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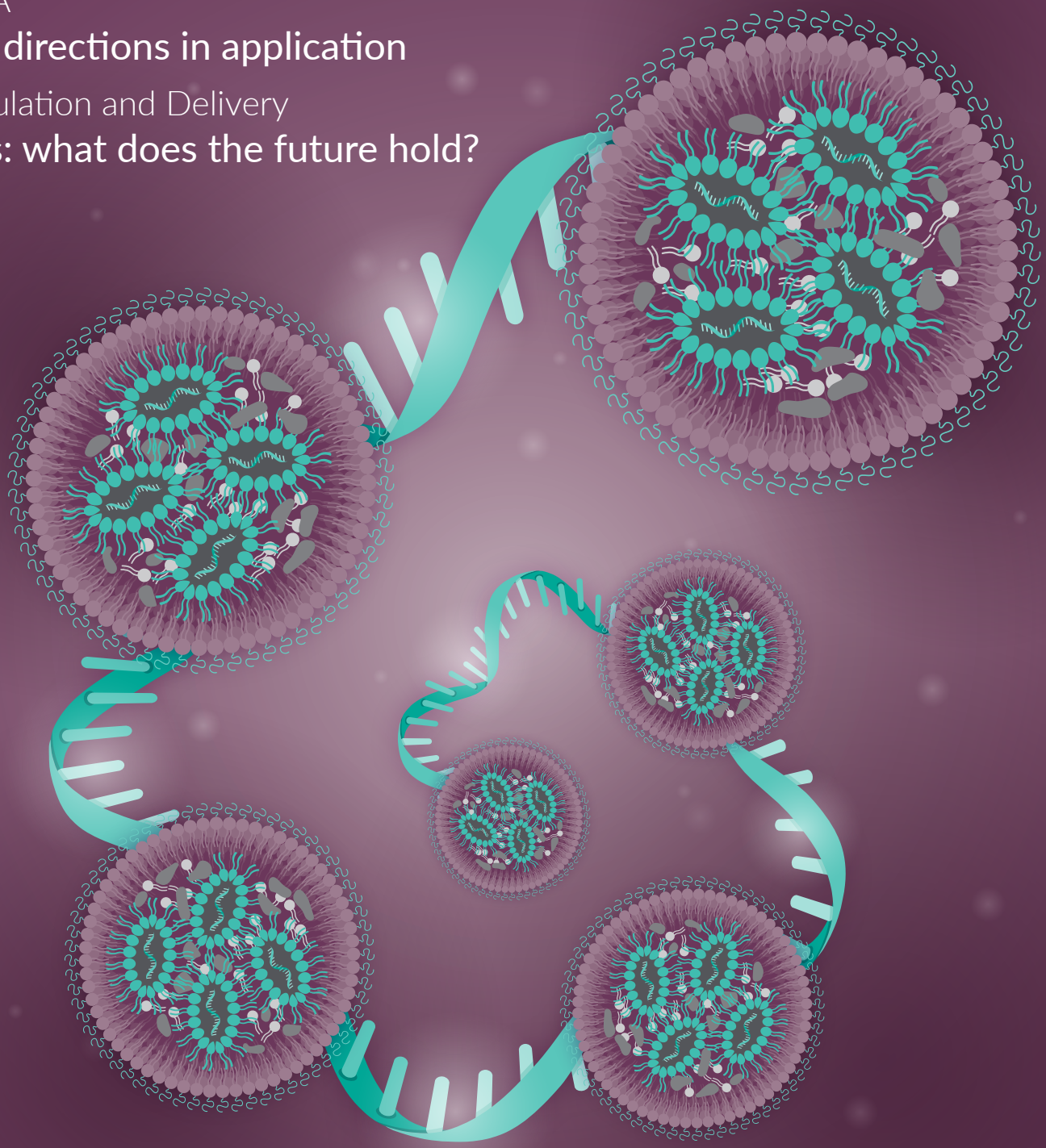

NUCLEIC ACID INSIGHTS

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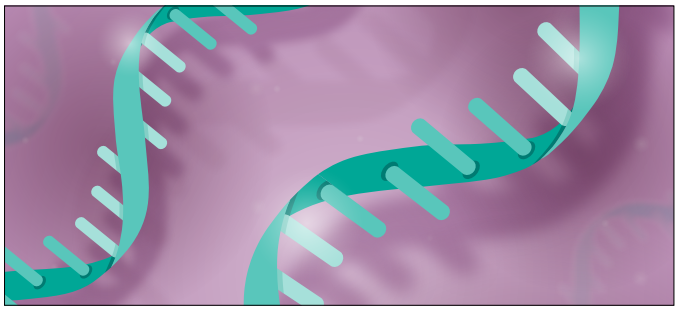

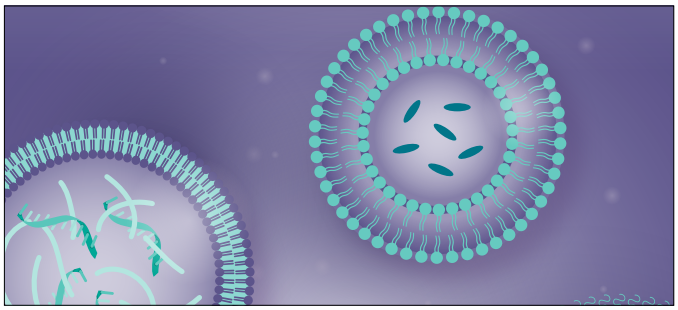

NUCLEIC ACID INSIGHTS

mRNA: NEW DIRECTIONS IN APPLICATION

SPOTLIGHT

Advancing mRNA-based cell therapies: the crucial role of mRNA optimization for therapeutic efficacy

Christian Bär, Ulrich Blache, and Sandy Tretbar



VIEWPOINT

"By refining mRNA design, we can overcome critical barriers, enhancing the therapeutic potential across various medical applications, including oncology, autoimmune diseases and beyond."

