MAY 2020

Volume 6, Issue 4

CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON:

Trends and advances in gene therapy delivery and gene editing

CELL & GENE THERAPY INSIGHTS

Volume 6, Issue 4

Trends and advances in gene therapy delivery and gene editing

CELL & GENE THERAPY INSIGHTS

Innovation Insights

-

May 2020
Volume 6, Issue 4

CELL & GENE THERAPY INSIGHTS

TRENDS & ADVANCES IN GENE THERAPY DELIVERY & GENE EDITING

SPOTLIGHT

EXPERT INSIGHT

High dose systemic gene therapy: emerging trends on safety and efficacy

Ying Kai Chan, Hansell H Stedman, Roland W Herzog, Guangping Gao & George M Church

Systemic delivery of adeno-associated viral (AAV) vectors has traditionally been used in the clinic for liver-directed programs such as hemophilia A and hemophilia B. With the approval of Zolgensma® for the treatment of spinal muscular atrophy type I (SMA1), high dose systemic gene therapy has become a promising approach for systemic and neuromuscular transduction for various indications. Here, we discuss emerging findings on safety and efficacy from recent clinical trials utilizing high dose systemic gene therapy. In particular, we highlight previously unappreciated observations related to safety, and discuss possible causes and future directions.

Cell & Gene Therapy Insights 2020; 6(4), 569–575

DOI: 10.18609/cgti.2020.066

Adeno-associated viral (AAV) vectors have garnered significant interest for treating a wide variety of diseases and tissues. Several programs have tested or are testing intravenous infusion of the vector to transduce the liver and treat diseases like hemophilia A and B. These liver-directed programs typically utilize doses ranging from mid-10^{11} vector genomes/kg to mid-10^{13} vg/kg and most of our clinical knowledge on systemic gene therapy come from these trials. In these clinical trials, there have been not been significant safety concerns, consistent with preclinical data and the relatively low immunogenicity of

AAV compared to other viral vectors. Instead, the main concern is durability of therapeutic benefit, as transgene expression was observed to decline in some patients weeks after vector administration [1]. Often, the decline was accompanied by asymptomatic elevations in liver enzymes and a measurable $CD8^+$ T cell response against AAV capsid, suggesting the immune system may have cleared out transduced hepatocytes.

More recently, systemic administration of significantly higher doses of AAV to enable systemic transduction or transduction of neuromuscular tissues, has been explored in clinical settings for treating neurological and neuromuscular disorders. These programs have tested doses ranging from 5×10^{13} vg/kg to 3×10^{14} vg/kg, which at the higher end is one to two orders of magnitude higher than typical doses used for liver-directed programs. Emerging results from various Phase 1/2 clinical trials revealed striking therapeutic benefit for some of these programs, but also highlighted safety findings not previously observed with liver-directed programs (see Table 1).

REMARKABLE SUCCESS IN THERAPEUTIC EFFICACY & INITIAL SAFETY SIGNALS

Spinal muscular atrophy (SMA) is a severe monogenic disease resulting from loss or dysfunction of the gene encoding survival encoding survival motor neuron 1 (*SMN1*). The disease is characterized by degeneration and loss of lower motor neurons, which leads to progressive muscle weakness and paralysis. SMA1 is the most severe form and can result in death for most patients by age 2. The *SMN1* gene paralogue *SMN2* encodes an identical protein, but has a single translationally silent nucleotide substitution that reduces the efficiency of splicing of exon 7, thereby reducing the levels of full length SMN protein below a critical functional threshold in patients with bi-allelic mutations in the *SMN1* gene [2,3]. Residual SMN expression from the *SMN2* gene has the potential to promote central immunological tolerance against the gene product, which minimizes the risk of cell mediated immune responses against the transgene product.

AveXis demonstrated striking therapeutic benefit in a Phase 1/2 clinical trial [4]: all children were alive at the conclusion of the study and most had significant motor function improvement, including the ability to sit without support (top row, Table 1). These compelling results eventually supported the approval of Zolgensma®. Of the 18 patients dosed, two experienced treatment-related serious adverse events (SAEs), both of which were related to large increases in serum aminotransferase levels above the upper limit of normal (ULN). The findings of this trial indicated the striking benefits of the therapy and the potential of high dose systemic gene therapy to treat other severe genetic diseases. However, they also highlighted, for the first time in the clinic, that very high vector doses can trigger liver enzyme elevations at levels that are of serious concerns, and that prophylactic immune modulation should be used to reduce this risk.

Interestingly, early data on Zolgensma® recipients suggest that the therapeutic benefit is durable. This is contrast to AAV gene transfer to cell types that, unlike motor neurons, are actively dividing in pediatric patients and thus may lose vector genomes over time. Furthermore, genetic defects in other diseases may result in greater loss of endogenous coding information and thus be more prone to immune responses to the therapeutic gene product. This may be especially important in consideration of genetic diseases with a high prevalence of deletional mutations.

THE DMD RACE & EMERGING TRENDS

Duchenne muscular dystrophy (DMD) is a severe muscle-wasting disease caused by genetic deficiency of dystrophin, a low abundance cystoskeletal protein that protects cell membranes from injury during forceful

DOI: 10.18609/cgti.2020.066

TABLE 1

Summary of recent clinical trials utilizing high dose systemic AAV gene therapy.

Indication	Program	Vector	Dose (number of patients)	Findings related to efficacy, activity or expression	Findings related to safety
SMA1	AveXis AVXS-101 (now Novartis)	scAAV9.CB.SMN1* *ubiquitous promoter	6.7 x 10^{13} vg/kg (3) 2.0 x 10^{14} vg/kg (12)	**Striking benefit:** all patients alive at the conclusion of the Phase 1/2 study, many were able to sit and roll; does not appear to have durability issues to date	**Two patients experienced treatment-related grade 4 events (SAEs), both related to liver enzyme elevations:** First patient in low dose cohort – 31 x ULN of ALT and 14 x ULN of AST. Prednisolone attenuated liver enzymes elevation and subsequently all patients in trial were given prophylactic prednisolone 1/12 in high dose cohort – 35 x ULN of ALT and 37 x of AST in spite of prophylactic prednisolone; patient was responsive to additional steroids. In addition to the grade 4 events, two other patients experienced <10 x ULN of ALT/AST
DMD	Sarepta/Nationwide SRP-9001	AAVrh74.MHCK7. micro-dystrophin	2 x 10^{14} vg/kg (4)	**Early positive findings:** muscle biopsies showed mean of 81% micro-dystrophin-positive fibers at 90 d	**No SAEs reported. 3/4 patients experienced elevated g-glutamyl transpeptidase (GGT),** which similar to ALT/AST indicates liver damage; resolved with more steroids
	Pfizer PF-06939926 (formerly from Bamboo)	AAV9.MSP. mini-dystrophin* *Pfizer has not disclosed the muscle-specific promoter used	1 x 10^{14} vg/kg (3) 3 x 10^{14} vg/kg (3)	Early positive findings – muscle biopsies showed mean of 38% (low dose cohort) and 69% (high dose cohort) mini-dystrophin-positive fibers at 2 mo	**2/6 patients experienced SAEs:** 1 patient experienced nausea and vomiting that required hospitalization for IV anti-emetics and fluids 1 patient experienced "activation of the complement system associated with acute kidney injury, hemolysis, and reduced platelet count." Patient received hemodialysis and 2 intravenous doses of a complement inhibitor. Renal function returned to normal within 15 days
	Solid Biosciences SGT-001	AAV9.CK8. micro-dystrophin	5 x 10^{13} vg/kg (3) 2 x 10^{14} vg/kg (3)	**Low or variable expression:** muscle biopsies showed "very low levels" (2/3) to 10% (1/3) at 3 mo for low dose cohort, and 10–70% for 2 of 3 patients in the high dose cohort that have been analyzed (no results available yet for the third patient)	**Trial was placed on clinical holds by FDA twice, 3/6 patients experienced SAEs:** First patient in low dose cohort – experienced SAE "characterized by a decrease in platelet count followed by a reduction in red blood cell count, transient renal impairment and evidence of complement activation." Solid amended study protocol to include intravenous steroids and to possibly treat complement activation with eculizumab 2/3 patients in high dose cohort – 1 experienced transient decline in platelet count and transient elevation of transaminases and bilirubin (>2 x of ULN). The other patient experienced broadly similar findings as the first patient in low dose cohort
XLMTM	Audentes AT-132 (now Astellas)	AAV8.Des.MTM1	1 x 10^{14} vg/kg (6) 3 x 10^{14} vg/kg (4)	**Striking benefit:** as of Oct 2019, all 6 patients in low dose cohort and first patient in high dose cohort achieved ventilator independence and able to rise to a standing position or walk	**4/10 patients experienced SAEs:** 1/6 patient in low dose cohort – Troponin I and CK increased, possible myocarditis at 7 wk; resolved with treatment 3/4 patients in high dose cohort – 1 patient experienced cholestasis which resolved with treatment; 1 patient experienced vomiting, nausea, fever and drop in platelet count, followed by troponin I and ST segment elevation indicative of mild myocarditis, which resolved with treatment; 1 patient experienced joint swelling which resolved without treatment
LGMD2E	Sarepta SRP-9003	AAVrh74.MHCK7.SGCB	5 x 10^{13} vg/kg (3)	**Early positive findings:** muscle biopsies showed mean of 51% SGCB-positive fibers at 60 d; 82% reduction in CK (biomarker for muscle damage) and improvement across all functional measurements at 9 mo. Sarepta plans to test 2 x 10^{14} vg/kg next	**2/3 patients had "elevated liver enzymes", one of which was designated a serious adverse event (SAE),** as the patient had associated transient increase in bilirubin." Resolved with more steroids

Key parameters and findings were summarized (current as of Dec 2019) based on publicly available information from conference presentations, press releases and publications. The majority of clinical trials utilized prophylactic oral steroids, unless otherwise noted. Doses may not be directly comparable between programs due to different assay methods. Due to space constraints, other factors that may impact safety and efficacy are not shown (e.g., AAV manufacturing methods and processes, patient demographic and clinical characteristics such as age, criteria and assays for patient screening and enrollment).
DMD: Duchenne muscular dystrophy; LGMD2E: Limb-girdle muscular dystrophy type 2E; sc: Self-complementary; SMA1: Spinal muscular atrophy type 1; XLMTM: X-linked myotubular myopathy.

muscle contraction [5]. The majority of mutations are multi-exon deletions that remove coding sequence while creating a single base shift in the remaining open reading frame. Most patients lose ambulation at ~10–12 years of age and ultimately die later in adulthood from respiratory insufficiency and/or cardiomyopathy. As the dystrophin coding sequence is much larger than the packaging capacity of the AAV vector, researchers have developed internally deleted transgenes encoding so-called "mini"- or "micro"-dystrophin, which have partially ameliorated disease progression following systemic delivery via AAV in preclinical DMD models. The potential immunogenicity of dystrophin in DMD patients prompted many groups to use muscle-specific transcriptional cassettes in an effort to reduce direct expression in antigen-presenting cells. Three companies – Sarepta, Pfizer and Solid Biosciences – have released non-peer-reviewed early results from clinical testing.

Intriguingly, the three programs had a relatively wide range of transgene expression and safety profiles (second row, Table 1). Sarepta reported no SAEs in the four patients dosed, while Solid and Pfizer reported five treatment-related SAEs out of a combined total of 12 patients. Notable clinical features of toxicity, in variable combinations in individual patients, include cardio-pulmonary insufficiency, complement activation, thrombocytopenia, hemolysis, hyperbilirubinemia, transaminase elevation, and kidney injury requiring ICU admission and hemodialysis.

Although all programs have reported evidence of recombinant mini/micro-dystrophin expression on selected muscle biopsies, the adverse events raise concerns about the risk–benefit tradeoffs for clinical trial participants. Insufficient data have been released to draw meaningful conclusions about the exact etiology of the adverse events, but immunotoxicity of one or more components of the vector are under consideration. There are differences in the vector capsids, promoters, and transgene products used in the three clinical trials, and the ages and mutational spectrum of the enrolled DMD patients are non-equivalent, complicating further interpretation. It is especially difficult to reconcile the discordant data for AAV9-based vectors – spectacular efficacy with manageable adverse events in Zolgensma®, but multiple SAEs at similar dose in DMD. Two differences between the SMA1 and DMD trials are notable: compared with the enrolled DMD patients, the SMA1 patients are substantially younger (approximately 0.5 to 2 years versus 4–13 years) and all have some level of expression of full length SMN from the *SMN2* gene, with the likely achievement of central immunological tolerance prior to administration of vector. None of the adverse events in the DMD trials were anticipated by preclinical studies in the DMD animal models in which dystrophin deficiency is caused by point mutations (mdx mouse, golden retriever muscular dystrophy GRMD dog). In contrast, a recent report of severe T cell immune responses in a dystrophin-null German shorthaired pointer muscular dystrophy dog model suggests that adaptive immune responses to the transgene product should be considered as a possible etiological factor [6].

OTHER PROGRAMS & MORE QUESTIONS

X-linked myotubular myopathy (XLMTM) is a severe neuromuscular disease caused by mutations in the *MTM1* gene. Most XLMTM patients require breathing support, with ~50% mortality by 18 months. Audentes used a muscle-specific desmin promoter and an AAV8 vector, identical to that used in a hemophilia B trial and structurally homologous to the AAVrh74 vector used by Sarepta in DMD (third row, Table 1). In Oct 2019, the company reported that all 6 patients treated in the low dose cohort and the first patient treated in the high dose cohort achieved ventilator independence and were able to rise to a standing position or walk, demonstrating striking therapeutic benefit. 4 of 10 treated patients experienced possibly/

DOI: 10.18609/cgti.2020.066

probably treatment-related SAEs, 2 of which involved troponin I elevation, a marker for myocardial injury. These findings suggested possible myocarditis, which appear quite different from the elevated liver-associated markers (ALT, AST, GGT, bilirubin) in other trials.

Subsequent to their DMD results, Sarepta reported results from the low dose cohort for their limb-girdle muscular dystrophy type 2E (LGMD2E) gene therapy program encoding SGCB (bottom row, Table 1). While the two Sarepta programs used different transgenes to treat different indications, they both utilized the same capsid (AAVrh74) and muscle-specific promoter (MHCK7), and presumably a similar manufacturing process. Sarepta reported early positive results, with an 82% reduction in CK (biomarker for muscle damage) and improvement across all functional measurements at 9 mo. Two of three treated patients experienced elevated liver enzymes, one of which was designated an SAE due to associated increase in bilirubin.

The XLMTM and LGMD2E results suggested that we are still early in learning about clinical applications of high dose systemic gene therapies, especially given the very small number of patients treated per dose cohort. Different observations on safety are still emerging and warrant careful monitoring and mitigation of risks. We do not know yet the relative contributions of innate and adaptive immune responses (e.g., antibodies or T cells to AAV capsid and/or transgene products) to the adverse findings. Furthermore, when thinking about possible key drivers of vector-associated toxicity, it is not possible to attribute adverse events to a particular capsid, promoter, indication or manufacturing process. Instead, the causes for SAEs appear to be multi-factorial, with amount of vector dose being one of the key factors.

TRANSLATION INSIGHT

Summarizing these clinical studies, high dose systemic gene therapy has revealed safety concerns that go well beyond the asymptomatic, transient ALT/AST elevations seen with the liver-directed AAV programs, to additionally include cardio-pulmonary insufficiency, bilirubin elevation, complement activation, acute renal injury, thrombocytopenia, hemolysis, and myocarditis. This long list implies that future vector modifications to improve the therapeutic index will define the pathway to value creation in the long-term management of these crippling neuromuscular diseases, as gene therapy addresses the magnitude of the currently unmet need. The safer the vector, the higher the likelihood that durable efficacy will be achieved at the maximally tolerated dose, with the lowest probability of transgene loss from deleterious immune responses.

All of the AAV vector capsids under development for systemic gene therapy in neuromuscular disease originally attracted interest because of their high liver tropism and potential use in liver-targeted gene therapy. In earlier hemophilia B trials, extensive preclinical testing in large animal models did not predict the ALT/AST elevations and concomitant decline in transgene expression seen in humans. Since the serious adverse events in the high dose systemic AAV trials have covered such a broad spectrum of organ toxicities, it is currently unclear which of the available preclinical models is most predictive. Focal and life-threatening systemic myositis has been seen following AAV vector administration in large animal DMD models [6,7] while acute multi-organ failure associated with disseminated intravascular coagulation was seen in two of five previously healthy macaques following administration of similar systemic doses of the almost identical AAV9 variants AAVhu68 and AAV-PHP.B [8,9]. Regardless of the mechanism(s) driving the SAEs outlined above, only detailed reports of the clinical studies will enable further dissection in animal models. Meanwhile, there remains plenty of room for improvement in every aspect of vector design to optimize dose-response, such as selectively myotropic capsids, stronger muscle-specific promoters, non-immunogenic transgene products to match the

physiological function of dystrophin and inclusion of oligonucleotides to inhibit innate immune responses [6,10–12].

In conclusion, high dose systemic gene therapy is showing exciting results in the clinic. As the number of such gene therapy programs continues to grow and our understanding improves, one should carefully balance risks and benefits in order to deliver transformative therapies to patients.

REFERENCES

1. Manno CS, Pierce GF, Arruda VR *et al.* Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nat. Med.* **2006;** 12**:** 342–7**.**
2. Lorson CL, Hahnen E, Androphy EJ, Wirth B. A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. Proc. Natl Acad. Sci. USA 1999; 96: 6307–11.
3. Monani UR, Lorsen C, Parsons DW *et al.* A single nucleotide difference that alters splicing patterns distinguishes the SMA gene SMN1 from the copy gene SMN2. *Hum. Mol. Genet.* 1999; 8: 1177–83.
4. Mendell JR, Al-Zaidy S, Shell R *et al.* Single-Dose Gene-Replacement Therapy for Spinal Muscular Atrophy. *N. Engl. J. Med.* 2017; 377: 1713–22.
5. Duan D. Systemic AAV Micro-dystrophin Gene Therapy for Duchenne Muscular Dystrophy. *Mol. Ther.* 2018; 26: 2337–56.
6. Song Y, Morales L, Malik AS *et al.* Non-immunogenic utrophin gene therapy for the treatment of muscular dystrophy animal models. Nat. Med. 2019; 25: 1505–11.
7. Kornegay JN, Li J, Bogan JR *et al.* Widespread muscle expression of an AAV9 human mini-dystrophin vector after intravenous injection in neonatal dystrophin-deficient dogs. *Mol. Ther.* 2010; 18: 1501–8.
8. Hinderer C, Katz N, Buza EL *et al.* Severe Toxicity in Nonhuman Primates and Piglets Following High-Dose Intravenous Administration of an Adeno-Associated Virus Vector Expressing Human SMN. *Hum. Gene Ther.* 2018; 29: 285–98.
9. Hordeaux J, Wang Q, Katz N *et al.* The Neurotropic Properties of AAV-PHP.B Are Limited to C57BL/6J Mice. *Mol. Ther.* 2018; 26: 664–8.
10. Wang D, Li S, Gessler DJ *et al.* A Rationally Engineered Capsid Variant of AAV9 for Systemic CNS-Directed and Peripheral Tissue-Detargeted Gene Delivery in Neonates. *Mol. Ther. Methods Clin. Dev.* 2018; 9: 234–46.
11. Sarcar S, Tulalamba W, Rincon MY *et al.* Next-generation muscle-directed gene therapy by in silico vector design. *Nat. Commun.* 2019; 10: 492.
12. Chan YK, Chan R, Graveline A *et al.* Engineering AAV vectors to evade innate immune and inflammatory responses. *Mol. Ther.* 2018; 26: 457–8.

AFFILIATIONS

Ying Kai Chan
Author for correspondence:
Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA, USA
and
Department of Genetics, Harvard Medical School, Boston, MA, USA
YingKai_Chan@hms.harvard.edu

Hansell H Stedman
Department of Surgery, Pennsylvania Muscle Institute, Penn Cardiovascular Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA
and
Corporal Michael J. Crescenz Veterans Affairs Medical Center, Philadelphia, PA, USA

Roland W Herzog
Department of Pediatrics, University of Florida, Gainesville, FL, USA
and
Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN, USA

Guangping Gao
Horae Gene Therapy Center, University of Massachusetts Medical School, Worcester, MA, USA
and
Li Weibo Institute for Rare Diseases Research, University of Massachusetts Medical School, Worcester, MA, USA
and
Department of Microbiology and Physiological Systems, University of Massachusetts Medical School, Worcester, MA, USA

George M Church
Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA, USA
and
Department of Genetics, Harvard Medical School, Boston, MA, USA

DOI: 10.18609/cgti.2020.066

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Ying Kai Chan and George M Church are co-founders of Ally Therapeutics. George M Church is also a founder of Editas Medicine and eGenesis Bio (for the full disclosure list, please see http://arep.med.harvard.edu/gmc/tech.html). Ying Kai Chan is currently an employee of Ally Therapeutics. Roland W Herzog is a scientific advisor of Ally Therapeutics, a recipient of royalty payments from Takeda Pharmaceutical Company and receives grant support from Luye R&D. Guangping Gao is a scientific co-founder of Voyager Therapeutics and AspA Therapeutics, and holds equities in the companies. Guangping Gao is an inventor on patents with potential royalties licensed to Voyager Therapeutics, AspA Therapeutics and other biopharmaceutical companies. Guangping Gao serves as a scientific consultant for several other gene therapy companies and is a scientific advisor of Ally Therapeutics. Hansell H Stedman is a co-founder of StrongHolt Therapeutics, is an inventor on patents licensed to and holds equity in this emerging biotechnology company.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *Feb 11 2020;* ***Revised manuscript received:*** *Apr 20 2020;* ***Publication date:*** *May 6 2020.*

CELL & GENE THERAPY INSIGHTS

TRENDS AND ADVANCES IN GENE THERAPY DELIVERY & GENE EDITING

SPOTLIGHT

INTERVIEW

A return to rational capsid design? Predicting the future of AAV vector R&D

R JUDE SAMULSKI received his PhD in Medical Microbiology and Immunology from the University of Florida. His graduate work (1978–82) involved the cloning of the adeno-associated virus (AAV) genome and the demonstration of AAV as a viral vector, including the first US patent involving non-AAV genes inserted into AAV. During his post-doctoral training at Princeton, he developed the AAV 2 ITR vector backbone, commonly used by most labs today as well as the initial establishment of an AAV production system. At the University of Pittsburgh Department of Biology, he was the first to demonstrate AAV transduction in rodent brain and muscle that culminated in the first clinical trials in the brain (Canavan) and muscle (DMD). In 1993, he was hired at the University of North Carolina (UNC) to establish a Gene Therapy Center. For over 25 years, Dr Samulski, as a Professor of Pharmacology and the Director of the Gene Therapy Center at UNC, has led a team of multiple Principal Investigators developing novel viral vectors and clinical gene therapy programs. He was recognized in 2008 by the American Society of Gene & Cell Therapy (ASGCT) as the first recipient of the Outstanding Achievement Award, was awarded the National Hemophilia Foundation's Investigator of the Year in 1999 and was the first non-MD to be placed on the University of Florida's Wall of Fame. He has served as past President of ASGCT and was invited to China to meet with the Chinese Minister of Health and soon after was recognized as one of China's Thousand Points of Light, a recognition bestowed on individuals whose contributions are benefiting mankind. In addition to being the lead inventor on over 300 patents in the field of AAV vectors and gene therapy, he is a scientific founder of ASGCT, Merlin, Asklepios BioPharmaceutical, NanoCor Therapeutics, Chatham Therapeutics, Bamboo Therapeutics, Viralgen, and other entities that continue to advance the field of human gene therapy and was selected as a seminal speaker at the Royal Society of Science in London in the Isaac Newton Lecture room on 'Delivering novel therapeutic in the 21st century' (October 24, 2018).

Cell & Gene Therapy Insights 2020; 6(4), 533–541

DOI: 10.18609/cgti.2020.062

Q What are you working on right now?

RJS: We've been working on a collection of gene delivery projects, focusing primarily on muscular diseases such as Duchenne muscular dystrophy and Pompe. These two programs have entered the clinic.

In terms of our earlier stage therapeutic targets, we're evaluating our vectors for delivery for neurological disorders such as Huntington's disease and Parkinson's disease.

Turning to research projects, we have a collection of efforts looking at what we refer to as vector development, which means improving the delivery system from the production capability all the way through to the viral capsid itself, and its ability to target and transduce cells. If you then strip away to the next layer of the vector modifications, you reach the transgene cassette that's inside of the capsid, where there are a few components that one can tinker with. These range from optimizing the promoter, to defining or inserting specific elements like introns that may provide more efficient expression, to optimizing the transgene for translational expression by codon optimization. Additionally, we are exploring the signals involved in the RNA message processing, which includes research into polyadenylation and message RNA stability.

When you consider these components all together, it's kind of like a Russian tea doll; you start at the large bioreactor level of producing the vector, then work your way to the capsid that carries vector, then to the promoters that are expressed in the vector, to the transgene, and so on. Our research projects have been primarily broken up in these terms at the research level.

Q Can you reflect on the past few years in AAV capsid engineering across the field?

RJS: The entire gene therapy community basically launched all of their efforts with the naturally occurring AAV isolates that originally were identified from patients (AAV serotypes 1–5) and then non-human primates (AAV 8, rh10, etc.). The original collection, which is typically defined as AAV serotypes 1 through 5, were all viruses that were identified in the 1950s and '60s through screening efforts relating to upper respiratory infections, looking for what may cause the common cold in order to develop potential vaccines. Through those efforts, they identified adenovirus as an infectious agent that came out of infected adenoid tissue. AAV was discovered as a contaminate and was referred to as adeno-associated virus.

So AAV ended up becoming the prototype that everyone studied in research and AAV2, which was the serotype that grew most efficiently in laboratory settings, became the gold standard from which all the vectors were initially developed. The first *in vivo* gene therapy approved in the USA uses AAV2 for Leber congenital amaurosis, so there's significant merit in those initial efforts.

However, since then, researchers have migrated to other natural isolates that have been identified in either human or non-human primate tissue. These collections go all the way up to AAV serotype 13, and include popular ones used in the clinic today, which are AAV6,

DOI: 10.18609/cgti.2020.062

AAV8, and AAV9, as well as RH10 (Rhesus monkey serotype 10). All of these viruses seem to have additional attributes; they either transduce certain tissues more efficiently, or they appear to target certain tissues more specifically. And now, they have also become part of the arsenal to test in the clinic – indeed, the second gene therapy approved in the US (for children with SMA) uses AAV serotype 9.

What you can see from this is that the first wave of vector development was primarily focused on the natural isolates. Ours was the first group to successfully develop what is referred to as a chimeric capsid, meaning you took pieces from one serotype and engineered them into the backbone of another, and tested it in the clinic for DMD. When you make a chimeric capsid, it's basically like changing the individual tiles of a Rubik's cube. You change very specific features of that new virus: whether it is less immunogenic compared to the natural isolates out there, or more muscle-tropic, or produces higher titer, etc. This approach has become very popular and is now one of the mainstays of testing new, so-called synthetic AAV capsids.

At the moment, none of these novel chimeric AAV vectors have been market approved by the FDA, but there are a number of them in the clinic – in patients with hemophilia and other immunological diseases, for instance.

I think what we're seeing most recently, though, is a circling back to what I refer to as 'rational design' capsids. The chimeric approach was originally a case of just shuffling things and seeing what came out, and then selecting for the one you want. While that was extremely attractive, it took us down a number of rabbit holes in terms of identifying chimeric capsids that only worked in the animal model that they were selected in, and as a result were not pantropic for mouse, monkey, and man. As a consequence of this, some people have now shied away from the library approach of making chimeric capsids, and have drifted back to what we refer to as the 'rational design' approach. I think this is where most, if not all, of the newer, more elegant delivery systems are going to come from.

Mavis Agbandje-McKenna of the University of Florida is primarily responsible for solving the crystal structures for the majority of the AAV serotypes. Thanks to her work, the amino acid position of every serotype that is naturally known is mapped at the x-ray structural level, thus enabling rational design. With this approach, what you are able to do is look at a structure, whether it's a loop or something else that's prominent on the virus surface, and you can engineer that loop or amino acid cluster only into the backbone of your so-called carrier serotype (e.g., AAV2). You can design specific attributes in a more educated manner – you can now know exactly why you're moving a piece of a serotype – e.g., because it confers high titer, or because it's more immunologically resistant. With the library approach, on the other hand, you got kaleidoscope of capsid components in the final product, and you didn't know what was responsible for the various vector attributes. You could spend a career trying to figure out which serotype contributed which amino acid, because most of the chimeric viruses were made of 6 or 7

"...what we're seeing most recently, though, is a circling back to what I refer to as 'rational design' capsids."

"We're hoping that over time, the novel capsids coming through may be viewed from a regulatory perspective as being more a part of the formulation than a part of the drug. The result of this could be that it's easier to take various capsids into the clinic, and to toggle between various serotypes without having to generate ... enormous non-clinical data packages..."

different contributions from the natural serotypes, if not more. So rational design, by definition, is choosing a subset of amino acids and specifically engineering them as replacements for amino acids that are in the selected capsid backbone that your production system has been optimized for, and all of your assays have been developed for.

