-specific capsid gene and the transgene, into the genome of the Alpha cell line (Figure 3). Using the latest cell line screening technologies, the producer clones are selected, characterized, and cryopreserved as a research cell bank (RCB) ready for handover to the customer.

Following good manufacturing practices (GMP) cell bank creation, the cells can be expanded to the desired scale and cell density. AAV production can then be switched on at an optimal time point by addition of a simple induction agent.

SCALE-UP STUDIES

To illustrate how the technology works in practice, scale-up studies were performed with Cytiva's model ELEVECTA cell line, with runs at 10, 50, and 200-liter scales.

Figure 4A shows how the viable cell concentration, as well as cell viability, increases from 10–200 liters, demonstrating that the performance of the cell line is not compromised when moving to larger-scale production.

FIGURE 3

Generation of an ELEVECTA producer cell line.

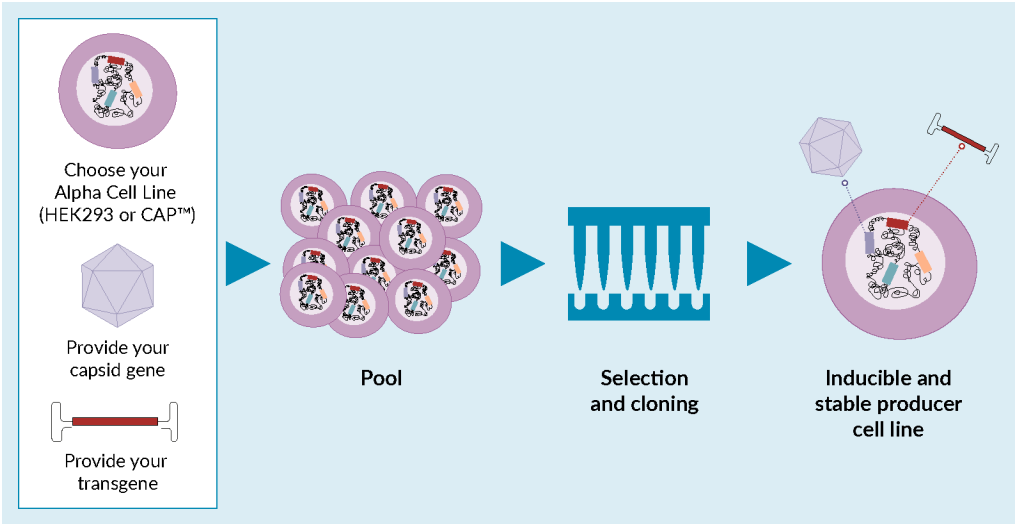


FIGURE 4

Scalable process from lab- to process-scale as demonstrated in 200 L Allegro™ STR bioreactor.

