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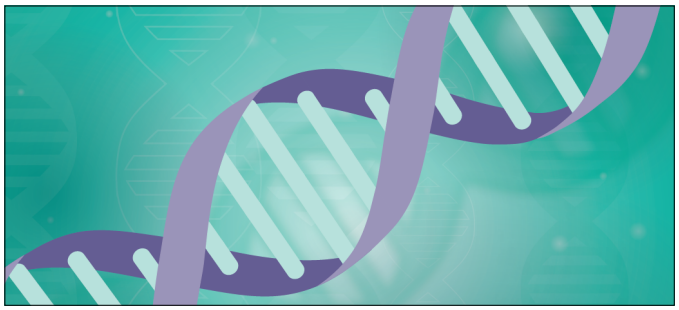
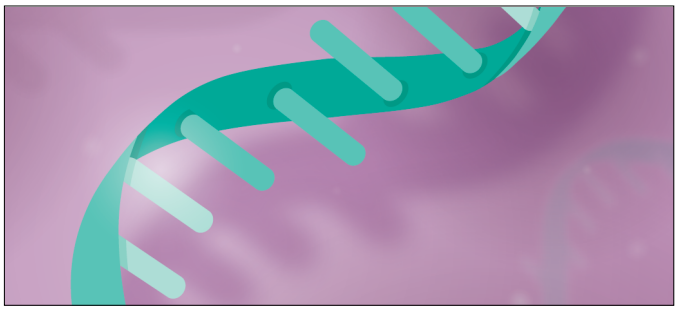


DNA
At the interface of synthetic biology and DNA



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LATEST ARTICLES

NUCLEIC ACID INSIGHTS

DNA
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SPOTLIGHT

COMMENTARY

Analytical control strategies for DNA starting materials in rAAV gene therapies and mRNA vaccines

Sonia Taktak, Josh Woods, Melissa M Anderson, Carlos Castaneda, Olivia Cunio, Haley Gassel, Elizabeth Ostrander, Alexis Schlueter, and Guojun Zhao

The importance of ensuring high-quality DNA starting materials has become increasingly significant due to advancements in gene therapies and mRNA products. In this paper, we explore the critical elements of a plasmid DNA (pDNA) control strategy, focusing on analytical quality and process-related impurities. Additionally, we examine the challenges and considerations in adapting these strategies to synthetic DNA starting materials. Establishing effective control measures early in the production stages is crucial for guaranteeing the final product's quality, safety, and efficacy. By presenting a comprehensive framework for DNA starting material control strategy, this paper aims to support the development of robust and reliable DNA manufacturing processes and testing strategies.

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INTRODUCTION

The central dogma of molecular biology describes the flow of information in a biological system, where (except for a few cases) the instructions encoded in DNA are first transcribed into RNA before being translated into proteins. This principle has sparked groundbreaking progress in the development of novel biopharmaceuticals whereby DNA is used for gene therapies or as a template in the messenger RNA (mRNA) manufacturing process. In gene therapy applications, the specific protein encoded can be a cure. In the case of prophylactic mRNA vaccines, the specific protein is an antigen that stimulates the immune system to recognize and fight the actual pathogen. As of 2025, there were over 45 FDA-approved cell and gene therapy products and 3 approved mRNA vaccines with significantly more in the clinical pipeline [1].

In alignment with this biological framework, regulatory guidance outlines how DNA is treated within the manufacturing

process of advanced therapies. Per EMA/246400/2021, Advanced Therapy Medicinal Product (ATMP) starting materials are defined as "all the materials from which the active substance is manufactured or extracted" [2]. This includes DNA used to transfer genetic material, such as DNA starting materials for viral vector production (recombinant adeno-associated virus [rAAV] gene therapy) or DNA templates for *in* transcription (mRNA) amongst others, see **Figure 1**. The DNA template for mRNA production may be a linearized plasmid DNA (pDNA) that has been produced in bacteria or may be derived enzymatically using a cell-free process [3]. It is a regulatory expectation that GMP principles be applied in the manufacturing process of DNA starting material [2,4]. Early in development, GMP-like grade starting DNA may be acceptable, however later in development or commercial, full GMP may be required. Careful consideration should be applied to the selection of the manufacturing site to ensure appropriate level of quality is applied during production and release.

For gene therapy products such as rAAV, production typically involves transient transfection of producing cells with two or three plasmids. These plasmids encode the components necessary to generate the rAAV vector: the capsid proteins, helper genes, and the therapeutic gene [5]. The therapeutic gene is encoded in the transgene region of the rAAV plasmid which consists of two inverted terminal repeats (ITRs) flanking a mammalian expression cassette, typically composed of a mammalian promoter, 5′ untranslated region (UTR), coding sequence of gene of interest (GOI), 3′ UTR, and polyadenylation signal. The final product consists of single-stranded DNA corresponding to the transgene region from the rAAV plasmid packaged in the capsid proteins [6,7].

For mRNA products, DNA is used as a template during the mRNA manufacturing process and is considered the starting material. A DNA template for mRNA *in vitro* transcription generally starts with a short RNA polymerase promoter at the 5′ site, followed by a 5′ UTR, the coding sequence of GOI, a 3′ UTR, a poly(A) sequence, and ends with a restriction enzyme site for linearization immediately downstream of poly(A) sequence, if present [8,9].

Ensuring adequate purity, identity, and integrity of the DNA can be challenging due to process complexity, sequence liability and instability. Establishing a comprehensive control strategy in early development for DNA starting material is important for manufacturing a high-quality product. The strategy should include evaluating the steps leading up to production of the DNA starting material, identifying the DNA key quality attributes that impact next steps and the ability of the DNA starting material to ensure that the final product meets the desired quality, safety and efficacy.

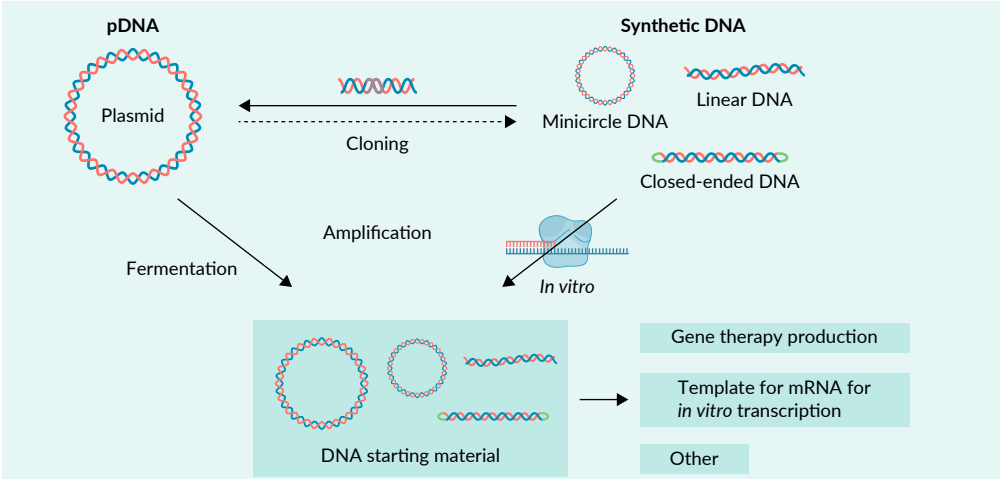
This commentary examines the conventional approach to pDNA starting material control strategy used in the production of rAAV gene therapies and mRNA vaccines, with a focus on analytical techniques for the key quality attributes of the pDNA starting material, i.e., topology, identity, integrity and process-related impurities. This commentary also addresses the challenges in applying these principles and strategies to synthetic DNA starting materials and how the analytical strategy feeds into the overall process control design. pDNA or synthetic DNA manufactured as a drug substance or drug product and cell therapies are out of scope.

PLASMID DNA

Large scale pDNA manufacturing has been developed since the 1990s in which pDNA is produced through bacterial fermentation, followed by extraction and purification [10,11]. For mRNA applications, an additional enzymatic digest and purification yield the desired linear form. pDNA is

FIGURE 1

DNA starting materials analytical control strategy.



