The Future of Cell and Gene Therapy:

Experts' perspectives

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The future of cell and gene therapy: Experts' perspectives

Five biggest trends of AAV-based gene therapies

Adeno-associated virus (AAV) was first discovered in the mid-1960s, then cloned for the first time in the early-1980s. However, it wasn't until 1995 that the first human patients were treated with AAV for cystic fibrosis. The first meaningful clinical efficacy followed in 2008 in the retinal diseases space – a journey that culminated in 2017 with the regulatory approval of Spark Therapeutics' Luxturna. In the intervening years, Glybera – an AAV-based gene therapy for the ultra-rare disease, hereditary lipoprotein lipase deficiency (LPLD) – gained market approval in Europe. However, it was subsequently withdrawn from the market due to its high cost. Most recently, in 2019, Zolgensma became the second AAV gene therapy to be approved by the US FDA, for spinal muscular atrophy (SMA). This potted history demonstrates that AAV has been on a long journey from initial discovery to successful clinical application. However, AAV-based gene therapy now stands on the cusp of bringing its' significant, often curative benefits not just to dozens of patients, but potentially thousands. Here, we explore five key trends and issues in the field today, which reveal a pathway to further product approvals and more widespread adoption by healthcare systems worldwide.

""We're starting to see increasing approvals of Luxturna and Zolgensma in other regions of the world, along with new and updated guidance relevant to gene therapy."

– Snehal Naik, PhD, Head of Regulatory Policy and Intelligence, & Regulatory Strategy Leader for Ocular Programs, Spark Therapeutics

"I think with a lot of these therapies; it's been decades of work building up to this becoming a very exciting place to try and make an impact on human health. That is what is happening now in the 2020s"

– Mark White, PhD, Associate Director of Biopharma Product Marketing, Bio-Rad

TREND 1: MOVING BEYOND RARE DISEASES

Almost without exception, AAV-based gene therapy's early clinical successes have come in rare and ultra-rare diseases – often serious monogenic disorders (requiring a single gene correction) impacting pediatric patient populations, for which there are no alternative treatment options available. High unmet medical need, expedited regulatory pathways, and the comparatively low-hanging fruit that single gene defects represent for gene therapy, all combined to make orphan indications a logical proving ground for the nascent AAV field. However, with clinical proof of concept achieved, the sector is now engaged in migrating AAV into larger, more commercially viable disease indications, including Parkinson's disease and Duchenne muscular dystrophy.

TREND 2: ADDRESSING THE TARGETED *IN VIVO* DELIVERY CONUNDRUM

One of the key characteristics of AAV that make it an attractive option for gene delivery *in vivo* is the differing tissue tropism of its various serotypes. Each of the dozen naturally occurring AAV serotypes discovered to date is suited to transduction of specific cell types, whether they are located in the CNS, heart, kidney, liver, lung, retina, etc.

Nonetheless, the successful clinical application of AAV has traditionally been limited to diseases that can be addressed through delivery to either the eye or the liver. Enabling systemic delivery and direct delivery to other tissues (e.g., muscle, brain) have proven to be thorny challenges to overcome. This is due to barriers such as insufficient tissue tropism to ensure tissue-specific expression across different organs in the body, the requirement for higher dosages in certain tissues/diseases, and AAV's inherent immunogenicity.

A key element to expanding the applicability of AAV to new diseases and patient populations will be allowing the safe, effective delivery of AAV vectors to the harder-to-reach cells in the body. In a significant recent breakthrough, PTC Therapeutics' Upstaza - a gene therapy that is delivered directly into the brain - was approved by the European Medicines Agency (EMA) in July 2022 for the treatment of adult and pediatric patients with severe aromatic L-amino acid decarboxylase (AADC) deficiency.

TREND 3. ENGINEERING A WAY AROUND THE DRAWBACKS OF AAV: OVERCOMING SAFETY & IMMUNOGENICITY ISSUES

AAV vectors have a number of limitations. For example, because AAV is naturally occurring in humans, up to 70% of the overall population have pre-existing antibodies against the virus. Furthermore, those who don't have pre-existing antibodies may only receive AAV gene therapy once as they will then develop antibodies, rendering redosing impossible. However, perhaps the most high-profile challenge today is related to safety. The prevalent approach to delivering the required degree of clinical efficacy in key target diseases such as hemophilia has been to increase dosage. Unfortunately, a number of Serious Adverse Events (SAEs) have resulted, leading to a recent spate of toxicity-related clinical holds imposed by regulators.

In a bid to address these longstanding issues, as well as to enhance aspects such as tissue tropism, an array of AAV capsid engineering approaches are being adopted. Whether they are aimed at shielding the viral vector from the immune system, or improving the specificity/efficiency of gene delivery allowing dose reductions and, therefore a reduction in Cost of Goods, next-generation engineered AAV vectors will be crucial to bringing *in vivo* gene therapies to broader patient populations.

"I'm very excited about the engineering aspects of AAV design, whereby these novel capsids can potentially have better safety and efficacy profiles. I'm hoping for many more improvements in design to help us produce better drugs in the future."

– Santoshkumar Khatwani, PhD, Director of Analytical Development, Sangamo Therapeutics

TREND 4: TACKLING CMC CONCERNS TO SATISFY REGULATORS

As any novel therapeutic modality progresses towards commercialization, regulators' requirements increase significantly. One of the greatest challenges facing AAV gene therapy developers today is a more stringent regulatory environment, particularly in the critical area of Chemistry, Manufacturing, and Controls (CMC).