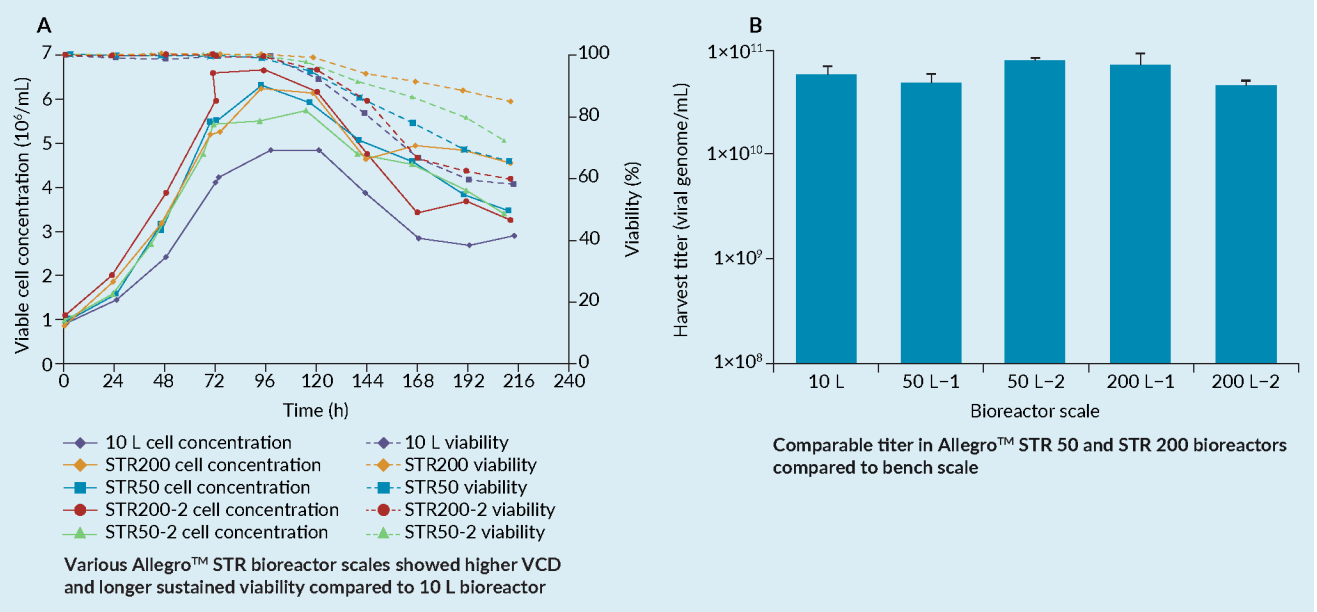


Figure 4B demonstrates that viral titers for different volumes remained consistent across all scales, with minimal batch-to-batch variability across different runs.

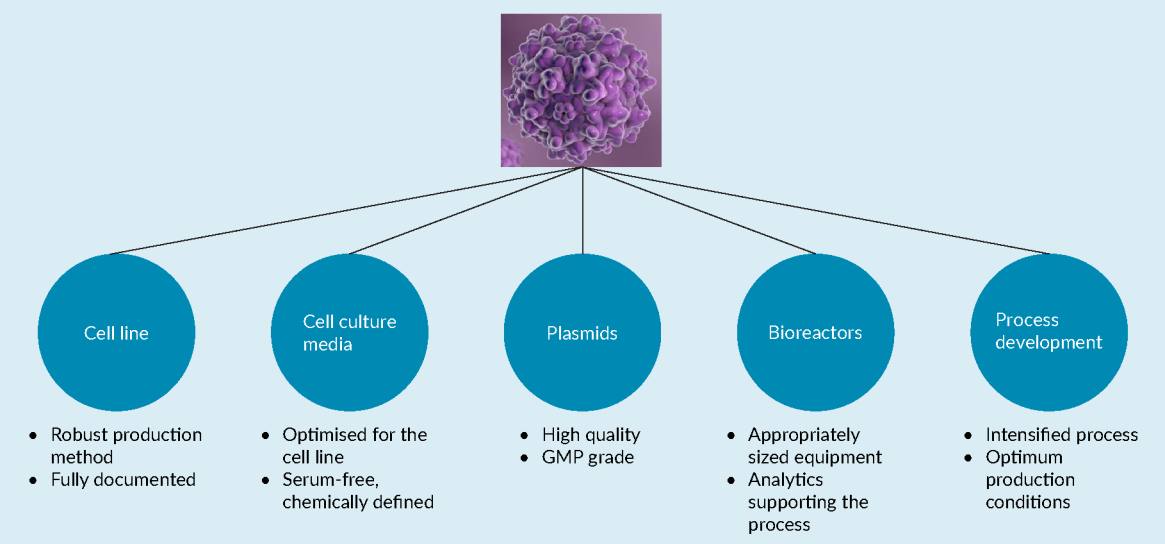
Upon handover of the customer-specific producer cell line, a product license agreement is set up before any GMP batches commence. The ELEVECTA producer cell line can then be used for multiple batches with no further cell line development needs.

A HOLISTIC APPROACH TO UPSTREAM PROCESSING

Upstream processing does not start with a bioreactor. It starts long before, in the cell culture

FIGURE 5

Holistic approach to upstream processing.



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lab, where the host cell lines are at the heart of the process and define the manufacturing path.

Figure 5 shows the many factors that contribute to success in upstream bioprocessing. The ELEVECTA producer cell line provides a firm foundation for large-scale GMP-grade manufacturing of AAV-based therapies.