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INTRODUCTION

In the rapidly evolving field of mRNA-based therapies, optimizing *in vitro*-transcribed (IVT) mRNA design and production is of paramount importance for achieving (cost)-effective and safe therapeutic outcomes. This is particularly true for non-viral chimeric antigen receptor (CAR)-T cell therapies, where high CAR expression

requires prolonged stability and minimal immunogenicity of the CAR–mRNA. By refining mRNA design, we can overcome critical barriers, enhancing the therapeutic potential across various medical applications, including oncology, autoimmune diseases and beyond [1,2].

KEY CRITICAL PARAMETERS FOR mRNA OPTIMIZATION FOR CAR-T CELLS

Minimizing RNA components for efficacy and safety

Reducing the foreign RNA load is vital for minimizing negative immune responses, especially in cell therapy applications. Initial investigations identified the minimal components required for detectable CAR expression: a 5′-cap, a CAR-encoding sequence (open reading frame), and a poly(A) tail. This basic RNA structure serves as a starting point for further enhancements in translation efficiency and stability, providing a streamlined approach to CAR–mRNA production.

Refinement of the 5′ cap for superior performance

The 5′ cap is essential for gene expression and mRNA stability and the choice of 5′ cap analogs profoundly affects mRNA performance. Commonly the 5′ cap is introduced co-transcriptionally during IVT by adding a cap analog such as the Anti-Reverse Cap Analog (ARCA). However, ARCA competes with the GTP nucleotides in IVT reactions resulting in either a low capping efficiency or a low translation efficiency if the amount of competing GTP is lowered. A solution is offered by CleanCap, a next generation cap analog with a cap 1 structure [3]. CleanCap outperforms other types of cap analogs, including ARCA, in transcription and capping efficiency, as well as translation of the IVT product. These findings underscore CleanCap as the preferred choice for CAR–mRNA generation, combining robust capping efficiency with enhanced protein expression. However, for upscaling and GMP manufacturing the patent landscape and licensing fees of preferred cap structures must be taken into account. To achieve low-cost mRNA production, it is essential to consider cap analogs with reduced licensing fees, though this often compromises mRNA performance.

Leveraging UTR sequences for improved stability and translation

Untranslated regions (UTRs) up- and downstream (5′ and 3′ UTR, respectively) play a crucial role in mRNA stability and translation, yielding enhanced CAR expression and prolonged CAR-T activity [4]. Specific sequences or secondary structures within UTRs can reduce exposure to ribonucleases, which in turn leads to reducing mRNA degradation and bolstering therapeutic efficacy. Moreover, the inclusion of internal ribosome entry sites and/or a well-defined Kozak-sequence around the start-codon aids ribosome binding and translation. Additional investigations into synthetic UTRs highlight further potential for refinement, e.g., by applying AI-based models for decoding of UTRs [5], particularly for applications requiring precise control of translation dynamics.

Balancing immunogenicity and translation efficiency

There is a broad spectrum of RNA nucleotide modifications available, e.g., N6-methyladenosine (m6A), pseudouridine (Ψ), 5-methylcytidine (m5C), and N1-methylpseudouridine (m1Ψ) [6], most of them being tested for use in mRNA therapeutics. In general, the use of modified nucleotides in IVT products reduces immunogenicity by limiting interactions with immune sensors like TLRs and RIG-1. While nucleotide modifications decrease protein expression

in T cells compared to unmodified mRNA, they offer advantages in stability and immune tolerance. Since nucleotide modifications can cause cell-type-specific effects, the need for tailored approaches to achieve optimal outcomes in different therapeutic settings is emphasized.