Regulators are requesting more and more data relating to AAV vectors' critical quality attributes (CQAs), placing strain on the still-evolving analytical toolkit. Defining the full/empty capsid ratio is a key recent example – a measurement which has gone from a novel discovery to a 'must-have' in regulators' eyes in a short period of time. As a result, expectations are that the next target for increased regulatory scrutiny will be the definition of exactly what is packaged inside the AAV capsid. Moreover, the fact that many AAV gene therapies are on accelerated clinical development pathways means that there is less time available than ever before to conduct product and process development.

Potency is another key area of focus here and has long been seen as a challenging attribute to characterize and measure for the gene therapy field. However, inadequate potency assays have been the reason behind a number of recent product failures at the Biologics License Application (BLA) stage.

Innovation in analytical technology will be central to allowing the gene therapy industry to sufficiently demonstrate the quality and consistency of its products.

TREND 5: THE DRIVE TOWARDS AAV PLATFORMS

With the ever-increasing costs of development and high-priced cell and gene therapy products having already encountered difficulties in securing managed healthcare insurance reimbursement, question marks have been raised over the long-term commercial viability of AAV gene therapy. This is particularly the case in the field's traditional stronghold - the rare and ultra-rare disease setting.

Today, academic and industry innovators and regulators alike are pursuing the idea of AAV-based platform processes, allowing the cost-effective development and delivery of novel gene therapies for the myriad orphan indications that could benefit from their curative potential.

This particular trend speaks to a broader one: a growing call for standardization across the AAV field and particularly, in manufacturing, which may help solve many of the aforementioned CMC-related issues.

"We are perhaps at something of an inflection point in the cell and gene therapy space. It's exciting to see what the future holds with some of the upcoming approvals and the expansion of gene therapy not just in the US and EU, but in the rest of the world as well."

– Chris Lorenz, Senior Vice President of Technical Operations, Astellas Gene Therapies

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CHRIS LORENZ is currently Senior Vice President of Technical Operations at Astellas Gene Therapies. Chris joined the company back in February 2016 when it was known as Audentes Therapeutics and was originally charged with starting up internal manufacturing operations. Since then, the TechOps organization has grown from a team of one to more than 250 spread across sites in California, North Carolina, and Japan, and has supported the development and manufacturing of dozens of AAV-based programs at both the preclinical and clinical stages. Prior to Astellas/Audentes, Chris worked for Grifols Diagnostics Solutions (formerly Novartis Diagnostics), and before that at Genentech. He holds a BS and MS in Chemical Engineering from Stanford University.

SANTOSHKUMAR KHATWANI graduated from the University of Kentucky in 2010 with PhD in Chemistry. Furthermore, he obtained postdoctoral training at the University of Minnesota. Dr Khatwani then joined BioVision Inc. in 2012 and served under different capacities until 2017 where he oversaw the manufacture, testing and release of several recombinant protein, enzyme and assays for various metabolically important enzymes. Currently Dr Khatwani is serving as Director of Analytical Development at Sangamo Therapeutics with strong focus on developing analytical solutions and CMC in support of product development at early and late phase of the clinical development.

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SNEHAL NAIK brings a confluence of regulatory affairs, early discovery, innovation, policy, and scientific expertise to her current hybrid role as Spark's Head of Regulatory Policy and Intelligence, and Regulatory Strategy Leader for ocular programs. In this capacity she established the regulatory policy function at Spark and is supporting global development of gene therapies. Snehal co-chairs the regenerative medicine committee at BIO, staffs efforts at the Alliance for Regenerative Medicine and the Innovative Medicines Initiative, and is an active member of the American Society of Gene & Cell Therapy and New York Academy of Sciences. Snehal graduated summa cum laude with an AB-MA in Biology from Bryn Mawr College, and holds a PhD in Molecular Genetics and Genomics from Washington University in St. Louis where she also completed the Cancer Biology pathway with the Siteman Cancer Center.

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The future of cell and gene therapy: Experts' perspectives

Regulatory expectations & guidelines around AAV gene therapy

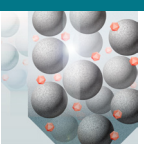
The first US Food and Drug Administration (FDA) regulatory guidance specifically for the cell and gene therapy field emerged in the 1990's, addressing preclinical R&D and manufacturing, and to a lesser extent, clinical aspects. Since then, the regulatory framework surrounding adeno-associated virus (AAV)-based gene therapies has been modernized considerably, particularly in the last five years. Here, we highlight some key aspects of evolving regulatory thinking and guidance around the space that have major repercussions for AAV-based gene therapy developers.

A spate of recent draft FDA guidance, which initially came out in 2018 and are now increasingly being finalized, follow two general directions. Firstly, there is an updating of the information that was previously described in the early preclinical and manufacturing guidance. Secondly, several disease-specific gene therapy guidances have emerged, covering hemophilia, rare diseases, retinal disorders, and central nervous system disorders. The latter cover some common considerations across the gene therapy field, but also others that are specific to the particular therapeutic area or indication in question.

Across the Atlantic, the European Medicines Agency (EMA) has followed a similar timeline and pathway with its development of advanced therapy medicinal product (ATMP) guidance. Again, ATMP-specific guidance that either updated or added to existing guidances began to emerge towards the end of the last decade. Notably, the EMA made a set of flowcharts [1] and checklists available covering quality, non-clinical, and clinical aspects. These are designed to help gene therapy developers plan their programs from the beginning, and to understand whether they are on track with what the regulators want to see at any given stage.

This reflects a general emphasis from regulatory agencies on advising gene therapy developers to think about regulatory considerations from the earliest stages of R&D. This is a necessary step, as the majority of biotech's in the sector are early-stage companies with a relative dearth of regulatory experience and expertise, particularly relating to requirements at the later stages of clinical development and commercialization.

