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NUCLEIC ACID INSIGHTS

BIOINSIGHTS

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Harnessing the potential of AI/ML to expand the horizons of the oligonucleotide therapeutics field

Artificial intelligence and machine learning (AI/ML) are already making their presence felt across the life sciences, but what can they offer the oligonucleotide therapeutics space as it battles to reach new therapeutic areas and opportunities? **David McCall**, Senior Editor, BioInsights, talks to **Arijit Bhowmick**, Director, Oligonucleotide Therapeutics, insitro, about the current state of the oligonucleotide therapeutics field, and how the powers of AI/ML can be harnessed to create better drugs, particularly oligos.

Nucleic Acid Insights 2024; 1(7), 219–225

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Q What are you working on right now?

AB: I am leading the oligonucleotide drug discovery work at insitro, which is primarily a machine learning (ML)/AI-based biology discovery and drug discovery and development company.

At insitro, we use clinical data, cellular data, and machine learning to gain deep therapeutic insights into diseases, which helps us to identify targets and carry out drug discovery to develop safe and effective medicines more efficiently.

My role at insitro is primarily to lead the oligonucleotide drug discovery efforts across targets and therapeutic areas, all the way from early-stage design, through drug discovery, to selecting candidates for clinical development.

Q As someone who has specialized in drug discovery in the oligos space for more than a decade, what are your high-level reflections on how the field has evolved over that period to reach the cutting edge of innovation as it stands today?

AB: I think this field is growing and now is the right time to be in it. Just to provide some historical context, oligonucleotide therapeutics only really exploded in the past 5 or 6 years. Before that, success was hard to come by. The first oligonucleotide drug, Vitravene®, was approved in 1998. Macugen® came along soon afterwards, but the field then fizzled out due to some early clinical setbacks. The entire oligonucleotides field subsequently took a beating as big pharma moved away from it.

That all started changing with the development of newer chemistries to improve PK/PD profiles of this class of drugs. Companies like Alnylam Pharmaceuticals and Ionis Pharmaceuticals, who really believed in the potential of oligo therapeutics, did some seminal work to understand the chemistry and biology of oligonucleotides, which I think benefitted the entire field and the industry in general. Eventually, 2018 saw the approval of the first siRNA drug from Alnylam and since then, there has been a tremendous interest in RNAi drugs both within big pharma and smaller biotechs, not only to treat rare genetic disorders but also in more prevalent disease spaces.

If you look at the oligonucleotide therapeutics field today, outside of RNAi, there are so many different modalities—mRNA, CRISPR/Cas9-mediated gene editing, RNA editing/writing, antisense oligonucleotides (ASOs), blocking/splice switching oligos, and aptamers, for instance—that have also seen significant innovation and progress in the last 5–6 years, and that continue to grow at a rapid pace today, which is very exciting. The success of mRNA vaccines during the COVID pandemic also propelled a lot of innovation in the oligo therapy space in general, and mRNA technologies in particular—saRNA, circRNA, conditionally expressed mRNA, mRNA-based cell therapy approaches, and tRNA therapeutics to name a few.

So, where does that leave us today? Well, if we think about oligonucleotide therapeutics in the context of their ability to treat diseases in different tissues or therapeutic/indication areas, then the biggest challenge that almost any oligo therapy platform faces today is extrahepatic delivery. What is truly promising for the field is that in the past couple of years, we have seen very exciting siRNA delivery data in the CNS, kidney, muscle, lung, and adipose tissue, primarily using lipid conjugates, antibodies or peptides.

"...if you look at any emerging biotech field through the years...there are always the two fundamental hurdles to overcome: safety and efficacy."

I think if you look at any emerging biotech field through the years—from the emergence of antibodies nearly half a century ago, through oligonucleotides, to cell and gene therapy—there are always the two fundamental hurdles to overcome: safety and efficacy. I believe that oligonucleotides, particularly siRNAs, have already proven themselves in both departments. They have shown themselves to be extremely safe, and furthermore, the dosing window can be extraordinarily long. With optimized chemistries one can achieve 8–12-month dosing for patients, which is nearly impossible to attain using classical small molecule or antibody drugs. This kind of efficacy and durability can dramatically relieve the burden on the healthcare system and encourage full patient compliance. Once we solve the extrahepatic delivery challenge, I can certainly see RNAi taking over small molecules and antibodies across a number of disease areas, from rare genetic disorders to more prevalent ones which affect a large population. So, RNAi is here to stay and will grow stronger.