Optimization in IVT protocols and mRNA purification

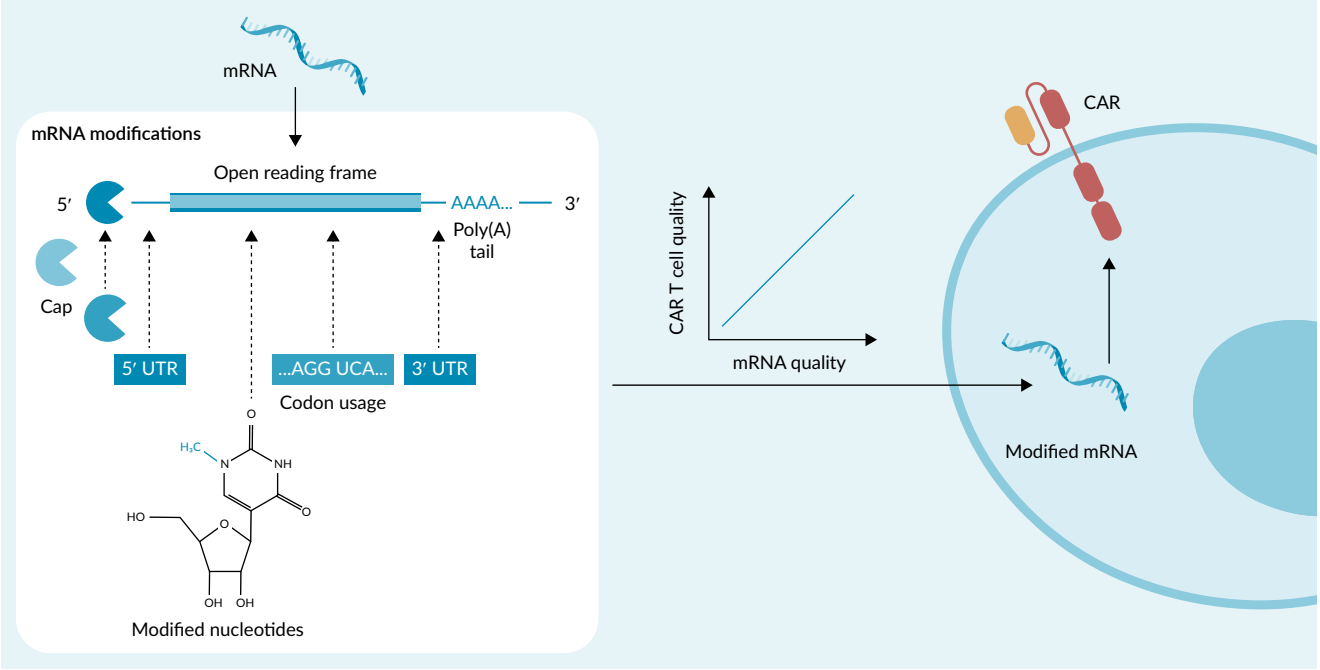
Beyond mRNA composition, the IVT process itself has a profound impact on mRNA functionality. For example, standard enzymes such as T7 RNA polymerase produce high amounts of double-stranded RNA which is highly immunogenic and should be avoided for CAR approaches. Optimized transcription protocols and the use of optimized engineered RNA polymerases can significantly reduce the double-stranded RNA amount [7] and, therefore, may reduce the necessity for complex downstream purification methods, such as reversed phase HPLC or oligo dT affinity chromatography [8]. Moreover, spin-column purification, compliant with GMP standards, has proved sufficient for high-quality mRNA production when paired with a robust IVT protocol [9]. These findings simplify manufacturing pipelines, making therapies more scalable and accessible.

IMPLICATIONS FOR BROADER THERAPEUTIC APPLICATIONS

Most advancements have so far been demonstrated in a cancer-related setting, but they hold even greater promise for autoimmune and fibrotic diseases, where transient CAR-T cells offer a safer therapeutic profile. By combining modifications such as CleanCap, optimal UTRs, and nucleotide modifications, alongside streamlined production protocols, mRNA-based therapies can achieve the precision, stability, and safety required for diverse clinical applications.

FIGURE 1

Key critical parameters for mRNA optimization for CAR-T cells.



Increasing mRNA quality leads to improved transient CAR-T cell quality.

CONCLUSION: A CALL TO ACTION

Optimizing mRNA design and production is not merely a technical challenge but a cornerstone of advancing mRNA-based therapies. Each improvement, from cap selection to nucleotide modification, contributes to the delicate balance of translation efficiency, stability, and immunogenicity **(Figure 1)**. As we refine these parameters, the potential to revolutionize cell therapies across oncology, autoimmune diseases, and beyond becomes increasingly tangible. It is imperative that future research continues to explore the interplay of these variables, ensuring that mRNA-based therapeutics fulfill their transformative promise.

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BIOGRAPHIES

Christian Bär completed his studies in Molecular Biology at Martin-Luther-University Halle, Halle, Germany and earned his PhD in Molecular Genetics from the University of Leicester, Leicester, UK in 2012, with a focus on tRNA modifications. During his postdoctoral training at the Spanish National Cancer Research Centre (CNIO) in Madrid, he gained expertise in cardiovascular gene therapy and (anti)-aging research. In 2016, he joined Hannover Medical School, where he specialized in non-coding RNA research, aiming to harness novel RNAs for therapeutic applications to combat heart disease. Since 2023, Bär has held a joint professorship in Regenerative Cardiology at Hannover Medical School, Hannover, Germany and the Fraunhofer Institute of Toxicology and Experimental Medicine, Braunschweig, Germany, where he continues to advance his pioneering work in novel RNA-based therapies.

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Ulrich Blache is a researcher at the Fraunhofer Institute for Cell Therapy and Immunology (IZI), Leipzig, Germany. Blache studied Cell Biology in Leipzig and Basel and received his PhD in Bioengineering from the ETH Zurich, Zurich, Switzerland in 2018. After spending postdoc time in tissue engineering, Blache joined the Fraunhofer IZI in 2020 where he is heading a research group focusing on the development of cell therapies.