There is another clear trend in US and European regulatory guidance and sentiment around encouraging gene therapy developers to lock down manufacturing process as early as possible. On a related topic, developers are



increasingly advised to make minimal changes to raw and starting materials through process development and scale-up. Both are key examples of hard-won learnings made by the gene therapy field in the past two decades that are now reflected in the regulatory framework.

However, both scientific understanding and technological innovation in the AAV gene therapy continue to evolve at a tremendous rate. In such an environment, there will always be a lag between the scientific cutting edge and the development of appropriate regulatory guidance. The AAV gene therapy field has struggled in recent times due to this lag – for example, in the area of potency assay development. Fortunately, the emergence of increasingly sophisticated process and analytical tools, which are customized to the specific requirements of AAV vectors, will help to close the gap moving forward.

"I think the really interesting piece is going to be having the regulations stay current, as the field evolves so rapidly."

– Snehal Naik, PhD, Head of Regulatory Policy and Intelligence, & Regulatory Strategy Leader for Ocular Programs, Spark Therapeutics

In terms of potential areas of focus for future regulatory guidance, it will be interesting to see if and when regulators provide specific guidance relating to analyzing the contents of AAV capsids. Additionally, the growing utilization of AAV vectors to deliver gene editing components will be one to watch. Recently, the US FDA has released modality-specific guidance for the gene editing and chimeric antigen receptor T cell therapy spaces – will we see this trend continue to the benefit of AAV-based gene therapies? For example, as the field migrates to larger indications from rare diseases, additional guidance may be required in terms of how to apply the existing regulatory framework.

Last but not least, the drive by all stakeholders to enable market and patient access to gene therapy on a global basis is set to continue in the regulatory sphere. Issues of regulatory disharmony between different jurisdictions have long existed. However, sector maturation and expansion of the gene therapy knowledge base are providing regulatory bodies with the tools to develop a global regulatory framework for the field.

"International harmonization or convergence could be especially enabling to the development of gene therapies, and in rare disease indications."

– Snehal Naik, PhD

The World Health Organization (WHO) recently released a draft document [2] relating to establishing common definitions and understandings around advanced therapies. Furthermore, the *International Council* for *Harmonisation* of Technical Requirements for Pharmaceuticals for Human Use (ICH) is working on non-clinical guidance around biodistribution specifically for gene therapies (ICH S12). Further convergence may be expected, to the benefit of all.

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The future of cell and gene therapy: Experts' perspectives

Five biggest trends of gene-modified cell therapy

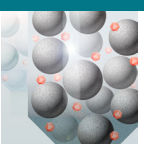
The gene-modified cell therapy field continues to grow apace, particularly in the oncology arena, which dominates both preclinical and clinical applications. For example, recent data from The Cancer Research Institute [1] suggests there are 2,756 cell therapies in development for cancer indications in 2022, up from 2,031 in 2021. Furthermore, this growth is reflected in the number of studies at every stage of development, from preclinical studies to pivotal clinical trials, and across every major immune cell type/modality, including chimeric antigen receptor T cell (CAR-T) cells, natural killer (NK) cells, T cell receptor T cells (TCR-Ts), and tumor-infiltrating lymphocytes (TILs). The American Society for Gene and Cell Therapy (ASGCT) concurs, stating in its Gene, Cell, & RNA Therapy Landscape Q2 2022 Quarterly Data Report [2] that in the year from Q1 2021, the overall gene therapy pipeline of products in preclinical to pre-registration studies increased by 16%. (*Ex vivo* genetically modified cell products comprised 73% of this total pipeline – a record high share).

The following key trends have emerged in recent years to shape the future of cellular immunotherapy, ensuring that more and more patients will be able to benefit from these game-changing treatments.

TREND 1: INDUSTRY TRAINS SIGHTS ON SOLID TUMORS

The six CAR-T cell therapies to have received US FDA approval to date (Kymriah, Yescarta, Tecartus, Breyanzi, Abecma, and Carvykti) cover between them two targets (CD19 and BCMA) and a relatively narrow range of hematologic malignancies, most notably B-cell non-Hodgkin lymphoma (NHL), B-cell acute lymphoblastic leukemia (ALL), and multiple myeloma (MM). An important point of recent focus for the developers of these approved products has been to drive their utilization earlier in cancer treatment. The fact that CAR-T cell therapies are now utilized in the second line is ensuring the R&D pipeline for hematologic malignancies such as NHL and acute myeloid leukemia (AML) continues to grow despite the competition. Overall, the most significant new trend in hematological indications is a recent concentrated focus on T cell malignancies. Regarding targets, recent evidence indicates that there is only a limited, incremental benefit to searching for additional targets. Instead, it looks like platform technologies may need optimization.

In terms of both unmet medical need and commercial potential, though, solid tumors represent a far larger opportunity for the sector. This has been reflected in a recent surge in the cellular immunotherapy R&D pipeline



for a wide range of solid tumor indications, including brain, renal/hepatic, colorectal, ovarian, pancreatic, prostate, thoracic, and head and neck cancers. In particular, since the emergence of data indicating it was a good indication for CD3 bispecific antibodies (being a 'cold' tumor turned 'hot' through T cell infiltration), prostate cancer has become an important early target indication for the field. However, toxicity issues such as those observed in Tmunity Therapeutics' PSMA CAR-T clinical program represent a speed bump in this area.

Looking to the future, Adaptimmune may deliver the first approved cell therapy to the solid tumor market in the coming 12 months (afamitresgene autoleucel, a TCR-T cell therapy for synovial sarcoma). In general, though, despite some encouraging early data, key questions remain. Chief among these is can the startling efficacy observed in hematologic malignancies be recapitulated durably in the immunosuppressive tumor microenvironment (TME)?

Target selection remains an issue because of the relative dearth of 'validated' targets for solid tumors in cell therapy. As a consequence, the field has moved to targets that have a question mark over their tumor specificity, in order to see how clean a target needs to be to be feasible for CAR-T cell therapy. Examples include claudin 18.2 and mesothelin.