Key quality attributes	Impact to product	Methods
Topology	Quality	AGE, CGE-LIF, restriction digests with AGE or CGE-LIF to identify/correlate topoisimers
DNA sequence	Efficacy	Short-read NGS (e.g., Illumina technology), long-read NGS (e.g., PacBio, Oxford Nanopore), Sanger
Poly(A) tail	Efficacy	Restriction digest with AGE, Bioanalyzer, Fragment Analyzer, PacBio long-read NGS, Sanger
Process-related impurities	Safety	qPCR (HC DNA), RT-qPCR or HPLC (HC RNA), BCA, ELISA or LC-MS (protein, antibiotic)

currently the starting material of choice due to its ease of development and large scale established manufacturing process. Once a platform manufacturing process for a backbone pDNA containing the GOI is in place, only the GOI needs to be replaced with a new GOI in the cassette or template to produce a new product. This enables rapid development and reliable production of new products.

Product development begins with designing and constructing the plasmid vector, which includes selecting a suitable backbone (including required regulatory elements) and GOI from the genetic sequence of the desired protein, as well as the cloning strategy. Plasmid can be constructed using different approaches. Popular cloning methods include restriction enzyme digestion and ligation, Gibson assembly, and Gateway cloning. The recombinant plasmid is then introduced into the microbial host, such as *E. coli*, via transformation, using techniques like electroporation or heat shock to ensure high-efficiency uptake. The transformed cells are selected for single clones, and only sequence-verified single clones are used to produce pDNA. The microbial host clones are cultured under controlled conditions to amplify the pDNA. Post-fermentation, the pDNA is harvested from the bacterial cells using centrifugation or filtration. The cells

are lysed, often using an alkaline solution, to release and preserve the pDNA, followed by neutralization to remove impurities. Purification steps include chromatography to separate pDNA from product related impurities, and ultrafiltration and diafiltration (UFDF) for concentration and buffer exchange, ensuring high purity of the circular pDNA [12].

After purification, circular DNA can be held or further processed into linear DNA template if needed. To produce the linear DNA template, the circular pDNA is linearized using a restriction enzyme followed by additional processing to generate the final linear DNA template. At each key step of the development and manufacturing process, rigorous characterization and quality control measures are implemented to ensure the purity, identity, and integrity of the pDNA.

The control strategy starts with the proper selection of a suitable cell line, as well as design and integrity of the plasmid backbone which can impact the ability to generate quality pDNA. The choice of bacterial strain depends on the nature of the DNA desired but should also focus on pDNA stability and copy number and needs to pass initial quality testing. Because the pDNA manufacturing uses a fermentation step, a cell bank must be established to ensure consistency and quality of the pDNA starting material production. The cell bank containing the gene of interest is intended to be used for subsequent manufacture of pDNA and should include tests for identity (sequencing), stability and purity at a minimum [4].

Finally, the ability of the pDNA to generate a product that meets the desired quality, safety and efficacy is the ultimate demonstration of the starting material suitability. By understanding what are the key quality attributes that could impact the final product, the proper control strategy can be derived. The four DNA attributes discussed below have been identified as key quality attributes of the pDNA starting material. Physical properties (pH, appearance, etc.) and safety (endotoxin, sterility, etc.) although also critical are not discussed.

Topology is a key quality attribute of pDNA and criteria should be end use-specific [4]. pDNA exists in three defined major forms; supercoiled (SC), linear and open circle. Additionally, multimers can be present for each form [13]. SC pDNA is considered a marker of high-quality DNA and has the potential to impact the quality of the subsequent product [14,15]. In early development, topology is analyzed to inform proper vector design, clone selection, in-process testing and batch release to assess DNA quality. Different separation methods have been used for topology assessment including gel electrophoresis methods, such as agarose gel electrophoresis (AGE) and capillary gel electrophoresis by laser-induced fluorescence (CGE-LIF).

In AGE, the various topoisomers of pDNA can be separated on an agarose gel based on DNA size and migration time. The gel is visualized with ethidium bromide under UV light and quantified by measuring relative percentages of band intensity for each topoisomer species present. In CGE-LIF, the plasmid is diluted and stained with fluorescent dye, then separated via a coated capillary in a gel matrix [16]. An electropherogram is produced with peaks correlating to the three topoisomer species. For both forms of electrophoresis, various restriction digestions can be performed to identify and correlate the peaks/bands to their respective topoisomers for each individual pDNA construct. CGE-LIF offers an advantage over AGE for analyzing pDNA constructs with numerous multimeric species, as it enables clearer identification by integrating all multimers of a specific topoisomer into a single migratory peak [16]. These analytical techniques used to assess topology are essential to demonstrate a sufficient amount of SC pDNA, indicating a high-quality starting material.

Integrity of the pDNA sequence is a key quality attribute of pDNA that directly

impacts the efficacy of the final product and is a regulatory expectation [3,4]. This includes the integrity of GOI, UTR, ITR and other DNA involved in rAAV production as applicable. Integrity of the GOI in the pDNA is essential to ensure the proper sequence is present in the drug substance. In pDNA used for rAAV-based gene therapy products, the ITR sequences are key for rAAV vector packaging and replication, while the UTRs enhance mRNA translation efficiency [17–19].

Potential truncations or sequence errors can be monitored by next-generation sequencing (NGS) and Sanger sequencing in cell banks and pDNA products. Short-read NGS, such as Illumina's technology, generates highly accurate sequence data of shorter nucleic acid fragments, typically 200–500 base pairs, that are ideal for identifying small nucleotide variants and short insertions or deletions. Short-read technologies can be complemented with long-read NGS to detect large and complex structural variants. Long-read sequencing, using platforms like PacBio and Oxford Nanopore, generates longer reads that are thousands of base pairs in length, allowing for assessment of structural variants and providing a more comprehensive view of the DNA starting material. Indeed, long-read sequencing technologies can be particularly useful for the resolution of difficult to sequence viral sequences (i.e., rAAV ITRs) which are prone to sequence rearrangements. While NGS provides detailed characterization of the starting material, Sanger sequencing remains a vital tool for determining the identity of pDNA. Its accuracy and compliance with 21 CFR Part 11 make it ideal for confirming plasmid sequences and ensuring the correct genetic constructs throughout production.

Integrity of the poly(A) tail, if present in the starting material, is a key quality attribute of pDNA that impacts the efficacy of the final product. The poly(A) tail of a mRNA transcript confers stability and is important for translation and nuclear export. In the pDNA template, the poly(A) tail can be lost during fermentation as polymerase slippage leads to heterogeneity in tail length. Consistency and stability of the poly(A) tail during manufacturing steps should be controlled [20].

There are several modern techniques to assess poly(A) tail integrity. In early development, these methods are used to screen microbial host cell colonies to identify quality candidates for a preliminary Master Cell Bank (MCB). The commonly used techniques include restriction digest with AGE, Bioanalyzer, Fragment Analyzer, and PacBio long-read sequencing. Restriction digest with AGE, involves using enzymes to cleave specific sites surrounding the poly(A) tail, and the resulting bands are separated by AGE. Any unexpected band size may indicate poly(A) tail truncation or heterogeneity. The Bioanalyzer is a microfluidics method which employs a restriction digest to release DNA fragments that include the poly(A) tail sequence. Multiple fragments on the electropherogram suggest poly(A) truncations. This method provides high resolution and sensitivity. The Fragment Analyzer method is a form of CGE, where pDNA samples undergo a double restriction digest, producing fragments detectable by fluorescence in the gel matrix. Poly(A) tail integrity is determined by integration of the electropherogram. PacBio long-read sequencing uses linearized double-stranded DNA to construct a circular library for high-accuracy consensus sequencing. The poly(A) tail length is determined by counting adenine residues. PacBio can identify low-level truncations that other methods might not detect.

Each method has its advantages for early screening and characterization and can be used orthogonally. For GMP testing and batch release, poly(A) tail integrity is often assessed through restriction digest with AGE or Sanger sequencing due to their reliability and high throughput. Sanger

sequencing, commonly used for pDNA identity testing, can also characterize the poly(A) tail to detect major truncations. Restriction digest via AGE and Sanger sequencing are both employed together to support a robust control strategy for poly(A) tail characterization.