In the mRNA space, with innovation in chemistry, delivery, and expression platforms, we will see this class of molecules being more widely used as vaccines, as well as therapeutics where we need gain of function or protein replacement. CRISPR-based gene editing technologies have also shown promise in the clinic and are poised to treat several diseases, particularly those that are liver-targeted. Overall, I feel this is the most exciting time to be in this space and oligonucleotides will soon be the most sought-after drug class, if they are not already!

Q Tell us more about how insitro is using oligonucleotides to develop next-generation medicines

AB: As I mentioned earlier, insitro has a unique approach to biology and drug discovery, which we call 'pipeline through platform'. We generate multi-modal phenotypic cellular data in our automated laboratories for our cell ML platform, and utilize high-content human clinical, imaging, and genetic data in our clinical ML platform to unlock novel therapeutic hypotheses. Our target discovery platform allows us to identify high conviction targets and de-risk these targets from a therapeutic intervention perspective. This helps us to accelerate drug discovery against those targets using different therapeutic modalities such as small molecules, biologics, and oligos. Specifically for developing oligonucleotide therapeutics, our platform not only allows us to get an unprecedented insight into the transcriptomic and phenotypic effect of knocking down a target, but it also allows us to investigate off-target effects thoroughly. This helps in de-risking the drug candidate and developing safe and effective medicines faster.

Importantly, this work is done with our integrated platform that also improves over time as more data is generated and models are improved.

Q Can you go deeper on how and where the application of ML/AI can unlock new opportunities for the oligonucleotide therapeutics space moving forward—and what will be some key challenges to be addressed along the way?

AB: AI/ML has already been employed to successfully design small molecule drugs. However, I think the application area in which it has yet to be used extensively is oligonucleotide drug design. There are already several companies working on small molecule drug design using ML/AI, for instance, but the same cannot be said for RNA therapeutics. Obviously, here we are talking about AI/ML applications in oligo drug candidate discovery specifically, and not target discovery, which we discussed before.

I think ML and AI can have a huge impact on the design of oligonucleotide drugs such as siRNAs, ASOs, and even mRNAs. For siRNAs and ASOs, there is a huge opportunity to look at available knockdown data from several studies and publications, combine that with target mRNA structure-function, and build a model to predict potency. Additionally, physics-based models can be applied to understand structure and interaction of these molecules with their activity partners, which may help in optimizing the chemistry to help potency.

However, there are several challenges that need to be addressed. Firstly, the target mRNA expression and RNA binding protein signatures vary across cell types. These affect siRNA accessibility and target knockdown, and it is very difficult to model these aspects of the mRNA. Additionally, it might be easier to model an unmodified RNA into a RISC or RNaseH, however, chemical modification patterns in siRNAs and ASOs will change these interactions. To model these interactions, we will need a lot of data across sequences and chemistry.

Another area where AI/ML can help RNAi design is to predict off-target effects and off-target potency, which is a crucial aspect of RNAi drug discovery. *In silico* pathway analysis of predicted off-target sites may help in selecting the safest drug candidates. All these innovations will eventually speed up oligo drug discovery efforts and help us to get to patients faster, which is the ultimate goal.

Outside siRNA/ASO drugs, AI/ML will also have a major impact on mRNA therapeutics and CRISPR-based gene editing as well. ML can be used to optimize an mRNA sequence and chemistry to ensure higher stability, enhanced protein expression, and reduced immunogenicity. A number of companies are already using ML to design LNPs for tissue-specific delivery of mRNA and CRISPR components. This will certainly help in developing potent mRNA therapeutics, and also open up ways to get them to tissues outside the liver.

"The application of artificial intelligence and machine learning to identify and de-risk targets will help in accelerating the oligo drug discovery process..."

Q Can you share your vision for the future of the field, particularly in terms of expanding its reach into new therapeutic areas and indications?