In terms of addressing the challenges of the TME, there is some convergence around PD-1 and TGF-β as dominant axes to be targeted. Some companies are prioritizing increasing potency and overcoming T cell exhaustion as strategies to overcome immunosuppressive effects. Additionally, cytokine enhancement is an important direction for current research. All of these approaches may have merit and in the long run, all may be needed. It will become a matter of how many elements can be deployed at once, and then interpreted meaningfully.

Solid tumors may also need to be addressed through multiple dosing, or combinations with drugs with which the cellular product needs to be compatible.

""To tackle solid tumors, a multi-pronged approach may be needed to obviate immune inhibition in the TME, through embellishing the therapeutic with biological response modifiers to co-opt endogenous immunity, render the immune cells resilient to multiple immune inhibiting mechanisms, use other approaches to combat mechanisms of resistance, or bring potentially curative cell therapies in at an earlier stage (pre-checkpoint inhibitors)."

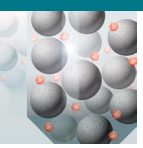
- Adrian Bot, MD, PhD, Founding Chief Scientific Officer & Executive Vice President of Research and Development, Capstan Therapeutics

Many combinations hold promise. While PD-1 checkpoint inhibitor combinations have shown limited utility in hematological malignancies, solid tumors should be a great place to test them further, providing modest CAR activity can be boosted by reinvigorating the T cells with a checkpoint inhibitor. On the other hand, there is some apprehension in the field relating to combining cell therapy with a given immune checkpoint blocking agent due to the fact that multiple pathways are operational.

One objective would be to overcome target heterogeneity by 'painting the target', which oncolytic virotherapies could achieve effectively. Another avenue is nanoparticle delivery of mRNAs, although specificity of targeting might be harder to achieve here. A further key approach could be repolarizing the TME from a negative (e.g., M2) to a positive (M1) environment.

It is possible that any agent that leads to tumor-specific lysis and inflammation may help, including chemotherapy or radiotherapy, CAR macrophages, and oncolytic virotherapies.

Finally, CAR-T cells would appear to work best below a certain tumor bulk level. Using another agent (e.g., an antibody–drug



conjugate or bispecific antibody) to debulk the tumor prior to T cell immunotherapy may therefore prove effective.

TREND 2: ALLOGENEIC CELL THERAPY ON THE CREST OF A WAVE

One of the most significant trends over the past 12–18 months is the increasing clinical application of allogeneic cell-based immunotherapies. This trend has been driven by the desire to produce a more consistent product, which can be used to treat multiple patients without the 'autologous baggage' associated with such patients being disadvantaged by ongoing pathology and previous treatment regimes. Furthermore, allogeneic products avoid much of the relatively time-consuming logistical complexity of the autologous cell therapy supply chain. The ability to leverage a generic cell source also facilitates cost-effective scale-up and consistent batch-to-batch compliance. These advantages have been reflected in the commercial sector recently, with several big pharma companies striking major platform deals with allogeneic cell therapy biotech's (e.g., Roche/Poseida Therapeutics).

'Off-the-shelf,' allogeneic CAR-T cells have the potential to overcome some of the critical issues associated with autologous approaches. In addition, the use of immune cells from healthy donors offers several advantages:

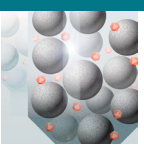
- A more uniform starting material, which allows for more predictable and reproducible manufacturing. Starting from healthy donor cells ensures more consistent performance of the cell product generated.
- Allogeneic therapies have the potential to provide a ready-to-use, immediately available immunotherapeutic drug, which does not require the patient to be healthy enough or physically equipped to be an immune cell donor, or to be able to wait for weeks or months for a bespoke cell lot to be manufactured.
- As well as being available to a broader patient population, allogeneic cell products would also be deployable in a broader range of points of care (not only a relative few highly sophisticated hospitals).
- A single manufacturing run allows dosing of many patients, as well as multiple dosing for individual patients, which offers the opportunity to reduce cost of goods.
- 'Off-the-shelf' CAR-T cells are not simply an allogeneic version of autologous therapies – they are a drug, and could be used as such (i.e., through re-dosing, combinations, etc.)

A recent transformative milestone for the field was proving the ability to make allogeneic T cells non-alloreactive, thereby breaking the donor-receiver compatibility barrier. Experience in transfusion and transplant has revealed the potential danger in infusing T cells from a donor into another person with an unmatched human leukocyte antigen haplotype. Donor T cells could be activated through their natural receptor, by healthy cells or tissues from the receiving patient, and trigger graft-versus-host disease (GvHD). Eliminating that receptor and activation route has allowed the use of T cells from any donor in a patient. This technical breakthrough means that T cell-based cellular products no longer need to be made bespoke to a patient, opening the door to mass production of allogeneic T cell therapy batches to treat many different patients, regardless of the donor.

"Well before allogeneic cell therapies were used for the first time, people said that graft versus host disease on one hand and immune rejection on the other would mean that it was impossible to dose them safely and achieve durable responses. We've shown that's not true."

– Dr Barbra Sasu, Chief Scientific Officer, Allogene Therapeutics

Allogeneic cellular immunotherapies are still in a relatively nascent stage of development,



but pioneering companies such as Cellectis, Allogene Therapeutics, and TC BioPharm are producing encouraging early clinical data. All eyes will be on clinical data read-outs over the coming 12 months for further evidence of comparable safety and efficacy to autologous cell therapies on the market and in development, and importantly, on the durability of response.

"Allogeneic CAR-T cells are essentially materializing the transition of cell therapies from the world of grafts, where they grew for decades, to that of industrialized 'off-the-shelf' pharmaceutical products."