Sandy Tretbar is a molecular biology and RNA biochemist by training who spent her PhD and postdoc time in Germany and the US. After her second postdoc in the immunology field, she joined the Fraunhofer IZI in 2018 as research associate and advanced as group leader in 2021. Now, she combines her long-standing RNA research experience with cell therapy development in immuno-oncology to investigate mRNA-based cell and gene therapies for the treatment of cancer and various other diseases.

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SPOTLIGHT

Addressing regulatory hurdles for individualized mRNA cancer immunotherapies: insights into the MHRA's new guidance for developers

INTERVIEW

"...there are two parts to the guidance—product design, and follow on product manufacture."

In this interview **Róisin McGuigan**, Commissioning Editor, *Nucleic Acid Insights*, speaks to **Francis Galaway**, Quality Assessor, MHRA, about the MHRA's newly published guidance on individualized mRNA cancer immunotherapies, along with recent advances and regulatory challenges in the space. They also discuss the role of innovative technologies, such as machine learning (ML), AI, and bioinformatics in supporting the development of personalized mRNA-based treatments.

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"Through internal discussions and consultations with other experts, we...saw the need to issue guidance to explain what steps developers should take and clarify what we, as regulators, expect from them."

Q Can you tell us about your current role and what you are working on right now?

FG I am a quality assessor in the Biologicals Team at the MHRA, where my primary role involves working on marketing authorization applications, with a specialization in advanced therapy medicinal products (ATMPs). I also provide scientific advice and guidance to companies, particularly on ATMPs and rare diseases. Recently, I have been focusing on developing guidance for individualized mRNA cancer immunotherapies, also known as cancer vaccines. Alongside this, I am also working on a couple of ATMP applications in gene therapies.

Q You have recently been working on the creation of a new guidance document on individualized mRNA immunotherapies. Firstly, can you talk a bit about the current developments and issues in the space that led to the initiation of this project?

FG There has been a massive expansion in the mRNA medicines field, largely driven by the success of mRNA-based vaccines during the COVID-19 pandemic. Many developers and innovators were inspired to explore other applications of this technology, including individualized therapies. This marks a big shift from treatments designed for large populations to those tailored for small groups or even a single individual.

Despite the advancements, this transition presents numerous challenges for both developers and regulators because products like these are unprecedented. We quickly recognized the complexity of this field and the significant hurdles developers face. Through internal discussions and consultations with other experts, we realized how intricate this area is, and saw the need to issue guidance to explain what steps developers should take and clarify what we, as regulators, expect from them.

Q Why was now the right time to create guidance in this area?

FG There is a significant number of clinical trials being initiated for individualized mRNA therapies, which prompted us to act. As we reflected on the expectations for developers in this field, we realized how complex this area is. Manufacturers often utilize advanced sequencing techniques, bioinformatics, and even aspects of machine learning (ML) and AI—and in addition, many other technologies are coming to fruition. For example, sequencing technology has advanced significantly over the years and is now being increasingly applied not just in development but also in the manufacturing of medicines. Additionally, analytical tools are advancing to have very

quick turnaround times. This progress is now intersecting with the success of mRNA vaccines and advancements in LNP technology. On top of that, the manufacturing process itself is incredibly intricate. It became clear that providing guidance was essential to support developers in navigating these challenges. With sufficient regulatory support and convergence of all these innovations, this will hopefully translate into meaningful outcomes for patients.

Q What are the key knowledge gaps or unmet needs that the guidance aims to address?

FG The first thing we addressed was the terminology and phrasing in this area. We spent a lot of time discussing what to call these mRNA-based immunotherapies, knowing they could not be labelled as 'cancer vaccines' since they are not prophylactic and are not used for infectious disease. Ultimately, we settled on 'individualized mRNA cancer immunotherapies'.

Alongside this, we made several other key decisions. Firstly, a significant gap we addressed was how to handle clinical trial design, since these individualized treatments differ for each patient. We concluded that it is not necessary to test every possible variation in clinical trials. However, we also set expectations to ensure these treatments are safe and effective.

We signposted that there are two parts to the guidance—product design, and follow on product manufacture. In product design, there are tissue and devices regulations for consideration and the importance of quality at each stage of product design. We highlighted the utility of human tissue and medical device regulations for bioinformatics and AI components. We also emphasized how ATMP regulations provide flexibility, allowing manufacturers to produce patient-specific batches under a single marketing authorization. Further, we outlined how developers could improve their analytical processes while remaining compliant with regulations and maintaining the safety of their medicines.

We also addressed topics such as post-market surveillance and post-market changes, particularly in the areas of software development and AI, where there are constant changes. Regarding AI, we included guidance on clinician and patient information. This section focuses on key terminology and outlines important considerations for using AI in these therapy pathways, including regulatory requirements for developing these products and additional measures to ensure their safe and effective use. We hope that this guidance will provide the principles for meaningful discussions between clinicians and patients, helping them understand the implications of these therapies.

Q Can you tell us more about the expert working group assembled to develop the guidance?

FG The MHRA formed an expert working group to focus on highly individualized therapies—not just mRNA cancer immunotherapies, but all individualized therapies that might be in development. We drew on expertise from across the UK, bringing together professionals from medicine, industry, and academia. A significant

portion of this expertise came from the ultra-rare disease space, as there is substantial experience in that area.