In the manufacturing of pDNA, process-related impurities that may be present from the manufacturing process should be understood and controlled to ensure the final product meets quality standards [3,4]. Host cell (HC) DNA and HC RNA may be measured by quantitative PCR (HC DNA), reverse transcriptase quantitative PCR (HC RNA), and high-performance liquid chromatography (HPLC) methods. Residual host cell protein or other potential protein impurities such as residual enzymes are assessed using enzyme-linked immunosorbent assays (ELISA), bicinchoninic acid (BCA) assays, liquid chromatography–mass spectrometry (LC-MS) amongst others. Process-related impurities should be assessed based on the manufacturing process, and are an important consideration for focus area in any biopharmaceutical control strategy.

The manufacturing of pDNA is an established process, amenable to the implementation of a platform approach to the control strategy and analytical methods. The pDNA starting material control strategy includes topology, sequence integrity, poly(A) tail integrity, if present, and process-related impurities. Platform release and characterization methods can be implemented to support rapid onboarding of new products when the products and processes share commonalities. Many of these control strategy approaches are now being applied to the next generation of DNA production methods with some necessary adaptation.

SYNTHETIC DNA (CELL-FREE DNA)

DNA starting material can also be manufactured with synthetic (not cell-based) processes. These approaches may be faster and can avoid the complexities and impurities associated with cell-based manufacturing. However, producing DNA via fully synthetic routes that matches cell-based DNA quality comes with a unique set of challenges which might require a separate analytical control strategy that must be tailored to each process. The basis for this strategy stems from the previously described cell-based analytical control strategy with synthetic specific evaluations that may include enzyme/polymerase purity, priming efficiency, process-related impurities and sequence integrity of DNA and resulting mRNA.

The earliest chemical synthesis of DNA was developed in 1950s by Alexander Robertus Todd [21]. Currently, synthetic DNA begins with phosphoramidite synthesis, a method developed by M H Caruthers in 1980s [22]. Due to limitations in chemical reaction efficiency, the length of synthetic DNA is typically limited by this phosphoramidite process and does not exceed 200 bases, as accumulation of errors is directly correlated with oligonucleotide length. Longer DNA fragments up to 3 kb can further be made from chemically synthesized fragments via high-fidelity processes such as overlapping PCR. These larger gene blocks can be taken through transformation and clone selection to provide high fidelity DNA for the plasmid process described above or an alternative to direct use for these DNA fragments is to continue down synthetic pathways to produce large amounts of high-fidelity DNA to serve as starting material for mRNA and gene therapy processes, bypassing plasmid production altogether.

Large-scale DNA production that begins with high-fidelity fragments can be accomplished through different approaches. One approach is high-fidelity PCR to make more DNA. High-fidelity PCR approach starts from synthesis of a well-designed DNA template and forward and reverse

primer pair. High-fidelity DNA polymerase ensures high quality of the final DNA products. Thermal cycling equipment capable of efficient heat transfer is critical for scale up. Another approach is by isothermal DNA amplification techniques such as rolling circle amplification (RCA) [23]. RCA is known for its robust amplification of nucleic acids. A typical RCA requires a circular DNA template, primers (either specific or random), DNA polymerases (Phi29 or Bst) and dNTPs. These are but two of several different processes capable of producing significant quantities of DNA starting material in a fully synthetic fashion. In addition to challenges associated with purification that could potentially influence the analytical control strategy, these and other processes introduce different process-related impurities compared to cell-based pDNA processes.

Perhaps the biggest challenge for fully synthetic DNA production is DNA sequence fidelity. A major advantage of DNA amplification by cell-based processes is the cell's ability to detect DNA damage, base substitutions, and base deletions. Inherent in the cells replicative machinery are repair mechanisms to correct for all these unwanted modifications to the desired starting material [24]. Not all fully synthetic processes incorporate such repair mechanisms. To ensure synthetic DNA process control, DNA fidelity should be closely monitored by analytical tools including NGS. Although the fidelity of synthetically derived DNA may not match that of pDNA, it is crucial to thoroughly characterize its fidelity to ensure no adverse impact on downstream processes when used as a starting material. This characterization may require not just thorough DNA analysis, but the drug substance should also be thoroughly characterized for sequence accuracy to ensure the DNA is free from substitutions, insertions, and deletions that can impact the subsequent drug substance.

Similar to pDNA, fully synthetic-derived DNA requires proper evaluation of the poly(A) tail, if present in the starting material. Truncations of the poly(A) tail are possible due to the nature of the DNA generation prior to amplification as synthetic DNA quality falls when attempting to produce repeating bases. While pDNA processes may provide control through clone screening and MCB generation, one goal of fully synthetic DNA production is to avoid those process steps to save time and resources. In the absence of established process control, understanding poly(A) tail truncation prior to amplification steps, in addition to proper evaluation after amplification may give insight to process impact to the tail. In fact, some enzymatically driven processes like RCA can preferentially amplify truncated products as the smaller DNA strand can accommodate more amplification cycles [25]. Proper process control monitored by thorough analytical evaluation including routine LC/MS analysis in addition to long-read sequencing may be necessary to ensure the desired tail length is generated.

Process-related impurities will differ depending on what synthetic process is evaluated. Synthetic DNA may not require analysis of some cellular-derived impurities (e.g., HCP, HC DNA, or HC RNA). However, other impurities could be introduced from raw materials. As enzymes used in synthetic processes are cell-derived, any residual DNA coming from those enzymes may need evaluation as any potential DNA could be indiscriminately amplified depending on priming strategy. Analytical tools including ELISA and NGS can provide the necessary information to derisk any raw material impurities.

Instead of HCPs, the enzymes used in amplification can be considered as potential protein impurities when assessing the DNA starting material. In the case of synthetic manufacture, the proteins present should be easy to define, and control of enzyme removal should be established. In addition to safety risks, it may be necessary

to assess process risk if the amplification process uses enzymes capable of disrupting DNA stability or future IVT. BCA assay may be appropriate to ensure enzymes used are sufficiently removed, but ELISA's can be developed if higher control of a particular enzyme is needed.

Finally, the quality of the mRNA drug substance made from the synthetic DNA template should be evaluated. Synthetic DNA might generate more dsRNA during IVT because it is often less homogeneous and may contain more regions prone to self-annealing compared to plasmid DNA. Additional process development to ensure all final drug substance quality requirements are met may be required.

Using fully synthetic DNA starting material production may confer advantages such as speed and greater process control. The basis for analytical control for synthetic DNA can start from pDNA processes, but risk assessed for the specifics that the fully synthetic process may remove or introduce.

TRANSLATION INSIGHT

The analytical control strategy for DNA starting material is critical for the successful production of gene therapies and mRNA products. Both plasmid and synthetic DNA require rigorous quality control measures to ensure the final product meets the desired standards. The strategy involves evaluating production steps, ensuring the quality of the starting material, and addressing impurities. Implementing this control strategy in early development also avoids the need for tedious and costly comparability studies, a regulatory requirement for late-stage changes. The ability to generate a product that meets the desired quality, safety and efficacy is the ultimate demonstration of the starting material suitability.

pDNA starting material analytical control strategy has evolved over time with the advent of new applications and technologies. Historically, the pDNA starting material control strategy was developed for use in manufacturing gene therapy products such as rAAV. Quality attributes such as topology, sequence integrity and process-related impurities were identified early on as key quality attribute of the pDNA to ensure the desired quality, safety and efficacy of the final product. With the advent of mRNA products, additional quality attributes needed to be considered for example with the addition of a poly(A) tail, present in some designs, additional analytical development was required to ensure proper poly(A) tail integrity. Now, with novel approaches such as synthetic DNA which aim to provide greater process control and speed the delivery of medicines to patients, the control strategy needs to be adapted again to meet the challenges of the new DNA designs and manufacturing processes.

Despite differences, the principles behind the control strategy remain the same which is to ensure the ability of the DNA starting material to generate a product that meets the desired quality, safety and efficacy. By understanding what are the key DNA quality attributes that impact the final product, the proper control strategy can be derived for each type of DNA starting material using a risk-based approach. With that in mind, industry and regulators can continue to focus on defining what quality attributes matter in the final product. From that understanding, the DNA control strategy can be derived to continue to ensure the final product meets the desired quality, safety and efficacy.