What this affords you, essentially, is a capsid platform backbone from which to work where you can continually develop more efficient delivery systems without having to start all over from scratch each time you come up with a novel capsid isolated from a library approach that you've never seen before.

So that's where the field is progressing. I think we have an issue at hand, though, which is that we're developing novel capsids quicker than we can test them in the clinic. This is purely because of the amount of time involved in taking a new gene therapy vector into the clinic. As a result, we're seeing new capsids that are superior to the ones being clinically tested, but because it takes too long and costs too much to just start over again, the majority of these will never see the light of day. So a number of people carrying out clinical studies at the moment are biting the bullet and sticking with the capsid they have – unfortunately they may never get the chance to test their superior capsids.

We're hoping that over time, the novel capsids coming through may be viewed from a regulatory perspective as being more a part of the formulation than a part of the drug. The result of this could be that it's easier to take various capsids into the clinic, and to toggle between various serotypes without having to generate these enormous non-clinical data packages that are required today.

Q Regarding the ongoing fight to overcome pre-existing immunity and enable redosing of AAV-driven gene therapies, are there any recent approaches that catch your eye as showing real promise?

RJS: These are two separate issues that need to be addressed, and different therapeutic approaches are being tested at the moment for each one.

DOI: 10.18609/cgti.2020.062

Regarding the challenge of pre-existing immunity to AAV, which excludes patients from clinical trials and therefore limits the ability of an approved drug to access all the patients who need it, this is being approached by traditional clinical practice that's already been established: you conduct a plasmapheresis of a patient by which you send blood through a column, which binds all antibodies including the AAV antibodies that are in that patient's blood. Consequently, when the blood is returned to the patient, you've purged them or their resistance. You then have a potential therapeutic window to deliver the AAV vector before the patient generates his/her normal antibodies again (this typically happens within 2 weeks). This approach is currently being validated in primates with AAV gene therapy. However, it's already a common clinical practice in various circumstances where a patient generates an antibody against their own protein. As a result, such an approach can be applied to gene therapy patients with pre-existing AAV Ab in order to be treated with the genetic therapeutic.

Turning to re-administration, the two basic approaches here are to either 'stealth' the virus so that it's not seen by the immune system, or to blunt or suppress the immune system so that it doesn't see the virus when we deliver the payload at the initial point of therapeutic administration.

These approaches are being evaluated by many, many groups. The traditional approach of blunting the immune system is obviously very well established already in the broad realm of organ transplants. There are a number of people who are mimicking those protocols to some degree to see if they can block the immune response to the primary administration of the AAV vector, thereby creating the potential to do repeated administrations. This is being done using traditional immunosuppressive drugs.

Others are taking approaches where they look at the dominant regions on the capsid that are viewed as being immunogenic, and then they try to shuffle them so that the immune system doesn't know which version of the virus is coming into the body – as is the case with different strains of 'flu. In my opinion, this approach is less likely to be successful simply because of how good the immune system is at picking up on the similarities between viral strains – for example, if you have a Hong Kong 'flu, which looks very similar to last year's 'flu strain, then you will get cross protection. So, it's not as trivial as it looks on paper.

Finally, there are groups that are trying to do combinations of these two approaches: they use some form of immunosuppression and novel capsid design to skirt around what may be creating existing antibodies with repeat administration.

All of these approaches are mainly in the preclinical phase at the moment. There is one set of studies from our colleagues in Florida where they used immunosuppression, and the data in a handful of patients does look like they did not mount an immune response to the capsids, which is obviously encouraging.

"...we're seeing new capsids that are superior to the ones being clinically tested, but ... the majority of these will never see the light of day."

I would not be surprised if within the next 3 years we see both the pheresis and immunosuppressive approaches providing the gene therapy community with ways to:

- Include patients in clinical trials who are currently being excluded
- Allow for repeat administration if necessary

Let me clarify on the issue of repeat administrations. To date, there's actually very little data suggesting it's required. The longest-term data in humans is with hemophilia patients, which was done by our colleagues from St Jude, is out over 10 years now, which is also consistent with all the animal studies that have been done.

However, when people study young animals – most notably, non-human primates – and they transduce juvenile or very young infant primates with AAV in the liver, they do find that when the animal and it's liver grow, the amount of therapeutic protein may not be sufficient for the adult-sized individual. There is then the possibility of requiring a repeat administration as the individual matures. Therefore, this issue is probably more urgent in the pediatric scenario than it is in the adult scenario.

Q Where in particular can future technological innovation help the field advance further?

RJS: I suspect I have a very different perspective on this at the moment than a lot of my colleagues. I think a lot of people are focusing on capsid development. But while I have been very bullish about that myself, and have been a proponent of developing novel capsids, I now see a different perspective unfolding. That is that when a capsid has been in patients, and all of the rigor that's required to demonstrate that it can be produced at GMP level has been applied, you have a critical data package that is invaluable in the sense that it provides the regulatory community with confidence that the given reagent and production system are able to advance uninterrupted.

In light of this, using a capsid that has been validated in humans is going to be more and more the go-to scenario for two reasons. The first reason I just described – the regulatory package – and the second is that if these things go off-patent and they no longer involve any monetary consequences, you can advance them more easily – as with AAV2 in the eye, for example.

Having said that, I think that where there's going to be the greatest impact is in the regulation of the vector transgene – being able to design promoters that will be efficient enough to compensate for capsid development. It's the promoter and the transgene that stay behind as the therapeutic drug for the life of the individual patient, not the capsid that delivered it during those first couple of hours – so if I can have a superior promoter, it more than compensates for not having the best capsid in the world.

"...the natural focus is to go to the regulation of the therapeutic ... This is where I feel we will be able to make the largest impact..."

And so I think the natural focus is to go to the regulation of the therapeutic and optimize

 DOI: 10.18609/cgti.2020.062

that so you can basically control all aspects of the efficiency and potency of the drug. This is where I feel we will be able to make the largest impact on these therapeutics.

In addition, you can see how the field is evolving quickly with microRNA and other elements that can ensure that if you don't want a drug expressed in a certain cell type, you can design the cassette so that it can be processed to be shut off, or not translated, or degraded, by all of the regulatory systems that have evolved over time, and that we are becoming familiar with, rather than have a cell specific targeting capsid.

> "The longest-term data in humans is with hemophilia patients ... is out over 10 years now, which is also consistent with all the animal studies..."

So, all of my efforts at this point, and I think the field as a whole will get there if it's not there already, are going to be at the transcriptional regulation level, and the translational level. That's where I think you'll see the next wave of technology advancements come forward. As a consequence of this, you have the opportunity to finally get into what I would refer to as some sophisticated gene regulations, where you have molecular switches that turn the gene on or off, and can tightly control the therapeutics.

If this can be accomplished in the next 3-to-5 years, then it affords us the ability to go after more complex diseases. For example, diseases where instead of a single gene defect you have a pathway, and that pathway may be dependent on a couple of genes in order for the final product to be made correctly, or to inhibit the product if it's something like a bad cholesterol, for instance. Being able to put in transgenes that you can regulate with a small molecules, or by biological switches that respond to a certain level of a signal and then turn your gene on or off, will really position the field to look at the complex diseases as the next natural target to follow the orphan monogenic disorders – diseases such as obesity, heart failure, and dementia.

Q Finally, what will be your chief goals and priorities in your own work over the coming 12–24 months?

RJS: One of my primary focuses right now is to ensure that the technology we and others are developing and perfecting doesn't just get applied in the popular orphan diseases – it is very important to me that we don't lose sight of those ultra-orphan diseases that can benefit from the same technology.

While I'm ecstatic and humbled that people look at me as a pioneer in developing AAV technology and bringing it to the research community to develop these drugs, I think it would be horrific if part of that history becomes that we left behind kids who had single gene defects but in the ultra-orphan diseases category. Obviously, therapies for these diseases are not commercially appealing and it's difficult to get funding for their development because of the small patient populations. But at the same time, we know the same technology in the same production model and with the same clinical protocols as might be applied for less rare diseases could rescue these patients just as easily.

Right now, I'm focusing on a foundation that I'm part of called the Columbus Children's Foundation. At the moment, with Krys Bankiewicz of the Brodno Hospital in Warsaw, we are treating children with amino acid decarboxylase deficiency (AADC) – there are about 90 of them in the world.

Krys has now injected something like 22 of these patients, so we're actually doing a countdown until this genetic disease is eradicated. You can see the potential excitement of being able to remove diseases off our list of 7,000, one by one, by focusing on things like this. It's very impactful, even though it may not be in the commercial sense, and I think it's testament to where we are with the technology and what potential it has.

I'll also continue working with AskBio and their programs in order to facilitate their more commercially- orientated approaches, which as I mentioned earlier are focused on muscle, liver, and brain.

I am also delighted to be involved with the Synpromics team in the UK – work that relates to the promoter technology we discussed previously.

So scientifically, I see myself pushing these things across the goal line as quickly as possible, where this technology has already been proven. That's just a simple matter of turnkey: switching out one transgene, putting in another, and then going back into the same paradigm of IV injection, liver transduction, promoter expression, secretion, etc.

I would not be surprised if we look back on all of this at some point in the future and see the original AAV vectors as the equivalent of the Ford Model T motor car. More than likely what comes after it will be very much more sophisticated biological nanoparticles that are chimerics between viruses, and synthetic delivery systems – all offering far greater control of both production and every aspect of drug delivery. I'm very optimistic that it's going to be a natural marriage between these disciplines. From a scientific approach, we will borrow and share what each system does best, and come up with a second wave of delivery technology that will probably dominate for another period of time.

AFFILIATION

R. Jude Samulski
Professor of Pharmacology and Director of the Gene Therapy Center, UNC, NC, USA

 DOI: 10.18609/cgti.2020.062

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Dr Samulski is Co-founder, President and Chief Scientific Officer of Asklepios BioPharmaceutical, Inc. and subsidiaries; Trustee, Board Director or Advisory Board Member of Columbus Children's Foundation, Engineered Stem Cell Inc., NanoCor Therapeutics, Inc., Optima Therapeutics Inc., Brain Neurotherapy Bio, Touchlight AAV Limited, Viralgen Vector Core S.L.*

Funding declaration: *The author received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Interview conducted: *March 28 2020;* ***Publication date:*** *May 15 2020.*

CELL & GENE THERAPY INSIGHTS

TRENDS & ADVANCES IN GENE THERAPY DELIVERY & GENE EDITING

SPOTLIGHT

INNOVATOR INSIGHT

FectoVIR®-AAV: a giant step for AAV large scale manufacturing

Alengo Nyamay'antu, Malik Hellal, Mathieu Porte & Patrick Erbacher

The number of advanced therapy medicinal products (ATMPs) to treat inherited genetic disorders is in constant growth, with a global 32% increase in new clinical trials in the last 4 years. ATMPs have demonstrated their success with already more than ten approved for commercialization. The success of AAV as the most promising viral vector for gene therapy is due to low immunogenicity, broad tropism and non-integrating properties. One major challenge for translation of promising research to clinical development is the manufacture of sufficient quantities of AAV. Transient transfection of suspension cells is the most commonly used production platform, as it offers significant flexibility for cell and gene therapy development. However, this method shows some limitations in large scale bioreactors: inadequate transfection protocol, reduced transfection efficiency and lower productivity. To address this concern, we present data on the novel transfection reagent FectoVIR®-AAV specifically developed to bring flexibility of transient transfection together with scalability and speed to market.

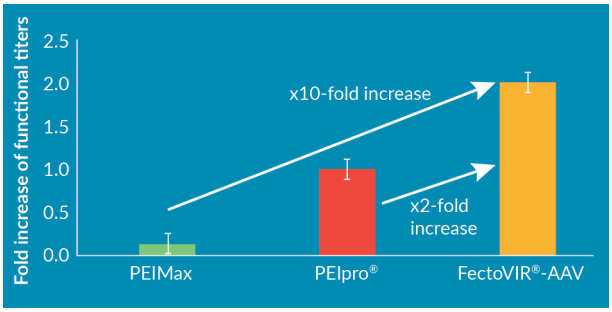
Cell & Gene Therapy Insights 2020; 6(4), 655–661

DOI: 10.18609/cgti.2020.077

Gene and gene-modified cell therapy treatments have demonstrated their potential in addressing unmet medical needs across a wide variety of human diseases, including cardiovascular, neurodegenerative, ocular, immunologic disorders and cancer. These therapies are based on the delivery of corrective DNA (DeoxyriboNucleic Acid) into target cells with viral vectors. These viral vectors can either be directly administered to patients, referred to as *in vivo* therapy, or first administered *ex vivo* to cells isolated from the patient

FIGURE 1

Improvement in both viral genome production and packaging efficiency with FectoVIR®-AAV results in a up 10-fold increase in functional rAAV-2 production in comparison with competitors.



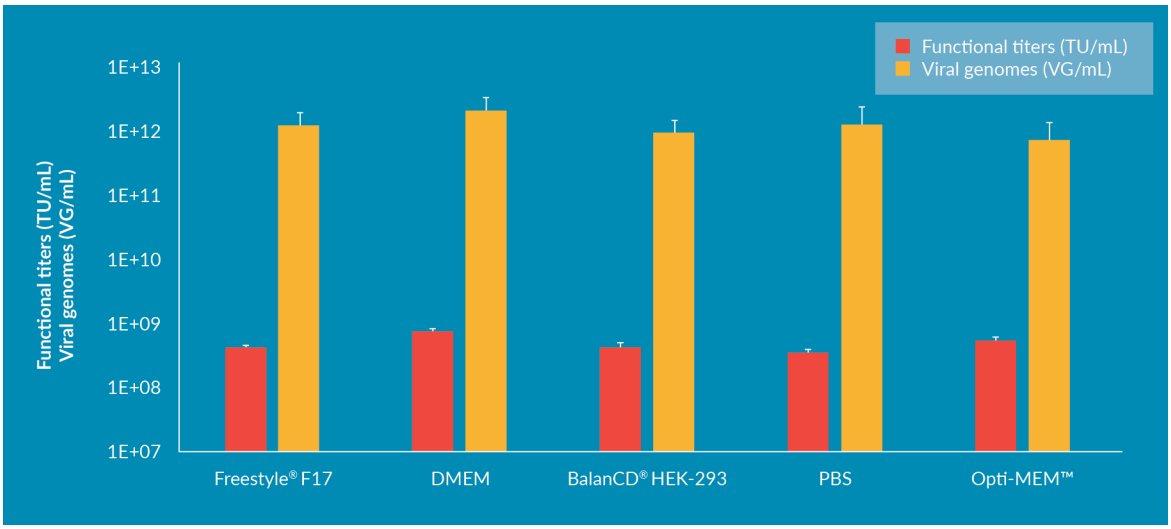
Suspension HEK-293T cells were transfected with PEIMax, PEIpro® and FectoVIR®-AAV using the recommended conditions. rAAV-2-GFP were harvested 72 h post-transfection of suspension HEK-293T cells with FectoVIR®-AAV/DNA complexes prepared in several synthetic media. Functional viral titers (TU/ml) of rAAV2-GFP were measured in an infectivity assay 72 h post-transduction of adherent HEK-293T cells: a serial dilution of harvested rAAV2-GFP is used to infect a given number of adherent HEK-293T cells. Three days post-transfection, the number of GFP-positive cells is measured by flow cytometry.

(autologous therapy) or from a donor patient (allogenic therapy). Based on the Alliance for Regenerative Medicine's 2019 annual Report, the number of approved gene therapy products has grown with the approval of two additional gene therapies for distinct indications (Zolgensma® for spinal muscular atrophy and Zynteglo® for beta thalassemia), and the number of gene therapies is expecting to double within the next 2 years [1]. The two viral vectors that are mostly used for gene and gene-modified cell therapy are recombinant adeno-associated virus (AAV) and lentivirus (LV). AAVs have particularly shown to be interesting because they are efficient for gene delivery to specific cell types, such as motor neurons (ex. Zolgensma®) and retinal cells (ex. Luxturna®).

Production of AAV viral vectors generally requires the co-transfection of three plasmids, with the first containing genes coding for the capsid proteins and necessary auxiliary genes, the second harboring the therapeutic gene of interest, and the third corresponding to the adenovirus-derived helper plasmid. These plasmids need to be delivered into mammalian cell lines, mostly human embryonic kidney 293 (HEK-293) cell lines and derivatives, which will be the host to synthesize recombinant

FIGURE 2

FectoVIR®-AAV is compatible with several commercially available synthetic media for suspension HEK-293 cells.

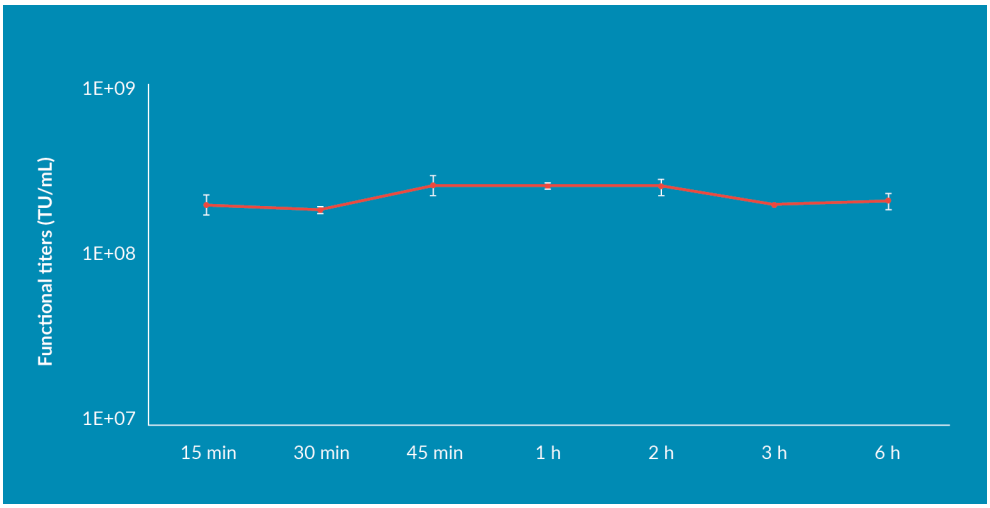


Recombinant AAV-2 were harvested 72h post-transfection of suspension HEK-293T cells with FectoVIR®-AAV/DNA complexes prepared in several synthetic media. Functional viral titers (TU/ml) of rAAV2 were measured in an infectivity assay 72 h post-transduction of adherent HEK-293 cells as follows: genomic DNA was extracted from harvested rAAV2-GFP using a viral DNA extraction kit. The number of viral copies was determined by the SYBR-green qPCR method using primers specific for inverted terminal repeat regions (ITR).

DOI: 10.18609/cgti.2020.077

FIGURE 3

Long-term stability of FectoVIR®-AAV /DNA complexes for reliable titer yields at industrial scale.



rAAV-2-GFP were harvested 72h post-transfection of suspension HEK-293T cells with FectoVIR®-AAV /DNA complexes prepared following the recommended conditions. With varying pre-incubation time of complexes (15 min to 6 h). Functional viral titers (TU/ml) of rAAV2-GFP were measured in an infectivity assay 72 h post-transduction of adherent HEK-293T cells.

AAV viral particles containing the therapeutic gene. The efficiency of the delivery process is essential to obtain a high number of producing cells. This delivery process is mainly dependent on the transfection method used. Transfection should ensure that plasmids are co-delivered into the highest number of cells and that the plasmids are protected from degradation before reaching the nucleus. In addition, the transfection method should be scalable and highly reproducible in order to support large scale viral vector manufacturing.

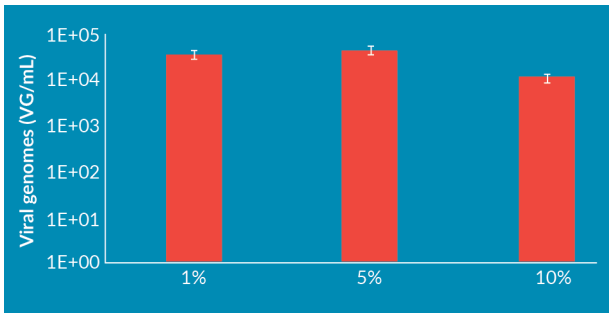
In a previous article [2], we discussed the advantages of using transient transfection for large scale viral vector manufacturing, which remains to this day the simplest and fastest approach to ensure production of viral particles. Of the existing transfection methods, the use of PEI-based transfection reagent is predominant in gene therapy as it combines affordability and compatibility for transfection of adherent and suspension cells. PEIpro® transfection reagent is a highly qualified PEI that has become the gold standard for large-scale production of viral vectors such as adenovirus, lentivirus and AAVs both in adherent and suspension systems [3–9]. The availability of this reagent at GMP (Good Manufacturing Practices) grade since 2018, has made PEIpro®-GMP the first transfection reagent that is compliant with international GMP guidelines and is suitable for ATMPs manufacturing and commercialization.

CURRENT NEEDS FOR LARGE SCALE AAV MANUFACTURING

The new challenge is now to develop production platforms that are capable of answering the growing needs for commercial manufacturing. Current conventional methods for virus production are based on adherent cell culture systems in the presence of serum, which are suboptimal set-ups when the aim is to put in place a validated manufacturing process to produce at large scale reproducible viral yields. Based on the manufacturing process adopted for protein and antibody manufacturing, the growing tendency among AAV viral vector manufacturers is to move on to suspension producer cells grown in chemically defined synthetic medium. By switching to suspension cell culture systems, the aim is 3-fold: reduce batch-to-batch variability by eliminating fluctuating parameters of cell

FIGURE 4

Optimized FectoVIR®-AAV transfection complex preparation for large scale transient transfection.



Total viral genome (VG/cell) of rAAV-2-GFP were quantified 72 h post-transfection of suspension HEK-293T cells in commercial synthetic medium. Transfection complexes were prepared in different volumes of complexation: 10%, 5% and 1% of final culture volumes.

culture (e.g. serum, cell seeding density), simplify downstream harvesting and purification processes, and increase AAV production to treat larger group of patients.

Because the gene and therapy field is progressing at a fast pace, so should technologies to support the feasibility of producing more at larger scale without compromising on quality. Large scale bioreactors for the culture of suspension cells are growingly used and this increase in scale can lead to lower yield that can be explained by: i) physical and mechanical constraints that impact cellular metabolism in large scale cell culture; and ii) time and volume constraints with the handling of bigger transfection volumes. With 20 years of expertise in transfection, Polyplus-transfection has tackled large scale transfection constraints by developing a novel transfection reagent. FectoVIR®-AAV is a novel class of animal free transfection reagent specifically developed for large scale transfection to improve scalability, productivity and flexibility for industrial manufacturing of AAV viral vectors in suspension cells.

IMPROVING LARGE SCALE TRANSFECTION: PRODUCTION YIELD

Transfection whether at small or large scale is dependent on the efficiency of the delivery molecule. As such, it is essential to identify a delivery molecule that is optimal for a given application. For AAV viral vector production in suspension cells, we screened a refined chemical library with specific criteria: scalability and improved production yields. These additional physico-chemical specifications were also essential to retain FectoVIR®-AAV as lead candidate: animal-free, scalable synthesis and GMP-grade compatibility. In comparison to the gold standard PEIpro® and other competitors used for viral vector manufacturing, FectoVIR®-AAV improved significantly rAAV2 production yield in suspension cells with up to 10-fold increase in functional titer yields (Figure 1). The increased production yield is reproducible at different scales, for the production of several AAV serotypes as confirmed by viral manufacturers who took part in the beta-testing. In addition, several of the most frequently used synthetic media for AAV manufacturing were also tested to prepare FectoVIR®-AAV/DNA pre-mixing complexes. As shown in Figure 2, FectoVIR-AAV® offers the flexibility that is needed during process development as it is compatible with the recommended synthetic media, as well as more standard media such as DMEM, Opti-MEM and even phosphate-buffered saline (PBS).

IMPROVING LARGE SCALE TRANSFECTION: SCALABILITY

Transfection at small scale requires the pre-mixing of DNA and transfection reagent to form complexes that are incubated at room temperature for a given amount of time, usually within a short 15–30-minute window to prevent their aggregation and allow efficient binding to the cell membrane and subsequent endocytosis. Once formed, these transfection complexes are immediately added to the cell culture. The pre-mixing volume usually represents 10% of the cell culture volume, and at small scale it is manageable to quickly add this volume.

DOI: 10.18609/cgti.2020.077

When moving on to large scale transfection, it consequently leads to working with large volumes during preparation of transfection complexes and during their transfer into the bioreactor. The implementation of a transfection protocol compatible with upscaling is therefore indispensable to maintain high viral titer yields. FectoVIR®-AAV transfection reagent addresses both time and volume constraints of large-scale transfection. As shown in Figure 3, the transfer time is no longer a limiting factor. FectoVIR®-AAV transfection complexes are stable after 15 minutes of pre-mixing and remain as such for up to 6 hours, thereby ensuring reproducible AAV titer yields when transfection complexes are quickly added to the cells at small scale, or when 1 hour or more is needed to add these complexes for large scale transfection. FectoVIR®-AAV has also been optimized to reduce the complexation volume (Figure 4). From the traditional 10% of the cell culture volume, transfection complexes can now be prepared in half the volume (5%) and even in a tenth of the volume (1%). For a 200 L cell suspension culture, it means that the transfection complexation volume can be decreased from 20 L (10%) down to 2 L (1%). This significant reduction in the minimum volume needed to prepare complexes alleviates technical constraints during the preparation and transfer of large complexation volumes into large scale bioreactors.

SHORT-TERM OBJECTIVE: MEETING QUALITY REQUIREMENTS

With the growing AAV manufacturing capacity demands, the need for novel technologies to aid viral manufacturers increase production is critical. It is also indispensable that all raw materials used for the production of therapeutic viral vectors meet quality requirements for future commercialization. Polyplus-transfection® has acquired a strong expertise in developing GMP grade transfection reagent, also recently marked by the launch of the first GMP compliant transfection reagent for therapeutic viral vector manufacturing (PEIpro®-GMP in 2018). The strategy for developing GMP-grade transfection reagent is a fully validated process in compliance with GMP guidelines to ensure traceability from starting material to the final product. Therefore, during initial identification of lead candidate FectoVIR-AAV®, we confirmed its GMP compatibility and the feasibility of its synthesis at large scale which now allows us to confirm that it will be commercially available starting Q2 2021, concomitantly with a residual test to ensure FectoVIR-AAV's traceability throughout AAV manufacturing process.

REFERENCES

1. Alliance for Regenerative Medicine Q3 2019 Data Report: https://alliancerm.org/publication/q3-2019-data-report
2. Nyamay'Antu A, Dumont M, Kedinger V, Erbacher P. Non-Viral Vector Mediated Gene Delivery: the Outsider to Watch Out For in Gene Therapy. *Cell Gene Ther. Ins.* 2018; 51–7.
3. Gilbert R. Key considerations for the use of suspension culture systems for viral vector manufacturing. *Cell Gene Ther. Ins.* 2020; 6(1): 143–8.
4. Bauler M, Roberts JK, Wu CC *et al.* Production of Lentiviral Vectors Using Suspension Cells Grown in Serum-free Media. *Mol. Ther. Methods. Clin. Dev.* 2020; 17: 58–68.
5. Collaud F, Bortolussi G, Guianvarc'h L *et al.* Preclinical Development of an AAV8-hUGT1A1 Vector for the Treatment of Crigler-Najjar Syndrome. *Mol. Ther. Methods Clin. Dev.* 2018; 12: 157–74.
6. Heldt CL. Scalable method utilizing low pH for DNA removal in the harvest of recombinant adeno-associated virus vectors. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2019; 1124: 173–9.
7. Cervera L, Kamen AA. Large-Scale Transient Transfection of Suspension Mammalian Cells for VLP Production. *Methods Mol. Biol.* 2018; 1674: 117–27.

8. Valkama AJ, Leinonen HM, Lipponen EM *et al*. Optimization of lentiviral vector production for scale-up in fixed-bed bioreactor. *Gene Ther*. 2018; 25: 39–46.

AFFILIATIONS

Alengo Nyamay'antu, PhD
Scientific Communication Specialist, Polyplus-transfection® SA

Malik Hellal, PhD
Senior Scientist in Chemistry, Polyplus-transfection® SA

Mathieu Porte, PhD
R&D Manager Bioproduction, Polyplus-transfection® SA

Patrick Erbacher, PhD
CSO, Polyplus-transfection® SA

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The authors are employees of Polyplus Transfection. They declare that they have no conflicts of interest.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

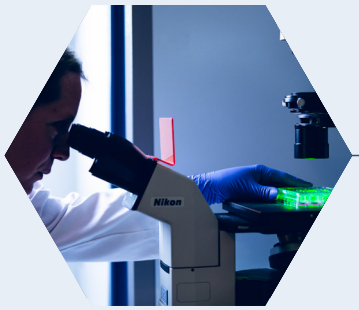
Submitted for peer review: *Apr 16 2020*; ***Revised manuscript received:*** *May 18 2020*; ***Publication date:*** *May 28 2020.*

DOI: 10.18609/cgti.2020.077

Polyplus transfection®

Expertise • Quality • Support

Leading company in transfection for Gene & Cell Therapy



First to provide GMP grade reagent for viral vector manufacturing

Supplier of PEIpro® product range: the gold standard for Gene & cell therapy

With 4 transfection product lines, Polyplus-transfection is the leading biotechnology company that supports Gene and Cell therapy, biologics manufacturing and life science research with innovative nucleic acid transfection solutions.