– David Sourdive, PhD, Executive Vice President CMC and Manufacturing, Cellectis

TREND 3: A BRAVE NEW WORLD OF GENE DELIVERY AND CELL ENGINEERING

The entire advanced therapy field is being transformed by innovation in gene delivery and genome editing technology. The engineered cell therapy space is no exception.

The traditional approach of utilizing retroviral/lentiviral vectors to transduce immune cells *ex vivo* continues to bear fruit, as improvements are made to their safety and efficiency. In addition, non-viral delivery platforms such as transposon systems [3–5] are emerging as viable alternative cell transfection tools. The rise of non-viral gene transfer is further enabled by next-generation cell electroporation and mechanoporation technologies.

The impact of genome editing is being felt throughout the field, but perhaps nowhere more so than in the allogeneic cellular immunotherapy space. Besides the application of gene editing in creating induced pluripotent stem cell (iPSC) master cell banks for therapeutic development, the majority of therapies in the current allogeneic CAR-T pipeline undergo at least one and often multiple edits. This has already had a transformative effect on the field, yet it is arguably just the beginning of a more profound revolution.

With advanced gene editing, it has become possible to perform genomic designs where pre-defined sophisticated scenarios are literally programmed into cellular products to be executed once infused into a patient. Furthermore, such "smart cells" can be endowed with supra-physiological properties, allowing them to perform tasks that normal cells cannot, and eventually, to succeed where the patient's own cells fail. For example, Cellectis is developing allogeneic CAR-T cells that are programmed using the company's own TALEN® genome editing platform and PulseAgile electroporation systems to overcome tumor defense mechanisms, whilst simultaneously triggering immunological scenarios changing the course of the disease.

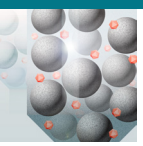
"TALEN® allowed Cellectis to treat the first patient ever with an off-the-shelf allogeneic CAR-T product in 2015, and is now the gene editing technology supported by the largest clinical experience in the field to date."

– David Sourdive, PhD

Finally, no discussion of the innovation in cell engineering can be complete without mentioning the advent of *in vivo* CAR-T cell therapy and its potential to disrupt the cell and gene therapy field. If the transition from *ex vivo* engineering of T cells to *in vivo* global reprogramming of the immune system can be achieved, many of the manufacturing/supply chain and commercial challenges associated with current autologous and allogeneic cell therapies alike will disappear. With CAR-T cell pioneers such as the University of Pennsylvania and its recent spinout, Capstan Therapeutics, driving progress in this space [6] it is clearly one to watch for the future.

TREND 4: THE INNATE IMMUNE SYSTEM'S DAY IN THE SUN MAY HAVE ARRIVED

To date, the engineered immune cell therapy field's successes in the oncology setting have



almost entirely been based on exploiting the adaptive immune system, arguably resulting in the innate immune system being somewhat neglected in the past. However, there has been a recent surge in R&D activity involving NK cells, γδ T cells, and macrophages in particular. This is driven in large part by lingering concerns over CAR-T cell therapy safety and durability, and the perceived need to leverage multiple pathways in order to successfully tackle solid tumors.

Building upon pioneering work by Dr Katy Rezvani and colleagues at the University of Texas MD Anderson Cancer Center, among other academic institutions, industry trailblazers such as Fate Therapeutics have delivered promising safety and efficacy data. The natural capability of NK cells to enable allogeneic use is one of several benefits they offer. However, NK cells face many of the same challenges as other immune cell types in firstly targeting/penetrating and then demonstrating durable activity in the immunosuppressive, hypoxic tumor microenvironment.

γδ T cell therapy developers have precipitated a recent move from the B-cell lymphoma space into lesions which, whilst being classed as hematological, have a solid tissue involvement. Examples include bone marrow and lymph node for AML and NHL respectively.

Ongoing efforts to improve understanding of the innate immune system's role in fighting cancer may lead to further advances and clinical applications, and significantly, the continuing expansion of the immune cell therapy armamentarium.

TREND 5: MANUFACTURING AND SUPPLY CHAIN INNOVATION IS RESHAPING THE PLAYING FIELD

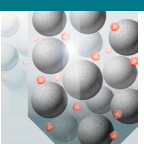
Novartis' recent unveiling of the T-Charge platform – a novel approach that can reduce autologous cell therapy processing time from two weeks to 24 hours – is just one example of the potentially game-changing impact that manufacturing innovation can have on the engineered cell therapy field. Indeed, with cost of goods control being a critical component of efforts to improve the affordability of these lifesaving, curative treatments, it is perhaps the single most vital aspect to ensuring their benefits become accessible to broader patient populations.

- Advances in a range of areas are delivering time and cost savings and increasing the robustness and reproducibility of cellular immunotherapy manufacture and product delivery to patients, including:

- Closed, automated manufacturing devices. As more and more solutions reach the market, offering improved flexibility and the potential to automate multiple process steps, the opportunity to manufacture closer to the point of care (and even at the patient's own bedside) grows – a vital step in defining the scale and nature of the role that autologous cell therapies can play in the future of healthcare.

- Analytics. Novel tools and assays enable more sensitive, accurate in-process monitoring and rapid release testing. They are also a critical component in the ongoing effort to bring the benefits of full manufacturing automation to the field.

- Cryopreservation and cold chain management. One of the obstacles to cell therapies becoming mainstream is the ability to deliver a product with a sustained shelf-life. A key approach to this problem is to freeze in the cleanroom and thaw at the clinic. Freezing/thawing in a reproducible manner is now a reality (as demonstrated by TC BioPharm, who recently commenced the EU arm of their phase 2/3 oncology trial with a fully allogeneic banked frozen-thawed γδ T cell product).