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AUTHORSHIP & CONFLICT OF INTEREST

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

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NUCLEIC ACID INSIGHTS

INDUSTRY INSIGHTS • NOVEMBER 2025

From discovery to delivery: recent developments shaping nucleic acid therapeutics

Jokūbas Leikauskas

With a background in science communication and digital publishing, Jokūbas focuses on advancing the nucleic acid therapeutics field by commissioning and shaping high-impact, open access content for *Nucleic Acid Insights*. As Commissioning Editor, he leads the development of interviews, expert articles, and industry perspectives that highlight emerging advances across mRNA, DNA, oligonucleotide, and drug delivery modalities. Jokūbas is driven to translate complex scientific topics into engaging, accessible content while maintaining strong connections across the nucleic acids community.

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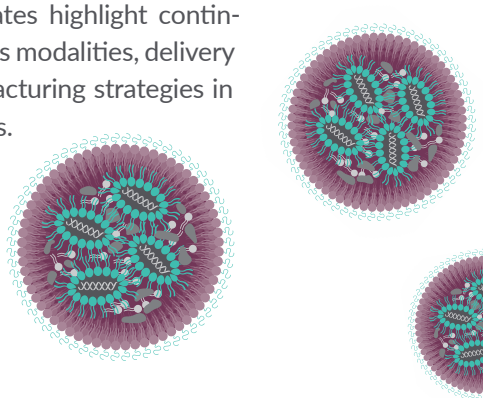
SUMMARY

Throughout November 2025, the nucleic acids field continued to advance through new partnerships, clinical progress, and technology development. Collaborations between **Biovectra** and **Revolution Biomanufacturing**, and between **Sanegene Bio** and **Eli Lilly**, reflected sustained investment in mRNA design and RNA interference (RNAi) platforms.

Multiple clinical programmes reported encouraging early findings, including antisense oligonucleotide (ASO), small interfering RNA (siRNA), and DNA-directed RNAi candidates from **Ausper Biopharma**, **ADARx**, **Rona Therapeutics**, and **Benitec**. Market activity featured significant financing events for **PeptiSystems** and **Benitec**. In tools and technologies, innovations included expanded enzymatic DNA architectures from **Touchlight** and next-generation lipid nanoparticle (LNP) systems from **Acuitas Therapeutics**. Collectively, these updates highlight continued diversification across modalities, delivery approaches, and manufacturing strategies in nucleic acid therapeutics.



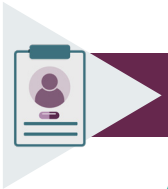
COLLABORATIONS AND PARTNERSHIPS

Biovectra and Revolution Biomanufacturing announced a partnership for integrated mRNA design and GMP production services [1]

Biovectra and Revolution Biomanufacturing have recently formed a partnership to provide an integrated pathway from mRNA construct design through GMP manufacturing. Revolution Biomanufacturing will contribute computational design and sequence optimisation, while Biovectra will provide GMP mRNA synthesis and supporting quality and regulatory activities. The companies stated that the collaboration is intended to streamline development timelines for therapeutic and vaccine programs that rely on custom mRNA constructs.

Sanegene Bio entered RNAi licensing and research collaboration with Eli Lilly and Company [2]

Sanegene Bio entered a licensing and research collaboration with Eli Lilly and Company to advance RNAi therapeutics. The agreement gives Lilly global exclusive rights to develop and commercialise these programmes, while Sanegene Bio will continue contributing discovery-stage work. Financial terms were not disclosed, but the companies noted that the deal includes upfront, milestone, and royalty components.



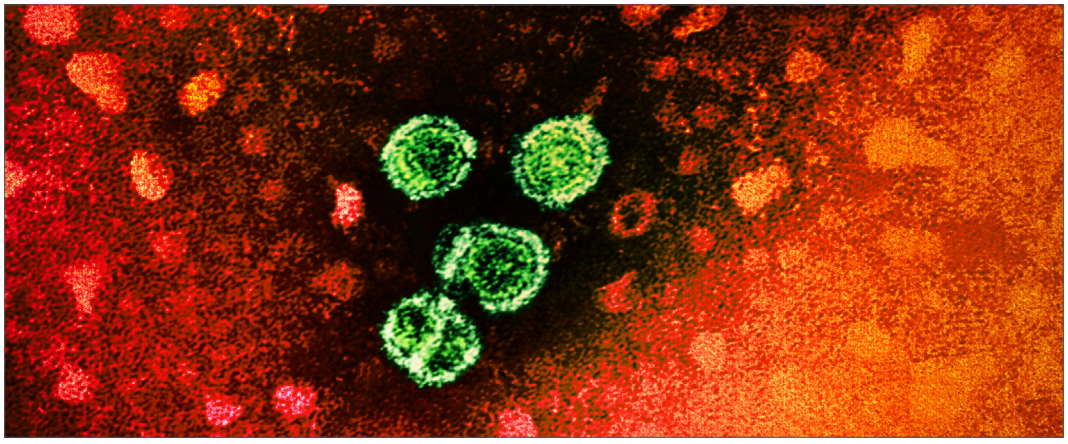
CLINICAL TRIALS AND RESEARCH

Ausper Biopharma reported updates on its Phase 1 trial of a novel ASO for hepatitis B [3]

Ausper Biopharma provided an update on the Phase 1 clinical trial of AHB-137, an investigational ASO therapy for chronic hepatitis B. The company announced completion of the single-ascending-dose portion and progress toward multiple-ascending-dose evaluation. Preliminary safety findings showed no serious adverse events at the tested doses, and the company plans to advance the programme based on these early results.

Benitec Biopharma reported progress on DNA-directed RNAi therapy [4]

Benitec reported interim results from its Phase 1b/2a trial of BB-301, a gene therapy designed using the company's DNA-directed RNAi platform to silence mutant PABPN1 and deliver a codon-optimized replacement. All six patients in Cohort 1 met the statistical response criteria, with improvements in dysphagic symptom burden, post-swallow residue, swallowing capacity, and pharyngeal closure over 3–12 months of follow-up. The US FDA granted Fast Track designation for BB-301 for oculopharyngeal muscular dystrophy with dysphagia; BB-301 also holds Orphan Drug Designation from both the FDA and EMA. Benitec treated the first patient in Cohort 2 in late 2025 and plans to meet the US FDA in 2026 to confirm the pivotal study design.



ADARx Pharmaceuticals announced positive interim Phase 1 data for siRNA therapy targeting complement factor B [5]

ADARx Pharmaceuticals reported interim Phase 1 results for ADX-038, a siRNA therapeutic targeting complement factor B (CFB) for complement-mediated diseases. ADX-038 was well tolerated, with headache and upper respiratory tract infection as the most common adverse events. The company noted that selective and durable alternative pathway suppression may support semi-annual dosing. ADX-038 is being advanced into multiple Phase 2 studies for immunoglobulin A nephropathy, complement 3 glomerulopathy, geographic atrophy secondary to age-related macular degeneration, and paroxysmal nocturnal hemoglobinuria.

Rona Therapeutics reported Phase 1 results for novel siRNA therapy [6]

Rona Therapeutics released Phase 1 results for RN0361, a long-acting siRNA targeting APOC3, at the American Heart Association 2025 Scientific Sessions. In the study, RN0361 produced durable, dose-dependent reductions in APOC3 and triglyceride levels, and was reported as well-tolerated across evaluated cohorts. The company highlighted the potential for RN0361 to be developed for dyslipidaemia and cardiovascular risk reduction.

MARKET TRENDS

PeptiSystems announced growth equity investment from Rubicon Healthcare Partners [7]

PeptiSystems announced a growth investment from Rubicon Healthcare Partners to support the global expansion of its manufacturing technologies for therapeutic peptides and oligonucleotides. Rubicon will become the company's largest shareholder following the transaction and aims to contribute strategic expertise and networks to PeptiSystems' international development. The investment is intended to accelerate commercial activities, expand organizational capabilities, and advance large-scale industrial applications of the company's flow-through manufacturing technology. PeptiSystems stated that the funding will also support customers transitioning to GMP production of peptide- and oligonucleotide-based therapeutics.

Benitec Biopharma announced pricing of $100M common stock offering [8]

Benitec Biopharma announced the pricing of a $100M equity offering comprising an underwritten public sale of 5,930,000 common shares and a concurrent registered direct offering of 1,481,481 shares to Suvretta Capital, each priced at $13.50. Underwriters received a 30-day option to purchase up to 889,500 additional shares. Benitec stated that net proceeds will support development of its DNA-directed RNA interference 'Silence and Replace' platform and associated therapeutic programmes, along with working capital and general corporate purposes.