- Viral vectors for Gene & Cell Therapy
- Life science research
- Protein and mAb production
- Non viral *in vivo* transfection

20 years 20 years expertise in transfection solutions

Available worldwide

www.polyplus-transfection.com

afaq ISO 9001 Qualité AFNOR CERTIFICATION

Contact us:

www.polyplus-transfection.com +33 (0)3 90 40 61 80 support@polyplus-transfection.com

CELL & GENE THERAPY INSIGHTS

TRENDS & ADVANCES IN GENE THERAPY DELIVERY & GENE EDITING

SPOTLIGHT

EXPERT INSIGHT

Polymer nanoparticles: potential for efficient, biodegradable, and cost-effective delivery of gene therapy to multiple tissues

Timothy C Fong, Steven Bodovitz & Kunwoo Lee

The field of gene therapy has had a resurgence of interest and activity in the last few years due to recent approvals and development of new gene editing technologies. Recent approvals include Luxturna®, which uses adeno-associated virus (AAV) to deliver DNA to treat inherited retinal disease and Onpattro®, which uses a lipid nanoparticle (LNP) to deliver RNA interference (RNAi) to treat hereditary transthyretin-mediated amyloidosis. New technologies that are starting to reach human testing include CRISPR-Cas9, CRISPR-Cas12a and various base editors, but the bottleneck is delivery. AAV is the most widely used viral platform for *in vivo* delivery, but it has significant limitations, including delivery of only nucleic acids, small payload capacity, potential for integration, pre-existing and acquired immunogenicity, and cost of manufacturing. LNPs are also limited to nucleic acid payloads and have not shown efficient *in vivo* delivery outside of liver and muscle. A new polymer-based nanoparticle system is being developed that has the potential to overcome these limitations and enable the next generation of gene therapy.

Cell & Gene Therapy Insights 2020; 6(4), 583–590

DOI: 10.18609/cgti.2020.068

INTRODUCTION

Gene therapy has undergone a resurgence in the last few years with the launch of several landmark drugs in which previously uncurable genetic diseases are now treatable or even cured. Novel gene therapeutic drugs can now restore vision in patients with inherited retinal disease (voretigene neparvovec, Luxturna®), reduce accumulation of amyloid deposits in peripheral nerves, heart, and other organs in patients with hereditary amyloidosis (patisiran, Onpattro®), and restore muscle function in patients with spinal muscular atrophy (nusinerse, Spinraza® and onasemnogene abeparvovec, Zolgensma®). Other gene therapies to treat genetic diseases of the hematopoietic system are in late-stage clinical trials or have been recently approved (EMA approval of Zynteglo®). These approvals have helped to accelerate the development of other gene therapies targeting a number of other genetic disorders, such as diseases of the central and peripheral nervous systems, lysosomal storage diseases, autoimmunity, cancer, and many others [1].

The approvals and expanding pipeline are driven by many strategies for blocking, adding, or changing genes. Strategies include gene knockdown with siRNA or antisense oligonucleotides (ASO) [2–5], expression of a novel chimeric or wild-type protein by gene insertion [6–10], replacement or correction of a segment of a mutated gene [11], base editing of the DNA or mRNA [12–14], or a combination of these approaches. Many more strategies are in development, including ceDNA, miRNA, samRNA, DNA/RNA base editors, and CRISPR-Cas9, CRISPR-Cas12a, and other ribonucleoproteins (RNP), but the bottleneck is the limited number of delivery methods that can target specific tissues and cells (Box 1).

BOX 1

Key opinion leaders' opinions on state of the art for gene therapy *in vivo* delivery.

"Delivery remains perhaps the biggest bottleneck to somatic-cell genome editing"
– Dr Jennifer Doudna, CRISPR Pioneer, *Nature* 2020; 578: 229–36.

"...somewhat disappointed with where we are..."
– Dr James Wilson, Gene Therapy Pioneer, Interview in STAT News, www.statnews.com, Nov. 21, 2019.

VIRAL VECTOR PLATFORMS

The earliest platforms to be developed for gene therapy applications were viral vectors, which have naturally evolved to have high efficiency of transduction to many cell types. Many different virus strains (adenovirus, murine retrovirus, adeno-associated virus, lentivirus, herpes virus, sindbis virus) have been used to develop vector systems. Adeno-associated virus (AAV) is the most common for *in vivo* delivery and lentivirus (LVV) is the most used for *ex vivo* delivery. Viral vectors, however, have had limited cell selectivity. One approach is to target viral vectors by direct injection: Luxturna®, for example, requires local subretinal injection to achieve good transduction because AAV does not cross into the retina after intravitreal injection. Another approach is systemic intravenous delivery to vascularized organs, such as liver, lungs, and spleen, but this only allows treatment of a narrow set of diseases. The ideal approach of targeting a specific population of cells after systemic administration is an active area of investigation and includes methods such as modifying viral capsid/envelope proteins [15–17] and *in vivo* selection [18–21], but cell-specific vectors have yet to reach the clinic.

After cell entry, some viral vectors, such as LVV, are designed to integrate their genetic cargo into the cell genome. Even non-integrating vectors like AAV have been shown to have low levels of integration [22–24]. For some applications (CAR-T, hemophilia, SCID), prolonged expression after integration of the therapeutic gene is the goal. However, integration at the wrong locus may result in malignant transformation of the modified cell as observed with X-linked SCID patients [25]. In addition to the potential for

DOI: 10.18609/cgti.2020.068

integration, viral vectors are limited in their capacity to deliver large cargos. AAV can only package about 4.3kb of DNA while LVV can package about 8.5kb for the gene of interest.

Another disadvantage of viral vectors is that many patients may have pre-existing immunity to the virus, as in the case for AAV, which may result in rapid clearance of the vector and reduce the effectiveness of the therapy [26]. Additionally, multiple administrations will eventually result in the generation of a neutralizing immune response even without pre-existing immunity [27].

Lastly, AAV and LVV have made gene therapy possible for some diseases, but the costs of manufacturing are high because viral vectors are produced in cells. Despite intense efforts to make industrial-scale production more efficient and cost-effective, viral production has limited yields of viral particles per cell, multistep purification processes, and variable final product characteristics (e.g., empty particles).

LIPID NANOPARTICLE PLATFORMS

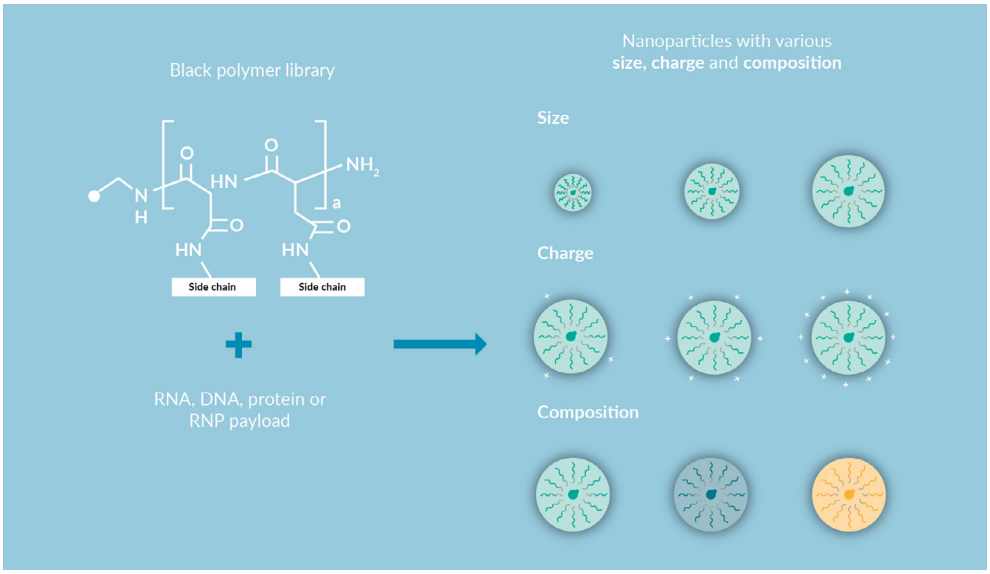
Lipid nanoparticles have been developed as an alternative to viral vectors and have demonstrated success in delivering RNA cargos to muscle by direct injection and to liver by intravenous injection [28–31]. Efficient delivery to other tissues via IV injection has not yet been as successful [32,33]. Like viral vectors, LNPs are limited to only nucleic acid cargos because the current manufacturing process requires LNP components to be dissolved in alcohol which denatures protein and RNP. However, the cost of manufacturing LNPs is much lower than for viral vectors. LNPs consist of four components: an ionizable lipid, a helper lipid, cholesterol and polyethylene glycol (PEG). The ratios of each of these components must be evaluated and optimized to produce the combination for efficient encapsulation and *in vivo* delivery of the cargo. Efforts are underway to develop targeted LNPs based on compositions using different lipid and PEG molecules and ratios of components [34,35]. One additional advantage of LNPs is that patients will be unlikely to have pre-existing immunity, although an anti-PEG response is possible with repeated administration [36].

CHEMICALLY SYNTHESIZED POLYMER PLATFORMS

Chemically synthesized, biodegradable polymer nanoparticles (PNP) have the potential

FIGURE 1

General structure of chemically synthesized block polymers and nanoparticle formation.



to overcome many of the disadvantages of viral vectors and LNPs. PNPs are typically comprised of block copolymer chains with attached side chain groups [37–43]. PNP characteristics (e.g., size, charge, cargo type, capacity) depend on the structure and type of side chains attached to the polyamide backbone (Figure 1). PNPs have been shown to efficiently encapsulate RNA, DNA, proteins, and RNP. In preclinical studies, PNPs have been shown to deliver RNA and CRISPR-Cas9 RNP to brain and muscle resulting in gene editing of these tissues, knockout of the target protein, and, importantly, reduction of clinical symptoms of the genetic disorder [11,44,45].

PNPs have several other advantages over viral vectors or LNPs (Table 1).

1. No limitation of size of cargo;
2. Self-assembling complex for one-step particle formation;
3. Potential for targeted delivery based on side chain composition and/or conjugation to peptides, monoclonal antibodies, single chain antibodies, carbohydrates, or other ligands;
4. Low likelihood of pre-existing immunity (but immunogenicity of repeated administration to be determined);
5. No integration of nucleic acid cargos into the host genome unless the cargo is designed for this purpose (e.g., transposon-based constructs);
6. Chemical synthesis that is easily scalable with low cost of goods.

Polymer-based nanoparticles have been investigated in academic laboratories for over a decade for gene therapy [40,41]. Early results were promising, but key challenges remained: how to formulate various gene therapy cargos and how to efficiently screen for targeted PNPs. In our laboratories, we have implemented an industrial process that combines deep polymer expertise with advanced computational analysis of polymer attributes and iterative screening. The process starts with synthesis of a library of polymers with diverse chemical properties that encapsulate RNA or RNP into PNPs of various sizes, charge, and stability in biological fluids. The polymers are screened on an array of primary human cells to assess stability, transfection efficiency, and toxicity and the best-performing polymers advance to *in vivo* testing. The results are analyzed using artificial intelligence/machine learning algorithms and the output guides the iterative process of synthesis of new polymers and screening until the identification of non-toxic polymers with efficient and specific cell and tissue delivery.

Experience with LNP development has shown that *in vitro* screening on immortalized cell lines is not highly predictive of *in vivo* function [46], but *in vivo* screening has

TABLE 1

Comparing gene therapy delivery platforms.

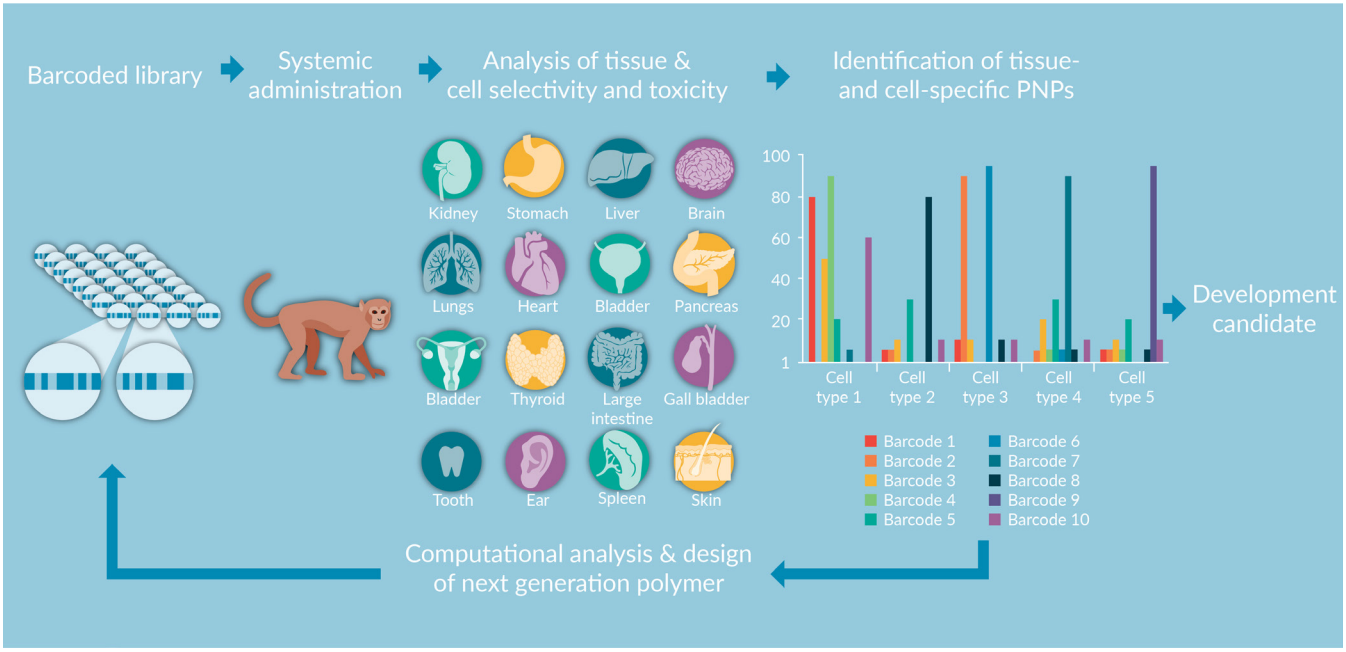
	AAV	Lipid nanoparticles	Polymer nanoparticles
Target tissues	Multiple (eye, CNS approved)	Liver only	Multiple tissue targeting
Manufacturing cost	Estimated $30K/patient*	Estimated $1K/patient*	Estimated $2K/patient*
Payload type	DNA only	DNA/RNA only	DNA, RNA, protein, RNP
DNA/RNA payload size	<5 kb	No upper limit	No upper limit
Off-target	High	Low	Low
Genome integration	Yes	No	No
Pre-existing immunity	High	Low	Expected to be low
Lyophilization/stability	No/low	No/low	Yes/high

*COGS estimated from analysis of internal and publicly available information on raw materials, ancillary manufacturing reagents and supplies, and labor costs to produce a dose of drug under GMP guidelines.

DOI: 10.18609/cgti.2020.068

FIGURE2

Schematic workflow for barcoded *in vivo* screening of polymer nanoparticles.



Candidate polymers that have demonstrated activity *in vitro* are individually complexed with a unique DNA barcode. A cocktail of polymers is then injected intravenously into an animal. One to two days after injection, organs and tissues are harvested and DNA is extracted, amplified by PCR, and sequenced. The relative level of each barcode within each organ is determined by the number of sequence reads and is indicative of polymer nanoparticle tropism for that organ.

been slow and expensive. Our solution is to screen multiple PNPs *in vivo* in a single animal using a barcode system (Figure 2) based on earlier efforts by investigators in this field who developed barcoded systems for *in vivo* screening [47–49]. One to two days after systemic injection of a cocktail of barcoded PNPs in mice, the organs and tissues are harvested and DNA is extracted, amplified by PCR, and sequenced by next-generation sequencing (NGS). The relative level of each barcode within each organ is determined by the number of sequence reads and is indicative of polymer nanoparticle tropism for that organ. The result of this process is that we have identified polymers of various sizes (40–250 nm) and charge with low to no toxicity that traffic to the brain, liver, spleen and lungs in mice. Moreover, advancing to barcoded screening *in vivo* in non-human primates will decrease the translational risk when progressing to human clinical trials.

We have demonstrated initial proof-of-concept of our polymer technology by identifying candidate PNPs that can deliver RNA or RNP cargo to the brain after intrathecal-lumbar injection. Our preliminary data suggest these PNPs are non-toxic and do not induce an inflammatory response at doses that demonstrate gene editing in the brain. We also have candidate PNPs that deliver RNA to innate immune cells and lungs and deliver RNP to liver after IV injection. As we screen larger and more diverse polymer libraries with *in vivo* barcoded screening, we expect to identify polymers that can efficiently deliver their payload to a greater number of different tissues and cells.

TRANSLATION INSIGHT

With the recent discoveries of new CRISPR proteins and the development of modified and chimeric CRISPR-Cas proteins, the last hurdle to propel gene therapies into mainstream clinical practice is to solve how these future treatments and cures will be safely, specifically,

and efficiently delivered to target tissues and cells. Identification and testing of PNPs that can deliver to specific regions of the brain and spinal cord in animal models of disease will have a significant impact on accelerating curative gene therapies using gene editing technologies like CRISPR-Cas9 for neurological, neuromuscular, and cognitive diseases. PNPs that specifically deliver mRNA and DNA to cells of the innate and adaptive immune system will potentially transform how we treat cancer, autoimmunity, and inflammatory diseases. And, specific delivery of gene correcting platforms like base editors to the liver or hematopoietic stem cells will allow the cure of many genetic disorders. We believe that our technology along with other published results [11,44,45,50–52] provide proof of concept that PNPs will be an important part of the gene therapy delivery solution.

REFERENCES

1. High KA, Roncarolo MG. Gene Therapy. *N. Engl. J Med.* 2019; 381(5): 455–64.
2. Aslesh T, Maruyama R, Yokota T. Systemic and ICV Injections of Antisense Oligos into SMA Mice and Evaluation. *Methods Mol. Biol.* 2018; 1828: 455–65.
3. Scott LJ. Givosiran: First Approval. *Drugs* 2020; 80(3): 335–9.
4. Solomon SD, Adams D, Kristen A *et al.* Effects of Patisiran, an RNA Interference Therapeutic, on Cardiac Parameters in Patients With Hereditary Transthyretin-Mediated Amyloidosis. *Circulation* 2019; 139(4): 431–43.
5. van Roon-Mom WMC, Roos RAC, de Bot ST. Dose-Dependent Lowering of Mutant Huntingtin Using Antisense Oligonucleotides in Huntington Disease Patients. *Nucleic Acid Ther.* 2018; 28(2): 59–62.
6. Maus MV, Grupp SA, Porter DL, June CH. Antibody-modified T cells: CARs take the front seat for hematologic malignancies. *Blood* 2014; 123(17): 2625–35.
7. Mendell JR, Al-Zaidy S, Shell R *et al.* Single-Dose Gene-Replacement Therapy for Spinal Muscular Atrophy. *N. Engl. J. Med.* 2017; 377(18): 1713–22.
8. Mikkilineni L, Kochenderfer JN. Chimeric antigen receptor T-cell therapies for multiple myeloma. *Blood* 2017; 130(24): 2594–602.
9. Scarfò I, Maus MV. Current approaches to increase CAR T cell potency in solid tumors: targeting the tumor microenvironment. *J. ImmunoTher. Cancer* 2017; 5(1): 28.
10. Schumann K, Lin S, Boyer E *et al.* Generation of knock-in primary human T cells using Cas9 ribonucleoproteins. *Proc. Natl Acad. Sci. USA* 2015; 112(33): 10437–42.
11. Lee K, Conboy M, Park HM *et al.* Nanoparticle delivery of Cas9 ribonucleoprotein and donor DNA *in vivo* induces homology-directed DNA repair. *Nat. Biomed. Eng.* 2017; 1: 889–901.
12. Gaudelli NM, Komor AC, Rees HA *et al.* Programmable base editing of A*T to G*C in genomic DNA without DNA cleavage. *Nature* 2017; 551(7681): 464–71.
13. Kim YB, Komor AC, Levy JM *et al.* Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions. *Nat. Biotechnol.* 2017; 35(4): 371–6.
14. Komor AC, Zhao KT, Packer MS *et al.* Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity. *Sci. Adv.* 2017; 3(8): eaao4774.
15. Börner K, Kienle E, Huang LY *et al.* Pre-arrayed Pan-AAV Peptide Display Libraries for Rapid Single-Round Screening. *Mol. Ther.* 2020; 28(4): 1016–32.
16. Joglekar AV, Sandoval S. Pseudotyped Lentiviral Vectors: One Vector, Many Guises. *Hum. Gene Ther. Methods* 2017; 28(6): 291–301.
17. Li C, Samulski RJ. Engineering adeno-associated virus vectors for gene therapy. *Nat. Rev. Genet.* 2020; 21(4): 255–72.
18. Davidsson M, Wang G, Aldrin-Kirk P *et al.* A systematic capsid evolution approach performed *in vivo* for the design of AAV vectors with tailored properties and tropism. *Proc. Natl Acad. Sci.* 2019: 201910061.
19. Deverman BE, Pravdo PL, Simpson BP *et al.* Cre-dependent selection yields AAV variants for widespread gene transfer to the adult brain. *Nat. Biotechnol.* 2016; 34(2): 204–9.
20. Lisowski L, Dane AP, Chu K *et al.* Selection and evaluation of clinically relevant AAV variants in a xenograft liver model. *Nature* 2014; 506(7488): 382–6.

DOI: 10.18609/cgti.2020.068

21. Powell SK, Khan N, Parker CL *et al.* Characterization of a novel adeno-associated viral vector with preferential oligodendrocyte tropism. *Gene Ther.* 2016; 23(11): 807–14.

22. Chandler RJ, Sands MS, Venditti CP. Recombinant Adeno-Associated Viral Integration and Genotoxicity: Insights from Animal Models. *Hum. Gene Ther.* 2017; 28(4): 314–22.

23. Gil-Farina I, Fronza R, Kaeppel C *et al.* Recombinant AAV Integration Is Not Associated With Hepatic Genotoxicity in Nonhuman Primates and Patients. *Mol. Ther.* 2016; 24(6): 1100–5.

24. Kaeppel C, Beattie SG, Fronza R *et al.* A largely random AAV integration profile after LPLD gene therapy. *Nat. Med.* 2013; 19(7): 889–91.

25. Cavazzana M, Six E, Lagresle-Peyrou C *et al.* Gene Therapy for X-Linked Severe Combined Immunodeficiency: Where Do We Stand? *Hum. Gene Ther.* 2016; 27(2): 108–16.

26. Shirley JL, de Jong YP, Terhorst C *et al.* Immune Responses to Viral Gene Therapy Vectors. *Mol. Ther.* 2020; 28(3): 709–22.

27. Verdera HC, Kuranda K, Mingozzi F. AAV Vector Immunogenicity in Humans: A Long Journey to Successful Gene Transfer. *Mol. Ther.* 2020; 28(3): 723–46.

28. Cullis PR, Hope MJ. Lipid Nanoparticle Systems for Enabling Gene Therapies. *Mol. Ther.* 2017; 25(7): 1467–75.

29. Finn JD, Smith AR, Patel MC *et al.* A Single Administration of CRISPR/Cas9 Lipid Nanoparticles Achieves Robust and Persistent *In Vivo* Genome Editing. *Cell Rep.* 2018; 22(9): 2227–35.

30. Hassett KJ, Benenato KE, Jacquinet E *et al.* Optimization of Lipid Nanoparticles for Intramuscular Administration of mRNA Vaccines. *Mol. Ther. Nucleic Acids* 2019; 15: 1–11.

31. Liang F, Lindgren G, Lin A *et al.* Efficient Targeting and Activation of Antigen-Presenting Cells *In Vivo* after Modified mRNA Vaccine Administration in Rhesus Macaques. *Mol. Ther.* 2017; 25(12): 2635–47.

32. Pardi N, Tuyishime S, Muramatsu H *et al.* Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. *J. Control Rel.* 2015; 217: 345–51.

33. Shi B, Keough E, Matter A *et al.* Biodistribution of small interfering RNA at the organ and cellular levels after lipid nanoparticle-mediated delivery. *J. Histochem. Cytochem.* 2011; 59(8): 727–40.

34. Wu XR, Zhang J, Zhang J-H *et al.* Amino Acid-Linked Low Molecular Weight Polyethylenimine for Improved Gene Delivery and Biocompatibility. *Molecules* 2020; 25(4).

35. Cheng Q, Wei T, Farbiak L *et al.* Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR–Cas gene editing. *Nat. Nanotechnol.* 2020.

36. Abu Lila AS, Ishida T. Anti-PEG IgM Production via a PEGylated Nanocarrier System for Nucleic Acid Delivery. *Methods Mol. Biol.* 2019; 1943: 333–46.

37. Cabral H, Miyata K, Osada K, Kataoka K *et al.* Block Copolymer Micelles in Nanomedicine Applications. *Chem. Rev.* 2018; 118(14): 6844–92.

38. Cordeiro RA, Serra A, Coelho JFJ *et al.* Poly(beta-amino ester)-based gene delivery systems: From discovery to therapeutic applications. *J. Control. Release* 2019; 310: 155–87.

39. Hadinoto K, Sundaresan A, Cheow WS. Lipid–polymer hybrid nanoparticles as a new generation therapeutic delivery platform: A review. *Eur. J. Pharm. Biopharm.* 2013; 85(3, Part A): 427–43.

40. Masago K, Itaka K, Nishiyama N *et al.* Gene delivery with biocompatible cationic polymer: pharmacogenomic analysis on cell bioactivity. *Biomaterials* 2007; 28(34): 5169–75.

41. Miyata K, Oba M, Nakanishi M *et al.* Polyplexes from poly(aspartamide) bearing 1,2-diaminoethane side chains induce pH-selective, endosomal membrane destabilization with amplified transfection and negligible cytotoxicity. *J. Am. Chem. Soc.* 2008; 130(48): 16287–94.

42. Rai R, Alwani S, Badea I. Polymeric Nanoparticles in Gene Therapy: New Avenues of Design and Optimization for Delivery Applications. *Polymers (Basel)* 2019; 11(4).

43. Sharma AR, Kundu SK , Nam J-S *et al.* Next generation delivery system for proteins and genes of therapeutic purpose: why and how? *Biomed. Res. Int.* 2014; 2014: 327950.

44. Lee B, Lee K, Panda S *et al.* Nanoparticle delivery of CRISPR into the brain rescues a mouse model of fragile X syndrome from exaggerated repetitive behaviours. *Nat. Biomed. Eng.* 2018; 2(7): 497–507.

45. Park HM, Liu H, Wu J *et al.* Extension of the crRNA enhances Cpf1 gene editing *in vitro* and *in vivo*. *Nat. Commun.* 2018; 9(1): 3313.

46. Paunovska K, Sago CD, Monaco CM *et al.* A Direct Comparison of *in Vitro* and *in Vivo* Nucleic Acid Delivery Mediated by Hundreds of Nanoparticles Reveals a Weak Correlation. *Nano Lett.* 2018; 18(3): 2148–57.

47. Guimaraes PPG, Zhang R, Spektor R *et al.* Ionizable lipid nanoparticles encapsulating barcoded mRNA for accelerated *in vivo* delivery screening. *J. Control Release* 2019; 316: 404–17.

48. Lokugamage MP, Sago CD, Dahlman JE. Testing thousands of nanoparticles *in vivo* using DNA barcodes. *Curr. Opin. Biomed. Eng.* 2018; 7: 1–8.

49. Xu M, Li J, Xie J *et al.* High-Throughput Quantification of *In Vivo* Adeno-Associated Virus Transduction with Barcoded Non-Coding RNAs. *Hum. Gene Ther.* 2019; 30(8): 946–56.

50. Hayakawa K, Uchida S, Ogata T *et al.* Intrathecal injection of a therapeutic gene-containing polyplex to treat spinal cord injury. *J. Control Release* 2015; 197: 1–9.

51. Perche F, Uchida S, Akiba H *et al.* Improved Brain Expression of Anti-Amyloid beta scFv by Complexation of mRNA Including a Secretion Sequence with PEG-based Block Catiomer. *Curr. Alzheimer Res.* 2017; 14(3): 295–302.

52. Uchida S, Itaka K, Chen Q *et al.* PEGylated polyplex with optimized PEG shielding enhances gene introduction in lungs by minimizing inflammatory responses. *Mol. Ther.* 2012; 20(6): 1196–203.

AFFILIATIONS

Timothy C Fong
CSO, GenEdit, Inc.

Steven Bodovitz
Director of Business Development and Operations, GenEdit, Inc.