REFERENCE

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ASK THE AUTHOR

Dovile Gruzdyte, Global Product Manager for Cell Line Development, Cytiva answers your questions on AAV production with the ELEVECTA cell line.

Q Does ELEVECTA work with any AAV serotype and gene of interest?

DG: So far, we have tested AAV 2, 5, 8, and 9, as well as some of the new capsid formats, and we believe that the technology works with all serotypes. As for genes of interest, as long as the packaging capacity is respected for AAV there should be no problem.

Q What allows for minimal batch-to-batch variability for the ELEVECTA cell line?

DG: The production process being so simple ensures that the production is robust. Since it's a monoclonal cell, there is minimal variability in production, and that's what makes this an excellent platform.

Q What scale-up studies did you perform during the development project?

DG: First, we created a stable polyclonal producer pool in roughly 50-milliliter volumes. After the single-cell cloning, we screened the top-performing clones in a miniaturized bioreactor system with volumes of 15 milliliters, then further tested the best-performing

clones in 3–10-liter benchtop stirred tank bioreactors, before performing process optimization to choose the best process conditions at this scale.

Cytiva also offers process development services, and those teams work closely with our cell line development teams. We encourage customers to opt for larger-scale cell line development so that the processes can be transferred to them at 50-liter scale or beyond.

Q What material needs to be provided to Cytiva to kick off the ELEVECTA project?

DG: We will need plasmids for the capsids the customer is looking at and the gene of interest. We will clone those into our proprietary backbone for stable integration into the host cell.

As we produce the material in the pool format, around 4 months into the project, we typically provide material to the customer for internal validation of downstream protocols, analytical methods, and infectivity assays.

BIOGRAPHY

DOVILE GRUZDYTE has held various engineering and management positions in the biotechnology sector. She has spent most of her career developing large-scale manufacturing enterprise solutions for biotechnology customers globally, for monoclonal antibodies and gene therapy production. She has a chemical engineering degree from Newcastle University and Delft University of Technology.

AFFILIATION

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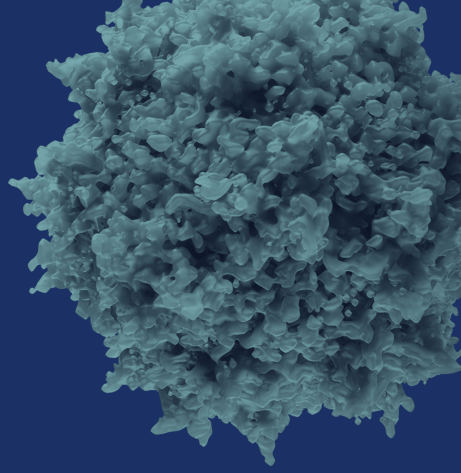

ELEVECTA™ producer cell line for AAV

No plasmids
No transfections
No helper virus

Discover the advantages at
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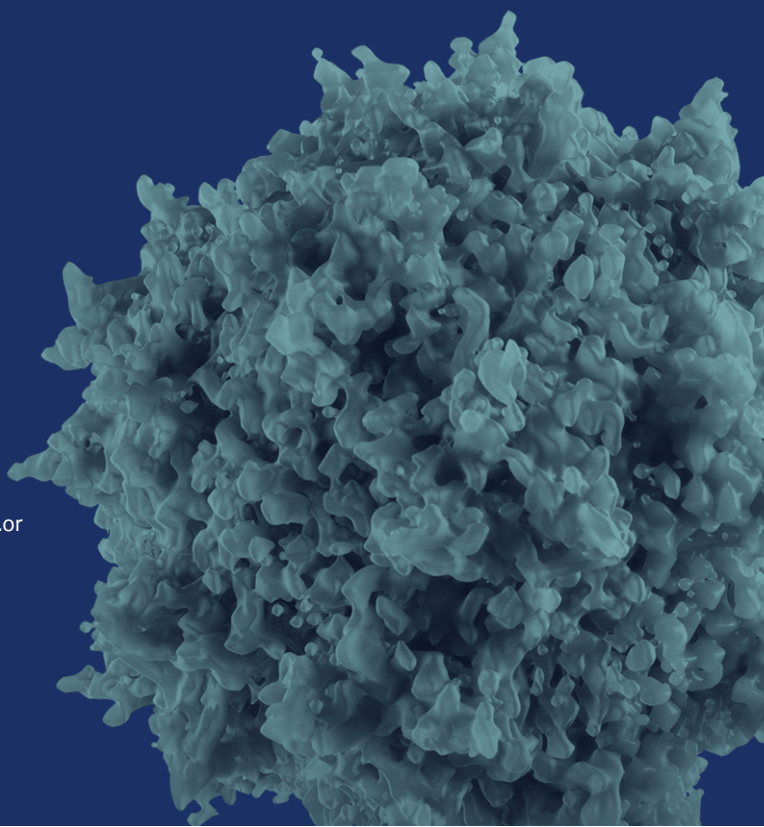