TOOLS AND TECHNOLOGIES

Touchlight expanded its doggybone DNA portfolio with new circular architectures [9]

Touchlight announced the expansion of its cell-free DNA platform with three new circular DNA format—sscDNA, hsscDNA, and dscDNA—designed to complement its existing megabulb DNA (mbDNA™) technology. mbDNA is a single-stranded DNA construct produced enzymatically, offering high purity, low immunogenicity, and knock-in efficiencies of 60–75% in primary T cells, with payload capacities up to 20 kb for applications such as homology-directed repair and episomal expression. The new architectures provide fully single-stranded, hybrid, or fully double-stranded circular DNA options, allowing user-defined sequences for systems incorporating transposases, recombinases, HITI, and episomal delivery. The expanded portfolio aims to improve stability, reduce immunogenicity, and enhance compatibility with diverse gene editing and expression platforms.

Acuitas Therapeutics unveiled next-generation LNP advancements at the 2025 mRNA Health Conference [10]

At the 13th International mRNA Health Conference, held in Berlin, Germany, Acuitas Therapeutics presented its next-generation LNP suite, designed to enhance potency, improve safety, and expand delivery beyond the liver for mRNA therapeutics. The company reported novel LNP candidates with up to four-fold increased potency in gene editing and vaccine applications, reduced liver exposure through optimized lipid structures, targeted immune-cell delivery using DARPin-conjugated LNPs, and mucous-penetrant formulations enabling airway delivery in cystic fibrosis models. Acuitas also highlighted a new pre-formed vesicle manufacturing approach that may reduce cost and improve flexibility for personalized mRNA-LNP therapies.

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4. Benitec Biopharma. Benitec Biopharma releases first quarter 2026 financial results and provides operational update. Nov 14, 2025.
5. ADARx Pharmaceuticals. ADARx Pharmaceuticals announces positive interim Phase 1 clinical data for its novel siRNA targeting complement factor B (CFB). Nov 7, 2025.
6. Rona Therapeutics. Rona Therapeutics presents Phase 1 data for RN0361, a long-acting APOC3-targeting siRNA, at the American Heart Association 2025 Scientific Sessions. Nov 9, 2025.
7. PR Newswire. PeptiSystems secures growth equity investment from Rubicon Healthcare Partners to accelerate commercial expansion. Nov 13, 2025.
8. Benitec Biopharma. Benitec Biopharma Inc. announces pricing of $100 million common stock offering. Nov 6, 2025.
9. Touchlight. Touchlight expands mbDNA portfolio with new circular DNA architectures for advanced therapies. Nov 5, 2025.
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NUCLEIC ACID INSIGHTS

mRNA
MANUFACTURING:
DOWNSTREAM/PURIFICATION

SPOTLIGHT

COMMENTARY

Enabling personalized mRNA therapeutics: the case for platform downstream processing

Federico Moreno-Sibaja, Sha Liu, Cinderella Nowak, and Lukas Gerstweiler

Personalized mRNA therapeutics demand rapid, sequence-independent manufacturing of clinical-grade material from single-dose batches. Strategies for construct design and *in vitro* transcription have become more standardized, however downstream processing remains poorly suited to microliter-scale operation, with common methods suffering from sequence/size dependence, toxic solvents, low recovery, or high losses during buffer exchange. This commentary advocates a true integrated small-scale downstream processing (DSP) platform built on oligo(dT) affinity capture followed by a fast flow-through affinity step that selectively removes residual impurities (mainly dsRNA). Implemented on convective supports (monoliths or fibres), this simple two-step, integrated purification train minimizes unit operations, maximizes yield, and is largely sequence-agnostic, ideal for individualized production under regulatory compliance. For large-scale needs, cost-effective alternatives (precipitation, membranes, or continuous oligo(dT) with non-affinity polishing are noted. Standardized, analytically robust DSP platforms are critical to realizing the full potential of personalized mRNA therapeutics.

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INTRODUCTION

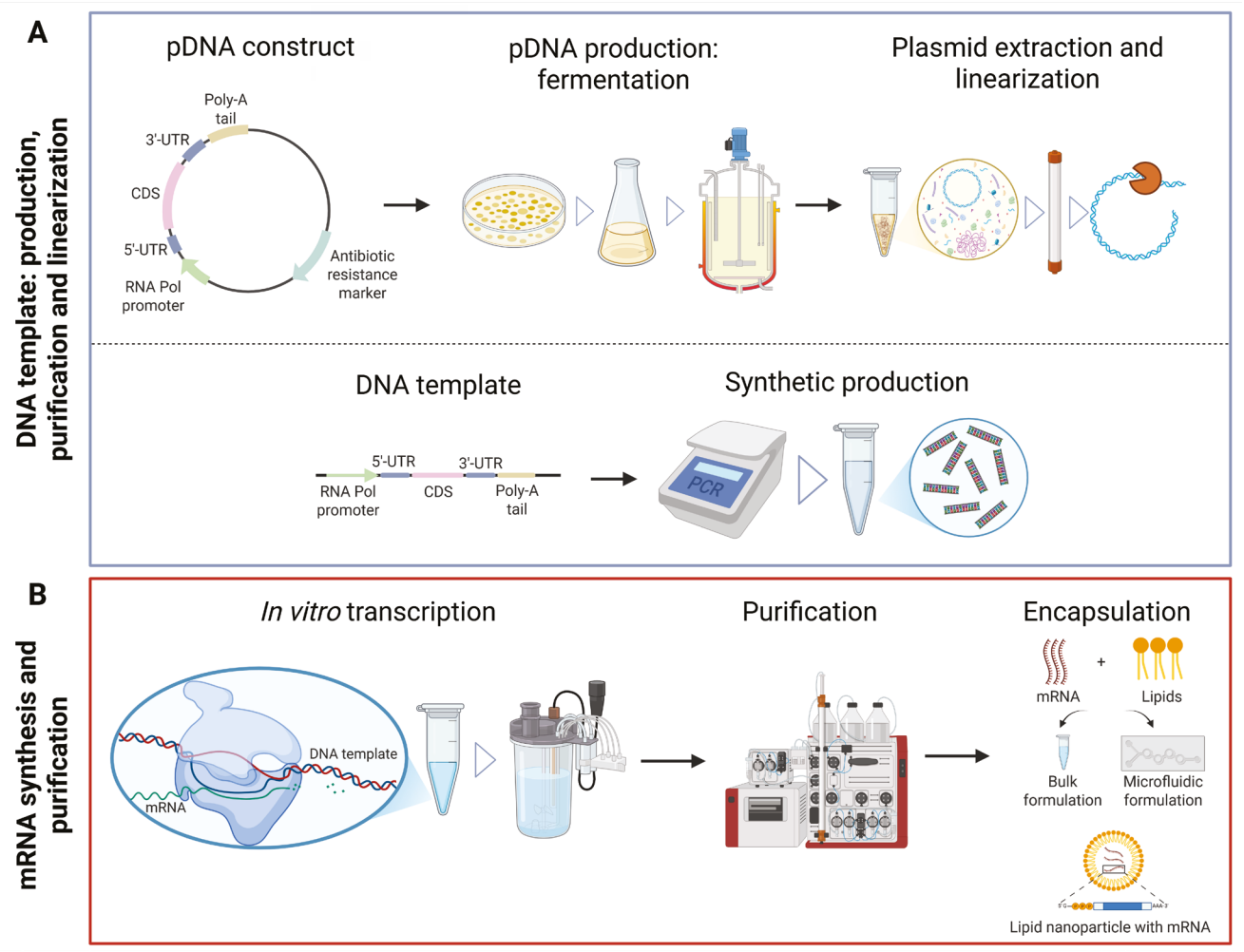
Messenger RNA (mRNA) therapeutics are emerging as credible alternatives to conventional vaccines. They offer rapid design cycles, broad programmability, strong efficacy and safety profiles, and the potential for cost-efficient manufacturing across immune and non-immune indications [1]. Because mRNA sequences can be designed and synthesized quickly using *in vitro* transcription (IVT), the modality is well-suited to individualized medicines such as personalized cancer vaccines [2].