AB: We have been talking a lot about the liver as an area where oligonucleotide therapeutics have been successful, but there are several other areas where we have yet to see that sort of success, and the key to unlocking them all is delivery. Delivering outside the liver in a successful way will be key, whether that involves ligand-mediated delivery, antibody-mediated, small molecule-mediated, conjugate-mediated, or LNPs—whatever works. That is the first thing that has to be solved. Once it is, it will open up a lot of therapeutic areas and indications.

One such area is the CNS where there is a lot of interest in using oligonucleotides as therapeutics. In the recent past, we have seen some promising data coming out of Alnylam's APP program—they showed potent knockdown and safety using their C16 siRNA platform in the CNS in both non-human primates and humans, which is very encouraging for the field. There are other companies—Denali Therapeutics, Switch, and Atalanta to name a few—who are developing innovative ways to deliver RNAi in the CNS.

Other tissues where there has been significant progress in delivery are the lung and more recently, in adipose tissue. Companies such as Avidity, Dyne, and Arrowhead have demonstrated very exciting data for siRNA and ASO delivery in these tissues using either antibodies, peptides or lipophilic conjugates.

The application of AI/ML to identify and de-risk targets will help in accelerating the oligo drug discovery process and hopefully we will also see the emergence of generative AI based models to aid oligo design. Additionally, I think we will see an increase in combination therapies with siRNAs, either using a combination of siRNAs against multiple targets or a combination therapy with an antibody or a protein, which will further expand the use of this class of drugs.

Finally, one aspect of oligo drugs that has not been studied extensively to date is oral delivery. I think there will be a lot of effort made in that direction to make orally delivered siRNA and ASOs bio-available. If successful, this could exponentially grow the use of oligonucleotide therapeutics. All in all, oligonucleotides have just gotten started.

Q Can you sum up one or two key goals and priorities, both for yourself in your own role and for insitro as a whole, over the foreseeable future?

AB: We have some really exciting data from our novel platform-derived genetic targets, and we are excited to push drug discovery efforts against those targets all the way to the clinic and help patients as quickly as possible. Several of these programs are rapidly progressing to later stages of preclinical development. A lot of my own goals are tied in with insitro's, but I would add that I am personally very interested in addressing the extrahepatic delivery problem using AI- and ML-based technologies, and would also like to use AI/ML for oligo design in future.

BIOGRAPHY

ARIJIT BHOWMICK heads insitro's oligo therapy drug discovery efforts across targets, modalities, and therapeutic areas—from early-stage design/screening to DC candidate nomination and development. Arijit has more than 11 years of experience in the field of oligonucleotide and RNA therapy. Prior to joining insitro, South San Francisco, CA, USA he worked at Regeneron Pharmaceuticals where he established and led the Nucleic Acid Therapeutics group under Regeneron Genetics Medicine (RGM), a group that focused on oligonucleotide-based drug discovery and technology development across modalities such as siRNA, ASO, mRNA, and gRNA design/optimization for gene editing. Before his time at Regeneron, Arijit was at Vitrisa Therapeutics, a privately funded bio-tech startup, where he led early-stage RNA based drug discovery efforts in the ophthalmology space. Arijit has a PhD in Structural Biology and Immunology and pursued his postdoctoral research in chemically modified RNA therapeutics technology development at the Albert Einstein College of Medicine, New York, NY, USA before joining the bio-pharma industry.

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NUCLEIC ACID INSIGHTS

OLIGONUCLEOTIDES: EMERGING MODALITIES

SPOTLIGHT

Delivering on the next generation of oligo therapies depends on overcoming, not ignoring, that sequence and chemistry are separable, but not independent

Chris Hart
Creyon Bio

"...the true potential of oligonucleotide based medicines has yet to be realized..."

VIEWPOINT

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Oligonucleotide based medicines (OBMs), including antisense oligonucleotides (ASOs) and short interfering ribonucleic acids (siRNAs), have emerged as a proven 'super modality', producing over a dozen approved drugs with many more in development. A number of the approved products have excellent target product profiles, including multiple blockbusters [1]. OBMs are short, chemically-modified nucleic acid polymers which can specifically modulate the expression of genes through a variety of mechanisms. Certain properties of OBMs set them apart from other therapeutic modalities, the most striking of which is that OBMs are fundamentally compositional (i.e., programmable) drugs in that the interaction with a target nucleic acid occurs through hybridization, and because non-hybridization interactions are avoidable. Further, adding targeting ligands to OBMs can potentially enable delivery towards specific cells and tissues in a programmable way. Importantly, the pharmacophore (elements that determine the target specificity), and the dianophore (elements that determine absorption, distribution, excretion, and metabolism, i.e., ADME), are separable [2]. It is important to recognize, however, that the impact of chemical modifications on OBM properties, particularly tolerability, can vary significantly depending on the underlying base sequence.