Kunwoo Lee
CEO, GenE

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Kunwoo Lee, PhD, is the CEO and co-founder, Timothy C Fong, PhD, is the CSO, and Steven Bodovitz, PhD, is the Director of Business Development and Operations of GenEdit, Inc. All are full-time employees of GenEdit.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *Mar 30 2020;* ***Revised manuscript received:*** *May 4 2020;* ***Publication date:*** *May 14 2020.*

DOI: 10.18609/cgti.2020.068

CELL & GENE THERAPY INSIGHTS

TRENDS AND ADVANCES IN GENE THERAPY DELIVERY & GENE EDITING

SPOTLIGHT

INTERVIEW

Gene editing as a key enabler of allogeneic cell therapy

TORSTEN MEISSNER obtained his PhD in biology at Free University, Berlin and moved on to do postdoctoral research in immunology and stem cell research at Dana-Farber Cancer Institute and Harvard University in Cambridge, Massachusetts. Torsten is currently an Instructor in the Department of Surgery at Beth Israel Deaconess Medical Center in Boston, a Harvard Medical School affiliated research hospital. His research combines genome, cell, and tissue engineering with the overall goal to take down the immune barrier to transplantation. Torsten is currently developing methods to generate immune-silent, living blood vessels from human induced pluripotent stem cells (iPSC) that can be used for disease modeling and vascular reconstruction.

Cell & Gene Therapy Insights 2020; 6(4), 517–523

DOI: 10.18609/cgti.2020.060

Q What are you working on right now?

TM: I'm currently an instructor in the department of surgery at Beth Israel Medical Deaconess Center, a Harvard Medical School affiliated research hospital in Boston. I did my post-doctoral training in my colleague Chad Cowan's lab when we were both still at Harvard University. My research focus was, and remains, to develop universal donor stem cell lines that withstand immune rejection upon transplantation. The immune barrier constitutes a

"Another game changer for me ... was switching to ribonucleoprotein complexes (RNPs) ... [It] can result in up to 95% targeting efficiency in primary T cells, with almost no cell death."

major roadblock to the clinical translation of stem cell-based therapies, which we are trying to overcome using genome editing.

Currently I'm associated with the lab of Elliot Chaikof, who is a truly remarkable vascular surgeon and bioengineer – he likes to joke that he has two jobs: a day job as a surgeon and a night job as a scientist! Together, we are trying to generate living blood vessels from stem cell-derived building blocks, vascular smooth muscle cells (vSMC) and endothelial cells (EC). The idea is to take the hypoimmunogenic stem cells that we developed in the Cowan lab to the next level to see if we can also use them to generate entire 3D tissues, such as blood vessels that can be used for vascular reconstruction.

This is a highly interdisciplinary project that involves genome editing, stem cell biology, vascular tissue engineering, and immunology. As an immunologist and stem cell scientist by training, this project is particularly exciting. Blood vessels are in direct contact with immune cells which travel through the blood vessels and thus patrol the entire body. Importantly, blood vessels also have barrier function; they are gatekeepers that control who and what goes in or out of circulation, by controlling leukocyte adhesion and transgression into the underlying tissue to clear infection.

With cardiovascular disease on the rise and the number one killer in the developed world, the application for such vessels are enormous, ranging from disease modelling, to drug screening and vascular reconstruction. This work is taking my previous approaches into the realm of tissue engineering.

Q What are the relative pros and cons of the various gene editing platforms?

TM: That's a tough question to answer, especially because the field of genome editing is evolving so rapidly. It's astonishing how far the field has come in such a short time, and as someone participating in its development, this fills me with great pride.

It has only been 7 years since CRISPR-Cas9 was successfully applied to human cells for the first time. There is no doubt that the adaption of the bacterial CRISPR-Cas9 system as a genome engineering tool has also been a watershed moment for the field of molecular medicine. Since the discovery of DNA as the site of storage of genetic information, scientists have dreamt of and actively pursued the ability to modify the genetic code – for example, to correct pathogenic mutations. CRISPR-Cas9 kicked off a genome editing renaissance – all of a sudden, everything seemed possible. A quick PubMed search I did this morning returned over 17,000 hits for CRISPR, as opposed to TALENs, for example (1037 hits). That alone shows

DOI: 10.18609/cgti.2020.060

"...over and over again, CRISPR-Cas9 has actually been proven to be remarkably specific, so it becomes more about quality control and strategies to include a safety net when things go wrong. It's definitely possible, with the right quality controls and safety network, to adapt CRISPR Cas9 to cell therapy."

how quickly CRISPR has taken the lead and outperformed other genome editing tools such as meganucleases, zinc fingers or TALENs.

CRISPR was quite simply a game changer. Why? It's ease of use. All previous systems relied on a custom-engineered endonuclease that had to be redesigned and built all over again from scratch for every single application. The outcome and efficiency also varied; zinc fingers rarely worked, and TALENs were a step up in efficacy but still laborious to build. With CRISPR you have an invariant endonuclease, the Cas9 protein. All you have to do is switch out the short guide RNA (sgRNA) that directs the endonuclease to the site of interest.

Another game changer for me, since I also work with primary human immune cells, such as T cells, was switching to ribonucleoprotein complexes (RNPs). These are made of the recombinant Cas9 protein and synthetic sgRNAs that you can order online. With previous plasmid-based technologies we observed high toxicity and cell death in particular in T cells, which hate being transfected with DNA. Switching to RNPs can result in up to 95% targeting efficiency in primary T cells, with almost no cell death. The results that we got at first try were jaw-dropping. And with such high efficiencies, it becomes a delivery problem rather than a technical problem: how to get Cas9/sgRNA complexes into the cells by nucleofection or viral transduction.

One particular challenge of the CRISPR Cas9 system is that it introduces double strand breaks into the genome, and the repair event (if no template for homologous recombination is provided) depends on the somewhat ill-defined and less understood endogenous repair pathways, such as non-homologous end joining (NHEJ) or microhomology directed repair. I believe it was George Church who referred to gene editing using CRISPR Cas9 as 'genome vandalism'. This sums up the risk and also the fear of uncontrolled collateral damage to DNA – so-called off-target events – that could potentially have adverse effects and result in compromised cell function or malignancy. And yet, over and over again, CRISPR-Cas9 has actually been proven to be remarkably specific, so it becomes more about quality control and strategies to include a safety net when things go wrong. It's definitely possible, with the right quality controls and safety network, to adapt CRISPR Cas9 to cell therapy.

Conceptually, I would like to mention the developments coming from David Liu's lab at Harvard University and the Broad Institute. He's developed more advanced genome editing systems such as the base editor, or his recent work on prime editing, which seem to greatly increase the possibilities of correcting pathogenic mutations and small deletions in the human genome. Both techniques have the advantage that they don't, at least in theory, introduce double strand breaks and can re-write genomic sequence in a defined way without the need for a DNA donor template, and without relying on homology-directed recombination (HDR). HDR only occurs in dividing cells and was thus a major hurdle of somatic gene therapy. Particularly for applications that involve the genome editing of postmitotic cells, such as neurons or cardiomyocytes, these technologies could have a great impact, once their full safety profile is established.

Q What will be the next steps in gene editing platform development – where do you see this field of innovation going next?

TM: The field is rapidly moving into the realm of clinical translation. We are already witnessing results coming in from the first clinical trials where genome editing has been used in T cells, such as Carl June's work with CAR T at the University of Pennsylvania. We're also seeing developments in hematopoietic stem and progenitor cells to treat various diseases such as inborn blood diseases like beta thalassemia and sickle cell, in clinical trials performed by CRISPR Therapeutics and Vertex Pharmaceuticals.

What I find very intriguing is that if you take a closer look at all the CRISPR companies that have sprung up, they are actually cell therapy companies using CRISPR-Cas9 genome editing as a tool to fix pathogenic mutations in the human genome. Interestingly, most of these companies also have a stem cell program. The fields of stem cell biology and genome editing have already merged under the umbrella of regenerative medicine and have become inseparable.

The next frontier will be *in vivo* editing, which has several inherent challenges of its own. First and foremost: safety. We don't want to put anything into a patient that could harm them. Next, efficacy – you need a certain percentage of cells, depending on your disease application, to have been edited to get a therapeutic benefit or effect. Then lastly, of course, there is the delivery challenge. How do we get the genome editing moiety in? One problem is the immunogenicity of Cas9 or the delivery vehicle, such as adeno-associated virus (AAV). A lot of groups have taken the approach of AAV, which has a broad range of tropisms for a variety of different cells, and other modalities such as lipid nanoparticles and cell permeable peptides are actively being explored too.

"The next frontier will be *in vivo* editing, which has several inherent challenges of its own. First and foremost: safety."

Other innovations are more of a technical nature. These include improving the range of target sequence specificity, by using

DOI: 10.18609/cgti.2020.060

orthogonal Cas9s or Cas9 homologues from different species and reducing the size of CAS9 to make it smaller, so it can actually be packed into an AAV, for example.

I was recently asked whether we will still be using CRISPR Cas9 to engineer the human genome 5–10 years from now, or some other modality. Clearly the answer is that we don't know. All I know is CRISPR Cas9 works – with all the precautions with regards to off-target events and delivery – and it's easy. You order Cas9 protein and order your guide RNA online, and they get delivered in a week. You mix those two together, then transfect your cells, and voila — it works in 2 out of 3 cases. I do this with undergrads; it's really amazing.

> "...we still don't have the right animal model to predict whether ... modified cells and their derivatives will be accepted..."

Q How do you see the application of gene editing evolving in the therapeutic realm, particularly as a key enabler of allogeneic cell therapy?

TM: This is the 'holy grail' to drive down the costs of cell therapy. It seems that almost every cell therapy company out there is interested in adopting a technology to prevent graft rejection, so that a cell product can readily be shared and administered to a larger pool of patients, and thus also create greater revenue.

Current approaches predominantly focus on two strategies: immune evasion and tolerance induction. Immune evasion aims to prevent the expression of human leukocyte antigens (HLA), which form the major histocompatibility barrier to transplantation. HLAs come in two flavors, HLA class I and class II. They are highly polymorphic surface molecules that provide cells with a molecular barcode that allows the immune system to distinguish self from non-self. In the setting of cell therapy, the HLA proteins form the major barrier to cell transplantation. We and other groups have already successfully applied genome editing to prevent surface trafficking of HLA class I by targeting β2microglobulin, an essential co-factor of class I surface expression, or by directly targeting the polymorphic HLA class I genes. HLA class II expression, which is also important for graft rejection, can be abolished by targeting its transcriptional master regulator, Class II Major Histocompatibility Complex Transactivator (CIITA).

The second strategy, tolerance induction, borrows heavily from pregnancy, where a hemi-allogeneic fetus that is only 50% identical to the mother is tolerated by the mother's immune system for 9 months instead of being rejected. We still don't fully understand this immunological paradox, formulated for the first time by Sir Peter Medawar in the 1950s, but through the work of multiple groups of immunologists, we have an idea of the factors which can inhibit the activity of immune cells that contribute to graft rejection. For example, PD-L1 which inhibits effector T cells, HLA-G which is normally only expressed by fetal trophoblast and keeps natural killer (NK) cells in check, and CD47, which as a 'don't eat me' signal for macrophages represents another immune checkpoint inhibitor.

"...almost every cell therapy company out there is interested in adopting a technology to prevent graft rejection, so that a cell product can readily be shared and administered..."

The idea is to reverse engineer trophoblast cells that have a unique HLA make-up (they are HLA-A and -B negative, express low levels of HLA-E and C, and express high levels of HLA-G) and at the same time overexpress tolerogenic molecules that can inhibit the immune system and prevent graft rejection (HLA-G, PD-L1 and CD47). Another feature of trophoblasts is they also overexpress PD-L1 and CD47 highly.

We and others have recently published strategies to use genome editing to remove HLA from the surface of human pluripotent stem cells (hPSC), and also to ectopically express the aforementioned tolerogenic PD-L1, HLA-G and CD47 in hPSC. Collectively, we have demonstrated that genome editing works, and can accomplish the goal of taking down the HLA barrier. We also show resulting hypo-immunogenic cells are still pluripotent and can be differentiated into a variety of different cell types, such as vascular smooth muscle cells and endothelial cells. Sonja Schrepfer at UCSF showed they can also be differentiated in cardiomyocytes, and we have differentiated them into pancreatic beta cells in collaboration with Doug Melton's lab at Harvard, although this work is not yet published. More recently, Andras Nagy's group in Toronto has published a combination of 8 factors that could overcome the allobarrier even in the presence of MHC, which is the murine version of the human leukocyte antigens.

The jury is still out on which of these approaches, and in what form, will be translated to humans. Cancer has been cured a thousand times in mice, so the question is whether this will work in humans. The biggest challenge I see in the development of allogeneic cell therapies is that we still don't have the right animal model to predict whether these modified cells and their derivatives will be accepted and not rejected by a human immune system. The immune system is complex and incredibly alert. It's able to detect even the smallest differences and changes in the human proteome that can arise, for example, during prolonged culture and cellular transformation. Minor histocompatibility antigens and neoantigens that arise during transformation can trigger a delayed graft rejection due to antibody-mediated triggering of NK cells or the complement cascade. These are processes that are extremely difficult to model with current animal models, which underlines the need to develop models further.

Q What will be your chief goals and priorities for your work over the coming 2 years?

TM: I'm currently applying for funding to set up my own lab. So far, I am indebted to the generous support of my mentors Chad Cowan and Elliot Chaikof, who is head of the

DOI: 10.18609/cgti.2020.060

Department of Surgery here at BIDMC – and financial support from the Harvard Stem Cell Institute. They have provided a seed grant sponsoring our efforts to generate living blood vessels from hypoimmunogenic human pluripotent stem cells

Previously I also did a lot of genome editing in primary immune cells, but that work is unfortunately on hold until I get more funding and can hire people to enable this line of work. The idea is to reprogram immune cells into cellular medicines. One obvious application is cancer immunotherapy, where genome editing has already been used to prevent T cell exhaustion and to engineer T cells with new specificities. In the setting of autoimmunity, it is conceivable to use gene editing to improve T regulatory function, for example.

Genome editing has propelled the field of cell engineering to another level. It almost seems that the limit to cell therapy is not its technical feasibility any longer, but rather your imagination.

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The author declares that they have no conflicts of interest.*

Funding declaration: *The author received no financial support for the research, authorship and/or publication of this article.*

Article source: *Invited.*

Interview conducted: *Feb 11 2020;* ***Publication date:*** *May 4 2020.*

AFFILIATION

Torsten Meissner
Department of Surgery at Beth Israel Deaconess Medical Center, Boston, USA

CELL & GENE THERAPY INSIGHTS

TRENDS AND ADVANCES IN GENE THERAPY DELIVERY AND GENE EDITING

SPOTLIGHT

INTERVIEW

Precise and non-disruptive gene editing based on programmable nickases

MANUEL GONÇALVES is a molecular biologist focusing on the development of gene delivery and gene editing systems. After a post-graduation period in a gene therapy company, Dr Manuel Gonçalves was awarded a fellowship from the Portuguese Foundation for Science and Technology to perform his PhD research on the investigation of hybrid viral vector systems for the stable genetic modification of human cells. In 2015, Dr Gonçalves became associate professor at the Department of Cell and Chemical Biology of the Leiden University Medical Center. In this capacity, he supervises a team whose research interests are converting viral vectors into delivery agents of gene-editing tools, studying the impact of epigenetic mechanisms on the performance of different gene-editing tools and strategies, and improving gene-editing approaches by guiding specific DNA repair pathways after introducing into target cells programmable nucleases or 'nickases'. In this context, his team has pioneered the investigation of viral vectors as delivery vehicles of TALENs and CRISPR-Cas9 nucleases.

Cell & Gene Therapy Insights 2020; 6(4), 427–435

DOI: 10.18609/cgti.2020.051

Q You started life in gene therapy in the viral vector field – how would you describe the cutting edge in this field at the present time?

"The *in vivo* route to gene therapy requires the production of vast quantities of functional (transducing) viral vector particles at clinical grade. This remains a crucial challenge."

MG: The cutting edge is multifaceted – important early-stage research lines and clinical applications are diversifying at a steady pace. We see, for instance, important developments in the engineering of new adeno-associated viral (AAV) vectors as well as adenoviral (AdV) vectors on the basis of an increasing number of natural serotype isolates. In the case of AAV vectors, structure-guide capsid designing, directed evolution, and *in silico* ancestral capsid reconstruction approaches are further contributing to obtaining AAV capsid variants with attractive properties: for example, the potential for escaping pre-exiting immunity *in vivo* and altered cell tropisms. AAV products based on pseudo-typing technologies (i.e., packaging of standard recombinant AAV serotype-2 genomes in capsids from a different serotype) have achieved market approval on the basis of unambiguous therapeutic effects in patients with spinal muscular atrophy type I (AAV-9).

Furthermore, despite genotoxicity risks inherent to their uncontrollable chromosomal integration, self-inactivating lentiviral (LV) vectors have come of age as therapeutic agents. They are delivering actual gene therapies involving autologous transplantation of T cells or hematopoietic stem cells (HSCs) genetically modified *ex vivo*. The range of LV vector-treatable conditions is steadily increasing, from primary immunodeficiencies (e.g., SCID-X1 and ADA-SCID) to certain metabolic disorders and, more recently, beta-thalassemia. Concerning the latter disorder, an advanced therapy medicinal product (ATMP) based on a LV vector expressing a modified beta-globin protein has been registered recently for treating transfusion-dependent beta thalassemia patients. Continuing in the realm of autologous transplantation of retrovirally-modified cells, we must also note the remarkable results obtained by CD19-targeted chimeric antigen receptor (CAR) T-cell therapies, with two ATMPs registered so far.

Finally, adenoviral vectors – especially those based on adenoviruses with a low seroprevalence in the human population – are at the forefront of clinical trials aimed at ailments that require short-term transgene expression, in particular cancer and infectious diseases (such as Ebola hemorrhagic fever and AIDS).

After a roller-coaster of hype and disappointment, gene therapy has matured, backed by solid science coming from both academia and industry. It is now a field that not only delivers genes, which is in itself a tremendous basic research asset, but has started delivering relief from suffering and even early death in some cases. That is inspiring to say the least! And in the whole trajectory of gene therapy, from conception to realization, viral vectors have been and are expected to remain at center-stage.

DOI: 10.18609/cgti.2020.051

"I see the field moving naturally along the current 'specialization' trend in which, based on their specific characteristics, AAV vectors and LV vectors will be applied in *in vivo* and *ex vivo* gene therapy protocols directed at tackling conditions requiring long-term or permanent transgene expression."

Q Where do you see the field moving next, particularly in terms of addressing remaining issues?

MG: It is sensible to bear in mind that as new advancements and technologies surpass specific problems, they will almost inevitably create new ones. The new problems may be less critical and different in nature than those associated with previous technologies, but they should be challenging, nonetheless. Aside from scientific and technological issues, regulatory and ethical aspects will come to the fore more frequently as genome modification technologies become more powerful and hence more consequential to stakeholders and society at large.

The upscaling capabilities and bioprocessing systems needed to generate clinical grade products are still not up to the task in terms of generating the amounts that are required for the world's clinical trials. Production is one bottleneck that still requires substantial optimization, so that these platforms can be applied in conditions that are not just restricted to a few patients.

In the short term, I see the field moving naturally along the current 'specialization' trend in which, based on their specific characteristics, AAV vectors and LV vectors will be respectively applied in *in vivo* and *ex vivo* gene therapy protocols directed at tackling conditions requiring long-term or permanent transgene expression. On the other hand, current adenoviral vectors seem most promising as vaccine and oncolytic agents.

The *ex vivo* gene therapy modality is set to profit from improved transduction protocols that better retain the stemness of HSCs, as well as from ongoing advances in milder conditioning regimens needed to create 'space' in the bone marrow niche for HSC engraftment. Less tractable issues, at least in the short-term, seem to be the development of methodologies permitting the *in vitro* expansion of *bona fide* HSCs or their derivation from induced pluripotent stem cells through directed differentiation. In addition, as the field works towards bypassing the complexity inherent to *ex vivo* protocols, I also see the emergence of HSC-targeted viral vectors that will allow testing the *in situ* genetic modification of HSCs. In this regard, LVs with pseudo-typed envelopes encoding therapeutic genes or AdV vectors fully deleted of viral genes (a.k.a. 'gutless' or high-capacity AdV vectors) encoding programmable nucleases are well-positioned candidates.

The *in vivo* route to gene therapy requires the production of vast quantities of functional (transducing) viral vector particles at clinical grade. This remains a crucial challenge. Among the three main viral vector platforms, large-scale production of AAV and LV vectors is more challenging when compared to the production of AdV vectors. Largely, this stems from the biology of adenoviruses – in particular, their very high vector genome replicon yields per producer-cell nucleus and high infectious-empty particle ratios. Traditionally, AAV and LV vectors have been generated through difficult-to-upscale transient transfection methods because the establishment of effective stable packaging cell lines has been challenging. Therefore, I believe that the ongoing efforts to setup and validate transfection-free production systems, either based on new-generation packaging cell lines or on transduction of producer cells with a heterologous viral vector containing vector and packaging constructs, is highly important. Finally, once administered, viral vectors have to face the double-edged sword that is the patient's immune system, comprising innate and adaptive arms. For this reason, I expect an increase in fundamental research directed at dissecting, for each target disease, the complex interactions between the innate and adaptive immune systems of patients and the gene therapy components, i.e., viral vector particles and transgene products. These insights are expected to guide the design of improved viral vectors and the development of safer and more efficacious *in vivo* gene therapy protocols.

Q Going into more depth on your current R&D activities, firstly, what are the chief considerations when converting viral vectors into delivery agents of gene editing tools?

MG: Viral vectors have an important dual role to play in the emergent field of gene editing, as they can be tailored for introducing into human cells programmable DNA cleaving enzymes as well as exogenous (donor) DNA containing the edits of interest. In the current viral vector toolbox, episomal viral vector systems are clearly preferable over their chromosomally integrating counterparts in that gene editing tools, such as zinc-finger nucleases (ZFNs), transcription activator-like effector (TALE) nucleases (TALENs), RNA-guided CRISPR nucleases (RGNs) and their derivatives, should best operate in an 'hit-and-run' fashion. That is, cleave the chromosomal DNA at the intended position and vanish shortly thereafter to minimize potential off-target activities. Clearly this is more easily and naturally accomplished in dividing target cells. Therefore, integrase-defective LV vectors, AAV vectors and AdV vectors are appealing scaffolds to build upon for gene editing purposes. This research is to some extent guided by the know-how gathered from their construction and application in 'classic' gene therapy contexts.

The considerations regarding the adaptation of viral vectors into agents for gene-editing tool delivery are manifold. Firstly, it is important to know whether these agents

"...in the whole trajectory of gene therapy, from conception to realization, viral vectors have been and are expected to remain at center-stage."

DOI: 10.18609/cgti.2020.051

are capable of introducing the gene editing tools into the nucleus of human cells in an efficient and intact manner. By gene editing tools, I mean not only the programmable DNA-targeting enzymes designed to cleave chromosomal DNA at specific positions, but also exogenous donor substrates designed to repair the targeted chromosomal lesion and, in doing so, install a specific genetic change amongst the 6.4 billion base pairs that make up a human genome.

"In the current viral vector toolbox, episomal viral vector systems are clearly preferable over their chromosomally integrating counterparts..."

Secondly, once in the nucleus of target cells, it is important to determine the specificity and fidelity of the gene editing process attained by each vector type. In particular, it is crucial to determine within target cell populations the frequencies with which the donor DNA becomes integrated at the intended target site versus at off-target sites. In addition to investigate the specificity of exogenous DNA insertion, it is equally important to determine whether these insertions take place in an accurate fashion at the target site or, instead, include genome-disrupting by-products, such as concatemers, vector backbone sequences, or small insertions and deletions (indels) at the junctions between endogenous and exogenous DNA. Regarding these aspects, it is becoming clear from our research and that of others that the structure of viral vector genomes, and therefore of the embedded donor DNA templates, can have a profound impact on the ultimate efficiency, specificity, and accuracy of gene editing.

Q What are the pros and cons of the various options available in terms of gene editing platforms? Why have you chosen the particular approaches that you have over the alternatives?

MG: Following up on my previous answer, our team together with collaborators has found that adenoviral vectors can package and deliver intact TALEN-encoding transgenes that go on to express functional TALEN proteins in human cells, e.g., primary mesenchymal stromal cells and, more recently, $CD34^+$ cells. In striking contrast, the transduction of the same transgenes by conventional and integrase-defective LV vectors leads to severe TALEN disruptions, consisting of extensive deletions within the repetitive amino acid sequences that make-up their DNA-binding domains. These rearrangements are likely to be caused by frequent reverse transcriptase template switching events taking place within the regions coding for the TALE repeats. Currently, TALENs can be introduced into target cells by LV vectors but not without substantial recoding and optimization of the respective expression units in order to substantially minimize the extent of repeat homology.

Moreover, earlier research had informed us that integrase-defective LV vectors express low amounts of transgene products, including ZFNs, due to their high susceptibility to epigenetic silencing mechanisms in transduced cells in which cellular histone deacetylases play a role. On the basis of these cumulative data and on the large packaging capacity of AdV capsids,

"...we are also interested in investigating the feasibility and utility of deploying different types of viral vector systems for introducing donor DNA into human cells."

we went on to demonstrate that the AdV vector platform is suitable for the co-delivery of *S. pyogenes* Cas9 nucleases and single guide RNAs into human cells.

In addition to the delivery of programmable nucleases, we are also interested in investigating the feasibility and utility of deploying different types of viral vector systems for introducing donor DNA into human cells. Related to this aspect and as I mentioned earlier, the structure of viral vector genomes can have a profound impact on the efficiency, specificity, and accuracy of gene editing procedures aiming at targeted gene addition. In this regard, it is of note that AAV, integrase-defective LV and AdV vector genomes reach target cell nuclei as linear single-stranded, linear double-stranded, and linear protein-capped double-stranded DNA templates, respectively.

We have found that the introduction of donor DNA into target cell nuclei in the context of integrase-defective LV vector genomes can result in efficient targeted gene knock-in in human cells exposed to tailored programmable nucleases (i.e., TALENs and RGNs). However, molecular analysis of a large panel of stably transduced cells revealed that substantial amounts of genome-modifying events were the result of random and inaccurate targeted donor DNA insertions. Interestingly, when compared to the integrase-defective LV platform, donor DNA delivery in the context of naturally protein-capped AdV vector genomes led to lower frequencies of targeted gene addition. And yet, the vast majority of genome-modifying events occurred at the proper location and in an accurate fashion. Complementary experiments in which the donor DNA in AdV genomes was flanked by the programmable nuclease target site, to assure its release from the context of protein-capped DNA, led to a significant increase in the frequency of random DNA insertions. These data suggest that linear free-ended donor DNA is more likely to be 'captured' at chromosomal breaks than donor DNA 'shielded' in protein-capped AdV vector genomes.

Q What, for you, are the next steps in genome editing platform development?

MG: Looking in particular at CRISPR-based systems, which clearly have taken the field by storm, they are moving at a very fast pace with the latest generation platforms presenting improved specificities or expanded genomic target coverage. These new tools are emerging through the application of powerful rational design and directed evolution protein engineering approaches, or the mining of vast metagenomic databases from which new CRISPR systems have been unearthed, some going on to be successfully adapted for biotechnological purposes. In parallel with this growing diversification of RGN platforms, perhaps a bit ironically, I also believe that the original *S. pyogenes* CRISPR-Cas9 system still has very high

DOI: 10.18609/cgti.2020.051

potential with a lot of room for improvement warranting further research and development.

In our own research, we are very much interested in further exploring the capabilities of this initial CRISPR system and in tweaking it so that we can optimize gene editing outcomes, including the efficiency, specificity, and accuracy of the gene editing process as a whole. In this context, one of our key aims is to explore the features of Cas9 nickases in order to move away from nucleases and achieve seamless and scarless gene editing so that, in the longer-term, one can hopefully translate these research findings into genetic therapies. This DSB-free gene editing research line is to some extent convergent with that of others on the development of base editors and, more recently, primer editors in which Cas9 nickase scaffolds are fused to heterologous effector moieties (in particular, deaminases and reverse transcriptases, respectively).

"...one of our key aims is to explore the features of Cas9 nickases in order to move away from nucleases and achieve seamless and scarless gene editing."

The impetus for this research stems from the very nature of programmable nucleases in general, be they TALENs, ZFNs, or CRISPR-based nucleases, as by generating double-strand DNA breaks (DSBs), these tools are intrinsically disruptive to the genome, regardless of whether the resulting DSBs take place at target or off-target sequences. Chromosomal DSBs can in fact cause a plethora of collateral by-products, some of them quite insidious – examples include mutagenesis at the other allele of a target gene that one does not want to disrupt, installation of large structural variants, genome-wide translocations and, upon cleavage at both alleles in homologous chromosomes, generation of unstable dicentric chromosomes. In addition to these genome-level events, at the cellular level, DSBs (whether targeted or otherwise) can trigger cell cycle arrest and apoptosis.

For these reasons, we are investigating ways to improve the precision and predictability of gene editing by investigating genetic manipulation approaches based on the formation of targeted single-strand DNA breaks (SSBs) by programmable nickases.