In recent years, IVT has moved toward more standardized practices, including model-informed DNA template design, rational selection of untranslated regions (UTRs), encoded poly(A) tails, and robust co-transcriptional capping. By contrast, DSP for personalized mRNA therapeutics has not yet fully matured into a true platform. Current

DSP approaches show a promising platform approach for capturing based on Oligo(dt) purification; however, polishing approaches rely on ion-pair reverse phase liquid chromatography that requires toxic acetonitrile and hence buffer exchange, and process performance is dependent on construct size and loading amount, or utilise cellulose, which shows relatively low recovery rates [3,4]. This is a poor fit for personalized products, especially in extreme cases where only a single dose is available. In such situations, only a very small amount of product and volume can be used, making extensive optimization impractical. Workflows might rely on manual pipetting, and losses from dead volumes and buffer exchange can significantly impact the already limited sample. There is a clear need for a small-scale DSP platform that consistently delivers highly pure, potent mRNA across diverse constructs while meeting regulatory expectations without rebuilding the process for each new sequence.

FIGURE 1

Schematic illustration of the mRNA production process.



(A) Pathways for the DNA template production process by either plasmid linearisation or synthetic production by PCR. **(B)** mRNA synthesis by *in vitro* transcription followed by purification and encapsulation. Created in BioRender [29].

mRNA MANUFACTURING

A high-level overview of mRNA manufacturing is shown in **Figure 1**. After designing and constructing the DNA sequence, IVT templates can be generated in two main ways [5]. Linear DNA can be produced by PCR, using reverse primers to encode the poly(A) tail [6]. Or using established pDNA workflows. Following purification of supercoiled pDNA, a restriction enzyme is used to linearize the plasmid downstream of the poly(A) tail to enable run-off transcription by T7 RNA polymerase [7].

Upstream development has advanced tremendously to enable platform processing for individualised mRNA therapeutics over the past decade. Major improvements include computer-aided DNA template design, co-transcriptional capping with trinucleotide cap analogues that routinely achieve more than 90% capping efficiency [8], optimized IVT reaction conditions [9], and engineered T7 polymerase variants that reduce the formation of unwanted by-products [10].

The purification, encapsulation for delivery, and formulation steps remain challenging [11,12]. IVT produces a heterogeneous mixture that can include full-length product, truncated transcripts, double-stranded RNA, over-extended RNA, DNA:RNA hybrids, and uncapped species [13]. How and why those impurities form is only partially understood, and literature also suggests sequence-specific mechanisms [13]. Many of these impurities share similar physicochemical properties, which makes separation difficult. Therapeutic mRNAs are also relatively large, which limits pore accessibility and mass transfer in traditional bead-based chromatography, reducing binding capacity and resolution and increasing product loss.

Progress is further constrained by analytics. Many commonly used assays are slow, semi-quantitative, low-throughput, or costly. Examples include dot-blot or ELISA-type methods for dsRNA and mass-spectrometry-based approaches for cap structure analysis, although recent publications describe automated high-throughput analytics for dsRNA and an HPLC method for capping efficiency measurement [4,14,15]. The field would benefit from robust, high-throughput, and standardized methods that can quantify critical quality attributes with the speed required for both personalized and large-scale manufacturing.

DOWNSTREAM PROCESSING: CURRENT & EMERGING TECHNOLOGIES

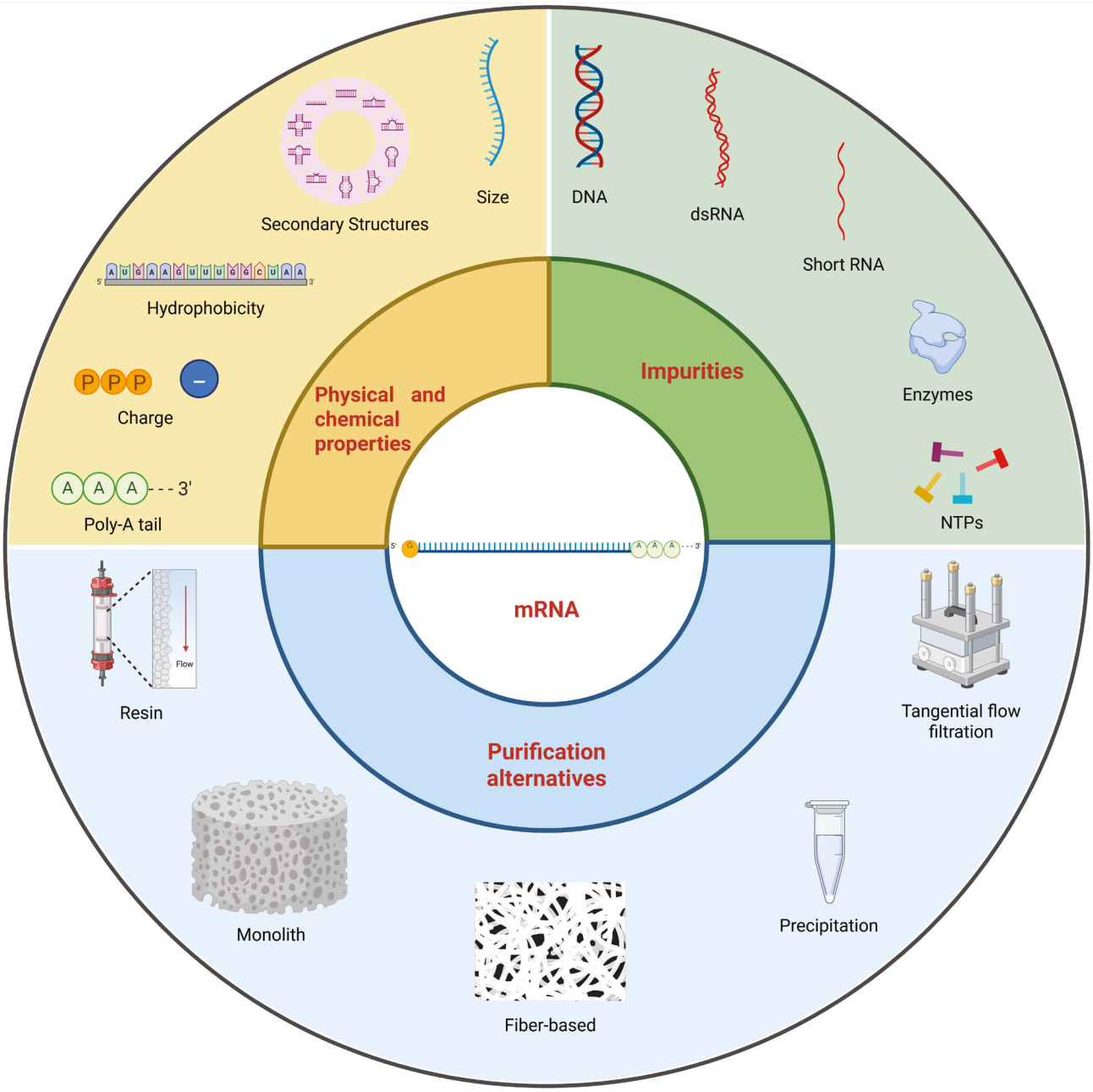
To remove impurities and purify mRNA **(Figure 2)**, a range of methods has been explored [12,16,17], including precipitation [18], tangential flow filtration [19,20], ion exchange [21], size exclusion [22], and oligo-dT affinity chromatography [23,24]. Precipitation and membrane-based operations are highly scalable for large batches, including continuous manufacturing, but they are difficult to scale down to very low volumes (<10 mL) without manual or automated pipetting [18,25].

Oligo-dT affinity chromatography, where the ligand hybridizes to the poly(A) tail at the 3′ end of mRNA, is one of the most promising platform operations and has been applied in continuous mode as well [26]. It enriches only transcripts with an intact poly(A) tail and effectively removes incomplete transcripts, NTPs, DNA, and short fragments, often achieving more than 95% purity. A notable limitation is that dsRNA can co-purify and therefore requires additional polishing. Reported polishing options include cellulose-based dsRNA depletion and ion-pair reversed-phase HPLC [17].

Chromatographic methods that rely on traditional bead-based resins also present challenges. The large size of therapeutic mRNA limits diffusion into pores and can exclude the molecule from many binding sites. This can result in low dynamic binding capacities, a need for low flow rates, reduced

FIGURE 2

Schematic illustration of physicochemical properties, IVT impurities, and purification alternatives for mRNA.