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CY38713-01Aug23-AD

CELL & GENE THERAPY INSIGHTS

VIEWPOINT

Leading a gene therapy team to market approval success

Ray Kester
Senior Quality Assurance Professional, Strategic Quality Leadership Resources, LLC

"Individuals need to understand the group vision and alignment, in addition to what their roles and responsibilities are at the individual level. Individuals are the ones that pull it off."

VIEWPOINT

On February 2, 2023, David McCall (Senior Editor, BioInsights) asked Ray Kester (Senior Quality Assurance Professional, Strategic Quality Leadership Resources, LLC) for insights into project management success, including those gleaned from his experience in guiding a team all the way to market with uniQure's second approved gene therapy product, Hemgenix. This article is based on that interview.

Cell & Gene Therapy Insights 2023; 9(7), 505–508

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MANAGING HEMGENIX'S PRODUCT VALIDATION PROGRAM

As uniQure reached the stage of clinical confidence and determined that it was time to move into its process performance qualification (PPQ) activities, the scale-up from the scientific laboratory to a commercial manufacturing facility was completed. On the commercial side, the following of standard manufacturing practices required some full consideration. uniQure recognized that they had both a team of great scientists and an in-house team of manufacturers, but up to this point, the two were yet to merge.

I was invited to visit and in 3 days we put together the framework plan of how we would work together. With both my operational/engineering and scientific backgrounds, in addition to an understanding of finance, we were able to put together a long-range strategy to achieve the work covering several years. Gathering and then working out how the pieces fitted together in the right place and timeframe was like completing a jigsaw puzzle to ensure the final product met specifications.

Often, gaining approval is thought of as being all about clinical evidence. That is certainly a vital part, but there are other critical aspects involved. The product needs to be able to be made, tested, and explained, with all of these elements performing together as an orchestra. Errors or breaches in the supply chain can lead to stumbles, but recovering from them and keeping moving forward is critical. Maintaining confidence through the use of encouraging language really matters—it can be vital for the success of a team, as can appreciating that different people come from different places, with different attitudes and different backgrounds of experience.

MANAGE A COMPLEX TEAM IN A TIME OF CRISIS

Prior to the COVID-19 pandemic, managing a successful gene therapy development program was about understanding standard protocols, and working with a team of skilled technical scientists and engineers. My initial goal is always to understand the background and the complexities faced in a given space, prior to forming a plan. I then strive to consult with the experts, use a project management format, and perform a work breakdown structure. Virtually every step requires documentation—therefore, I create a master template for that, which also serves to communicate the work process steps and expectations to the team, including the portfolio management and risk management functions. Having a standard format of communication with each function means that everyone understands their expectations—it helps keep everyone on the same page.

At the time when the pandemic struck, we had already established a standard work pattern for everybody involved in the project. We did need to move to the virtual world instead of meeting in conference rooms, but the workflow itself did not change. We held the same meetings at the same times, on the same days, and with consistent weekly reports compiled regardless of our physical locations. Similarly, we set up common file structures and locations so everyone could still use them. As the pandemic eased, we remained in that same mode, and it continued to bring benefits to the whole organization right the way through to the stage of regulatory agency filings. We had 15-minute daily meetings, or 'scrums' as we called them, to assess any problems requiring solutions that same day. Leadership participation, confidence, and encouragement allowed for persistence in spite of the pandemic.