However, chromosomal SSBs are not per se very potent stimuli for the activation of the homology-directed DNA repair (HDR) pathway commonly used for precise targeted gene addition (knock-in). For example, if one wants to perform gene knock-ins by recruiting the HDR pathway after site-specific SSB formation, the efficiency is normally extremely low. Interestingly, we found that if one coordinates nicking at the genomic target position with nicking of donor DNA templates, the efficiency of the SSB-induced gene editing process is substantially increased, with the advantage that, for the most part, one is not disrupting the other allele or triggering collateral effects in the form of translocations or other structural variants. We have dubbed this precise and non-disruptive gene editing strategy in trans paired nicking.

Q In the long-term, how great a role will gene editing play in the therapeutic sphere?

MG: It goes without saying that gene editing is already having a major impact in basic research. Following on the tracks of gene therapy, it is my belief that with continuing improvements in the delivery of gene editing tools on the one hand, and in the precision of gene editing processes on the other, we will start seeing these technologies permeating the therapeutic sphere in the form of ATMPs directed at treating a growing number of diseases.

Historically, it was said that in gene therapy the three most important parameters to consider were delivery, delivery, and delivery. However, in the case of gene editing, somewhat similarly to real estate activities, these parameters need to be coupled to three others: location, location, and location! In particular, one has to make sure that, once in the cell, a specific gene editing reagent generates a break or a nick at the right place and that the subsequent chromosomal insertion of the exogenous genetic information is done in a high-fidelity fashion. Indeed, an often neglected aspect when assessing gene editing outcomes is that even when one finds the exogenous DNA at the target location, frequently, this DNA is incorporated in an inaccurate manner due to, for instance, the prior involvement of non-homologous recombination processes. Thus, delivery, location and fidelity are all crucial gene editing parameters that need to be carefully assessed and brought together.

Q You are also engaged in studying the impact of epigenetic mechanisms on the performance of different gene-editing tools and strategies – can you tell us more about what drives this particular field of study, and your findings from it to date?

MG: Perhaps unsurprisingly, we found that target DNA cleavage by both TALENs and RGNs is most frequent at euchromatin than at heterochromatin. More interesting was the realization that, between these two programmable nuclease platforms, RGNs based on the *S. pyogenes* CRISPR-Cas9 system appear to be the least hindered by the compact nature of heterochromatin. This is despite the fact that, in contrast to TALENs, native *S. pyogenes* RGNs did not evolve to assess and cut genomic DNA in eukaryotic cell nuclei.

In my opinion, a relevant follow-up question is whether the off-target nuclease activity profiles of specific programmable nuclease reagents change according to the epigenomic characteristics of different cell types or cell differentiation stages.

In subsequent research, we sought to investigate whether alternative higher-order chromatin conformations influence the proportions between wanted and unwanted gene editing outcomes resulting from HDR and non-homologous end joining (NHEJ) pathways, respectively. We found that wanted HDR events increase in relation to mutagenic

"...delivery, location and fidelity are all crucial gene editing parameters that need to be carefully assessed and brought together."

 DOI: 10.18609/cgti.2020.051

NHEJ events when euchromatic target sites transit to a closed heterochromatic status. The degree of this relative increase in wanted gene editing outcomes at heterochromatin seems, to some extent, dependent on whether the donor DNA is of viral, non-viral or synthetic origins.

Q Finally, regarding the application of the toolboxes you are developing: where will they have the greatest utility, and what are your current and planned future activities?

MG: We have two main research avenues. One is to investigate the performance of different viral vector delivery platforms to introduce gene editing tools into human cells. Another, as I discussed earlier, is to investigate the utility of nickases in bringing about non-disruptive and high-fidelity gene editing.

Through this research, adenoviral vectors were identified as a robust platform for the delivery of various gene editing reagents, and it was further determined their amenability to achieve highly specific and accurate targeted chromosomal insertion of exogenous donor DNA. More recently, together with collaborators, our group has demonstrated that in trans paired nicking expands gene editing to chromosomal regions that were previously impossible to modify seamlessly using nucleases (due to their recurrence in the genome or essentiality for cell function).

Looking ahead, we hope to integrate the two previously mentioned research lines in order to test the performance of seamless gene editing strategies in human cells. This research aims at establishing well-defined 'disease-in-a-dish' cellular models using induced pluripotent stem cells and testing *ex vivo* gene repair protocols in adult stem/progenitor cells.

We are particularly interested in directing our research efforts to the investigation of genetic therapies for Duchenne muscular dystrophy and haemoglobinopathies. Therefore, we focus on muscle progenitor cells and HSCs as target cells. Crucially, this research is done in close collaboration with colleagues and research groups from our institute and elsewhere.

AFFILIATION

Manuel Gonçalves
Department of Cell and Chemical Biology, Leiden University Medical Center, Netherlands

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The author declares that they have no conflicts of interest.*

Funding declaration: *The author received no financial support for the research, authorship and/or publication of this article.*

Article source: *Invited.*

Interview conducted: *Feb 14 2020;*

Publication date: *Apr 23 2020.*

CELL & GENE THERAPY INSIGHTS

TRENDS & ADVANCES IN GENE THERAPY DELIVERY & GENE EDITING

SPOTLIGHT

INTERVIEW

Next steps in targeting the CNS with AAV-driven gene therapy

BRUCE GOLDSMITH is Passage Bio's CEO and President, and he also serves on its Board of Directors. Dr Goldsmith has extensive experience in the biopharma and biotechnology industries through the many roles he has held across the research and corporate spectrum. Prior to Passage, Dr Goldsmith was a Venture Partner at Deerfield Ventures and Interim CEO of Civetta Therapeutics.

Cell & Gene Therapy Insights 2020; 6(4), 577–582

DOI: 10.18609/cgti.2020.067

Q Can you tell me what you're working on right now?

BG: As I look to 2020 and the near-term, one of my main focuses is on advancing our pipeline with multiple IND filings. We are approaching our first this quarter, and then we have two more filings in the second half of this year.

Our other focus is on building the company. It's a relatively simple mandate, but there are many, many aspects to it. Building the company includes thinking about the culture we have already established – how we operate, what we focus on, and our primary mission.

In fact, when it comes to our primary mission – which has been set by our founder, Jim Wilson, as well as Tachi Yamada and Steve Squinto – we've been focusing on the needs of the

"...we think about the interplay between the delivery, the promoter, the transgene, and capsid, to essentially optimize what we hope is a differentiated therapeutic benefit."

patients we hope to serve once we advance our pipeline into the clinic and beyond. That focus continues to resonate throughout in terms of how we are advancing and building the company. Additionally, we've been drawing inspiration from our relationship with the University of Pennsylvania, which is fundamental both to the formation of the company and to our drive forward.

Moreover, we will continue throughout this year to hire people who have expertise in clinical development and manufacturing, and we are building our clinical and manufacturing capabilities in line with what the University of Pennsylvania Gene Therapy Program (GTP) is offering. Of course, because of the COVID-19 crisis, we're obviously very much concerned about how we maintain the health and safety of our employees as we move the organization forward.

Q Gene therapy for rare central nervous system (CNS) diseases represents something of a departure for you in terms of your biopharma career to date – how great an adjustment has it been?

BG: Backing up a moment, the thing that really attracted me to Passage Bio was compelling science as a foundation for drug development. This has been a theme throughout my career, whether that was at Johnson and Johnson, Allos Therapeutics, Lycera, or even the work I did at Deerfield Management. It was all driven by drug development and compelling science, and this is no exception.

The roles I played in those companies required a deep understanding of the disease states we were trying to address. Whether it was a rare disease in oncology like peripheral T-cell lymphoma, or autoimmune disease, or very complex mechanisms of action, trying to drive forward from a basic science perspective has been a key aspect of my career. The compelling science and the link to understanding disease states were absolutely critical. I think this comes back to my interactions with clinicians, basic scientists, and patients with extreme unmet medical needs, combined with a number of rare disease experiences throughout my career.

So yes, this is something of a departure for me, as I've done mostly small molecule development. But my career has always been about drawing on my background as a scientist. As it happens, my PhD is from the Department of Biology at University of Pennsylvania with a concentration in neuroscience. So, it's fantastic to have the opportunity to circle back to this area, and to collaborate with Dr Jim Wilson and his amazing team.

The other thing I've done throughout my career, when I have had gaps in particular technology knowledge, is to ensure I am working with excellent people and can draw on their knowledge. I pride myself on my ability to absorb knowledge and to ask detailed questions.

DOI: 10.18609/cgti.2020.067

Therefore, the focus on science, on patients, and on the clinical and commercial aspects are still very much within my wheelhouse.

Q Going into more depth on Passage Bio's approach, what specific advantages can next-gen AAV vectors offer?

BG: Our general approach is to choose the appropriate vector to solve the specific scientific and clinical issues that are arising. While next-generation vectors are a component of that – in fact, we're using a next-generation vector for two of the initial indications that we are pursuing: GM1 gangliosidosis and Krabbe disease – the third program utilizes AAV1, which was initially discovered some time ago.

The way we think about next-generation vectors throughout the pipeline is all about optimization rather than wholesale use. So, while they do have advantages, we think about the interplay between the delivery, the promoter, the transgene and capsid, to essentially optimize what we hope is a differentiated therapeutic benefit. One example is that we de-risk and differentiate based on investment in thinking about capsid tropism – it may be that a next-generation capsid has really unique tropism that optimizes one aspect of the intended target profile. However, we don't think of this in isolation – instead, we look at it as a whole. In other words, where appropriate, we use next-generation vectors, and where a more traditional approach may work, we will use that. Our partnership with the University of Pennsylvania GTP allows risk mitigation through finding the right AAV and approach for the indication that we're pursuing.

Q How are you optimizing the engineering of your vectors as development continues towards use in the clinic?

BG: The relationship with the University of Pennsylvania's GTP is absolutely integral to our efforts, and they are performing all of the research to enable IND submission. We work hand-in-hand with them throughout this process. It's a very collaborative effort. But I want to emphasize that the GTP does all the de-risking and optimization, including the initial de-risking from a manufacturing and engineering perspective, as well.

The other component is our strategic relationship with Catalent's Paragon Bioservices, a specialty contract development and manufacturing organization, to also help remove potential manufacturing hurdles.

Therefore, if the vector we choose – as well as the transgene promoter, and so on – is optimized for our desired outcomes, but we can't manufacture it, then we will invest time and effort via our collaboration with Penn to optimize those engineering issues.

Q Turning to delivery methods and routes to the CNS and to the brain in particular, can you tell us more about Passage Bio's approach? Why do you consider it to hold promise for improved safety and efficacy in what is such a difficult target tissue?

BG: The difficulty of the target tissue really has to do with the need to address CNS diseases through either high systemic delivery, intraventricular delivery, or, in our case, intra-cisterna magna delivery (ICM).

And when thinking about delivery – again, with the benefit of the University of Pennsylvania's and Jim Wilson's extensive work in this field behind us – we have looked at all the studies on the gene distribution post-ICM versus other approaches, as well as safety. ICM has been used for many years and has been shown, especially with the use of guided fluoroscopy and MRI, to be quite a safe procedure that bypasses the blood–brain barrier effectively.

In our view, delivery to the cerebrospinal fluid through ICM injections really does provide the best access for the capsids to transduce the target cells. It also avoids, for example, the high AAV delivery that may be needed using a systemic approach, which could lead to other side effects. In general, we and others believe that the CNS space is immune privileged. The potential for neutralizing antibodies may be lower, and this may increase the overall safety and efficacy of the delivery of the drug.

Because of these reasons, we believe that ICM delivery is an efficient and appropriate way to move forward due to its significant advantages. This is something we've studied extensively using various animal models in an effort to assess any risks and to ensure that this is, indeed, an optimized method of gene delivery.

Q Can you tell us a little bit about the disease you've selected for your lead indication for clinical development?

BG: The lead indication for which we will file an IND in this quarter is GM1 gangliosidosis, a recessive lysosomal storage disease that results in very severe damage to the CNS, as well as selected peripheral tissues.

GM1 presents as a spectrum of disease, with the most severe and common type being early infantile GM1. Unfortunately, this is a disease with rapid progression and a life expectancy of only 2 to 4 years. Symptoms include reduced muscle tone and progressive CNS dysfunction, and infants with this disease require significant supportive care very early in life. The reason we focused on infantile GM1, in particular, is because this is a very homogenous population of infants. We hope to get the most impact and benefit for these patients by early intervention. Additionally, from a clinical endpoint perspective, we believe this is the ideal population to study and to define whether we are actually seeing benefits.

"...in the context of COVID-19 ... we are considering what safety measures will be needed for patients and their families..."

We will be studying biomarkers for beta galactosidase gene delivery, as well as very early clinical endpoints. The reason why we, together with Penn, chose GM1 was based on this current patient and treatment landscape. We believe that while there may be other

 DOI: 10.18609/cgti.2020.067

> "...delivery to the cerebrospinal fluid through ICM injections really does provide the best access for the capsids to transduce the target cells. It also avoids, for example, the high AAV delivery that may be needed using a systemic approach, which could lead to other side effects. In general, we and others believe that the CNS space is immune privileged."

treatments potentially advancing in the pipeline, our approach can offer significant advantages and differentiation.

When we think about selecting any indication, we focus on developing therapies in diseases not only where there are high unmet medical needs, but also where we believe we can have a transformational impact differentiated from any other therapies that are also advancing.

Q What clinical challenges do you expect to face as you approach this first-in-human study, and how are you preparing to meet them?

BG: This is going to be Passage's first clinical trial. I've been in groups that have launched first clinical studies before, and it's always a challenge. However, the one difference is that the organization, as a whole, is new. The reason why we've recruited the management team we have – either through manufacturing in the case of Alex Fotopoulos, or through clinical with Gary Romano – is because they and their respective teams all have had significant leadership experience with trial start-up and operations, whether it's delivering the drug to site, or writing clinical protocols and contracting with the sites to move them forward.

We know it's going to be a challenge, but we also feel that the team we've brought together has been fantastic in terms of preparation. I will note that we're also working incredibly closely with the University of Pennsylvania throughout this process because Penn is essentially responsible for the IND. We're working with them in a seamless fashion to ensure that all of the work is aligned and synergistic.

We also recognize that in rare orphan diseases, patient recruitment is always a challenge. In the era of COVID-19, of course, we face another rather exceptional challenge. Regardless, we're continuing to work with advocacy groups, such as Cure GM1 and the National Tay-Sachs & Allied Diseases Association, to try to identify patients.

We're working right now with sites to prepare for logistics, and with Catalent to address the manufacturing needs. As this is in the context of COVID-19, we're also very focused on making sure we have enough lead time, and we are considering what safety measures will be

needed for patients and their families, as well as the physicians who will be treating them. It's an extremely challenging situation to be faced with, but it's also an opportunity for us to succeed in the face of an unprecedented obstacle.

Q Can you summarize what the next 12–24 months hold in store for you and Passage Bio?

BG: Building on what I talked about earlier, the priorities are the IND filings and clinical trial launches. We have three INDs planned for submission this year for GM1, frontotemporal dementia (FTD), and Krabbe disease. These trials will start within the next 12–24 months. GM1 is planned to have the first clinical initiation this year, and FTD and Krabbe are planned to have their trial initiation in the first half of 2021.

We will also progress three additional preclinical programs in our pipeline that we've already licensed from the University of Pennsylvania. In fact, we have an additional six programs we can bring in through our collaboration with Jim Wilson and the GTP, which will build out the overall pipeline for Passage Bio to 17 programs. That will occur between now and 2025.

The year 2021 is going to be a data-rich year. It will showcase not only the initial proof of concept for Passage's programs, but also its relationship with the University of Pennsylvania. We're really looking forward to transforming ourselves from a research-stage company into a clinical-stage company, and delivering what we hope will be effective translational therapies to the initial patients we will be treating.

AFFILIATION

Bruce Goldsmith
CEO, Passage Bio

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The author declares that they have no conflicts of interest.*

Funding declaration: *The author received no financial support for the research, authorship and/or publication of this article.*

Article source: *Invited.*

Interview conducted: *Apr 17 2020;* ***Publication date:*** *May 14 2020.*

DOI: 10.18609/cgti.2020.067

CELL & GENE THERAPY INSIGHTS

May 2020

Innovation Insights

CELL & GENE THERAPY INSIGHTS

Innovation Insights

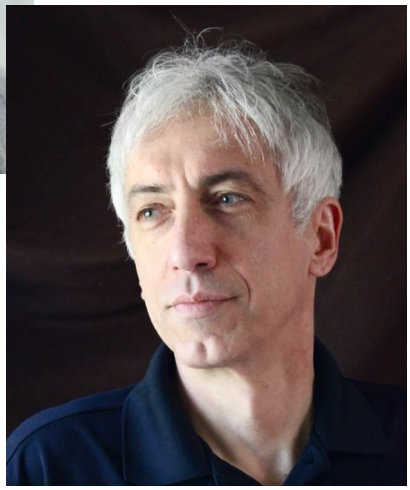
-

May 2020
Volume 6, Issue 4

CELL & GENE THERAPY INSIGHTS

Innovation Insights

-



Exosome-based therapeutics: ready for prime time

EDITORIAL

Jonathan Finn PhD & Konstantin Konstantinov PhD

INTRODUCTION

Efficient delivery of bioactive molecules to target cells is critical for drug development. This is especially true for next-gen therapeutics, such as gene therapy or gene editing, where large, complex molecules and/or genetic information needs to be delivered to the right location in a specific cell. Decades of investment into understanding how

Cell & Gene Therapy Insights 2020; 6(4), 597–605

DOI: 10.18609/cgti.2020.070

endogenous delivery systems (e.g., viruses) work has led to the development of numerous classes of viral vectors that are currently revolutionizing gene therapy, such as lentivirus and AAV-based therapeutics. Unfortunately, these virus-based delivery systems face many of the same challenges as their parental viruses, namely, they induce robust immune responses that severely limit their application, both in patients that have pre-existing neutralizing antibodies due to prior exposure or in the inability to re-dose after the first therapeutic exposure.

In a similar way to how humans have harnessed millions of years of evolution to develop viral-based vectors, exosomes represent a new delivery modality that has the potential to revolutionize drug delivery. Exosomes are nanoscale (30–200 nm) extracellular vesicles (EVs) that are released by cells across all kingdoms of life, with roles in both normal physiology and disease pathology. These endogenous nanoparticles are composed of lipids, proteins, nucleic acids and carbohydrates, and have a number of diverse functions including maintaining cellular homeostasis and intercellular communication. Exosomes have garnered much attention in recent years, with the number of published papers increasing exponentially over the past decade (>3000 in 2019 alone) (Figure 1), due in part to their exciting potential to specifically deliver a range of biologically active macromolecule cargos.

While there is much interest in translating exosomes to the clinic, progress in the field has been restrained by two major challenges:

1. Reliably engineering exosomes with defined, drug-like properties;
2. Manufacturing GMP exosomes reproducibly at a sufficient scale.

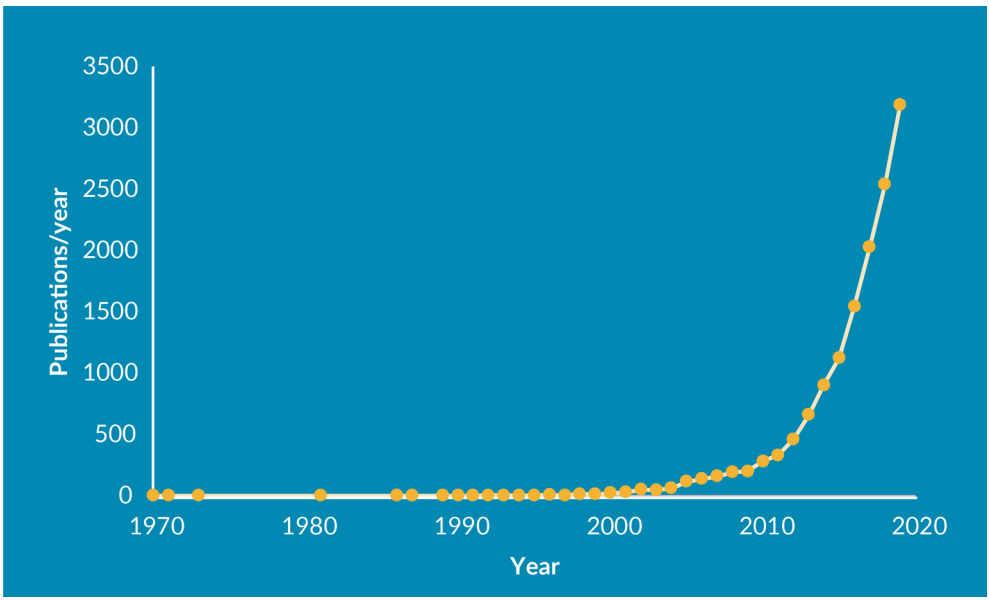
In this editorial we highlight advances made by Codiak BioSciences that address both of these challenges and describe how exosome-based therapeutics are now ready for clinical development.

PRECISION ENGINEERED EXOSOMES FOR SPECIFIC APPLICATIONS

Two distinct approaches are being taken to develop exosome therapeutics:

FIGURE 1

Exosome publications over time.



DOI: 10.18609/cgti.2020.070

1. Using natural exosomes from producer cells (usually derived from MSC's or stem-cell progenitor derived cells); or

2. Engineering exosomes with specific, drug like properties.

While there is merit to both approaches, for example, the use of stem cell derived exosomes requires less time and effort to conduct research-grade experimentation, the creation and use of rationally and intentionally designed exosomes with highly defined and reproducible properties and a known mechanism of action (MOA) is both a compelling alternative to working with naturally derived, highly heterogenous exosomes with poorly defined MOA and a more viable foundation for the advancement of important new drugs.

Over the past decades there have been a number of attempts to develop methods to genetically engineer exosomes by directing fusion proteins to the exosome surface or lumen using membrane targeting sequences or exosome enriched proteins (e.g., GPI motifs [1], lipid anchors [2], pDisplay [3], LAMP2B [4], CD9 [5], CD63 [6], or MFGE8 [7]). While these methods have helped advance our understanding of exosome biology and function, we found that they generally result in heterogenous expression with insufficient levels of biological activity for most therapeutic applications. Driven by a need for a robust exosome engineering platform, we set out to identify new proteins that could be used as 'scaffolds' to efficiently engineer exosomes with drug-like properties. We quickly appreciated that to accomplish this objective, we would need a method for generating an exosome preparation pure enough to ensure our analysis was based on exosomes and not contaminating impurities. In the exosome field at large, the most common method for exosome purification is based on serial ultracentrifugation steps. We found that this approach was not sufficient for the reproducible isolation of high purity exosomes as it co-purifies many non-exosomal proteins, membrane fragments, and nucleic acid. It was only through the incorporation of a density gradient that we were able to reproducibly make high purity exosomes, which we use as our reference standard (Figure 2). Using these highly purified exosomes and LC-MS based proteomics we identified novel scaffold proteins that are highly enriched in exosomes.

One scaffold we have identified, PTGFRN, is a type I transmembrane glycoprotein that is naturally found on the surface of exosomes; however, through overexpression we could increase its copy number from ~40 to over 5,000 copies per exosome. By molecular fusion to PTGFRN, we have been able to decorate the exosome surface with a wide range of molecules, including antibody fragments, cytokines, reporters, enzymes, receptors and vaccine antigens. Importantly, by fusing targeting ligands to PTGFRN, we are able to direct exosome tropism, having successfully targeted a number of cell types both *in vitro* and *in vivo*.

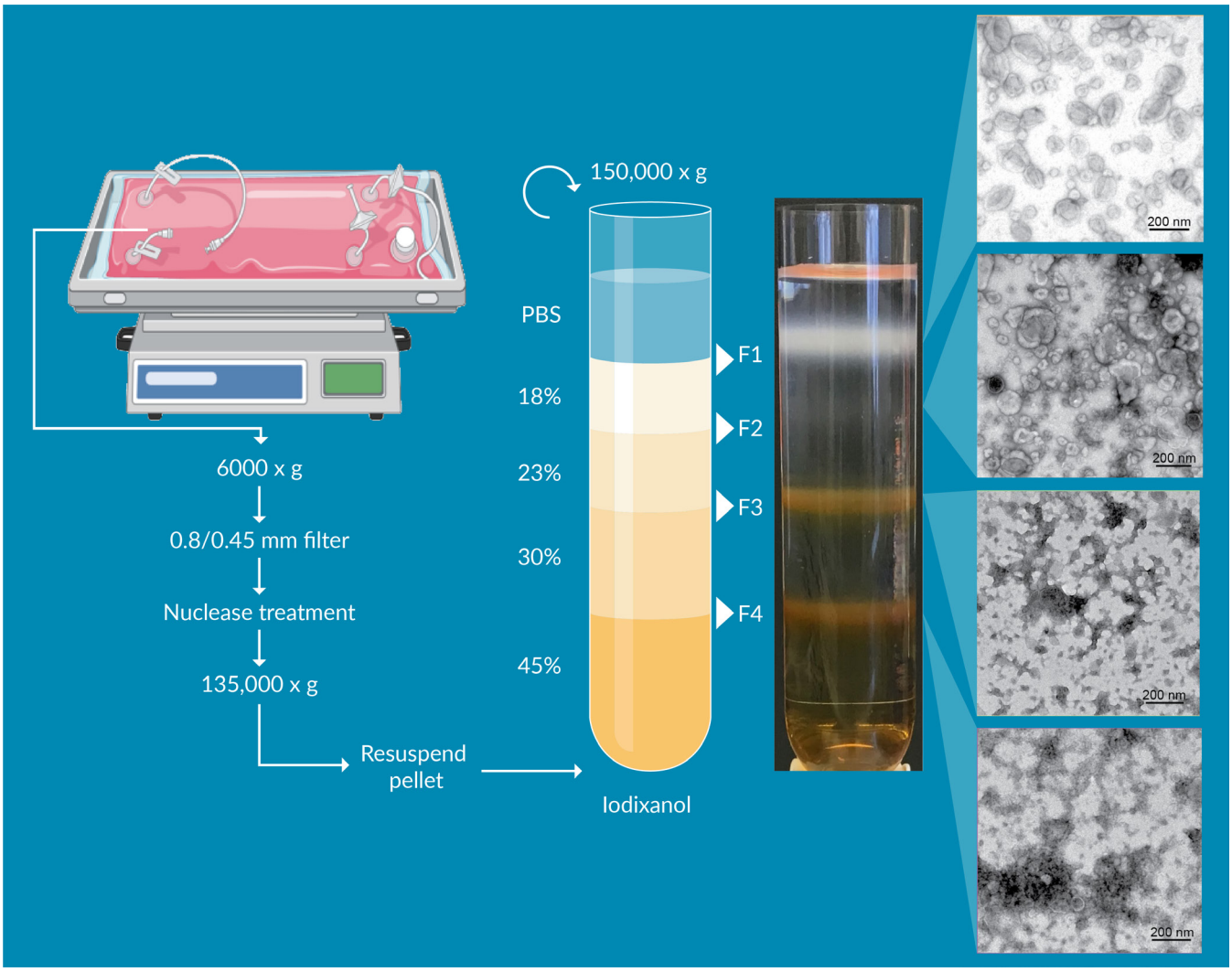
One of our lead programs, exoIL-12™, uses PTGFRN to decorate the exosome surface with IL-12, a very potent NK and T-cell stimulating cytokine. In doing so, we have been able to dramatically increase the potency (multi-log) compared with recombinant protein by increasing the local tumor residence time and decreasing the toxicity by reducing systemic exposure.

Similarly, BASP1, a member of the MARKS family, is able to direct fusion proteins to the exosome interior (lumen) with high efficiency. A wide range of proteins have been successfully loaded into exosomes using BASP1, including reporters, genome editing nucleases, vaccine antigens, affinity ligands, and even gene therapy vectors such as AAV.

In addition to using molecular biology to engineer exosomes and producer cell lines, we have also developed methods to exogenously load exosomes with therapeutically relevant payloads. One such example is our lead program, exoSTING™, where exosomes (engineered to target tumoral macrophages) are loaded with a small molecule cyclic dinucleotide (CDN) STING agonist. STING agonists have demonstrated potent tumor regression in several preclinical tumor models

FIGURE 2

Research scale exosome purification method.



Exosome producer cells are grown in suspension until the appropriate cell density has been reached. Supernatant is collected and residual cells and debris are removed by centrifugation and filtration, followed by a nuclease treatment to reduce viscosity and any residual genomic DNA. The supernatant is then subjected to a high speed ultracentrifugation to pellet the exosomes as well as other contaminating debris. Pellet is then resuspended and loaded onto iodixanol step gradient before another ultracentrifugation. Fraction 1 (F1) contains highly purified exosomes, while the other fractions are contaminated with protein, membrane fragments, and nucleic acid. *wavebag clip art was created with Biorender.com

and are currently being explored in the clinic, however the low tumor retention and poor membrane permeability of free STING agonist have limited their efficacy. We have found that association of STING agonist with exosomes dramatically improves the PK and PD properties and leads to >100-fold increased potency in *in vivo* tumor models. We are also exploring novel methods for the incorporation of small molecules, oligonucleotides (e.g., ASO, siRNA), and peptides into exosomes, such as chemical conjugation or membrane incorporation using hydrophobic moieties. We have had particular success loading exosomes with cholesterol-conjugated ASO molecules, being able to incorporate thousands of ASO molecules per exosome and achieve potent mRNA knockdown both *in vitro* and *in vivo*. Together with our ability to direct exosome tropism to various cell types by using targeting moieties, the incorporation of ASOs into exosomes targeted to specific cell types and tissues could significantly broaden the therapeutic utility of ASOs and siRNAs.