"In 10 years', time, hospital pharmacies will be dispensing numerous different freeze-thawed cell therapies. Each one can't have its own unique/bespoke protocol for thawing, so the industry needs to collectively develop unified systems and standards for such processes."

– Dr Michael Leek, Co-Founder and Executive Chairman, TC BioPharm

Digitizing the cell therapy supply chain. For autologous cell therapies in particular, optimized track-and-trace and orchestration platforms are a must-have to mitigate supply chain risk and ensure every patient has the chance to receive the best possible cell product.

Raw and starting materials. Standardization in apheresis/leukapheresis collection is increasingly viewed as a vital step towards ensuring a more consistent cell therapy product, whilst alleviating the burden of multiple different products/protocols on the point of care or apheresis center.

Meanwhile, the emergence of iPSC-derived products from biotech companies including Fate Therapeutics and Notch Therapeutics encourages that as allogeneic cell therapies become more mainstream, the issue of insufficient donors will not prove to be an insurmountable bottleneck for the field.

"Cell therapies need to become 'pharmaceuticalized': this means acceptable costs of goods, seamless distribution, and efficacious, reproducible product."

– Dr Michael Leek

The cell-based immunotherapy field has come a remarkably long way in just a decade. However, as these trends suggest, the sector should prepare itself now for an even faster pace of evolution and a still greater degree of innovation over the ten years to come.

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MICHAEL LEEK is the Executive Chairman & Founder at TC BioPharm. Michael was previously CEO of both TC BioPharm and an orthobiologicals company, and he also worked with a clinical team developing an autologous infusion of stem cells to initiate regeneration of new hepatic tissue. He was jointly responsible for moving Intercytex from a pre-clinical research company, to an AIM-listed biotech with several cell therapy products in the clinic. Michael is also a Fellow of the Royal Society of Medicine and Honorary Lecturer at the University of Aberdeen, School of Medical Sciences. In 2017, he was presented with the 'Scottish Life-Sciences Entrepreneurial Business Leadership' award.

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Harnessing analytical technologies to modify your AAV development workflow

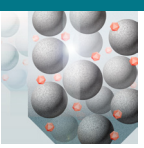
In '*Five biggest trends of AAV-based gene therapies*', we highlighted some key challenges relating to the development and manufacture of AAV-based gene therapies, many of which require developers to alter their workflows. Here, we delve deeper into these challenges and look at how gene therapy developers can make the changes required to address them. In particular, we explore the hurdles in measuring and reducing immunogenicity in the clinic, in better understanding potency by leveraging multiple analytical techniques, and in cultivating a robust understanding of critical quality attributes to ensure safety and efficacy.

Significant concerns remain around the immunogenicity of adeno-associated virus (AAV) vectors, particularly where they are delivered systemically. There are lingering question marks around pre-existing immunity, the durability of response, and the ability to re-dose. But it is safety issues that are front and center in the gene therapy field at present.

The hitherto standard approach of increasing the dosage of viral vector genomes to drive expression in the target cells may lead to off-target toxicity, particularly in the liver. However, it is important to remember that AAV-based gene therapy is still in its relative infancy as a technology area. As more experience is gained and knowledge mined from clinical trials and real world data, the 'sledgehammer' approach of increasing dose is becoming more refined and precise. Novel AAV vectors are being engineered to more specifically target small subsets of cells *in vivo*, and to more accurately define the site of gene expression.

This push towards more targeted AAV vectors that allow dose reduction is partly about the biology of making the vector more efficient, but it is also about the manufacturing. In particular, the gene therapy field's ability to identify, measure, and leverage the viral vector product's critical quality attributes (CQAs) is central to this endeavor's success. Here are some specific areas where innovation in analytical tools and techniques is providing valuable new insights into the quality and consistency of AAV vector manufacture.

- Vector characterization and purity. Accurately measuring viral protein (VP) ratio, empty/full/partially full capsid ratio, and residual host cell DNA packaged in the capsid and are key for regulators and industry alike. Regulators, manufacturers, and tool providers are all critical stakeholders in



establishing standards for the application of novel tools that offer the improved precision which industry requires.

- Measuring empty/full ratio is an area of strong focus for industry currently. However, a lack of standardization in terms of which analytical method to use means that different laboratories and companies may achieve strikingly different results with the same sample. As a consequence, sponsors have tended to favor direct methods such as analytical ultracentrifugation (AUC). However, AUC is both time consuming, and requires a particular skillset within the QC group.

"Are there methods that are more real-time, more rapid, more precise than AUC? I think that's where we need to continue to push the envelope, but ultimately, converge on one method so we can truly compare apples to apples across industry. Then, when we do see safety or efficacy signals, we're using the same calibration curve, if you will."

– Chris Lorenz, Senior Vice President of Technical Operations, Astellas Gene Therapies

"In many cases we're measuring and documenting things where we don't know the range of what's acceptable. Empty/full is a good example. What's important right now for the regulators is that you document what it is and how you measured it. If you document it well, you can do some retrospective studies, if necessary, and learn as you go."

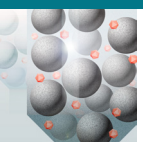
– Mark White, PhD, Associate Director of Biopharma Product Marketing, Bio-Rad

- Viral genome (vg) titer is a critical CQA for AAV-based gene therapies. The traditional qPCR-based vg titer quantification method is steadily being replaced by a more sophisticated analytical toolkit that includes droplet digital PCR (ddPCR).