We also needed expertise from outside the MHRA in areas such as bioinformatics and AI, as these are highly specialized fields where we do not have all the knowledge in-house. This working group was crucial for informing us about the correct terminology to ensure it resonates with all stakeholders. Notably, the group was chaired by Professor Sir Munir Pirmohamed, who also chairs the UK Commission on Human Medicines (CHM).

Q What key advice and takeaways would you highlight for developers?

FG As mentioned earlier, one of the key developments is that we now have a fixed set of terminology to use, ensuring everyone is working within the same frame of reference. While the regulatory pathway is complex, we outlined how to follow it in this guidance, aiming to streamline the process for developers.

Additionally, we are classifying these immunotherapies as gene therapies or ATMPs, which brings important flexibilities, particularly for manufacturing. We expect the manufacturing process to help control the manufacture to a single set of specifications, despite the variability in starting materials. Even though each batch is patient-specific, with different mRNA sequences, they can all fall under the same marketing authorization provided that they are for the same indication and formulation.

Regarding clinical trial design, we clarified that if the formulation and overall design remain consistent, developers can extrapolate results from trials using a specific set of patient-specific sequences to support a marketing authorization that covers a broader range of patient batches. This means the developers do not need to test every possible patient sequence individually.

Turning to bioinformatics and AI analysis, we emphasized that these processes must be validated or accredited, and highly reproducible. For example, we would not permit continuous learning, which is a feature of some AI systems.

Q Your own area of expertise is in manufacturing—could you expand on the current evolution of manufacturing in the mRNA immunotherapies space, particularly in terms of tools and tech?

FG The success of mRNA vaccines during the COVID-19 pandemic has sparked an explosion of activity in this space. Since then, the technology has advanced rapidly in numerous ways. One of the most notable developments is the rise of LNPs as a highly effective delivery system for mRNA, solving what had been a significant challenge.

We also have much faster and more accurate analytical techniques. For example, it is now possible to rapidly sequence a tumour sample and identify new antigens that could target cancerous cells. Additionally, DNA synthesis plays a key role in this rapid manufacturing process, but in the future it may be possible to synthesize mRNA directly.

All of this is happening alongside a decade of progress in designing and controlling manufacturing processes that can handle highly variable starting materials.

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"I hope that alongside traditional mass-produced medicines, patients will increasingly have access to individualized treatments."

CAR-T therapies are a classic example of this, as they rely on autologous material from patients, which is inherently variable. These therapies have turned into a real success, demonstrating that such variability can be managed effectively.

With these advances coming together, many companies and innovators now have the potential to develop medicines that integrate novel technologies into individualized products that are manufactured relatively quickly. For us as regulators, this has required some innovation in how we apply the existing regulations. However, we think there is sufficient flexibility, which is why we have classified these therapies as ATMPs.

It is also worth noting that many of the principles we developed for these cancer immunotherapies can likely be applied to other individualized medicines as new technologies continue to emerge.

Q Where do you see the mRNA therapeutics space heading in the next few years, and what evolutions or potential challenges do you anticipate?

FG It is difficult to make any predictions because the field is changing so rapidly. I hope that alongside traditional mass-produced medicines, patients will increasingly have access to individualized treatments. Treatments tailored specifically to each patient could lead to better clinical outcomes. Ideally, with the advancements in rapid manufacturing and quick turnaround times, we might even see these treatments being produced locally—perhaps within hospitals. The main goal is to make the entire treatment is individualized for a patient and their specific needs. If this vision becomes a reality, it could significantly improve the safety and effectiveness of treatments and create a more holistic standard of care within the same hospital or clinical unit.

However, as with the development of any therapeutic, there are also potential pitfalls. Firstly, it is crucial to get the initial leaders right in terms of the regulatory pathway, as this will set the tone for the rest of the industry. That is partly why we wanted to issue guidance early on. It is easy to make mistakes or misunderstand regulatory expectations, and if things go wrong for the first few developers, it could discourage others from pursuing this field. It is also incredibly difficult for companies working in this area, as they need to integrate various areas of expertise and technologies. But with the right support and guidance, I'm hopeful they will get it right.

Additionally, from a quality assessor's perspective, one of the biggest challenges will be potency assays. Finding ways to ensure efficacy and safety without true potency assays will be difficult, but we are working to help developers navigate this. Ideally, we would like to see more robust potency assays to improve the manufacturing process and ensure the quality of the product patients receive.

Regarding the use of ML and AI, I should also mention that we are collaborating with other regulators globally to harmonize best practices. In particular we are working with the US FDA and Health Canada on guidelines for ML. We are also collaborating with

regulators worldwide, particularly on the terminology, to ensure we are all working within the same frames of reference and have consistent expectations.

The MHRA's draft guidance on individualised mRNA cancer immunotherapies is now in its final consultation phase, and can be accessed here: **Draft guidance on individualised mRNA cancer immunotherapies**.