DOI: 10.18609/cgti.2020.070

Through the combination of cell engineering and exogenous loading, we are able to generate molecularly defined exosomes with known mechanisms of action. Collectively, we refer to these methods of generating precision exosomes as our 'engEx™' platform, and it has become the engine through which Codiak Biosciences is able to rapidly generate custom exosomes both for exploratory and therapeutic applications.

MANUFACTURING & ANALYTICAL PLATFORM

From the inception of the company, we realized that manufacturing was going to be critical for enabling clinical translation of our engEx exosomes. Traditionally, evolving advanced biology research into a technology for manufacturing of new therapeutic modalities has been a challenging and lengthy endeavor. Scientific concepts typically originate in academia, where only small amounts of material are required for research purposes. As the field matures and attracts industrial interest, the need for larger quantities of material to support pre-clinical and clinical activities often becomes a major impediment. While the direct scale up of the methods established in academia is often seen as the fastest path to addressing the demand for clinical material, such methods are not usually designed for scalability and compatibility with routine GMP manufacturing. Exosome biotherapeutics are a textbook example of this situation. Traditionally, small preparations of exosomes are generated using differential centrifugation – a method involving multiple centrifugation steps that separate exosomes from cells and other impurities [8]. This technology has been implemented in a wide variety of forms and has helped to enable discovery and early research. However, using differential centrifugation to produce large amounts of exosomes of high quality and purity, including GMP material for clinical trials or commercial supply, is not practical and, therefore, radically different

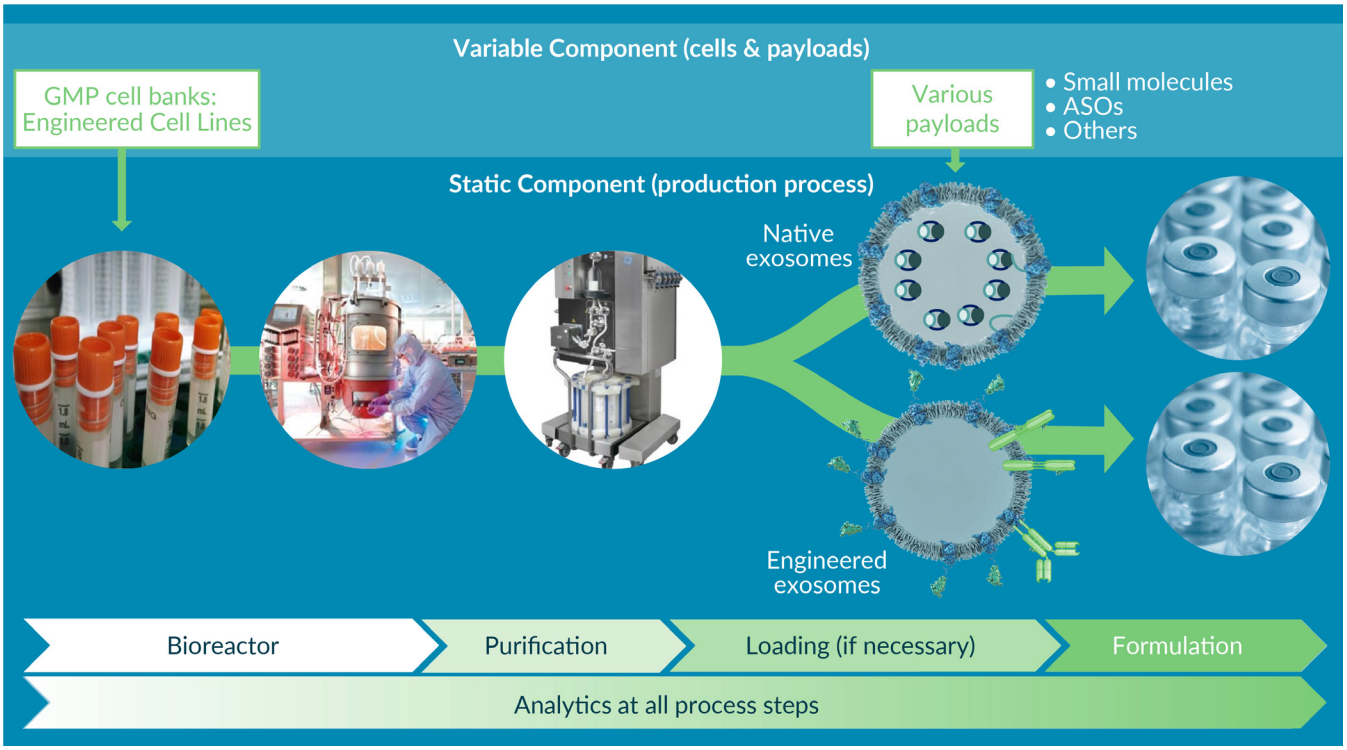
TABLE 1

Targeted characteristics of the exosome manufacturing technology.

Targeted characteristic	Justification and technical directions
High degree of scalability	The required amount of material may vary significantly according to the indication: exosome biotherapeutics might serve both large patient populations and orphan indications; doses may also vary significantly
Platform profile	The manufacturing and analytical technology should work with various engEX constructs without the need for major re-development
Compatibility with Contract Manufacturing Organization (CMO) infrastructure and GMP requirements	Most small- and mid-size companies need to manufacture toxicology and clinical material at CMOs. In such cases, the exosome manufacturing technology should be compatible with the existing CMO infrastructure. The production methods should comply with the established regulatory requirements
High productivity and process reproducibility	The manufacturing technology needs to use human cell lines (to mitigate immune response risks) with high exosome productivity and stable expression profile. High density 3D cell culture (preferably suspension) should be targeted
High exosome purity	Traditional exosome enrichment methods often yield high impurity levels. The active biological molecules associated with these impurities may complicate data interpretation, process reproducibility and patient safety. Development of alternative unit operations yielding high purity is required
No high-risk materials in the production process (e.g., serum or animal derived materials)	To reduce risks to patient safety, process contamination, and process variability, cell culture media, purification buffers, and other materials should be free of animal derived components
Robust portfolio of analytical methods	The complexity of exosomes demands advanced analytical methods covering various classes of attributes. The methods selected for GMP lot release and characterization need to be compatible with regulatory requirements in a phase-appropriate manner
Reasonable manufacturing cost	New modalities, such as cell and gene therapy, are often associated with high manufacturing cost. Cost considerations should be proactively built in the design of the large-scale manufacturing technology

FIGURE 3

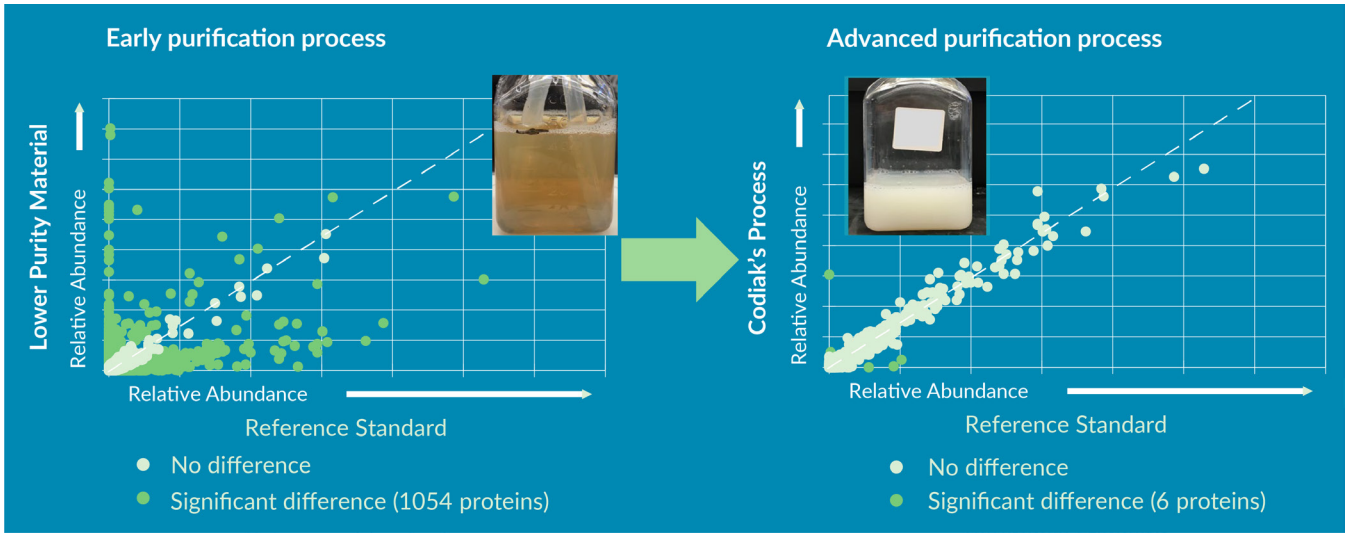
Flow chart of Codiak's exosome manufacturing process.



technical solutions are required. Until recently, progress in exosome production has been limited, with attention given to alternative small-scale purification methods, such as size exclusion chromatography and tangential flow filtration. Large scale cell culture technologies for exosome production have also lagged behind. To address these challenges, Codiak

FIGURE 4

MS proteomics-based comparison of exosome purity to reference standard: material of suboptimal purity (left); material derived from an optimized purification process (right).



Each dot represents a specific protein, and its position indicates the relative abundance of this protein. If the sample and the reference standard match perfectly, all points will line up on the 45-degree dotted line. Note the color difference between the less pure (brown) and the highly pure material (white).

DOI: 10.18609/cgti.2020.070

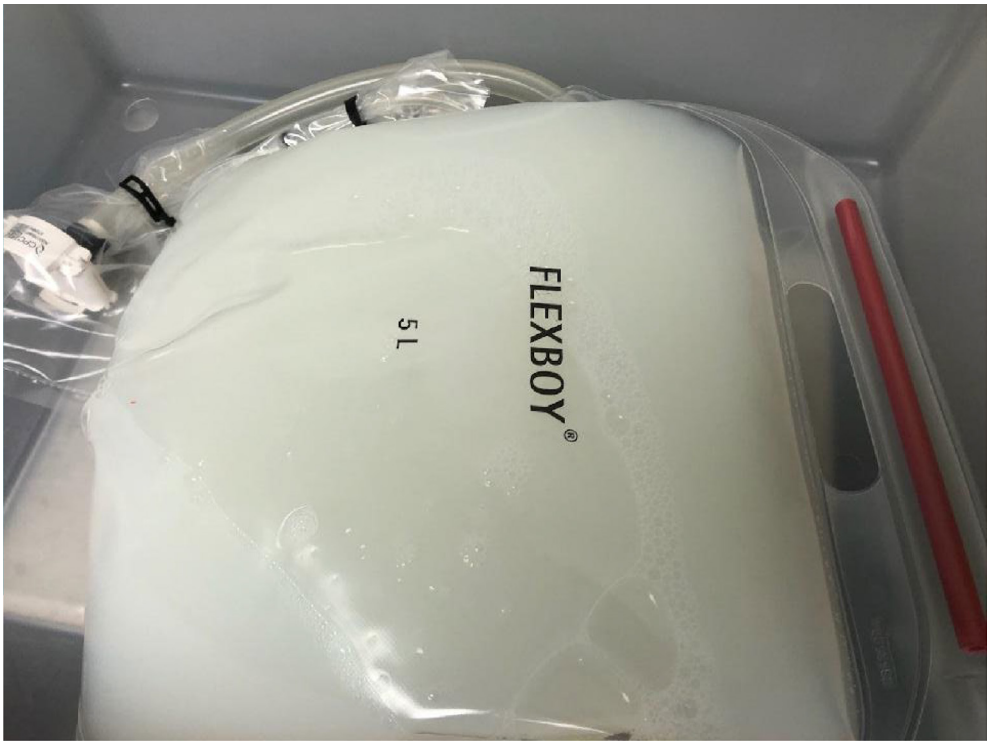
BioSciences has designed a fully integrated exosome manufacturing platform, encompassing scalable upstream and downstream operations and supported by a robust portfolio of analytical methods. Our original design goals are summarized in Table 1.

The above guiding principles yielded the exosome manufacturing platform outlined in Figure 3. Its large-scale implementation marks an important step towards industrialization of exosome manufacturing, following a path similar to the recent advances in the field of well-established molecular biotherapeutics, such as recombinant proteins and antibodies. The upstream part of the process utilizes immortalized human cells grown in suspension, engineered to produce the desired engEx construct. The cell culture, fed with chemically defined media, constitutively secretes exosomes in the supernatant. The harvest from the production bioreactor is processed downstream in a sequence of filtration and chromatography steps. The results achieved with an optimized purification process are shown in Figure 4, demonstrating a high degree of similarity to our reference standard. Depending on the engEx constructs, the purified and concentrated exosome bulk is either directly formulated and filled in vials, or is first loaded with the desired therapeutic payload, followed by formulation and vial fill. This manufacturing platform has been implemented in two GMP forms utilizing functionally closed, single-use technology: a 2,000L fed-batch process for exoSTING and a 500L high-density perfusion process [9] for exoIL-12, yielding large amounts of purified exosome bulk (Figure 5).

The efficiency gains with the development of highly reusable upstream, downstream, and analytical platform technologies are substantial. This paradigm enables significant acceleration of the time to IND (a broadly used performance indicator in the biotech industry), which in our recent experience positions exosome biotherapeutics not far behind the timelines reported for well-established molecular biotherapeutics [10]. Looking forward,

FIGURE 5

A sublot of highly purified exosomes derived from a GMP 500L perfusion bioreactor.



The material is formulated and filled in a 5L single-use bag.

the above described manufacturing and analytical technology is expected to evolve rapidly, driving exosome biotherapeutics towards growth, clinical success, and industrialization.

SUMMARY

Exosomes represent an entirely new class of delivery vehicles that have the potential to revolutionize drug delivery by enabling targeting to specific cell types and tissues, potentially overcoming potency, immunogenicity and toxicity issues which have hampered many efforts to address promising targets and modalities. The exosome field has now advanced and we believe that progress in exosome engineering and large-scale industrial manufacturing has enabled the clinical development of exosome-based therapeutics. There is still much research to be done in the exosome field, including a deeper understanding of endogenous exosome function, improvements to the efficiency of loading additional cargo types, and continued advances in modulating exosome tropism. Codiak BioSciences has made promising advances in all of these areas. We believe and hope that these advances will enable new avenues for therapeutic applications, including genome editing, AAV delivery, and novel vaccine platforms.

REFERENCES

1. Kooijmans SAA, Aleza CG, Roffler SR *et al.* Display of GPI-anchored anti-EGFR nanobodies on extracellular vesicles promotes tumour cell targeting. *J. Extracell. Vesicles* 2016; 5: 31053.
2. Lai CP, Kim EY, Badr CE *et al.* Visualization and tracking of tumour extracellular vesicle delivery and RNA translation using multiplexed reporters. *Nat. Commun*. 2015; 6, 7029.
3. Ohno SI, Takanashi M, Sudo K *et al.* Systemically injected exosomes targeted to EGFR deliver antitumor microrna to breast cancer cells. *Mol. Ther*. 2013; 21: 185–91.
4. Alvarez-Erviti L, Seow Y, Yin H *et al.* Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat. Biotechnol.* 2011; 29: 341–5.
5. Yim N, Ryu SW, Choi K *et al.* Exosome engineering for efficient intracellular delivery of soluble proteins using optically reversible protein–protein interaction module. *Nat. Commun.* 2016; 7: 12277.
6. Stickney Z, Losacco J, McDevitt S, Zhang Z, Lu B. Development of exosome surface display technology in living human cells. *Biochem. Biophys. Res. Commun.* 2016; 472: 53–9.
7. Zeelenberg IS, Ostrowski M, Krumeich S *et al.* Targeting tumor antigens to secreted membrane vesicles *in vivo* induces efficient antitumor immune responses. *Cancer Res.* 2008; 68: 1228–35.
8. Jeppesen DK, Fenix AM, Franklin JL *et al.* Reassessment of Exosome Composition. *Cell* 2019; 177: 428–45.
9. Konstantinov KB, Goudar C, Ng M *et al.* The "Push-to-Low" Approach for Optimization of High-Density Perfusion Cultures of Animal Cells, *Adv. Biochem. Engin. Biotechnol.* 2006; 101: 75–98.
10. Lee GW, Sarno K, Speidel J *et al.* Seamless Transition from R&D to Manufacturing. *Bioprocess Int.* 2018; 16(9): 2–7.

AFFILIATIONS

Jonathan Finn, PhD
Vice President of Discovery Research, Codiak BioSciences

Konstantin Konstantinov, PhD
Executive Vice President, Manufacturing and Process Sciences, Codiak BioSciences

DOI: 10.18609/cgti.2020.070

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Dr Konstantinov has a number of patents pending including: Production Of Extracellular Vesicles In Single-Cell Suspension Using Chemically-Defined Cell Culture Media; Loading Of Extracellular Vesicles Through Imparting Of Mechanical Shear; and Production Of Extracellular Vesicles In Single-Cell Suspension Using Chemically-Defined Cell Culture Media.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Revised manuscript received: *Apr 29 2020;* ***Publication date:*** *May 20 2020.*

CELL & GENE THERAPY INSIGHTS

Innovation Insights

-

COMMENTARY

Exosomes at scale as next-generation cell free medicines: fact and fiction

Ian Dixon

In 2020, exosomes (also known as extracellular vesicles [EVs]) are emerging from academic laboratories and into biotechnology company led clinical trials. But this progress has, until now, been held back by the absence of a robust, scalable and proprietary purification technology. Despite this, a handful of biotechnology companies are promoting their manufacturing capabilities and progress into clinical trials. This article considers the fundamental question behind claims of scalability of EV manufacturing and its implications for the development of this promising therapeutic modality.

Cell & Gene Therapy Insights 2020; 6(4), 597–607

DOI: 10.18609/cgti.2020.072

INTRODUCTION

Exosomes or extracellular vesicles (EVs) have emerged as a hot topic among scientific researchers and medical professionals. EVs are now gaining traction in the biotechnology sector as next-generation, cell-free medicines.

Research into the mechanism of action for stem cell therapies has demonstrated that secreted paracrine factors (including EVs) are the essential regenerative elements [1], and points to the potential benefits of using off-the-shelf EVs from allogeneic adult stem cells (and other sources) to treat a wide range of conditions including COVID-19 related acute respiratory distress syndrome (ARDS).

Every month we read about another EV transaction or another biotechnology company entering the EV field. Some companies claim to be able to manufacture their product at 'large-scale' – but how much of this is hype and hope?

EVS

EVs are membrane-bound (assembled using cellular bi-lipid layer membrane) nanoparcels (around 40–200 nm in diameter) naturally produced by cells from almost all forms of life including plants. EVs can be thought of as a highly conserved mechanism by which cells both communicate and share resources. EVs are found in all body fluids [2].

Unlike other short-acting trophic/paracrine factors secreted by cells, EVs have been shown to 'reprogram' recipient cells via various mechanisms including the transfer of nucleic acid sequences. As a result, EVs can have long-term effects on target cells (and therefore tissue/organs/individuals) such as epigenetic reprogramming, reducing senescence and pro-regeneration cell phenotype changes.

While EVs are a natural part of our bodies, as we age the amount of 'good' circulating EVs declines – as does our regenerative and healing capacity [3]. Hundreds of publications point to the regenerative potential of EVs from adult stem cells, tested in animal models of human conditions such as cardiac repair, neuro-regeneration, autoimmune disease, osteoarthritis, fibrosis and sensory loss.

Unlike many single-acting drugs (e.g. monoclonal antibodies and small molecules), EVs are complex structures: both delivery vehicles and the goods delivered (Table 1).

This rich functionality of EVs explains both the important role of EVs in biology (across so many forms of life) and the potential value of EVs as medicines.

EV MEDICINES FOR HUMANS

At present there are no EV regenerative medicines approved for sale or at late-stage clinical trials – EVs are still experimental medicines.

EVs as medicines have so many facets – sources, forms and risk-profiles (Box 1).

Just as enzyme replacement therapy grew from a Big Idea in the early 1980s into a true biotechnology success story (both for patients and investors) over a few decades, EV

TABLE 1

EV medicine functions and advantages.

EV function	Advantages	
Delivery vehicle	▶ Non-immunogenic even if allogeneic (unmatched) ▶ Readily cross blood–brain barrier ▶ Protects nucleic acids (e.g. DNA, mRNA and siRNA) against metabolism/degradation ▶ Can be targeted to certain cells ▶ Can be lyophilized and formulated	Highly versatile and robust
Goods delivered	▶ Resources (e.g. amino acids and lipids) for cell proliferation ▶ mRNA, miRNA and lncRNAs from cell ▶ Enzymes and proteins in and on the EV from its originating cell ▶ Small molecule drugs, biologics, siRNA, gene therapy etc. that have been purposefully manufactured into the EV	Adaptable to many uses as a medicine

DOI: 10.18609/cgti.2020.072

replacement therapy and other medical uses of EVs holds promise in many potential markets and in many forms.

If an EEV is delivering a payload of an 'active ingredient' then it may be considered an ATMP, but NEVs are likely seen as biologics. EVs from some sources will be regulated and sold as OTC nutraceutical/cosmeceutical products.

From Table 2 there are three main different types of EVs, each with a different inherent risk profile, as described in Table 3.

EV medicines will address many needs. EVs are ideally suited to uses such as vaccines, delivery of nucleic acids, targeting small molecules to certain cells and as NEVs as a regenerative medicine.

EV MEDICINE 'MANUFACTURE' STEPS 101

Given that EVs are naturally produced by cells, when we talk about EV medicine 'manufacture' we are really talking about purification and not synthesis. Unlike liposomes, EVs are a natural secretion from cells via a process called 'exocytosis'.

Using stem cell derived EVs (NEVs) as an example, the cardinal manufacture steps are shown in Figure 1.

These steps are very similar to the manufacture of recombinant proteins or viral products, except that such products can usually be separated by ligands that are known to be specific for the desired product.

To manufacture commercial therapeutic EV product, the purification system needs to be capable of processing cell secretome at scale – initially hundreds of liters and ultimately thousands of liters.

MANUFACTURING REQUIREMENTS FOR EVS AS MEDICINES

Unlike nutraceutical/cosmeceutical EV products, future ATMP and biologic EV products will need to meet stringent manufacturing requirements akin to those applied to biologic products.

ATMP are using the EVs to deliver an active ingredient, so very high levels of EV purity will be required from the in-process manufacturing and analytics. Biologic NEVs products also need to have high levels of purity (low levels of contamination) to become part of the medicines supply-chain and gain acceptance within the medical and pharmaceutical industry.

Aside from purity, all medicines need to meet stringent requirements for sterility – so ideally the manufacture will occur within closed systems.

BOX 1

EV sources

Human sources

- Blood and blood plasma
- Blood platelets
- Adult stem cells (e.g. MSCs)
- Other human cells (e.g. T cells and iPSCs)

Non-human sources

- Plants (e.g. ginger)
- Bovine milk
- Worms

MANUFACTURING IS THE PROBLEM FOR EV MEDICINES

So far, the promise of EVs as next-generation cell-free medicines has been limited by the so-called 'manufacturing problem' – the ability to purify EVs in large-scale as a well-defined proprietary biologic-type product (Figure 2) [4].

Progress of EVs from human stem cells (hNEVs) as medicines has two positive influences:

1. EVs are endogenous, naturally occurring factors in our bodies – i.e. they are likely to be safe and work;

TABLE 2

Many forms of EV 'medicines'.

Type of EV	EEVs	NEVs	HEVs	NEVs
Regulated as	ATMP	Biologic	Human blood product or ?	OTC
Sourced from	Human cells	Human cells	Human blood or human blood plasma	Human cells, animal products (e.g. milk) or plants (e.g. ginger)
Type of product	Antiviral, anticancer, autosomal conditions, orphan conditions	Regenerative medicine, etc.	Regenerative medicine, etc.	Nutraceutical/ cosmeceutical
$ value per dose	High	High	Medium	Low
ROA	i.v., intranasal, nebuliser, i.p., topical etc.	i.v., intranasal, nebuliser, i.p., topical etc.	i.v., intranasal, nebuliser, i.p., topical etc.	Oral and topical

ATMP: Advanced technology medical product; EEV: Engineered EV; NEV: Naïve EV; HEV: Human EV; OTC: Over the counter; ROA: Route of administration.

2. Allogeneic MSCs have been used in hundreds of clinical trials [5] and EVs are a key part of the MOA of MSCs [6] – i.e. hNEVs are likely to be safe if properly manufactured.

The need for manufacturing scale at the start of development

It is relatively easy to make doses of experimental EVs to inject mice that have a mass of 25 g, but scaling up to humans (around 80,000 g or around 3,000 times the mass of a mouse) has been an ongoing challenge and hold-up in the shift from discovery to development.

But making material for small Phase 1 studies is not the real challenge compared to reaching commercial scale. As a rule of thumb, each development step further increases the amount of material by a factor of 10. So 'scalable' for a Phase 1 trial is probably only 1/10th scaled for Phase 2, 1/100th the scale of a Phase 3 study, 1/1,000th the scale of a limited commercial launch, and (hopefully) 1/10,000th to 1/100,000th the size of a fully commercial product.

Entering Phase 1 testing without the ability to scale up manufacture by 10,000–100,000x at the time is pointless, as the process (and thus the product) will inevitably need to change (and therefore be retested) before the product reaches the customer.

Starting Phase 1 testing with experimental products made using prototype processes doesn't make sense for biotechnology investment.

TABLE 3

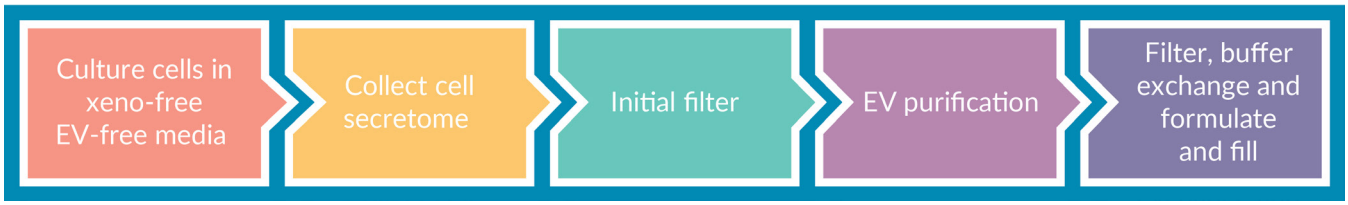
General risk profiles of types of EV medicines.

Designation	Described	Commentary	General risk profile
EEVs	Engineered EVs	EVs (i) from modified cells, (ii) loaded up with 'API' or (iii) both	Higher
NEVs	Naïve EVs	Naturally produced by cells (unmodified)	Medium
HEVs	HEVs	Human tissue source (e.g. blood)	Lower

DOI: 10.18609/cgti.2020.072

FIGURE 1

Key EV medicine manufacturing steps.



EV medicines have unique demands on manufacturing

As a new class of medicine, EV have unique structures, biology and manufacturing challenges.

For EV medicines, the purification challenge can be described as follows:

- Removal of contaminating cellular debris;
- Efficiency of isolation method – yield, time and cost;
- Wide range of sample types as starting material;
- Isolation methods exploit various unique features of exosomes, e.g. size, size distribution, morphology, quantity and biochemical composition;
- Reliability and technical complexity of characterization assays post purification to evaluate quality, purity and confirm identity of EVs and differentiate them from other microvesicles or co-purified biomolecules; and
- Commercial application issues – yield, time, cost, purity (GMP), shelf life, safety and efficacy.

For EV medicines there is also the issue of fractionation of EVs influenced by the isolation protocol. Does the purification technology isolate most of the EVs from the source material or does it isolate a fraction – and is the isolated fraction 'better' or 'worse' than the bulk of the EVs in the source material?