Being able to establish a clarity of vision so that everybody understands their roles and responsibilities is key. Putting that into the project plan is essentially synchronizing the clocks to enable project delivery. So, all individuals across the value chain—including those working in regulatory affairs, supply chain, and receipt of goods—understood

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what was expected of them. During the pandemic, the supply chain turned everything upside down. However, due to good planning, we had the ability to identify alternative suppliers where possible, and to do risk assessments for substitutions of materials that would continue to follow GMP. Finding ways to deal with shortages was a common topic in our communications.

GUIDING PRINCIPLES FOR PROJECT MANAGEMENT

You must have both confident persistence and persistent confidence. This involves knowing that the science works, and simply requires follow-through and problem-solving. Trial and error has financial implications when something does not work the way we want it to, but we cannot hold tomorrow hostage with today's failure when we have confidence that a solution can ultimately work. The executive team at uniQure was remarkably consistent in their delivery of both confident persistence and persistent confidence.

Another key guiding principle is the standard work process. I standardize the expectations of what it takes to complete common tasks so that it complies with standard operating procedures (SOPs). That way, people recognizing and following their SOPs becomes standard behavior. Additionally, it is key to build enough time into a process through a portfolio management approach. Having work broken down into distinct steps in this way allows for things to not go perfectly every time.

GETTING THE MOST OUT OF TALENT

It is about understanding vision, alignment, and focus, then understanding what each individual needs.

A successful project starts with leadership. The leadership must be able to express confidence in the team and select people who understand the nature of the journey they are embarking upon. Individuals need to understand the group vision and alignment, in addition to what their roles and responsibilities are at the individual level. Individuals are the ones that pull it off.

The model that I originally defined and continue to refine is the three Rs: respect, recognition, and reward. Some people value their recognition extremely highly. Others want to be included in decisions, which is about respect. I acknowledge that I may not have the most qualified opinion in the room, so I ask others for their input. Rewards come in many different varieties for different people and can be fiscally- or satisfaction-based.

My method is to engage the intellectual curiosity that naturally exists in teams, keeping conversation healthy and inquisitive to cut through bias. It is common for me to be the least educated in the room, surrounded by PhDs with multiple post-docs. These individuals know things that nobody else on the planet knows. When I am in a room with a dozen people, who have all had decades of learning, I always ask the person who is speaking if they can tell me more. I do not expect to entirely understand their answers myself, but often, all of the other ears in the room pay attention because they are natural learners. In this way, everybody becomes engaged in understanding how a particular step works. This creates a background of inquisitiveness in the room, and an intellectual collaboration is set in motion. As a result, we can find answers to complex questions that we never knew existed.

My top tips are to create a conversation, strive to eliminate bias, and communicate to find solutions to problems that we did not know existed when we started the conversation. None of that is possible without consistency in leadership.

BIOGRAPHY

RAYMOND KESTER is the president and CEO of Strategic Quality Leadership Resources. Ray has a lifelong career in leadership roles in engineering, operations, quality within the biotech industry including leading numerous products from early stage through regulatory approval and commercialization. With a passion for leadership, he developed and refined a leadership model that amplifies the capability of each person and dramatically enhances team performance. His model and methods have been used by many in the industry to achieve regulatory approval and commercial success.

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CELL & GENE THERAPY INSIGHTS

INTERVIEW

How a new generation of FDA reviewers can meet the future demands of cell and gene therapy

Regulatory agencies around the world are currently dealing with the departure of long-term staff members who have witnessed the evolution of the cell and gene therapy field over decades. However, there are also benefits to a fresh approach. **David McCall,** Senior Editor, BioInsights, speaks with former Senior Investigator at the US FDA, **Deborah Hursh,** about what recent reorganization at the FDA means for new agency staffers and sponsors alike.

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Q What are you working on right now?

DH: I retired from the US FDA at the end of 2022. Since then, I have been finalizing a few things with my research laboratory, which was essentially closed down. We just had a manuscript published in *Cytotherapy* and I placed the last of my fellows elsewhere. I then started my own sole proprietorship cell therapy consulting company, which has been a truly novel experience for me. That is what I am doing currently.

Q Regulatory agencies around the world are currently having to deal with the departure of key long-term staff members—individuals who have witnessed the evolution of the cell and gene therapy field over decades. Can you comment on the impact of losing institutional memory at the US FDA in particular?