BIOGRAPHY

Francis Galaway is a Quality (CMC) Assessor in the Biologicals team at the MHRA, London, UK. He specializes in the licensing of advanced therapy medicinal products (ATMPs) and represents the MHRA on several international working parties for cell and gene therapies. He is experienced in a range of collaborative regulatory pathways including Orbis and the centralized EMA procedure as well as the UK's International Recognition. Francis spent 8 years at the Wellcome Sanger Institute working on intercellular interactions and the immune system. His PhD studies were conducted at the Astbury Centre for Structural Molecular Biology, Leeds, UK on novel viral platforms for gene therapy applications.

Francis Galaway, Quality Assessor, MHRA, London, UK

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FORMULATION AND DELIVERY:
LNPs: WHAT DOES THE FUTURE HOLD?

SPOTLIGHT

Exploring challenges, opportunities and state-of-the-art tools in the lipid nanoparticle space

INTERVIEW

"...the future of the pharmaceutical industry in the lipid nanoparticle space looks highly promising..."

Róisin McGuigan, Commissioning Editor, *Nucleic Acid Insights*, speaks to **Ketaki Deshmukh**, Senior Scientist, Novo Nordisk, about advanced characterization tools for LNPs, challenges in extrahepatic delivery, scalability issues, and the potential impact of AI and machine learning (ML) on future LNP development.

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Q What are you currently working on?

KD I am a Senior Scientist at Novo Nordisk, specializing in the optimization of LNP formulations through high-throughput screening techniques. My primary focus is on enhancing the hepatic and extrahepatic delivery efficiency of novel lipids. Additionally, I am involved in developing robust analytical methods and assays to define and maintain the critical quality attributes and critical process parameters of LNPs.

In my role, I collaborate closely with cross-functional teams to design and execute experiments aimed at improving the efficiency and safety of LNP-based delivery systems.

I also leverage advanced analytical tools and methodologies to comprehensively characterize LNPs, ensuring compliance with stringent regulatory and quality standards.

Beyond my core responsibilities, I actively contribute to cross-departmental projects, aiming to integrate AI and ML-driven approaches to further enhance the precision and efficiency of LNP formulation processes. My work is instrumental in pushing the boundaries of drug delivery systems, ultimately aiming to improve patient outcomes and expand the therapeutic potential of novel treatments.

Q What are the key challenges for extrahepatic delivery using LNPs and the most promising avenues for achieving targeted delivery?

KD Achieving extrahepatic delivery is always challenging, especially in the context of gene editing or gene delivery. LNP-mediated extrahepatic delivery is challenging primarily due to the presence of apolipoprotein E, which binds to LNPs and facilitates their uptake by hepatocytes. Secondly, the structural design of most LNPs mimics natural lipoproteins, causing liver cells to recognize them as nutrient sources, further driving their preferential uptake by hepatocytes. Furthermore, upon intravenous injection, nanoparticles are often captured by the reticuloendothelial system, particularly in the liver and spleen, further limiting their distribution to other tissues.

Certain organs, such as the brain and tumors, pose additional challenges due to the blood–brain barrier and the blood–tumor barrier, which significantly impede nanoparticle delivery to their interior. To address these hurdles, scientists are actively developing various technologies to enhance extrahepatic delivery, including active and passive targeting strategies.

In passive targeting, the lipid composition of the LNPs is modified. Since these nanoparticles are composed of four primary lipid components, you can add extra components or manipulate existing ones to potentially alter their targeting properties and favor specific tissues. For instance, adjusting the size and charge of nanoparticles can influence their tissue distribution and improve extrahepatic delivery. By using PEGylation or similar modifications, LNPs can evade detection and clearance by the immune system, leading to longer circulation time and more opportunities for passive accumulation in target tissues.

Active targeting involves modification of LNPs to actively bind to specific tissues or cells. This strategy enhances the selectivity and precision of delivery by using ligands, antibodies, or other targeting molecules. LNPs can be conjugated with ligands such as antibodies, peptides, or small molecules that specifically recognize receptors or markers on the surface of the target cells.

Q What would you identify as the current state-of-the-art in terms of advanced characterization tools available for LNPs?

KD Advanced characterization tools for LNPs encompass a range of sophisticated techniques designed to comprehensively analyze their physical, chemical, and biological properties.

Cryo-electron microscopy (cryo-EM) is one of the most widely-used tools. This powerful imaging technique enables high-resolution visualization of LNPs in their native hydrated

"[A] critical challenge is ensuring the long-term stability of nanoparticle formulations."

state. Cryo-EM is used to determine the size, shape, and internal structure of LNPs, providing detailed insights into their morphology and assembly.

Dynamic light scattering (DLS) combined with multi-angle light scattering (MALS) techniques provide precise measurements of particle size, polydispersity index, molecular weight, and structural integrity. DLS and MALS are invaluable for the development, optimization, and quality control of LNP-based delivery systems. In order to understand the surface charge of the particle, Zeta potential analysis can be used, providing information about LNP stability and the potential for aggregation. Optimizing the Zeta potential is essential for optimizing the formulation and ensuring the stability of the particles.

Furthermore, small-angle X-ray scattering (SAXS) can be used to analyze the internal structure of LNPs. By studying the scattering patterns of X-rays, SAXS provides insights into the structural organization and phase behavior of lipid components within LNPs.

Mass spectrometry (MS) is used to identify and quantify the lipid components and encapsulated drugs within LNPs. MS provides precise molecular weight information and helps assess the composition and purity of LNP formulations.