Ultracentrifuge: the unsuitable standard

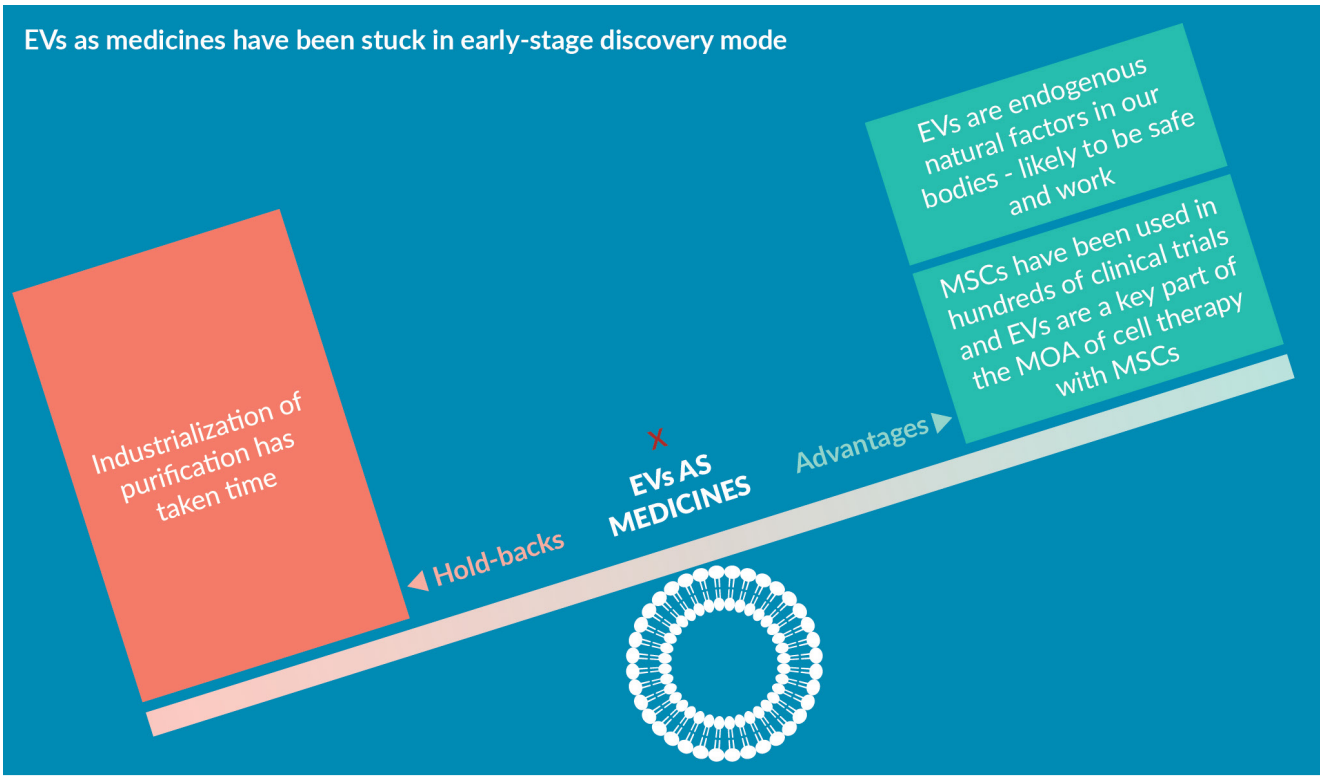
For research materials in small-scale the 'gold standard' purification step is ultracentrifugation (UC) – spinning samples at 100,000 g for 4 to 8 hours [4]. But for a therapeutic product, UC has many limitations [7]:

- It is an open system and batch process;
- It segregates material based upon density, and other materials in the secretome have similar or same densities;
- It has limited capacity in each batch and is time consuming;
- UC changes the osmotic environment;
- It is not a proprietary step, so the product produced is not proprietary unless there is something special about the originating cells.

Previous publications have highlighted the clinical use of 'EVs' in a limited number of small (4–40 participant) human studies [8] in treating conditions such as cancer, chronic kidney disease (CKD) [9] and graft-versus-host disease (GvHD) [10]. In general, these anticancer materials have used 'EVs' from UC to deliver a payload (e.g. peptide or small molecule) into cancer cells. The CKD study 'EV' treatment used NEVs from MSCs using

FIGURE 2

EV medicines have been stuck in discovery due to the 'manufacturing problem' and despite positive influences.



UC. In 2020, we have a better understanding of the deficiencies of UC and the limitations of 'product' it delivers – but UC has been the most available approach so far, and small-scale laboratory experiments previously published increase the likelihood that subsequent researchers continue to use UC.

Most importantly, the materials provided by UC has be shown to contain detrimental protein impurities [11]. The finding adds further evidence that therapeutic EVs should be isolated by alternative methods to ultracentrifugation, such as affinity-based purification protocols.

ALTERNATIVE PURIFICATION TECHNOLOGIES

Other purification technologies used with EVs also have limitations (Table 4).

Exosomes purified by size exclusion chromatography alone, meanwhile, have also been shown to contain contaminants.

Affinity chromatography to the rescue

In a recent review of exosome manufacturing and purification, UK researchers highlighted affinity-based methods as the most promising exosome isolation method, both in terms of purity and scalability [4].

Affinity-based purification of exosomes works by selectively recognizing and sticking to the exosome's membrane surface. The process works like a highly selective version of the hooks and loops on Velcro™. When the 'hook' component is immobilized on a stationary surface, any passing exosomes will be caught by the 'loops' found exclusively on their surface.

Affinity purification techniques are already used in large scale by the biologic therapeutics industry and the blood products industry. Many of the latest blockbuster drugs are monoclonal antibodies. During their manufacture, these therapeutics are efficiently purified on large scale using an affinity-based

DOI: 10.18609/cgti.2020.072

TABLE 4

Limitations of other EV purification techniques.

EV purification technology	Key limitations
SEC	Protein aggregation and clogged membrane, labor intensive, low yields, not proprietary
IAC	Absence of universal biomarkers, antibodies are expensive at large scale
TFF and depth filtration	Purity, clogging, not proprietary
Orthogonal purification	Yield, not proprietary
Two phase separation	Contamination of the result
Acoustic separation	Open system, yield and contamination
Microfluidics	Yield and scale

IAC: Immunoaffinity chromatography; SEC: Size exclusion chromatography; TFF: Tangential flow filtration.

hook called Protein A, which selectively recognizes and captures the antibody. The purified antibody can then be released from Protein A in a step called elution.

On a small scale, the hook component can be coated onto the surface of magnetic beads. When the beads are added to an exosome-containing flask, the exosomes will stick to the beads, and a magnet can then be used to pull out the exosome-covered beads from all other impurities in the mixture. On a larger scale, the hooks can be immobilized onto solid particles that are packed into chromatography columns. As the exosome-containing mixture is pumped through the column, the exosomes stick while all the other impurities are simply washed through the column and are discarded. The captured exosomes are then released in pure form at the elution step.

THE NEED FOR A PROPRIETARY STEP IN THE PURIFICATION PROCESS

Academics are generally driven by motives other than commercial, but biotechnology companies will spend substantially to progress a product through to Phase 2 and registration studies, and a pharmaceutical company partner will oftentimes want to see intellectual property (IP) protection for products they take forward into registration and sales.

For biotechnology companies, a proprietary process is important – potentially essential. Biotechnology companies that use secretome from 'standard' adult stem cells (e.g. MSCs) need a proprietary step in their purification process to have a proprietary product (the product is the process – the process is the product) and IP rights (e.g. exclusivity of manufacture and clinical data) – otherwise later competitors can piggyback on the first-mover's expensive investment into generating clinical data and enter the market with a 'generic' EV product with reduced investment.

Companies like Exopharm use a proprietary affinity chromatography (AC) approach with its LEAP ligands which are the subject of patent applications (progressing through national phases). With LEAP-AC, Exopharm is an example company that has a proprietary process suited to handle hundreds and then thousands of liters of filtrate containing EVs.

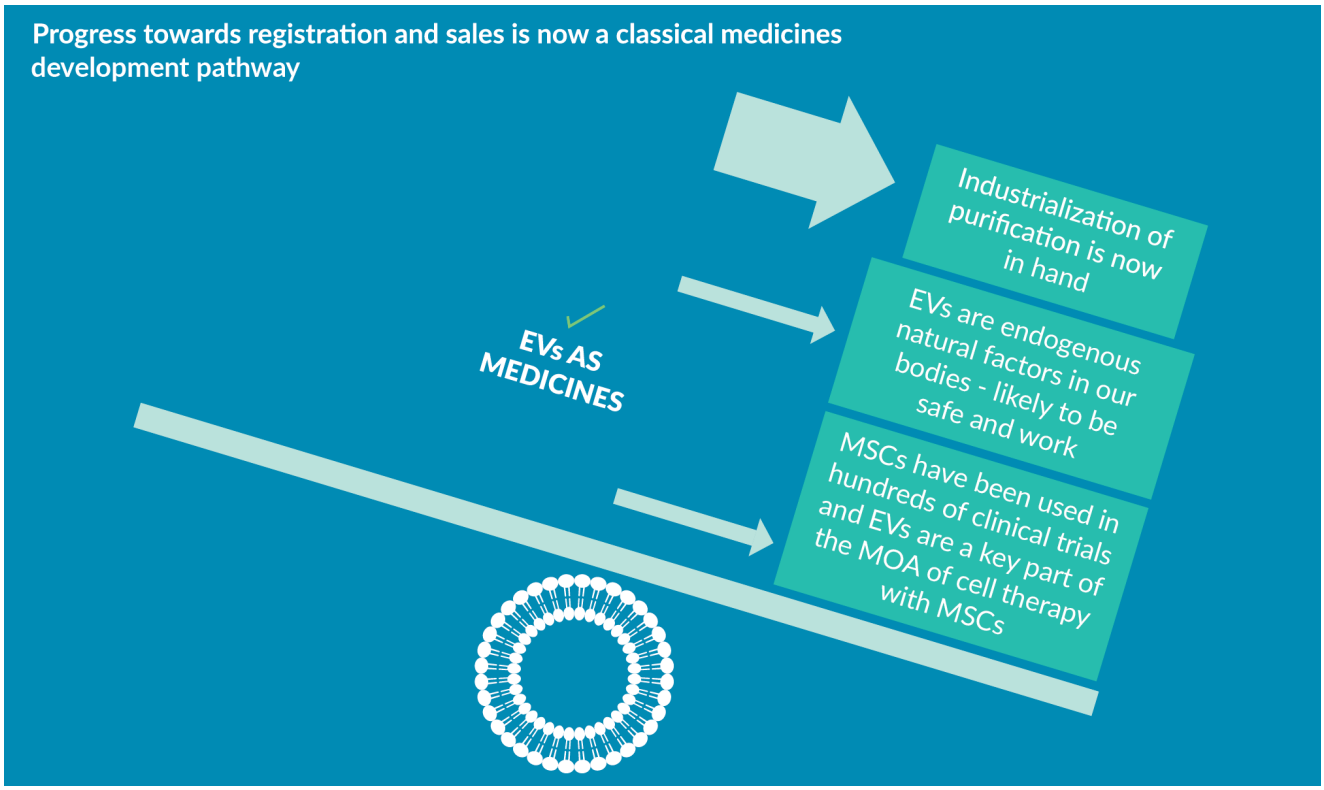
TABLE 5

Biotechnology companies in EV medicine field.

Companies progressing EVs as therapeutics (December 2019)	Companies planning human clinical trials of EV products	Companies commenced dosing of humans by January 2020
14	5	Exopharm Ltd

FIGURE 3

Development of EV medicines is now fully underway with the industrialisation of purification.



COMMERCIAL EV MEDICINES IN 2020: MYTH OR REALITY

In the period leading up to 2020, much of prototype and proof-of-concept work on EVs as medicines has been delivered by both academic and biotechnology groups – the numbers of PubMed papers about EVs is growing exponentially [12] and the science is building a strong foundation.

But is this translating into innovative medicines for patients?

The Boston Exosome Based Therapeutic Development Summit 2019 was an inaugural forum for biotechnology EV medicine people to gather and talk about the race into clinical trials. At the time of the Summit, none of the companies had human dosing underway with proprietary regenerative medicine EV products.

In December 2019, Nature Biotechnology published an article [13] listing 14 companies progressing EV medicine products. But at April '20 Exopharm seems to be the only one that has commenced human dosing in its PLEXOVAL I study in wound healing and safety (Table 5).

Now that companies like Exopharm have affinity chromatography technologies to industrialize the purification of EV medicines, these products can now progress into development activities aimed at registration and sales (Figure 3).

LOOKING FORWARD

As with any genuinely new class of medicine, translation can be slower than desired or expected. Recently, CAR-T therapies have shown us that rapid progress from experimental discovery product to registration and sales is possible (despite manufacturing challenges and limitations) if the outcomes for many patients are strong and obvious.

Successful commercialization of EV medicines will combine the following factors:

- Biotechnology companies with commercial objectives and adequate funding;

DOI: 10.18609/cgti.2020.072

- A proprietary manufacturing technology that can scale-up and deliver proper products with commercially attractive cost of goods sold (COGS);
- 'Out-of-the-box' clinical outcomes in selected uses;
- Off-the-shelf products i.e. long stability, allogeneic, route of administration resolved;
- Fitting the Pharmaceutical industry supply chain and logistics model;
- Standardization of analytical tools and solid understanding of potency, dosing and mechanism-of-action (MOA).
- The first effective experimental CAR-T cells were developed in 2002 and the first CAR-T therapy was approved by the FDA in 2018 (16 years gestation).

Based upon suitable enabling manufacturing technology, it is now feasible that a first FDA-approved EV medicine could arrive by 2025/2026 (17 years gestation) if the patient outcomes and safety profile are compelling (Figure 4).

CONCLUSION

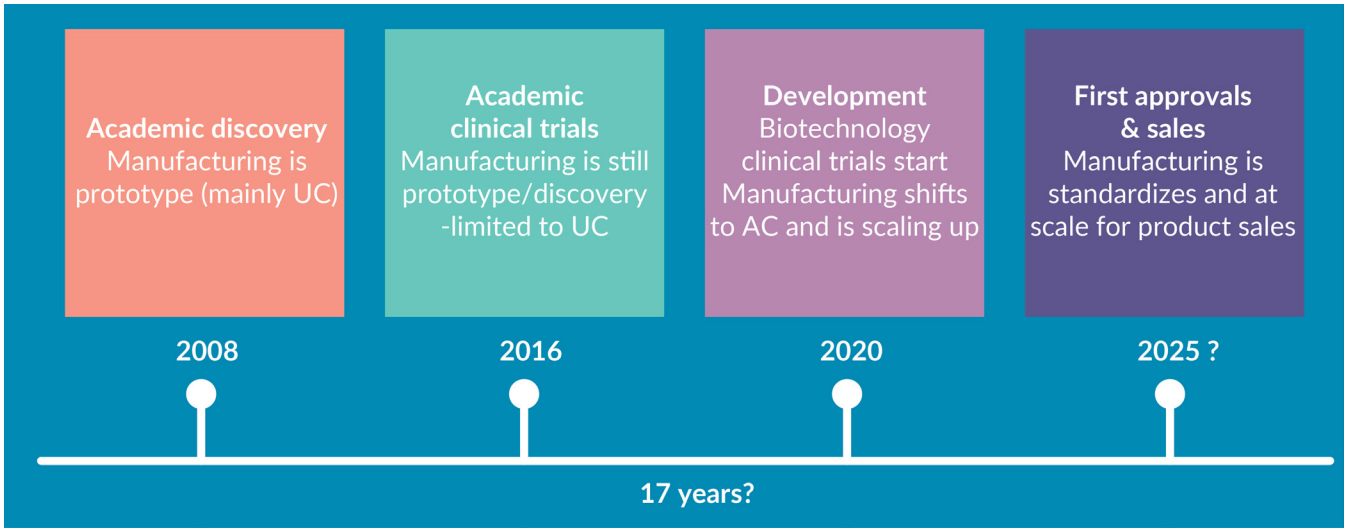
History shows us that great innovations have to be 'industrialized' to become mainstream. Henry Ford took the promise and excitement that followed the first automobile (1885) and industrialized it with the first Model T in 1908 (23 years). In 1983 Motorola introduced the first commercial mobile phone, but the first iPhone was released in 2007 (24 years). Closer to home, in 1975 Kohler and Milstein made the first monoclonal antibodies, then in 1988 humanised monoclonal antibodies were developed. The first humanized mAb was approved by the FDA in 1997 (daclizumab) – a gestation period of 22 years.

There are many reasons to believe that EV medicines will also soon become a safe, important and mainstream part of world-wide healthcare. This outcome will be underpinned by the industrialization (i.e. scale, purity, costs, consistency and standardization) of the EV manufacturing technology (the 'making') and clever selection of the applications best suited to EV medicines (the 'products') – together serving the needs of doctors, patients and the industry.

Companies with scalable proprietary manufacturing technologies can drive this EV medicine revolution and support the further and ongoing innovation that will occur once the EV platform has been derisked.

FIGURE 4

EV medicine – a view of the discovery, development and sales timeline.



REFERENCES

1. Caplan AI. Mesenchymal Stem Cells: Time to Change the Name! *Stem Cells Transl. Med.* 2017; 6(6): 1445–51.
2. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science* 2020; 367(6478).
3. Eitan E, Green J, Bodogai M *et al.* Age-Related Changes in Plasma Extracellular Vesicle Characteristics and Internalization by Leukocytes. *Sci. Rep.* 2017; 7(1): 1342.
4. Colao IL, Corteling R, Bracewell D, Wall I. Manufacturing Exosomes: A Promising Therapeutic Platform. *Trends Mol. Med.* 2018; 24(3): 242–56.
5. Kabat M, Bobkov I, Kumar S, Grumet M. Trends in mesenchymal stem cell clinical trials 2004-2018: Is efficacy optimal in a narrow dose range? *Stem Cells Transl. Med.* 2020; 9(1): 17–27.
6. Zhang ZG, Buller B, Chopp M. Exosomes - beyond stem cells for restorative therapy in stroke and neurological injury. *Nat. Rev. Neurol.* 2019; 15(4): 193–203.
7. Li P, Kaslan M, Lee SH, Yao J, Gao Z. Progress in Exosome Isolation Techniques. *Theranostics* 2017; 7(3): 789–804
8. Lener T, Gimona M, Aigner L *et al.* Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper. *J. Extracell. Vesicles* 2015; 4: 30087.
9. Nassar W, El-Ansary M, Sabry D *et al.* Umbilical cord mesenchymal stem cells derived extracellular vesicles can safely ameliorate the progression of chronic kidney diseases. *Biomater. Res.* 2016; 20: 21.
10. Giebel B, Kordelas L, Borger V. Clinical potential of mesenchymal stem/stromal cell-derived extracellular vesicles. *Stem Cell Investig.* 2017; 4: 84.
11. Forteza-Genestra MA, Antich-Rossello M, Calvo J, Gaya A, Monjo M, Ramis JM. Purity Determines the Effect of Extracellular Vesicles Derived from Mesenchymal Stromal Cells. *Cells* 2020; 9(2).
12. Srivastava A, Amreddy N, Pareek V *et al.* Progress in extracellular vesicle biology and their application in cancer medicine. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* 2020: e1621.
13. Zipkin M. Exosome redux. *Nat. Biotechnol.* 2019; 37(12): 1395–400.

AFFILIATIONS

Ian Dixon
Founder and CEO, Exopharm Ltd

DOI: 10.18609/cgti.2020.072

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Dr Dixon is a shareholder, director and employee of Exopharm and is paid by Exopharm and will benefit if Exopharm's shares increase in price. Dr Dixon has conflicts of interest in relation to the material contained and the sentiments expressed. Dr Dixon is a co-inventor of the Exopharm LEAP technology described in the article and has pecuniary interests in its commercialisation. Dr Dixon is also a founder and shareholder in Cynata Therapeutic Ltd, a stem cell company. The content written by Dr Dixon has been augmented by material prepared by Exopharm, including material written by Dr James Mitchell Crow and Dr Karen Holden, both employed by Exopharm and paid by Exopharm. The views contained herein are those of the author alone and are presented to the best of the knowledge of the author at the time of writing. It is possible that the author is not aware of events or activities relevant to this article.*

Funding declaration: *The author received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Revised manuscript received: *May 8 2020;* ***Publication date:*** *May 20 2020.*

CELL & GENE THERAPY INSIGHTS

Innovation Insights

-

INTERVIEW

An educated approach: AI-enabled neoantigen vaccine R&D

ROMAN YELENSKY is Gritstone Oncology's first employee and serves as chief technology officer, with responsibility for the EDGE™ tumor antigen identification platform. Previously, Dr Yelensky was vice president at Foundation Medicine, which he joined at its inception. At Foundation Medicine, he co-led sequence data analysis for FoundationOne™ and led validation studies supporting clinical laboratory accreditation and testing of more than 100,000 patients. Dr Yelensky established Foundation Medicine's FDA-regulated products program, leading to FDA approval of the first NGS-based companion diagnostic. He holds a PhD in bioinformatics and genomics from the Massachusetts Institute of Technology and has co-authored more than 75 manuscripts, including most recently on EDGE in *Nature Biotechnology*.

Cell & Gene Therapy Insights 2020; 6(4), 591–595

DOI: 10.18609/cgti.2020.069

Q What are you working on right now?

RY: I'm the Chief Technology Officer for Gritstone Oncology. I was actually Gritstone's first employee and I've been here for over 4 years now. From the beginning, my primary area of responsibility has been the development and application of our EDGE™ platform for tumor antigen discovery.

The platform was published about a year ago, and we're continuing to improve it and working on the next version. We are applying the platform along with other laboratory work and computational analyses in order to discover novel tumor specific antigens, particularly neoantigens, for use in cancer immunotherapy.

Q Can you frame for us what the platform is, and how it supports Gritstone's internal R&D pipeline?

RY: The EDGE platform at the conceptual level is fairly easy to understand – it's a neural network model that allows us to predict which peptides or protein fragments are presented on the surface of tumor cells. When they're tumor specific, which of course neoantigens are, they can be targeted by various types of cancer immunotherapies. What we primarily pursue ourselves are neoantigen vaccines, but these can also be targeted with cell therapies as well as other approaches.

The distinguishing feature is the way EDGE was trained. Over the course of several years we collected a large set of human tumors, on which we performed what is known as immunopeptidomics. This is when you take a tumor and use mass spectrometry to sequence the peptides presented on the surface of the cells. In this way, we created a very large training data set of tumor-presented peptide – specifically, those presented on human leukocyte antigen (HLA) molecules. Our data set is made up of well over a million peptides from hundreds of tumor samples, and that (along with other published datasets) was used as the input to the neural network training, also known as deep learning. This neural network now allows us to look at a novel sequence and predict whether that sequence will be presented on tumor cells.

Q More broadly, how do you envision platforms such as EDGE enabling the cancer therapeutics field as a whole?

RY: The whole question of predicting which peptides are presented on the tumor cell surface is quite a general one. Every protein gets degraded into peptides, and some of those peptides can be presented on tumors. When you couple that with a degree of tumor specificity – if this peptide is only present or predominantly present in tumor cells – those can be targeted by various kinds of cancer immunotherapies.

> "...our primary approach, which is the development of neoantigen vaccination, needs to be combined with checkpoint inhibition."

They can also be targeted with cell therapy. To this end, we have a partnership with a leading cell therapy company here in Cambridge, Massachusetts called bluebird bio, with which we deploy EDGE to find tumor-specific HLA presented peptides for their use in cell therapies. They can also be targeted by a newer class of antibody drugs

DOI: 10.18609/cgti.2020.069

"...we are now able to do single cell sequencing to study both the tumor cells and the T cells that may be interacting with them. There are exciting new approaches that allow you to even do this single cell sequencing with spatial resolution, so you can combine traditional H&E analysis and antibody staining with single cell genomics."

(newer to the solid tumors at least) known as bispecific antibodies. One side of the antibody binds to the tumor using one of these tumor-specific peptides, and the other binds to T-cells leading to so-called synthetic immunity.

EDGE is really a platform for predicting HLA presented peptides that may be tumor-specific and can be targeted by a variety of approaches. This is something we have been pursuing more generally, as well as utilizing it for our neoantigen vaccines.

Q Tell us about the rationale and potential underpinning Gritstone's combination therapy development partnership with Bristol-Myers Squibb and their immune checkpoint inhibitor, Opdivo?

RY: This is an important partnership for us. We believe that our primary approach, which is the development of neoantigen vaccination, needs to be combined with checkpoint inhibition. The therapeutic hypothesis is that we have many patients who don't respond to the current checkpoint inhibitors because they don't have neoantigen reactive T cells that can recognize their tumor. Therefore, the thinking is that we can give our neoantigen vaccine to educate the immune system and stimulate T cells that can recognize the tumor. Once the T cells get to the tumor, they still need to be able to attack and destroy it, and the current belief in the field is that without PD-1 checkpoint inhibition, this won't be effective. It requires a combination approach: you need to generate these neoantigen reactive T cells that can recognize the tumor, and once they get to the tumor, you need to inhibit PD-1 checkpoint so those T cells can then eliminate it.

Q What can you tell us about Gritstone's manufacturing facility for personalized cancer therapeutics?

RY: Something a bit different about Gritstone is the fact we invested in our own biomanufacturing quite early in our development. We actually started building our

"...for an organization to be truly 'AI-enabled' they need to have a certain degree of confidence, flexibility, and capability to apply the right AI tools to the right problems if and when they arise."

biomanufacturing facilities before we were even in the clinic, and this turned out to be a very wise decision.

It became clear from working with CMOs, although many of them are excellent at creating various biologics, that they're more set up for an off-the-shelf therapeutics approach, where you book a manufacturing slot many months in advance and it takes many months to create the therapy. This works fine if you're making a batch of drugs, but if you're making a drug for each patient you really need a lot more control over both the time and the cost. That's why we made the decision early on to invest in our own biomanufacturing. This decision really made our personalized planned neoantigen approach possible, so we can make vaccines for patients in our own 40,000 square foot biomanufacturing plant facility in Pleasanton, California.

Q What for you are the critical next steps in terms of novel innovation and future enabling tools for the immuno-oncology space?

RY: My focus and background is genomics and informatics, and in translational work, so what I'm most excited about are the emerging tools that allow us to study in unprecedented depth what is happening to the tumor, and in the patients when we give them our therapies.

For example, we are now able to do single cell sequencing to study both the tumor cells and the T cells that may be interacting with them. There are exciting new approaches that allow you to even do this single cell sequencing with spatial resolution, so you can combine traditional H&E analysis and antibody staining with single cell genomics. This kind of analysis may help us to understand what is going right when patients are responding, and what's going wrong when they aren't.

I find these next generation genomics tools as applied to translational samples really promising, and it's going to be informative for next steps with these therapies. Because of course, the response rate to the PD-1 checkpoint inhibitors is still quite low: maybe 20–30% of patients respond. But why do the rest of the patients not respond? A key part of our hypothesis is that they need neoantigen reactive T cells. But are there other factors improving clinical benefit? We and others will have to study what happens when we give these combination therapies, which will inform how they may be improved further. We are currently collecting on-treatment biopsies and will be asking these kinds of questions.

Q Can you describe your vision for what the fully AI- or neural network-enabled R&D organization of the future might look like?

DOI: 10.18609/cgti.2020.069

RY: I was recently on a panel with several other AI leaders within biotech and pharma, and what was amazing to see was that AI means something different for every company. For us, it is the prediction of these HLA presented peptides. For another company it may be their preferred approach for analyzing high throughput screening data, or it could be molecular dynamics because they do a lot of structural biology, or it could be analysis of imaging.

Therefore, there's not really a single right answer. But in my view, for an organization to be truly 'AI-enabled' they need to have a certain degree of confidence, flexibility, and capability to apply the right AI tools to the right problems if and when they arise.

AFFILIATION

Roman Yelensky
Chief Technology Officer, Gritstone Oncology

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Dr Yelensky is an employee of and shareholder in Gritstone Oncology. Gritstone Oncology has received funding for neoantigen research from private and public investors. Dr Yelensky is also an inventor on multiple patents or patent applications related to neoantigen immunotherapy.*

Funding declaration: *The author received no financial support for the research, authorship and/or publication of this article.*

Article source: *Invited.*

Interview conducted: *Apr 21 2020;* ***Publication date:*** *May 15 2020.*

CELL & GENE THERAPY INSIGHTS

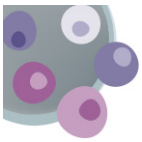
COMMERCIAL INSIGHT: MAR 2020

Commercial Insight: cell & gene therapy

Providing a critical overview of the sector's commercial development: M&As, licensing agreements & collaborations, financial results, IPOs and clinical/regulatory updates, with commentary from our Expert Contributors.

Cell & Gene Therapy Insights 2020; 6(4), 641–654

DOI: 10.18609/cgti.2020.076



CELL THERAPY – Mark Curtis. Director, Manufacturing Partnerships, AVROBIO

Despite setbacks as a result of COVID-19, the biotech space is showing resilience and there have been recent financings. ElevateBio announced a significant round of $170 million to continue building out its infrastructure and to progress a series of cell and gene therapy programs, while Sigilon raised $80 million to keep working on its encapsulated cell therapy platform. As we enter the COVID era, biotech and pharma companies have rushed to push therapeutics and vaccines into the clinic, including cell therapy companies. AlloVir, an ElevateBio company, announced a partnership with Baylor to develop T cell therapies that can be targeted to SARS-CoV-2. Celularity also recently announced it would be using its off-the-shelf natural killer (NK) cell platform to target the virus and was just given FDA clearance to start a Phase 1/2 trial of an allogeneic NK cell therapy in patients with COVID-19. It's great to see the healthcare community coming together to tackle the pandemic.