Most OBM discovery efforts have borrowed methods and approaches from small molecule discovery and attempted trial-and-error screening campaigns to advance lead compounds toward clinical candidates and ultimately, approved new drugs. These methods are often developed around the misunderstanding that the base sequence and the chemical modifications are independent and can therefore be optimized independently. These stage-gate processes then usually focus on finding active compounds based on sequence and using a fixed nucleic acid chemistry or chemical architecture. Typically, various sequence motifs or simple sequence-based heuristics are applied in an effort to avoid some compounds with unfavorable pharmacology and, hopefully, bias towards more favorable results.

This has led to a strong historical emphasis in the field on identifying chemistries and heuristics that have general benefit across all sequences. Modifications like locked nucleic acids (LNAs) were heralded as being generally toxic [3], phosphorothioate backbones have been claimed to be the driver of immune response [4,5], and methoxy ethyl (MOE) ASOs and siRNAs have been claimed to be generally safe [3,6,7]. And yet, LNAs have been advanced successfully into Phase 2 trials without a safety signal [8] and used as part of successful n-of-1 treatments [9]. Chemistries put forward as 'more tolerable', such as 5-10-5 MOE or 3-10-3 cEt gapmers, have had significant adverse events or observed pre-clinical liabilities [10–12], and siRNAs have also suffered notable clinical setbacks and challenges [13]. Singularly optimizing only chemistry or only sequence without understanding the collusion between the two is like playing 'whack-a-mole'. Failing to embrace the interrelatedness of sequence and chemistry prevents OBMs from realizing their full potential of being an 'information drug' that are foundationally engineerable.

Thus, the true potential of OBMs has yet to be realized, perhaps because of an overcommitment to the idea that the pharmacological properties imparted on these OBMs by the chemical modifications used is not influenced by the sequence. A further potential contributing factor is the false hope that these polymeric molecules could be optimized in the same ways that small molecules are, with simple heuristics or brute-force trial-and-error screening. This also justifies the current regulatory stance, which affords little ability to update understandings based on previous OBMs. Currently, each new OBM drug is evaluated independently with each molecule effectively being treated as a completely new chemical entity, because there hasn't yet been enough useful prior information created to exploit or to change our priors or expectations of safety and efficacy.

However, if we leverage the polymeric nature of OBMs, they should be engineerable. Critically, though, the interplay between sequence and chemistry must be accounted for. We should not assume there are simply 'safe' chemistries or 'unsafe' sequences. OBMs have a small set of compositional units that are linked together to create novel compounds. Because of this limited diversity of the components of OBMs, the effects of the units and the interactions between the units can be described and well understood, and ultimately used to understand the properties of novel compositions. This can then be incorporated into the assessment of the tolerability risk of each novel oligonucleotide-based therapeutic.

Modern AI/ML techniques have the potential to help resolve this seemingly intractable problem, but appropriate data needs to be the focus that allows us to understand how these units interact. We need to solve the sequence and chemistry collusion problem. Retrospective and collated data from happenstance studies will be inadequate to explore the complex and large design space of OBMs. Traditional drug discovery efforts depend on stepwise processes optimized to create drugs that are 'good enough' by advancing only a few compounds serially through increasingly expensive and stringent pharmacology studies. Ultimately, for OBMs, this precludes learning how the library of compositional units interact and manifest with molecular properties that are either favorable or not. Polymers like OBMs should be engineerable, if you understand how the components interact and work together to create the emergent properties of the full molecule. However, we must focus on creating purpose-built data rather than hope that retrospective data collected as part of one-drug-at-a-time campaigns will be sufficient to solve a foundationally different problem from those faced by other drug discovery efforts.