High-performance liquid chromatography (HPLC) is yet another critical tool for quality control and ensuring the consistency of LNP batches. It is mainly used to analyze the encapsulated drug, and any degradation products in the formulation.

Finally, flow cytometry is also used to analyze the physical and chemical characteristics of LNPs at the single-particle level. It is used to study cellular uptake and intracellular trafficking of LNPs, often in combination with fluorescent labeling.

All of these advanced characterization tools could be used in the optimization and development of LNP formulations, as well as during process development and scale-up.

Q What are the key current challenges in LNP process development?

KD **Firstly, one major issue is scaling up LNP production from laboratory to industrial scale while maintaining product consistency and reproducibility.** Due to the complex nature of LNP formulations, achieving minimal batch-to-batch variability during large-scale manufacturing is particularly challenging.

Another critical challenge is ensuring the long-term stability of nanoparticle formulations. This includes preventing the degradation of both the lipid components and the encapsulated therapeutic agent. Many LNP formulations are highly sensitive to temperature, requiring stringent cold chain logistics, which can increase costs and complicate distribution.

Q What are the key parameters to consider when looking at successful scalability of LNPs?

KD **Successful scalability requires the optimization of several critical parameters.** This includes optimizing upstream and downstream processes such as

"It is crucial to implement efficient and easy-to-scale upstream and downstream processes from the early stages of product development."

formulation composition, effective mixing, homogenization, solvent removal, sample concentration, and filtration. These steps are essential to achieve the desired properties and functionality of LNPs.

It is crucial to implement efficient and easy-to-scale upstream and downstream processes from the early stages of product development. Implementing advanced characterization techniques, as mentioned earlier, during process development and scale-up can help maintain product quality and safety. These measures will facilitate the development of standardized protocols and quality control measures for LNP production, addressing some of the challenges discussed earlier.

Q When and how do you predict the industry will move forward in the LNP space and leverage nanoparticle delivery?

KD I think the future of the pharmaceutical industry in the LNP space looks highly promising, with significant advancements expected in targeted delivery, stability, and the integration of advanced technologies. The industry is likely to focus more on tissue-targeted and extrahepatic deliveries to address a broader range of diseases, including cancer, genetic disorders, infectious diseases, and vaccines.

Additionally, AI and ML will play crucial roles in optimizing novel lipid chemistry, LNP formulations, predicting biological interactions, and streamlining drug development processes. These technologies will also contribute to enhancing formulation stability, reducing the need for cold-chain logistics.

Regulatory guidelines for LNP-based therapeutics are expected to become more harmonized, facilitating faster approval processes. Innovations in cost-effective production methods will further reduce the overall cost of LNP-based therapies, making them more accessible to a wider patient population. It's going to be an interesting era for gene editing and gene delivery.

Q What are your own key goals and priorities for the next few years?

KD As I touched on earlier, my goal is to advance extrahepatic and targeted delivery systems by leveraging AI and ML to optimize LNP formulations, predict biological interactions, and expedite the drug development process. Additionally, I aim to develop LNPs with enhanced stability to withstand a broader range of storage conditions, thereby reducing the reliance on cold-chain logistics and lowering the overall cost of LNP-based therapies.

BIOGRAPHY

Ketaki Deshmukh holds a PhD in Nanomaterials from esteemed university Birla Institute of Technology And Science, Pilani, Pilani, India. Her academic foundation, combined with industry experience, provides her with a holistic perspective on the challenges and opportunities in lipid nanoparticle-based drug delivery.

Ketaki Deshmukh, Senior Scientist, Novo Nordisk, Lexington, MA, USA

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NUCLEIC ACID INSIGHTS

VIEWPOINT

Is the literature review paper dead? How AI is transforming the research landscape in DNA research

Jon Ashley

VIEWPOINT

"...the use of AI...should be considered an effective resource rather than a tool to be feared."

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In academia and research, the review article has acted as an effective way to summarize, challenge, and push the future direction of research though scientific discourse [1]. Reviews can be used to identify gaps in the research and highlight recent discoveries, methods, and tools as well as helping to collate data. This is important when working in fields that are fast-paced and evolving. Reviews can also help summarize fields of research to help researchers understand and grasp new areas outside of their expertise.

This is particularly true within the DNA research domain, which is a constantly evolving field. Areas such as DNA nanostructures [2], enzymatic synthesis of DNA [3], DNA based storage systems [4], and new oligonucleotide-based therapeutics [5] have emerged as hot areas of research with accelerated growth over the last few years, and findings from these fields can quickly render previous findings obsolete. However, with the proliferation of review articles, which have increased exponentially [6],

there are some issues that have distorted the value of review papers. Review papers tend to be more highly cited than research articles leading to distorted measures of impact such as journal impact factors. Authors can in some cases cite review papers based on information provided from original research articles rather than citing the original source of research. Authors sometimes prefer to cite review articles over research articles to create the impression that the research conducted was more novel than it actually is. Review articles can also suffer from author selection bias, which can distort the true state of the field by highlighting the advantages of a technology, method or tool, while downplaying its limitations. There have also been highlighted cases of review papers where the authors have excessively self-cited their own work, which can also distort the true state and impact of a field [7].