GENE THERAPY – Richard Philipson. Chief Medical Officer, Trizell Ltd, UK

Novartis appears to have emerged relatively unscathed from a difficult period for its gene therapy unit AveXis. The announcement of no significant sanctions from FDA, following concerns about data from an animal study last year, follows hot on the heels of positive long-term clinical efficacy data, suggesting durable benefits from its one-off treatment for spinal muscular atrophy. Elsewhere, Freeline has been granted Orphan Drug Status in Europe for its treatment for Fabry disease, and Provail's treatment for frontotemporal dementia gets Fast Track Designation in the USA. Freeline's treatment, FLT190, is already in the clinic, with results from its Phase 1/2 study expected next year.

Clinical Regulatory

NOVARTIS DEMONSTRATES LONG-TERM BENEFITS OF ZOLGENSMA® FOR SPINAL MUSCULAR ATROPHY

Zolgensma® is known as the world's most expensive drug, with a single shot costing over $2.1 million – but it is hoped that the drug will be able to permanently correct the genetic mutations that cause spinal muscular atrophy (SMA).

SMA is a rare genetic disease linked to two genes encoding survival motor neuron protein: *SMN1* and *SMN2*. Mutations in *SMN1* cause all subtypes of SMA, and the number of copies of *SMN2* affect the severity of the conditions, with multiple copies associated with less severe symptoms. The disease causes muscle weakness and decreased muscle tone, leading to problems with breathing, eating and swallowing, which ultimately lead to a drastically reduced life expectancy for many patients; infants with severe disease often do not survive beyond their first few years.

However, the latest data is promising – and arrives at the same time as Zolgensma® is approved for SMA patients under 2 years old in Japan. Data from the STR1VE-US, SPR1NT and START studies were revealed at a session presented by the Muscular Dystrophy Association, after its 2020 conference was cancelled due to COVID-19.

Interim data from SPR1NT showed that patients are achieving motor milestones when treated with Zolgensma® while pre-symptomatic. The majority of patients (7/8) with two copies of *SMN2* managed to sit independently within the WHO window of normal development, with the 6 remaining patients within the cohort having not yet passed the developmental window. Most patients did not require feeding support and are within an appropriate weigh range, with no patients requiring ventilatory support, Novartis reported.

Data from STR1VE-US, which is now completed and has formed the basis of regulatory filings for Zolgensma®, showed that in a cohort of 22 patients, 91% met the co-primary efficacy endpoint of event-free survival at 14 months, and 59% met the co-primary efficacy endpoint of functional sitting for ≥30 seconds at 18 months of age. Thirteen patients managed to sit for 30 seconds or more at the age of 18 months at the first study visit, and a fourteenth patient achieved the sitting milestone at 16 months, although this was not confirmed at the 18 month visit. 68% of patients did not require non-invasive ventilatory support at any point during the study, and 82% did not use ventilatory support at 18 months. STR1VE-US also included an 'ability to thrive' endpoint that included swallowing, feeding, and maintaining an appropriate age-related weight: 41% achieved this goal at 18 months.

DOI: 10.18609/cgti.2020.076

Long-term follow-up data from the Phase 1 START study, conducted in SMA type 1 patients, showed that at the end of the 24-month study all 12 patients taking the targeted therapeutic dose were alive and free of permanent ventilation, and 10 of these patients enrolled in an ongoing observational long-term follow-up of the START study. Novartis also reported that six out of ten patients do not require treatment with Biogen's Spinraza, another expensive SMA drug.

Expert Pick

Novartis has been through a difficult period with Zolgensma®, its AAV-9 based gene therapy for spinal muscular atrophy, following accusations of data manipulation that first went public last August, with FDA stating at the time that it would consider civil or criminal penalties against the company. However, recent news suggests that AveXis, the company's gene therapy unit, will not face sanctions for alleged discrepancies in mice testing data in its drug application package. This comes at the same time as a clinical update from a range of studies, reporting that patients who receive the one-off treatment show rapid, significant and clinically meaningful therapeutic benefit, including in patients treated pre-symptomatically, with sustained durability for up to 5 years post-dosing. Overall, a much brighter outlook and its potentially transformational treatment.

SCORPIONS HELP DEVELOP A CAR T THERAPY WITH A STING IN THE TAIL

Despite the huge impact CAR T therapy has had in hematological cancer, the approach has so far seen limited success in solid tumors. With the help of some scorpion venom, scientists at City of Hope National Medical Center, California, hope to change that.

The team have designed a novel CAR based on a toxin found in scorpion venom, chlorotoxin (CLTX), that has previously been shown to blind to glioblastoma (GBM) cells. The chlorotoxin-directed CAR T cells were able to cause regression of glioblastoma xenografts and prolonged survival in mice, with no evidence of antigen escape and without triggering adverse reactions. Now, the team are working on a Phase 1 clinical study to test the safety of these CLTX-CAR T cells in collaboration with the National Cancer Institute.

Tumor heterogeneity is a key challenge in targeting GBM, so the team used patient tumor cells to compare CLTX binding with the expression of known antigens that are being

studied as CAR T targets including IL13Rα2, HER2 and EGFR. They found that CLTX bound to a large number of patient tumors, while expression of the other antigens varied widely between patient samples.

Study author Michael Barish commented: *"We are not actually injecting a toxin but exploiting CLTX's binding properties in the design of the CAR. The idea was to develop a CAR that would target T cells to a wider variety of GBM tumor cells than the other antibody-based CARs."*

And this isn't the only medical application scorpions are assisting with – components of scorpion toxin are also under investigation in the treatment of chronic pain and arthritis.

Expert Pick

After years of clinical failures in treating glioblastoma, making progress on standard-of-care requires thinking outside the box. Researchers at City of Hope are doing just this with the design of a novel CAR T based on the venom carried by scorpions. Instead of taking an antibody-based approach researchers created a CAR that contains chlorotoxin (CLTX), which they found can broadly bind to glioblastoma cancer cells, including long-lived subsets thought to be at the core of relapse in patients with glioblastoma. In mouse studies CLTX-CAR-T cells were shown to bind glioblastoma cells in vivo while sparing healthy cells, and prolong survival of mice. The next step is figuring out CLTX's mechanism of action.

GENE THERAPY CANDIDATE FLT190 GRANTED ORPHAN DRUG DESIGNATION

FLT190, developed by Freeline therapeutics, has been granted orphan drug designation by the European Commission as a potential treatment for Fabry disease.

Fabry disease is a lysosomal storage disorder characterized by mutations in the *GLA* gene that cause an absence of the enzyme alpha-galactosidase A (alpha-GAL A), which leads to build up of the fatty molecules Gb3 and LysoGb3. FLT190 is an *in vivo* liver-directed adeno-associated viral (AAV) gene therapy; unlike currently available treatments such as enzyme replacement therapy, which requires regular infusions, FLT190 is intended to be given as a single dose that reinstates normal production of alpha-GAL A.

Preclinical data found that a single injection of FLT190 led to over 1,000-fold higher production of alpha-Gal A compared to controls in mice, with Gb3 and LysoGb3 levels significantly reduced, and no adverse effects observed.

DOI: 10.18609/cgti.2020.076

Now, FLT190 is under investigation in an international Phase 1/2 trial involving adult males with classic Fabry Disease: MARVEL1. The trial is currently recruiting at sites within Europe and is focused on the safety of the drug, and its ability to trigger liver cells to produce high levels of alpha-GAL A. Endpoints include clearance of Gb3 and LysoGb3 from the blood and urine, alterations in kidney and skin biopsies, kidney and heart function, alpha-GAL A immune response, viral shedding, and quality of life. FLT190 will be administered via IV infusion and patients will be monitored for 9 months before entering a long-term follow-up period. Participants will be both patients previously treated (dose escalation) and patients new to the drug (dose expansion) and the trial is predicted to end by March 2021.

The preliminary data to emerge from the dose-escalation component of MARVEL1 is promising – data presented at the 16th annual WORLDSymposium, Orlando, Florida, showed that the therapy was well-tolerated and patients saw a three- to four-fold increase in blood alpha-GLA activity after a week, which was sustained until week 20.

Chris Hollowood, Freeline Chairman commented:

"Receiving orphan drug designation from the European Commission signifies our continued progress and commitment as we develop FLT190 as a potential one-time treatment for patients with Fabry disease."

ALLOVIR AND BAYLOR COLLEGE OF MEDICINE JOIN FORCES TO TACKLE COVID-19

Allovir, a biotechnology company focused on allogeneic cell therapy, and Baylor College of Medicine have formed an alliance to develop T cell therapies to help improve outcomes in immunocompromised people who are exposed to the virus behind the current COVID-19 pandemic.

AlloVir intends to build on its existing investigational off-the-shelf allogeneic cell therapies, which are designed to restore natural T cell immunity to immunocompromised patients, in order to treat and prevent virus-associated disease. To date, AlloVir's most advanced T cell therapy offering is Viralym-M, which can target six viral pathogens that affect immunocompromised patients including cytomegalovirus, adenovirus, and Epstein–Barr.

AlloVir and Baylor hope to apply the same approach to SARS-CoV-2 by creating an off-the-shelf therapy targeted at SARS-CoV-2 and potentially at similar viruses, such as SARS-CoV, MERS-CoV and endemic coronaviruses. Although a timeline for the program hasn't been publicized, the aim is to use the SARS-CoV-2-specific T cells as a monotherapy, and incorporate coronaviruses into AlloVir's ALVR106, a therapy which targets common community-acquired respiratory viruses.

PREVAIL GAIN FAST TRACK DESIGNATION FOR GENE THERAPY CANDIDATE TO SLOW FRONTOTEMPORAL DEMENTIA

Prevail Therapeutics has announced that its experimental gene therapy program targeting frontotemporal dementia with a *GRN* mutation (FTD-GRN), PR006, has received FDA Fast Track Designation.

Frontotemporal dementia is the second most common cause of dementia in the over 65s, second only to Alzheimer's disease, and affects 50,000–60,000 people in the USA, with no treatment available. FTD-GRN

makes up 5–10% of patients with frontotemporal dementia and is caused by mutations in the *GRN* gene that cause reduced levels of the protein granulin. Although granulin's role in the brain is not well characterized it is thought to affect the survival of neurons, and reduced levels lead to lysosomal dysfunction and ineffective protein degradation.

Earlier this month, Prevail announced that the FDA had accepted the company's Investigational New Drug (IND) application for PR006, allowing the company to initiate its PROCLAIM Phase 1/2 clinical trial to investigate the safety and tolerability of the adeno-associated viral vector-based therapy – the trial will also look at key biomarkers and efficacy endpoints in patients with FTD-GRN, and dosing is planned to begin this year.

"The FDA's decision to grant Fast Track Designation for PR006 is an important step forward in our mission to deliver a potentially disease-modifying gene therapy to FTD-GRN patients as quickly as possible. FTD-GRN progresses rapidly and there are currently no therapeutic options available. We believe PR006 has the potential to fill this unmet medical need and make a significant impact for patients," commented Asa Abeliovich, Founder and Chief Executive Officer of Prevail.

Ones to Watch

The granting of Fast Track Designation for Prevail's treatment for frontotemporal dementia with a GRN mutation (FTD-GRN) endorses the significant unmet clinical need in this devastating condition. FTD-GRN, inherited in an autosomal dominant pattern, affects an estimated 3 to 15 per 100,000 people aged 45 to 64, and accounts for 5 to 10% of all cases of frontotemporal dementia. PR006 is one of three programs in the company's pipeline, all focused on neurodegenerative diseases caused by lysosomal dysfunction. Whilst the AAV-9 based therapy has not yet reached the clinic, an active IND suggests that the first clinical trial in patients will start imminently.

CAR M TECHNOLOGY FROM UPENN SEES SUCCESS IN MICE

University of Pennsylvania spin-out company Carisma Therapeutics has reported that its novel human chimeric antigen receptor macrophage (CAR M) technology has shown promising findings in a mouse model of ovarian cancer.

A previous study conducted by the UPenn researchers who developed the technology, published in *Nature Biotechnology*, found that anti-HER2 CAR M treated mice had decreased tumor burden and prolonged survival in two ovarian tumor xenograft models. The team hope that CAR M therapy, which involves genetically engineering human macrophages to direct their phagocytic activity against tumors, can overcome some of the limitations CAR T therapy has shown in treating solid tumors. The unique functions of macrophages, including the fact they are naturally drawn

DOI: 10.18609/cgti.2020.076

to solid tumors, are better equipped to survive in the tumor microenvironment, and can activate the adaptive immune system, could potentially make them a key tool in cancer immunotherapy to combat solid tumors.

In their latest study, Carisma tested HER2-directed CAR Ms in two separate mouse models of metastatic ovarian cancer and found that survival improved, and the therapy was effecting positive changes to the tumor microenvironment. The company is now working towards an IND filing and is developing plans for a Phase 1 clinical trial evaluating a HER2-targeted CAR M.

Michael Klichinsky, Co-Inventor of the CAR-M technology and Scientific Co-Founder and Vice President of Discovery of Carisma Therapeutics, commented:

"This is the first time that human macrophages have been engineered to express CARs and have successfully been shown to infiltrate tumors, influence the surrounding tumor microenvironment, reduce tumor burden through phagocytosis and increase overall survival in animal models of solid tumors."

Ones to Watch

Carisma Therapeutics is an outlier in the world of CAR-based cell therapies for oncology, in that it uses a macrophage as the cellular vehicle, rather than a T cell. Carisma's founding team and management believe that macrophages may be better suited to the tumor microenvironment than T cells, which have struggled to produce the same results in solid tumors as they have in cancers of the blood. Carisma has recently generated data in a mouse model of metastatic ovarian cancer suggesting CAR M's will have a future in the use of cell-based immunotherapies for oncology applications.

ROCKET PHARMA PUBLISH PROMISING RESULTS FROM DANON DISEASE PROGRAM

Danon disease is a rare X-linked lysosomal glycogen storage disease caused by mutations in *LAMP2*, with key characteristics including cardiomyopathy, skeletal muscle weakness, organ dysfunction, and intellectual disability. Currently available treatments – such as heart transplant – focus solely on symptoms, and the condition results in a significantly shortened lifespan for those affected.

However, a gene therapy could be on the horizon. Rocket Pharmaceuticals have recently published a new study in *Science Translational Medicine,* co-authored by Eric Adler, Director of Cardiac Transplant and Mechanical Circulatory Support at UC San Diego Health, California, entitled "AAV9.LAMP2B Reverses Metabolic and Physiologic Multiorgan Dysfunction in a Murine Model of Danon

Disease" that demonstrates improved cardiac function and survival in *LAMP2* knockout mice systematically injected with a recombinant adeno-associated serotype nine (AAV9) capsid containing the human LAMP2B transgene (AAV9.LAMP2B).

A single IV infusion of AAV9.LAMP2B at varying dose levels was given to two groups of male mice; a 2-month-old cohort was used to test the ability of the therapy to prevent disease development, while a 6-month-old cohort was used to determine the ability of the therapy to reverse established disease. In the 6-month cohort, improvement in cardiac and hepatic phenotypes and improvement in autophagy in the heart, liver, and skeletal muscle tissue were observed. For the mice receiving the 3 highest doses, improved survival was also seen – although as the mice were euthanized at 9 months further studies on survival are needed. In the younger mice, expression of LAMP2B RNA equivalent to or higher than wild type was seen in the heart, liver, and skeletal muscle, and the protein was expressed in a dose-dependent fashion in the heart. Another promising finding was that, although previous studies have raised concerns over liver toxicity being caused by high doses of systemically delivered AAV, no evidence of treatment-related liver damage was found in the mice.

An ongoing Phase 2 dose escalation study of AAV9.LAMP2B as a treatment for Danon disease is also underway at UC San Diego Health, and is enrolling 12–24 candidates to assess the safety and efficacy of increasing dose levels in pediatric and young adult male patients.

Jonathan D Schwartz, Chief Medical Officer and Senior Vice President of Rocket, commented:

"Presently, there are no specific or compelling treatment options for Danon disease. These impressive data provide rationale that RP-A501 could have a significant impact for patients contending with Danon. We look forward to continued collaboration with Dr. Adler and colleagues to investigate the potential of RP-A501 in our ongoing clinical trial, from which we expect to report data in the second half of this year."

SIGILON RAISES OVER $80 MILLION FOR HEMOPHILIA CELL THERAPY TESTING

Hemophilia is a promising target for cell and gene therapy developers, but so far, success has been limited. Sigilon Therapeutics is hoping to change this with its lead program, a cell therapy for hemophilia A utilizing encapsulated cell technology. The company has just completed a series B round of fundraising that has netted $80.3 million in funding, which will help get the treatment into clinical studies later this year.

Previous attempts to transplant working cells into hemophilia patients and restore clotting factors have failed due to immune rejection and tissue scarring – but Sigilon believe their Afibromer matrix technology can protect cells, while optimizing their delivery and promoting long term stability. The technology encases therapeutic cells in implantable spheres that shield them from immune attack and fibrosis, while allowing the cells to receive oxygen and nutrients, and to release the therapeutic protein they contain – such as insulin for diabetes, or a clotting factor for hemophilia – while preventing cell-to-cell contact. In preclinical studies, delivery of Sigilon's SIG-001 treatment for hemophilia A into a mouse model resulted in a steady production of the blood-clotting protein Factor VIII, and controlled bleeding.

Sigilon Therapeutics CEO Reogerio Vivaldi, commented:

"It will be the first time this technology platform will be tested in humans after being extensively tested preclinically in many different species. In parallel, we will be starting IND-enabling studies in other indications, particularly in lysosomal storage disorders."

DOI: 10.18609/cgti.2020.076

CELL MEDICA REBRANDS TO PURSUE CAR-NKT THERAPIES

Cell Medica has rebranded as Kuur Therapeutics as it redirects its focus from autologous Epstein-Barr virus-specific T-cells to autologous and allogeneic CAR natural killer T-cell (CAR-NKT) therapies.

Slow enrollment has seen Kuur call a halt to two clinical trials of a previous candidate last year – and the success of its CAR-NKT candidates has led to a new direction. IP Group, Schroder Adveq and Kuur's CAR-NKT partner Baylor College of Medicine have provided an undisclosed investment to help newly rebranded Kuur pursue its goals.

Two Phase 1 studies are now underway. GINAKIT2 is exploring autologous CAR-NKT cells in neuroblastoma, and is enrolling patients at the third dose level, and ANCHOR, which is assessing allogeneic CAR-NKT cells in CD19 malignancy, has just had its Investigational New Drug (IND) application approved, with patient treatment expected to start this year. A third therapy for hepatocellular carcinoma is also in preclinical development with an IND submission expected next year.

Kuur's new CEO, Kevin S Boyle, Sr, commented:

"I am excited to lead Kuur Therapeutics at such a pivotal moment. We are making final preparations to take our off-the-shelf program into the clinic and believe the allogeneic approach holds huge promise for unlocking the potential of CAR therapies for large patient populations. Compared with patient-specific autologous CAR products, it is immediately available for treatment and less expensive to manufacture."

Licensing agreements & collaborations

CODEXIS AND TAKEDA FORM GENE THERAPY R&D COLLABORATION

Protein engineering and biotherapeutics development company Codexis has formed a partnership with Takeda to leverage its protein engineering platform to develop new gene therapies for rare diseases. The indications the duo plan to tackle include lysosomal storage disorders and blood factor deficiencies.

The strategic collaboration and license agreement will see Codexis using its CodEvolver protein engineering platform to generate novel transgenes with enhanced stability, activity and cellular uptake. Takeda will then use these transgenes to generate candidates to treat rare genetic disease, and be responsible for clinical development and

commercialization of any resulting gene therapy products. The work will initially focus on three programs, with the option for Takeda to initiate up to four additional programs for separate target indications. Codexis will be eligible for an upfront payment, reimbursement for R&D, development and commercial milestone payments, and low- to mid-single digit percentage royalties on sales of any commercial product developed.

John Nicols, president and chief executive officer of Codexis, commented:

"Our CodeEvolver platform technology enables the rapid engineering of novel genetic sequences that encode more efficacious proteins. The prospects of these improved sequences for the development of differentiated gene therapies for patients with rare diseases therefore holds great promise. Takeda's expertise in developing novel treatments for patients with rare genetic disorders, and its commitment to developing the best possible gene therapies, makes them an ideal partner for our growing Novel Biotherapeutics business unit."

SERVIER AND CELLECTIS FINE TUNE COLLABORATION TO FOCUS ON CD19 CAR T THERAPIES

Servier originally made a deal with Cellectis, a company focused on immunotherapies based on gene edited allogeneic CAR T cells, back in 2014, obtaining rights to their off-the-shelf CAR T therapies. Now, Cellectis has announced that the deal has been updated to give Servier additional rights to its CD129 CAR T therapies, with Servier providing €25 million up front, up to €370 million in additional milestones, and an increased royalty rate.

Sevier, alongside sublicensee Allogene, is already trialing UCART19 in acute lymphoblastic leukemia and ALLO-501 in non-Hodgkin lymphoma. The amended deal grants Servier an exclusive worldwide license to develop and commercialize all Cellectis CAR T cell products targeting CD19, and also returns the rights to five undisclosed allogeneic CART T targets to Cellectis.

ASKBIO AND UNC SET SIGHTS ON ANGELMAN SYNDROME

Clinical stage gene therapy company Asklepios BioPharmaceutical (AskBio) has teamed up with the University of North Carolina (UNC) at Chapel Hill to develop a therapy for Angelman syndrome using the company's adeno-associated virus (AAV) technology.

The collaboration aims to combine AskBio's extensive AAV capsid promoter library and proprietary cell line manufacturing process with UNC researcher's preclinical work suggesting that people with Angelman syndrome could see their symptoms improved using gene therapy.

Angelman syndrome, caused by loss of function of the *UBE3A* gene, commonly results in severe intellectual and developmental disabilities, and often comes with other symptoms such as frequent seizures, sleep problems and scoliosis.

Mark Zylka, director of the UNC Neuroscience Center, commented:

"Individuals with Angelman syndrome face lifelong challenges, and our gene therapy approaches hold the potential to correct this disorder at its genetic roots. We are incredibly excited to partner with AskBio, as they have been vanguards of clinical gene therapies for rare diseases."

The financial terms of the agreement have not been disclosed.

DOI: 10.18609/cgti.2020.076

TORQUE THERAPEUTICS AND COGEN IMMUNE MEDICINE INTEGRATED INTO NEW BIOTECH

Venture capitalist firm Flagship Pioneering has merged two of its sister biotechnology companies, Torque Therapeutics and Cogen Immune Medicine, to form a combined company now known as Repertoire Immune Medicines. Torque CEO John Cox is to head up the new combined effort.

Torque's work was focused on "deep primed adoptive cell transfer" which it hopes will be able to overcome some of the hurdles cell therapies face in treating cancer, such as tumor microenvironment, and Cogen's focus was on understanding the immune system. Together as Repertoire they plan to combine these areas to find ways to harness the immune system to prevent and treat cancer, automimmune disorders and infectious disease, using their DECODE discovery and DEPLOY product platforms.

Repertoire also plan to utilize three discovery technologies to understand the drivers of the immune response: MCR, cell-based reporter assays to experimentally quantify MHC-specific peptide display and de-orphan T cell receptor (TCR) clonotypes across peptide-MHC libraries; CIPHER, MHC multimer-based assays to detect and measure TCR clonotypes, peptide-MHC reactivity and phenotypes on a single-cell level; and CAPTAN, internally developed deep-learning computational tools to classify platform hits and leverage large data sets to predict TCR reactivity.

The company currently has a Phase 1/2 clinical trial underway testing their PRIME$^{IL\text{-}15}$ product either alone or in combination with Keytruda® in patients with relapsed or refractory solid tumors and lymphomas.

"Repertoire is pioneering a new class of therapies based on high throughput, high content interrogation of the intrinsic ability of T cells to prevent, or cure diseases," commented Noubar Afeyan, CEO of Flagship Pioneering and co-founder and chairman of Repertoire Immune Medicines.

Finance

REDPIN BAGS $15.5 MILLION TO PROGRESS CHEMOGENETICS PLATFORM

Chemogenetics company Redpin Therapeutics has secured $15.5 million in the initial closing of its Series A financing round, which was led by 4BIO Capital, Akrin Bio Ventures and Takeda Ventures Inc, along with existing investors from the seed round.

The investment will allow Redpin to continue to develop its ion channel-based chemogenetics platform to address disorders associated with neural circuit dysfunction including epilepsy and Parkinson's disease. The approach uses ion channels as neuromodulation tools to selectively activate or inhibit disease-causing neurons using low doses of the anti-smoking drug varenicline (CHANTIX®), to regulate only dysfunctional neurons, while leaving normal functioning cells alone; this could offer significant benefits compared to current systematic drugs used to address local neuron dysfunction, which can have limited efficacy and cause off-target adverse effects.

Dmitry Kuzmin, Managing Partner at 4BIO Capital, who will join Redpin's Board of Directors, commented:

"Our goal is to support and grow advanced therapy companies with the potential to cure chronic disease. Redpin has a highly compelling, validated chemogenetics approach that could have significant potential in the targeted treatment of neuropathic disorders. The strength of Redpin's science alongside the world-class knowledge and expertise of the Company's founders and management team make us fully confident in the future success of the Company towards this goal."

ELEVATEBIO RAISES $170 MILLION IN NEW FINANCING TO TACKLE CELL AND GENE THERAPY BOTTLENECK

ElevateBio is a holding company with the goal of tackling the manufacturing bottleneck in cell and gene therapy by providing a centralized R&D and manufacturing site for its portfolio companies, known as ElevateBio BaseCamp. ElevateBio has unveiled two companies since its launch last year: AlloVir and HighPassBio. Now, with an additional $170 million in series B financing, the company intends to make its R&D and manufacturing hub fully operational, and to fund the clinical development of six new programs. The programs will include treatments from AlloVir and HighPassBio, and at least three new cell and gene therapies from as-yet undisclosed portfolio companies, including AAV and T cell-based technologies.

ElevateBio has also entered a 10-year deal with Massachusetts General Hospital to manufacture cell and gene therapies, as well as to build cell and gene therapy biotechnology companies. It plans to begin Phase 2 and 3 studies for AlloVir's Viralym-M program, which targets six viruses that commonly affect patients who receive stem cell transplants, and to move a program targeting diseases caused by community-acquired respiratory viruses into the clinic this year. Additionally, the company intend to be performing cGMP manufacturing at their BaseCamp from as early as 2021.

LEGEND FILES IPO TO FUND CAR T PLANS

Chinese CAR T developer Legend Biotech has submitted IPO paperwork to the US Securities and Exchange Commission, revealing its plan to list stock in the US. The Genscript Biotech spin-out company has a promising CAR T pipeline, led by the Johnson & Johnson (J&J)-partnered anti-BCMA autologous CAR T therapy JNJ-4528.

J&J paid $350 million upfront for a global license to JNJ-4528 in 2017, with the companies set to evenly split profits generated outside of China. Since then a Phase 1B

DOI: 10.18609/cgti.2020.076

trial has reported a 69% complete response rate in multiple myeloma patients who had received a median of five prior treatments, with all of the patients responding to the drug. Legend expects filings for approval in the USA and EU for JNJ-4528 later this year.

But this isn't the only arrow in Legend's quiver – the company also has several allogeneic cell therapies; one against hematological cancers that is currently in the clinic, and another being tested in gastric and pancreatic cancer. Its pipeline also includes autologous CAR Ts against CD19xCD22 and CD33xCLL-1.

Movers & shakers

CATHERINE M VACZY AND MICHAEL T REDMAN JOIN GENPREX LEADERSHIP TEAM

Genprex, a gene therapy company with a focus on cancer and diabetes, has appointed Catherine M Vaczy as Executive Vice President and Chief Strategy Officer, and Michael T Redman as Executive Vice President and Chief Operating Officer.

After a time spent as a practicing attorney representing life science and technology companies, Ms Vaczy went on to gain over two decades of experience serving as a founder and senior executive in the life sciences space, most recently serving as a strategic advisor to early stage biotechnology companies. She also co-founded and served as a senior leader for NeoStem, Inc. (now Caladrius Biosciences) for 10 years, and formed part of the senior leadership team of Nasdaq-listed ImClone Systems Incorporated (sold to Eli Lily and Company). At Imclone she was involved in securing a $1 billion co-development deal for the company's blockbuster drug, Erbitux®.

Mr Redman has held a range of key executive roles in clinical-stage life science companies over the last 30 years, with a focus on strategic business development and manufacturing and clinical operations. From 2007 to 2019 he served as President, CEO and Director of Oncolix, Inc., a publicly traded clinical-stage biopharmaceutical company focused

on developing therapies for women's and children's cancers. During his tenure at Oncolix, he advanced the company's lead drug into human clinical trials, completed the in-licensing of a promising radiopharmaceutical drug for the treatment of bone-related cancers, and took the company public. Prior to this he co-founded and led Opexa Pharmaceuticals, an immunotherapy company, and held management positions with Zonagen (now Repros Therapeutics, Aronex Pharmaceuticals, Biovail Corporation and American Home Products (acquired by Pfizer).

Genprex has recently received Fast track Designation for its lead drug candidate and licensed a gene therapy for diabetes. Both Ms Vaczy and Mr Redman are anticipated to play key roles as Genprex works towards new clinical trials and development partnerships.

- Written by Roisin McGuigan,
Cell and Gene Therapy Insights

DOI: 10.18609/cgti.2020.076