Created in BioRender [29].

productivity, low resolution, and higher product loss, depending on process conditions [24,27]. New convective stationary phases that are not diffusion-limited, such as monoliths (for example, CIMmultus, Sartorius) and fibre-based media (for example, Fibro, Cytiva), provide a more suitable backbone for mRNA purification and have demonstrated higher dynamic binding capacities [24,27]. From a platform perspective,

however, binding capacity still varies with transcript length, and predictive models that forecast binding behaviour a priori have not been fully developed. Many chromatographic steps, such as AEX, SEC and HIC, are also sequence and size-dependent and require case-by-case development. Chromatographic-based polishing has recently been reviewed in detail [17].

For individualized single-dose mRNA therapeutics, a challenge arises from process integration of the individual unit operations, the need for at least some process development depending on the sequence/size of the construct, and the very small available sample volumes. While concentration and buffer-exchange steps are practical at medium and larger scales, high losses can occur due to the dead volumes of filters in TFF systems. Manual pipetting and spin-filter approaches are practical in laboratory settings but raise compliance concerns for clinical-grade material.

A practical path forward is a two-step affinity scheme that is simple to seamlessly integrate and automate. In this approach, oligo-dT serves as the primary capture, followed by a fast flow-through affinity step that binds residual impurities. Because the impurity pool (dsRNA, uncapped, fragments, aggregates) after oligo-dT is typically small, often below 5%, an impurity-targeted step can be efficient in processing a larger product stream [26,28]. Oligo-dT capturing followed by flow-through affinity chromatography to deplete impurities without further buffer adjustment between the steps has also tremendous advantages in terms of process integration, yield, and speed of processing, as the number of unproductive unit operations between purification steps is decreased, overall process yield and process speed are improved. Several companies are investigating this strategy. For example, Invitrogen commercialised AVIPure dsRNA clear resin, a protein-ligand resin marketed for dsRNA clearance. Although such specialty resins can be costly, the expense is less consequential for small-batch, individualized products and the approach supports a platform that is largely sequence independent. Similar affinity media could be developed for minor species such as RNA:DNA hybrids, although it is unreported whether they co-purify by oligo-dT purification, or even uncapped mRNA. Cleanability constraints of affinity ligands would not be a problem for individualised mRNA production, since the purification train/resin would be used only once as a single-use consumable. Key open questions include seamless integration into oligo-dT chromatography step, recovery and yield across diverse mRNA constructs, and the robustness regarding buffer compositions.

OUTLOOK

Personalized mRNA therapeutics will only reach their potential if downstream processing converges on standardized, sequence-agnostic platforms that work reliably at very small scales. We propose convective media as the backbone for such platforms, combined with oligo-dT capturing followed by flow-through affinity chromatography to pair selective capture with targeted impurity removal in a simple, modular train. This minimizes unit operations and reduces non-productive buffer exchange steps, leading to a robust and fast process. This approach now needs rigorous validation across transcript sizes, sequence contexts, and manufacturing sites, supported by robust analytics and clear criteria for lot release.

For large-scale production, continuous multi-column oligo-dT is a promising and already described route; alternatively, precipitation or membrane operations can serve as the primary capture, followed by low-cost, non-affinity chromatography for polishing. While multi-affinity trains should still be explored, the cost

of specialty affinity resins may be prohibitive at large scale, so these cost-lean alternatives could provide better overall economics without compromising quality.

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AUTHORSHIP & CONFLICT OF INTEREST

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NUCLEIC ACID INSIGHTS

mRNA
MANUFACTURING:
DOWNSTREAM/PURIFICATION

SPOTLIGHT

Formulation and delivery of RNA-based vaccines and therapeutics: addressing outstanding questions around safety and purity

INTERVIEW

"...moving away from this dependence on LNPs is an exciting advance in the field."

In this interview, **Róisin McGuigan**, Editor, *Nucleic Acid Insights*, and **Jesse Erasmus**, Director of Virology at HDT Bio, discuss current challenges and opportunities in RNA safety, manufacturing, and targeted delivery, and the key ongoing obstacles facing viral and LNP-based delivery modalities.

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Q How did you get into the RNA field, and what are you currently working on?

JE I am a virologist with some experience in immunology, and I now specialize in RNA vaccine development. My career started with my PhD back in 2012. I

went to the University of Texas Medical Branch, where I worked on host-restricted alphaviruses as vaccine platforms.

We were taking alphaviruses that were restricted to insects only (did not replicate in humans) and used an insect cell system to grow chimeric viruses that we could then use as vaccine antigens. During that time, I saw firsthand the complexities around manufacturing of these viruses in cell-based systems. I wanted to move into a space where we could get away from cell-based manufacturing processes.

When we made these viruses, we would transcribe RNA and put it into a cell to produce the virus. The logical next step was to go straight back to the RNA and put that RNA directly into a vaccinee to produce the antigen of interest. That's how I got into the RNA vaccine space—rather than through immunology or biochemistry, I was approaching it from a virology perspective.

We wanted to continue to develop alphaviruses as an RNA vaccine platform, and so I moved up to Seattle back in 2016. I initially worked at a nonprofit company founded by Steve Reed, who is also the CEO of HDT. We started working on developing self-amplifying or replicon RNA, which are also RNAs derived from alphaviruses as a vaccine platform. That's what we have been doing ever since.

Q Could you frame for us what you see as the key challenges and outstanding questions for the field when considering the safety of RNA-based vaccines and therapeutics?

JE Firstly, I'll get a little controversial. The field is currently stuck on some dogma around what is thought to be necessary for the development of safe and effective RNA therapeutics, including beliefs around the structural requirements of a formulation that needs to be used to deliver RNA.

This goes back to the application of RNA delivery technology that was originally optimized for intravenous (IV) administration and liver-targeted delivery. If you think about it from the viewpoint of someone developing the first approach to deliver an RNA, you want to go for the lowest-hanging fruit. They picked a tissue that's easy to target, and a disease of that target tissue, with an RNA that is small and relatively stable—in this case, an siRNA used to knock down a gene in the liver. This ultimately led to Onpattro®, which was approved back in 2018.

Between 2018 and the end of 2019 when COVID emerged, the field didn't make any huge leaps and simply applied the same lipid nanoparticle architecture. People changed out the ionizable lipid component, but it was the same four-component structure including an ionizable lipid, a helper lipid, a cholesterol, and a PEG lipid. In this case it was used to deliver a slightly larger RNA molecule encoding an open reading frame of virus antigen, with a switch from IV delivery to intramuscular.

The concept was that moving away from that original ionizable lipid would achieve more localized biodistribution, and get away from the liver. However, a small change to the composition by changing the ionizable lipid does not necessarily dictate biodistribution. It's a core lipid that complexes with RNA on the inside of the lipid nanoparticle and it does enhance endosomal escape in non-hepatic cells, but it does not decrease uptake in liver cells. It's really the surface chemistry and size of a nanoparticle that dictates its biodistribution, and LNPs are PEGylated neutral nanoparticles.

"...the main challenges right now are trying to balance safety and tolerability with potency."

If you look at the biodistribution data for existing LNP-mRNA vaccines on the market, even though they are delivered into the muscle, they still broadly distribute throughout the body. You get a lot of uptake in the liver as well as other off-target tissues, including the heart, spleen, and kidneys, and RNA copies have even been detected in the eye. Essentially, you get a systemically distributing nanoparticle. This makes sense given that that nanoparticle was originally optimized for specific delivery following systemic administration.

At HDT, we approached this problem from a different angle. We didn't try to build upon LNPs. The first paper describing an mRNA used as a gene expression modality *in vivo* was published back in 1990. One year before that, there was a paper showing that you can take alphavirus genomes and deliver them into a cell, and use those genomes to launch expression of heterologous genes. That field emerged from the virology side, and launched the idea that replicons could be used as gene delivery modalities.

As this came from the virology space, they were using viral delivery. They would package these RNA molecules into virus particles which could mediate very efficient *in vivo* delivery. You could deliver picogram quantities of RNA and elicit the responses that we're seeing today with 10–100 μg RNA doses.

The potency of delivery was orders of magnitude better when using viral delivery—but of course, viral delivery comes with a lot of downsides. Requiring cells to produce your drug product results in complexities around purifying and demonstrating safety of that product. We approached this from that angle and considered how we could mimic an alphavirus particle in terms of its tropism and biodistribution.