As foreseen and well articulated by Francis Collins, advances in modern biology driven in part by the genome revolution have delivered in providing the detailed molecular and genetic understanding of pathogenesis, yet drug development has not overcome long timelines and high failure rates [14]. OBMs offer perhaps the most direct path towards redefining drug discovery as drug engineering, bringing with it the potential to dramatically increase the efficiency of translating the discoveries of modern biology into new meaningful treatments for patients.

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BIOGRAPHY

CHRIS HART is the CEO and Co-founder of Creyon Bio, Carlsbad, CA, USA and an experienced leader with decades of experience leveraging computational methods, machine learning and AI, and deep biological insights to solve problems. Prior to creating Creyon Bio, he built the functional genomics department at Ionis Pharmaceuticals where he was responsible for the company-wide genomics and bioinformatics efforts, including execution and strategic leadership of exploratory drug discovery programs that included programs in rare and common diseases. He worked at the Science and Technology Policy Institute advising the White House Office of Science and Technology Policy on matters ranging from personalized medicine to international health research funding. Chris earned his PhD from the California Institute of Technology, Pasadena, CA, USA and conducted post-doctoral training at Yale University, New Haven, CT, USA.

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NUCLEIC ACID INSIGHTS

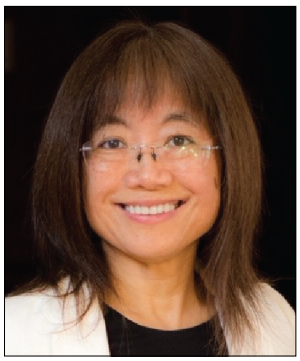
FORMULATION AND DELIVERY:
EXTRACELLULAR VESICLES

SPOTLIGHT

Revolutionizing *in vivo* genome editing with targeted, non-viral delivery

Joseph Nabhan, Gary Hao, and Qin Yu
Vesigen Therapeutics





VIEWPOINT

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On August 8, 2024, **David McCall**, Senior Editor, BioInsights, spoke to Vesigen Therapeutics' **Joseph Nabhan**, Chief Scientific Officer, **Gary Hao**, Vice President, CMC, and **Qin Yu**, Vice President, Research, about the potential for novel vesicular delivery platforms to enhance the genome editor delivery toolkit and enable the expansion of *in vivo* gene editing therapeutics into new tissues and indications. This Viewpoint article is based on that conversation.

The field of therapeutic delivery has witnessed significant advancements in recent years, with a growing focus on precision medicine and the development of more targeted drug delivery systems. One such innovation, developed by Vesigen Therapeutics, is a new class of extracellular vesicles (EVs) known as ARMMs ([ARRDC1]-mediated microvesicles). These vesicles provide a solution for the delivery of complex therapeutic payloads, including gene editing tools, proteins, and RNA.

THE ADVANTAGES OF ENGINEERED VESICLES AS A DELIVERY VEHICLE FOR GENOME EDITORS

The field of genome editing has advanced rapidly, yet delivery methods for these powerful therapeutic tools have struggled to keep pace. Currently, most genome editors are delivered using lipid nanoparticles (LNPs) or AAV vectors. While both methods have shown promise, they each have significant limitations. For example, LNPs often accumulate in the liver, particularly in hepatocytes, making it difficult to effectively target extrahepatic tissues. AAVs, on the other hand, are limited by payload size, carry risks of toxicity and immunogenicity, and can lead to persistent genome editor expression, increasing off-target effects.

Vesigen Therapeutics' [ARRDC1]-mediated microvesicles (ARMMs) technology offers a promising alternative **(Figure 1)**. Building on the natural biology of extracellular vesicles (EVs), ARMMs are engineered using the ARRDC1 protein to drive vesicle biogenesis directly from the cell surface and intraluminal loading. This enables the efficient and targeted delivery of therapeutic payloads—including proteins, RNAs, and ribonucleoprotein complexes such as Cas9/gRNA gene editing complexes—directly to specific cells. Additionally, ARMMs can be engineered to incorporate fusogens on their surface to bypass the issue of endosomal trapping reported with LNPs. ARMMs can thus release payloads directly into the cytosol of recipient cells, which allows for safer and more efficient delivery to a broader range of tissues.