DH: There certainly has been a departure of long-time staff members in recent times. Many of these people are of the post-World War II generation—what in the USA is called the 'baby boomer' generation. I frankly think it is time for us to move on and free up space and resources for younger people. It does come with some loss of perspective and institutional memory of how the regenerative medicine field developed. There is also a loss of a certain way of doing business. However, sponsors can have confidence in the fact that the regulations and the regulatory framework stay constant. That should give us consistency. The new reviewers will not reinterpret how everything is done.

Also, I personally mentored a lot of younger reviewers, and I hope I imparted both my knowledge and my outlook on how to do review work to these people. Other long-time reviewers who have left have done the same. The agency has been really successful at recruiting very talented young people specifically in cell and gene therapy. These new recruits have up-to-date skills and a modern scientific outlook. I am optimistic that the newer reviewers, once they get into the swing of things, will make the agency a better place.

Q What were the drivers behind the recent FDA reorganization and what will it mean for both agency staff and sponsors?

DH: The recent successes in gene therapy have greatly increased the workload. It changed the old Office of Tissues and Advanced Therapies (OTAT) from being an office that primarily handled investigational submissions (INDs) to one that needed to do significant Biologics License Application work, which is much more complex and requires larger CMC review teams. The COVID19 pandemic also increased the workload by about 30% and reduced the amount of time that staff could actually work due to family obligations. Frankly, it was overwhelming, and it drove a lot of people to leave. It certainly figured in my leaving.

The FDA completed the latest round of their user fee negotiations in 2022 and these negotiations reflected both stakeholder and agency realization of all of the above. It resulted in the promise of a lot of new positions that were targeted toward cell and gene therapy. However, to accommodate all these new positions, the office structure had to change to ensure there was adequate supervisory oversight over this group of new reviewers. Hence the creation of the superoffice now called the Office of Therapeutic Products (OTP).

This superoffice structure creates an office with more supervisory positions. This will create a lot more promotion opportunities for younger reviewers, aiding in staff retention. In the short term, I predict that things will be a bit rocky as the staff grows and the new supervisors learn their roles. The reorganization also took what was a pretty flat structure and made it a lot

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more hierarchical, so there may be some siloing that was not there before. Nevertheless, the increased staffing was necessary, and the restructuring was probably the only way that this could happen in a timely manner.

> "...the office structure had to change to ensure there was adequate supervisory oversight over this group of new reviewers. Hence the creation of the superoffice..."

Q As a former Principal Investigator at the agency, what is your view on how the FDA can prepare to meet the future demands of such a rapidly advancing field?

DH: I can only speak for the Center for Biologics Evaluation and Research (CBER). CBER has always maintained a lot of active laboratory programs. Part of that is because the lot release for biologics was in many cases done by the center in its own labs. Also, the center culture was to maintain an active research program to keep skills up to date and provide research on things that were applicable to regulatory science that might not be done by academic labs. CBER has 80 Principal Investigators, each of whom runs their own research program and is supported by both intramural and extramural funding.

For OTAT, now OTP, the labs provide expertise on specific vector types and cell types. They investigate new technologies such as gene editing and epitope prediction. For example, my lab focused on stem cells and issues related to genome stability, and more recently, manufacturing scale-up. This level of research ensures there are a lot of staff with really up-to-date technical knowledge to back up the full-time review staff. I felt CBER was always very forward-looking in its efforts to keep research focused on emerging critical issues for advanced therapy products.

All of this was supported by a really large investment in state-of-the-art equipment. CBER has Illumina and PacBio sequencing platforms, high throughput imaging, and confocal microscopy, mass spectrometers, as well as an amazing number of flow cytometers. There is also significant bioinformatics capability and high-capacity computing resources. This is all accompanied by agency, center, and division seminar series that continually bring in people who either have new products or new technologies. I have a lot of confidence that CBER has the infrastructure and the institutional will to keep science at the center up to date.

Q The iPSC-derived cell therapy field is seeing an upsurge in activity, including in the number of clinical trials underway. What specific trends and challenges do you see for this burgeoning area?