- Last but certainly not least, potency. Traditionally, AAV gene therapy potency assays have been demonstrated by a combination of three different attributes: infectivity, expression, and finally, a functional potency assay for the final vector product itself. However, there are many new technologies that are increasingly in use today. For example, $TCID_{50}$ has traditionally been used as a method of indicating the infectious titer of the assay, but today, there are technologies available that use Laser Force Cytology (LFC), which are capable of demonstrating viral titer much more quickly and with comparatively minimal effort. There are many more potency assay platforms available that are automated, including ELISA platforms such as Mesoscale Discovery (MSD), Gyrolab, or Ella, all of which have allowed faster turnaround times and improved accuracy.

- Of course, potency remains a particularly difficult area for gene therapy. The challenges start with the fact that cell-based bioassays are utilized, which means there will be some associated variability in results. Success in developing a functional AAV potency assay is partially dependent on firstly selecting or engineering an appropriate cell line, and then establishing the assay as early as possible in process development.

- The 'holy grail' in AAV potency assays is developing a single functional *in vitro* method. However, due to incomplete understanding of disease biology (particularly in rare and ultra-rare diseases) the field is currently reliant on the potency matrix approach, where two or more different orthogonal methods are combined. These often include *in vivo* potency methods, which are suboptimal. The aforementioned emerging analytical tools are beginning to change the way industry thinks about potency, but there is still work to be done here.



"One of the ways you can improve the potency assay is to have accurate and highly precise dosing assay (e.g., vg titer) as it is used as input in the potency assay to calculate multiplicity of infection (MOI). Digital PCR-based technologies have significantly improved the input vg titer that is used in the potency assay."

– Santoshkumar Khatwani, PhD, Director of Analytical Development, Sangamo Therapeutics

The AAV analytical toolkit continues to grow and improve – for example, charge detection mass spectrometry (CDMS) and mass photometry have arrived to offer alternatives to AUC. Increasing the range of options available is a positive for the field, as is the fact that certain methods (eg. liquid chromatography/mass spectrometry) allow a deeper understanding of the viral protein identity as well as the post translational modifications of these viral proteins. Ultimately, these methods may lead the field to identify new CQAs that have not yet been understood, further enhancing the quality and consistency of tomorrow's gene therapy products.

Finally, it is important to note that any analytical data is only as valuable as the software that supports it, making the considerations for software selection a vital piece of the jigsaw. For instance, compliance with 21 CFR part 11 is a prerequisite.

"Gene therapy is maybe 20 years behind where antibodies are, as far as standardization goes. We get to take advantage of some of the standardization in the antibodies space and bring it over to gene therapy. But other things are so new that we're building it as we go. It's dynamic and exciting to be part of that process."

– Mark White, PhD, Associate Director of Biopharma Product Marketing, Bio-Rad

The right combination of repurposing and innovation in the analytical tools area can provide AAV-based gene therapy researchers and developers with the insights they need to address the field's greatest challenges.

CONTRIBUTORS

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SNEHAL NAIK brings a confluence of regulatory affairs, early discovery, innovation, policy, and scientific expertise to her current hybrid role as Spark's Head of Regulatory Policy and Intelligence, and Regulatory Strategy Leader for ocular programs. In this capacity she established the regulatory policy function at Spark and is supporting global development of gene therapies. Snehal co-chairs the regenerative medicine committee at BIO, staffs efforts at the Alliance for Regenerative Medicine and the Innovative Medicines Initiative, and is an active member of the American Society of Gene & Cell Therapy and New York Academy of Sciences. Snehal graduated summa cum laude with an AB-MA in Biology from Bryn Mawr College, and holds a PhD in Molecular Genetics and Genomics from Washington University in St. Louis where she also completed the Cancer Biology pathway with the Siteman Cancer Center.

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Bulletin 3365

Tips for meeting regulatory guidelines for AAV development

Regulatory guidance for AAV-based gene therapy has evolved rapidly over the past five years in particular. Here, we delve deeper into the resultant pain points for developers and manufacturers, offering advice on how best to alleviate or avoid them in order to streamline regulatory compliance.

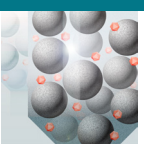
THE IMPORTANCE OF STARTING EARLY WITH A MULTIDISCIPLINARY APPROACH

New directions in AAV vector design and capsid engineering may have profound effects that reach beyond clinical safety and efficacy. Novel constructs may carry important considerations for process and product development. It is therefore crucial that all the stakeholders in gene therapy R&D – from discovery research to analytical development, and from manufacturing to regulatory affairs – are involved from the get-go. This type of multidisciplinary approach flies in the face of the traditional, siloed biopharma development model. However, it has been a hugely beneficial characteristic of adeno-associated virus (AAV) gene therapy from the field's earliest days. And in today's environment, where standardized approaches are rare, the regulatory bar is higher, and truncated development timelines are the norm, it is more important than ever.

This is especially true in the area of chemistry, manufacturing and controls (CMC), with its growing regulatory burden for industry. Whether the specific task at hand is process improvement, identifying critical quality attributes (CQAs), demonstrating comparability, or developing a potency assay matrix, responsibility cannot lie solely with manufacturing, or with the quality assurance and quality control team. It must be a partnership - for example, nonclinical, translational, and clinical development departments must all ask themselves: 'how can I generate data to help support the comparability strategy?'

It is vitally important to have such conversations upfront. Potency assay development provides an excellent example as to why. Traditionally, potency was somewhat neglected until later in clinical development, when regulators required a validated assay to be in place. However, today, regulators expect to see a potency assay at a much earlier stage. Furthermore, it is important to get an early handle on potency assay for internal decision-making purposes. For instance, if one wishes to introduce a new element to an AAV vector construct, a potency assay is necessary to fully understand the impact of this change.

Investing upfront in process, analytical, and formulation development will help alleviate the regulatory burden later in development. For example, ensuring your early-phase clinical trial vector material is as similar as possible



to the material you might use in pivotal studies, or the commercial product will allay any concerns regarding comparability.