More specifically, the use of ARRDC1 as an intraluminal loading handle facilitates efficient packaging of therapeutic payloads into these non-viral delivery vehicles. Unlike other EVs including exosomes that are derived from multi-vesicular endosomes, ARMMs are formed directly at the cell surface. This biogenesis process ensures consistent product quality. Vesigen has also shown that ARMMs are stable for at least 6 months at 4 °C. As naturally occurring vesicles derived from human cells, ARMMs are less likely to provoke an immune response compared to synthetic LNPs or viral vectors like AAVs. Their transient nature further enhances safety by decreasing the risk of prolonged presence of genome editors in the body, which could lead to unintended off-target effects.

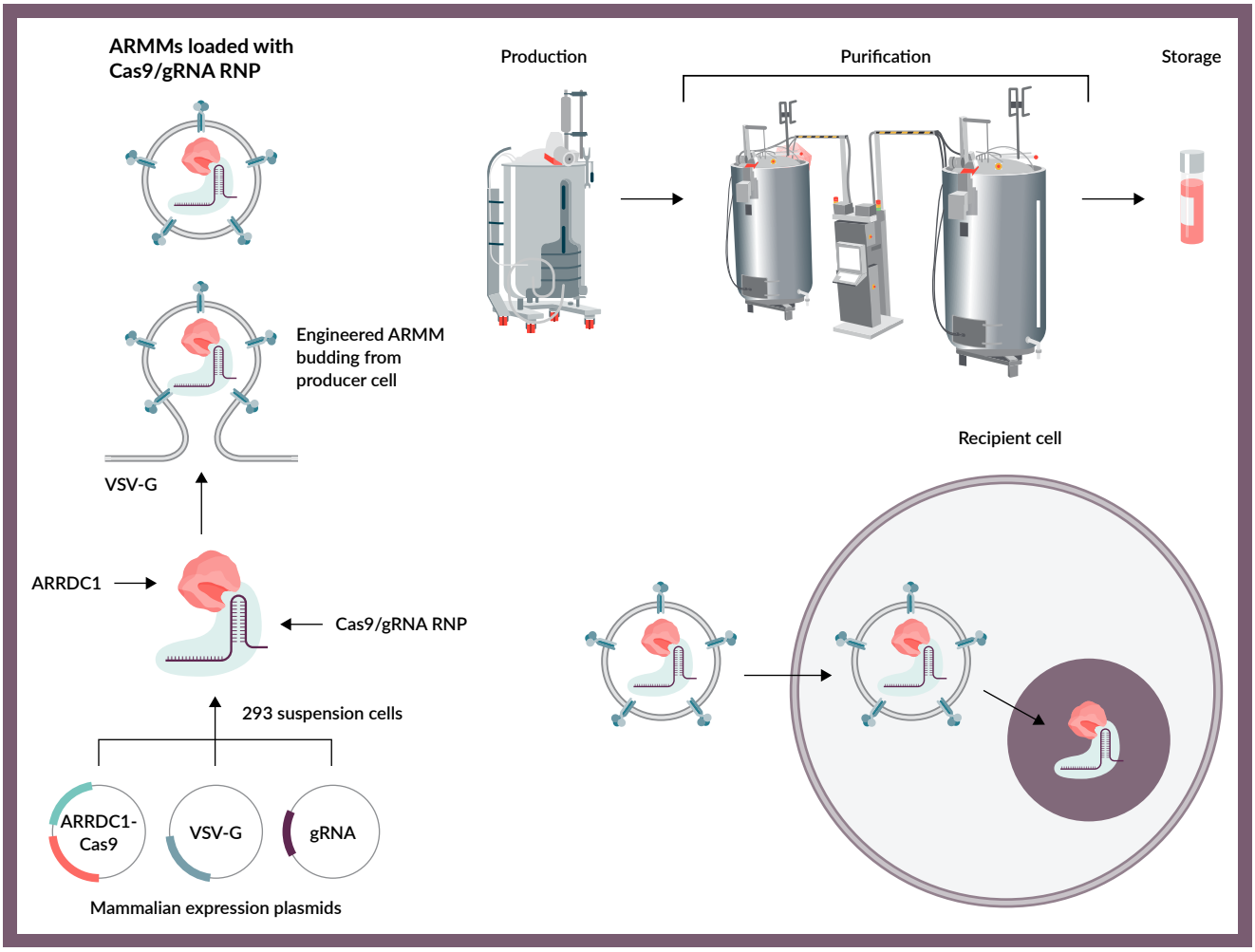
Another significant advantage of ARMMs is their engineering versatility. ARMMs can be easily modified to display targeting moieties, such as single-chain variable fragments (scFvs) or variable domain of heavy-chain antibodies (VHHs), allowing for precise targeting of specific cell types, such as T cells, B cells, and hematopoietic stem cells. ARMMs can also be directed to target solid tissues. These capabilities open up new possibilities for delivering therapeutics to hard-to-reach tissues and for developing personalized medicine approaches tailored to an individual patient's needs.