DH: This is an area that is now moving pretty rapidly, but there are some systemic issues. One of these issues is the lack of high-quality, affordable reagents that can be obtained in sufficient quantities—the complex media, the growth factors, the cytokines, etc. that are necessary to do both expansion of iPSCs and the step differentiations. We also do not

yet have a good handle on the relationship between in vitro culture and genome stability. We have a hard time measuring this and also understanding what the data we can collect actually means. Both sponsors and the agency have that problem.

The fact that all of these products remain in culture for a really long time makes this very difficult. There is a lot of selection pressure in these sometimes months-long in vitro cultures. That goes beyond just genome stability, and includes questions of epigenome stability and probably metabolic shifts as well. This is an area ripe for research.

Then there are issues on how these products will be made at sufficient scale to market them. I am not convinced that we have a clear idea of how this is going to work while complying with cGMP, either for scale-up or for scale-out. However, I am encouraged because there is a lot of active research on many of these issues.

Q What advice can you distil from your more than two decades 'in the trenches' of CMC review and research to pass on to the new generation of regulatory agency staff?

DH: The most important piece of advice that I was given at the beginning of my career was to understand the difference between things a reviewer needs to know versus information that is just 'nice to know'. Some things are regulatory requirements, that a reviewer has to resolve in order to comply with the regulations, but other questions are issues of scientific interest. These things are interesting, but not necessarily something a sponsor needs to invest their time and resources in. It is important not to ask these questions or at least, to limit them.

New reviewers should also use the expertise around them, going beyond the mentor that is usually assigned in an onboarding. I used to go door-to-door to ask more experienced reviewers how they handled this or that question, and found that my colleagues were always very welcoming and helpful.

Q What would you pick out as some of the key pitfalls that sponsors tend to fall into with the CMC component of their IND applications, and what would be your related advice?

DH: CBER offers a lot of pre-meetings, which you should use to ask specific questions about potential problems with your product design or manufacturing – and then you should apply the information that you receive! As a reviewer, I spent a large amount of time on these submissions and crafting answers to questions in order to guide sponsors towards doing things the correct way. You would be surprised at how often sponsors come in with an IND and have not addressed any of the things that were related to them in their pre-submission meeting.

The worst situations arise when a sponsor comes in the door with an IND and has no previous experience with the agency. That happens less frequently than it used to, but these are

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the most challenging submissions. Basic critical information is usually missing in these cases, and it necessitates a lot of back and forth during a time sensitive period. Nobody enjoys that.

The last piece of advice would be to really understand the level of detail that is required. A narrative and a flow chart of manufacturing as well as a detailed description of every step is what you should be submitting. You need to submit all of the Certificates of Analysis (CoA) and sometimes even the Certificates of Origin (CoO), if they are human-, animal- or tissue culture-derived materials. Then, the in-process and final assays that you propose should have at least some release criteria and not just serve to collect information.

There should also be some demonstration that you can actually make your product reliably and reproducibly, with the same impurity profile. That is really important for cell therapy because there is always an impurity profile. You need to know what the impurities are, and you need to know that you are going to get roughly the same impurity profile every time you make the product. If you cannot do that, then you are going to get a lot of pushback from your reviewer.

BIOGRAPHY

DEBORAH HURSH is an expert on Chemistry, Manufacturing and Control (CMC, or Product Quality) issues for cell and gene therapies. She served as a CMC reviewer and Principal Investigator of a research laboratory at the Center for Biologics Evaluation and Research (CBER) of the US FDA for over 20 years. She has a PhD in Molecular, Cellular and Developmental Biology from Indiana University, and did post-graduate work at Harvard and the National Cancer Institute prior to joining CBER in 2000. At FDA, Dr Hursh evaluated products derived from stem cells and participated in policy development in the areas of stem cells, assisted reproduction and xenotransplantation. She chaired the organizing committee for a CBER advisory meeting on oocyte and embryo modification for the prevention of transmission of mitochondrial disease and was the CMC subject matter expert for the 2022 FDA advisory meeting on xenotransplantation. She was the review chair of the Humanitarian Device Exemption review committee resulting in the approval of the Miltenyi CliniMACS CD34 Reagent System, a Class III device used to select stem cells. Her research lab studied issues relevant to the safety and effectiveness of cell therapy products, including those derived from MSCs and PSCs.

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