TALK TO THE REGULATORS EARLY & OFTEN

Of course, it is not enough to simply start early. It is of critical importance to seek dialogue with the regulators as early and as often as possible, both to ensure you are on the right track and to leverage the considerable experience and know-how that agencies such as the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) have built up during the past decade in particular. Over this period, the major regulatory agencies have demonstrated a clear willingness to engage with developers, as well as a high degree of flexibility. Many of the CMC issues that have recently derailed late-stage AAV product candidates might have been avoided through earlier, more collaborative discussions with the regulators.

DEALING WITH PROCESS & ANALYTICAL METHOD CHANGES

While steps can be taken to minimize alterations to process, materials, and analytical methods, particularly in later development, some degree of change is inevitable. Without it, improvements cannot be made and the patients would not benefit from these technological advancements. So how to minimize the impact and potential delays this may cause?

First and foremost, it is imperative to gain a strong understanding of any changes, which is dependent on robust analytical development. Again, making an early start in this regard is preferable, as is ensuring assays are developed sooner rather than later and demonstrated to be fit-for-purpose as appropriate for the clinical phase of the drug product. However, it is also important for a sponsor to begin investigating CQAs utilizing characterization tools and techniques that are not necessarily destined for quality control (QC) application, but rather to build internal knowledge of the product and analytical method alike. This may inform both clinical and product development decision-making later on.

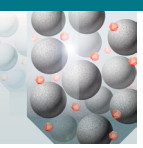
The companies that navigate this change management process most efficiently typically employ a very tight feedback loop between process development and analytical development/manufacturing QC. This is key to balancing risk – for instance, in adopting a novel analytical method that might be an improvement on a more established one, but which is not as well-known to regulators. This is an area where analytical tool providers can make a valuable contribution by introducing standardization and providing additional information and bridging studies to support regulatory CMC. They can also share experiences and lessons learned from other applications of the technology.

> *"We get a lot of questions on some of the assays that we're developing around, 'how are these going to be treated as they go through the regulatory environment?' It's great when we can say 'we've got a couple of customers we know have already brought it through'. It decreases the fear that they might be doing something brand new and potentially get tripped up later in QC."*
>
> **– Mark White, PhD,** Associate Director of Biopharma Product Marketing, Bio-Rad

Establishing and maintaining a suitable program for vector materials retain library from early batches onwards can also prove invaluable at later stages – if bridging studies are required to build out and validate a potency assay matrix, for example, or to ascertain if/how stability changed as more mature methods were introduced.

TACKLING REGULATORY DISHARMONY WITH A STREAMLINED APPROACH

Whilst regulators around the world are working more closely than ever to find common



ground in regulations for advanced therapies, the reality is that there is divergence. For example, differences have been observed recently between the US FDA and EMA in terms of advice relating to clinical trial designs, and the use of a sham control arm or a randomized control arm within the same trial. Disharmony such as this can lead to the requirement for sponsors to conduct costly and time consuming additional clinical studies in order to satisfy both regulatory bodies.

Area of regulatory divergence exist on the manufacturing side, too. For example, simple differences in terminology must be given due consideration, particularly when assembling dossiers for regulatory submission.

Again, early discussions with the regulators are a crucial component in successfully and efficiently navigating any issues. It is important to clearly and convincingly put forward the rationale for a given study design and explain why it will provide all of the data each regulator will require. From a global perspective, one of the advantages of the gene therapy field is that many regions and jurisdictions look to the FDA and EMA to set their own guidance and regulatory frameworks. Ensuring that a program meets both US FDA and EMA requirements should provide a solid foundation for regulatory submissions elsewhere in the world.

IT TAKES A VILLAGE… LEVERAGING PRE-COMPETITIVE COLLABORATIONS TO SOLVE THE MAJOR CHALLENGES IN AAV

There are many unknowns when you are blazing a trail in a novel and highly innovative field of scientific endeavor such as AAV-based gene therapy. It is not solely a question of understanding the therapeutic modality itself and related safety issues such as immunogenicity; the biology and natural history of many rare and ultra-rare diseases that are targets for gene therapy is relatively unknown, for instance. This in turn may limit the value of predictive tools such as animal models – often in gene therapy, the true test only really comes in the clinic.

At the same time, the body of both nonclinical and clinical data is growing at a faster rate than ever before. And increasingly, driven by bodies such as the US FDA and National Institutes of Health as well as industry associations and individual companies, the opportunity to pool data and resources to get to the bottom of the most challenging issues in the field is being investigated.

In the past year alone, several late-stage AAV developers have reported similar issues in both the potency assay and safety areas. Driven by a shared desire to put patients first, some of these companies have since shared data through something of a pre-competitive consortium model, in order to collectively learn how they may each move forward.

"I think the first thing is to be collaborative. We've heard about that across the various departments in your own organization as well as across the industry, including all of the instrument and assay providers. Because it is really going to take everybody pulling in the same direction to do this right."

– Snehal Naik, PhD, Head of Regulatory Policy and Intelligence, & Regulatory Strategy Leader for Ocular Programs, Spark Therapeutics

"We've seen high doses with remarkably good safety, and we've seen low doses that have had some safety signals. It's clearly not unidirectional. We need a better understanding of why and what that is, and perhaps we will get there faster by coming together as a field and sharing what we're seeing."

– Chris Lorenz, Senior Vice President of Technical Operations, Astellas Gene Therapies

The increasingly stringent regulatory environment for AAV-driven products is bringing many of the long-standing issues and limitations for this technology into sharp relief. Gene therapy's traditionally more collaborative, less siloed approach must be retained and enhanced if we are to successfully solve unmet medical need and serve the patient.

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