ADDRESSING CURRENT CHALLENGES IN EV MANUFACTURING

If EVs are to fulfill their potential in enabling commercialization of *in vivo* gene editing therapeutics, one of the primary obstacles to be addressed is the high cost of manufacturing. Current EV production systems are relatively low in terms of productivity compared to other therapeutic platforms, making it difficult to produce the large quantities required for widespread clinical use. Vesigen has invested significant upfront effort to establish robust

FIGURE 1

Schematic depicting generation of engineered ARMMs loaded with Cas9/gRNA ribonucleoprotein (RNP).



HEK293 suspension-adapted cells grown in bioreactors are transfected with plasmids encoding the indicated intraluminal payloads (ARRDC1-Cas9 and gRNA) and VSV-G fusogen. Purification of ARMMs is carried out using tangential flow filtration and anion exchange chromatography before storage at 4 °C. Treatment *in vitro* or *in vivo* results in functional delivery of the genome editing complex.

upstream and downstream processes that use standard unit operations to enable scale-up to support manufacturing. Vesigen is further addressing this issue by developing technologies to increase productivity of ARMMs manufacture while engaging with regulatory agencies to help shape specific guidelines for EV-based therapies in general.

Reducing the amount of therapeutic material required to achieve efficacy is a related and equally critical goal particularly for systemic administration. Here, Vesigen is exploring strategies to enhance the efficacy of ARMMs by reducing their uptake by the liver, when targeting other cell types. By decreasing the clearance of ARMMs, we aim to increase the therapeutic efficacy of ARMMs while simultaneously reducing the required dosage. These innovations are paving the way for the broader application of ARMMs in gene editing delivery and other therapeutic technology areas.

THE FUTURE OF *IN VIVO* GENE EDITING

While *in vivo* gene editing is still in its infancy, the potential it carries for revolutionizing the treatment of complex diseases

is immense. We envision a future where multiple delivery platforms coexist, each tailored to specific cell types and diseases. The versatility, safety, and engineering potential of ARMMs make them strong contenders for mainstream use in gene editing-based therapeutic applications, with the ultimate goal of addressing a broad range of diseases.

BIOGRAPHIES

JOSEPH NABHAN is Chief Scientific Officer at Vesigen Therapeutics, Cambridge, MA, USA. Joe is a co-inventor of the ARMMs technology upon which Vesigen was established. Prior to joining Vesigen, Joe held scientific and leadership positions in several pharmaceutical companies including Astellas Pharma, Pfizer, and Millennium Takeda leading drug discovery efforts across multiple therapeutic areas. He received his PhD from McGill University, Montreal, QC, Canada.

GARY HAO is Vice President of CMC at Vesigen Therapeutics, Cambridge, MA, USA. He has over 17 years of industry experience in CMC development of biotherapeutics including biologics, RNAs, viral vectors, and extracellular vesicles. He obtained his BS degree from Nankai University, Tianjin, China and PhD degree from Cornell University, Ithaca, NY, USA. During his career, he worked as key contributor to approved products (BAVENCIO and BRIUMVI). He published more than 20 papers and holds three patents.

QIN YU is Vice President of Research at Vesigen Therapeutics, Cambridge, MA, USA. She has over 18 years of experience in biopharmaceutical drug discovery and development, including 10 years of scientific leadership role in the cell and gene therapy field. Her scientific expertise primarily focuses on viral and non-viral vectors for therapeutic delivery, with her most recent roles centering on extracellular vesicle-based delivery technology. She obtained her PhD in Virology from University of Alabama at Birmingham, Birmingham, AL, USA.

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