Virus particles, particularly those that are produced in cell culture, tend to bind strongly to negatively charged heparan sulfate molecules on the surface of cells via charge-charge interactions. Positively charged moieties on the surface of an alphavirus particle bind rapidly to negatively charged molecules on the surface of cells, which leads to rapid uptake at the local injection site. That's the mechanism whereby viral-delivered replicon RNAs work.

We started with a nanocarrier—which we call LION—that is cationic on its surface. We can vary the ratio of the negatively charged RNA to the positive charge and optimize for rapid uptake *in vitro* and *in vivo*.

You might wonder why we are talking about the surface of a nanoparticle, and how we are modulating that surface charge with an RNA molecule. We bind the RNA molecule to the surface of this nanoparticle, and you might conclude that this would lead to an inability to protect that RNA, because we're no longer encapsulating it in a core. However, as was also shown by others in the field before us, cationic nanoparticles complexed with RNA are still protected from enzymatic degradation once you expose them to an RNAse or a similar enzyme. How we make our RNAs and how we understand safety is in the context of these cationic nanocarriers that actually bind RNA at their particle surface.

Turning back to the question of the key challenges, the main challenges right now are trying to balance safety and tolerability with potency. The field has evolved to often optimize for potency first. Then when you enter into clinical trials you evaluate safety and tolerability, and set your maximum tolerable dose.

Right now, most of the vaccines on the market are operating at or near the tolerable dose. They are trying to achieve a dose as high as possible that is safe and tolerable. We have hit

"We think off-target innate immune responses are the primary driver of reactogenicity, and may even be pathogenic, so what kind and where in the body are the innate immune responses necessary for a robust adaptive immune response?"

that ceiling, and it is very hard to now incorporate multivalent vaccines where you need to add more RNA to account for more antigens, or to go after more self-antigens like in the context of cancer/oncology, where higher doses may be needed to drive more robust CD8 T-cell responses against a self-antigen.

Other key questions include what innate immune responses are necessary, and where in the body are those responses necessary, and perhaps unnecessary? We think off-target innate immune responses are the primary driver of reactogenicity, and may even be pathogenic, so what kind and where in the body are the innate immune responses necessary for a robust adaptive immune response? Then there is within-host and between-host variation in innate immune response. We need to understand which individuals a modality is and is not optimal for, and how we can improve more generalizable approaches.

Moving down into CMC-related issues, there are questions around what process-related impurities and general product attributes are linked to tolerability, and any adverse safety events associated with these.

Finally, a major challenge is simply general communication and public perception—especially right now in an era of politicized misinformation. We need to get ahead of that and try to fully educate the general population about these modalities.

Relatedly, we cannot lump our understanding of the technology into one category. It is a very diverse field, with a diversity of approaches and technologies. We are continuously learning and evolving and improving the ways that we develop these types of modalities.

Q Where and how does the mRNA manufacturing process impact safety and immunogenicity, and where do you see opportunities to improve upon safety in this area?

JE Let's break down manufacturing of RNA drug substance into upstream and downstream. In the upstream part of the process, one can optimize the *in vitro* transcription conditions, the raw material identity and purity, and also template sequences that are associated with off-target transcripts. Those are all areas of active investigation by many in the field.

Once you have optimized and selected for those conditions and are moving into the downstream part of that process, you have to deal with residual solvents, RNA aggregates, double-stranded RNA (dsRNA), nucleotides, proteins, and exogenous nucleic acids coming from your template DNA and from the way that that template DNA was manufactured. With a traditional *E. coli* fermentation system you will have residual *E. coli* RNA and DNA, and with more synthetic approaches like enzymatic amplification of template, you may have residual proteins.

You need to understand how to remove and characterize those and also understand how those components impact both the within-host and between-host innate immune responses present at the individual as well as at the population level.

Q What current and emerging tools are there available for the removal of product-related impurities such as dsRNA?

JE What is emerging now is novel ligand chemistries for affinity capture. There are novel detection reagents to characterize dsRNA molecules, and novel antibodies that bind various conformations of dsRNA. Long-read direct RNA sequencing is also emerging, where you can characterize the abundance, length, and identity of these molecules in your RNA drug substance. There is also mass spectrometry.

There are quite a number of emerging ways to characterize that particular impurity, but at HDT we are also focusing a lot on how to reduce the production of that material in order to eliminate or reduce the need to remove it downstream. Ultimately, it will most likely come down to a combination of both.

Q What are the ongoing obstacles when using LNPs to deliver mRNA—and how is HDT addressing this issue?

JE This comes down to when you start creating and characterizing the drug product, which is the formulated RNA presentation. We don't have a lot of experience with LNP drug product, but from what I've heard and read in the field, one of the key quality attributes that is emerging is these lipid-RNA complexes that can form. They seem to be stability-indicating, and there are ways to detect them and to reduce their production.

Downstream of that, another core difference between LION and an LNP at the drug product stage, is that with LNPs you mix your RNA with ionizable lipids. You complex them in an organic solvent like ethanol, and combine the other three components, resulting in five components combined in an ethanol phase. This complexes and forms the LNP encapsulating the RNA, and then you have to remove the solvent. Downstream of that purification step there is an additional assay required to characterize residual solvent. That adds some complexity, but obviously, these things have been established and solved.

With LION, we don't need to build a nanoparticle around RNA. Our nanoparticle is manufactured completely separately and independently and is stable at 2–8 °C for several years. LION can be combined with RNA at the drug product stage, and then it can be combined in buffer. There's no need to dialyze to remove that organic solvent, or for an assay to characterize residual solvent. Basically, what you put in to make your drug product is what you get out, and there's no need to modify the final product.

This greatly simplifies the way that we manufacture the product. It's going to be more amenable to emerging technologies like continuous manufacturing modalities, where at the final stage you just mix these two components prior to fill/finish. Characterizing that combined product does add complexity, but all of those problems have really been worked out in the LNP field so we don't anticipate that to be too much of a hurdle.

Q What are the most exciting developments you're seeing in the delivery space at the moment?

JE I'm biased, so I'd have to plug our technology at HDT. We believe moving away from this dependence on LNPs is an exciting advance in the field. We have characterized the biodistribution of our technology and shown highly localized delivery to the injection site. We do see a small percentage of molecules draining to the lymph node, but beyond that we don't see delivery to other tissues.

We have targeted, localized delivery depending on your route of administration. We have characterized this fully for an intramuscular route, and we are characterizing it for intradermal as well. Where you inject the material is where you get delivery. We don't see any delivery to liver, so have solved this problem of extrahepatic delivery.

Of course, there's always ways to make additional progress in this field, and the next problem that we would like to solve is targeted delivery beyond the local injection site. If you want to target, for example, every muscle in the body, or the eye following an intramuscular delivery, or if you want to be able to deliver material systemically but target specific cell types that that molecule can come into contact with during circulation, that's another problem.

Trends we are seeing in the field include ligand-directed LNPs. Similar approaches could be applied to a cationic nanocarrier like LION, where you can conjugate an antibody that is specific to a certain receptor. That is a low-hanging-fruit approach to targeted delivery.

Another emerging area in the field of LNPs is how many variations around that four-component structure you can conceive. The four-component LNP space is pretty huge. There are companies screening hundreds of thousands of compositions and empirically defining where those compositions biodistribute (characterizing their tropism). Then there are companies now combining those large datasets with machine learning, to train models that can now take a desired target and spit out a predicted composition that would get you to that target.

In all cases, where that's going is you are going to be able to enrich for certain targets, but I don't think we will ever fully be able to achieve 100% delivery of payload to a desired target cell or tissue. There will always be some residual off-target delivery. This is the next major problem to solve in the field. For now, our plan at HDT is to build upon product indications and build upon that safe, localized delivery, as there is a lot you can achieve with such a platform with this safety profile that delivers locally at the injection site.

BIOGRAPHY

Jesse Erasmus is the Director of Virology at HDT Bio, a biotech company in Seattle. Jesse received his PhD from the University of Texas Medical Branch, Galveston, TX, USA and completed his postdoctoral training at the Infectious Disease Research Institute and the University of Washington, Seattle, WA, USA. His research is focused on the development of platform technologies, beginning with the first application of insect specific viruses as platforms for vaccines and diagnostics at UTMB and more recently with the development of non-viral delivery of replicating RNA as a gene-expression platform for vaccines and therapeutics.

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