Artificial intelligence (AI) is already having a transformative effect on academia and research in terms of increasing accessibility and productivity of researchers with the introduction of large language models (LLMs). In particular, LLMs can summarize and explain information in a variety of styles to suit the reader, making research more accessible to lay audiences. While ChatGPT, Co-pilot, and Gemini have dominated the headlines, the introduction of AI tools that are tailored for academia has rapidly grown. Earlier versions of LLMs were incapable of providing sources for the information given and in some cases, even generated fake references [8,9]. However, this has changed with the introduction of newer LLM models, which are capable of searching the internet in real time and providing real citations to back up their answers. Perplexity [10], Consensus [11], and Scite AI [12] are AI-based search engine tools that can be used to summarize a field, search for answers to questions through the research prompt, and provide a list of references to back up their findings.

The answers provided by Perplexity, Consensus, and Scite AI tend to provide more concise summaries with a limited number of sources. This is due to the maximum token limit at which AI tools can perform unless the reader subscribes to the premium service. These tokens are chunks of text that a LLM can process, and the maximum token limit can vary from each AI tool. The token limitation suggests that literature reviews performed by the majority of AI tools are currently not as comprehensive as published review papers. Storm [13], which was developed by Stanford University, can provide a more comprehensive article, which resembles a review article/Wikipedia page. SCISPACE [14] offers users a suite of several AI tools, including a literature review generator, AI writer, citation generator, and data extractor tool, providing researchers with everything they need to write and search for papers. NotebookLM [15] allows tailoring of the answers given by uploading multiple papers as sources to its server, so that in effect, researchers can summarize research from a number of papers without reading each individual paper. Jotlify [16] is another AI tool which is capable of turning research papers and summarizing them through an audio file, which extends the review article to a new format, while Mapify [17] can be used to summarize topics or papers in the DNA research field into the style of a mind map.

AI is not only transforming the way we search the literature for research but also providing a means to simplify and explain complex scientific concepts in DNA for non-experts and student readers. This is transforming the way in which universities teach and assess future generations of researchers within the natural sciences. While some types of assessments are now in danger of becoming obsolete, such as essays and lab reports, new deeper learning-based assessments could be introduced to encourage student learning through AI

use, and the development of prompt engineering as a critical research skill will be a feature of higher education in the next few years.

Although these AI tools can be used to summarize and review DNA research findings, they currently lack the robustness, accuracy, and oversight to replace review articles, which benefit both from being rigorously peer reviewed and from mainly using primary sources only. In addition, AI tools will likely carry over selection/information biases from the source they cite, can make mistakes, and sources still need to be checked for accuracy [8,9]. Despite this, AI tools are rapidly improving over time and may soon replace existing academic search engines such as Scopus, Web of Science, and Google Scholar as the primary means for searching the literature. The current subscription-based business models of these AI tools make them inaccessible to cash-limited individuals and universities due to the sheer number of AI tools out there. Nonetheless, overall, the use of AI is transforming the way we search the literature and perform research within the field of DNA, and should be considered an effective resource rather than a tool to be feared.

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BIOGRAPHY

Jon Ashley is a Senior Lecturer at the School of Pharmaceutical and Biomolecular Sciences in Liverpool John Moores University, Liverpool, UK. He is currently a Community of Analytical Measurements (CAM) fellow and a chartered Chemist (CChem). Dr Ashley's research focuses on the development of aptamers and molecularly imprinted polymers and their application as molecular recognition elements in nanosensors and as therapeutic drugs. Dr Ashley's current research focuses on the development a new all in one multifunctional nanosensor platform for the detection of therapeutic drugs in blood samples. The nanosensor utilizes both an aptamer and nanoMIP which incorporates both cooperative binding mechanism and fluorescence quenching transduction which can potential overcome the issues of non-specific binding of blood matrix components.

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AUTHORSHIP & CONFLICT OF INTEREST

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WEBINAR DIGEST

Precision in production: optimizing monitoring and quality control for high-value plasmids

Oleksandr Karpenko, Bioprocess Analytics Field Application Scientist, Repligen Corporation

Gene therapy analytics directly influence product quality, ultimately saving precious time and resources. However, traditional UV spectroscopy, commonly used for bioprocess analytics, can pose a range of challenges. This poster introduces a streamlined analytics process for gene therapy products utilizing variable pathlength technology (VPT) in place of traditional analytical tools. Case study data demonstrates that VPT-based technology can accurately determine pDNA purity ratios in human gene therapy products, automating the R-value calculations and eliminating the need for dilutions.

TRADITIONAL UV SPECTROSCOPY VERSUS VARIABLE PATHLENGTH SPECTROSCOPY

Traditional UV spectroscopy, commonly used for bioprocess analytics, has limitations such as lengthy assay times, labor-intensive procedures, and susceptibility to errors. VPT, also known as slope spectroscopy, addresses these issues by adjusting pathlengths to maintain a constant concentration (**Figure 1**). As a result, VPT eliminates the need for dilutions, significantly increasing process efficiency and reducing cycle times by providing instant analytical results, which ultimately enhances process understanding with real-time insights.

CASE STUDY: UTILIZING SLOPE SPECTROSCOPY TO DETERMINE PDNA PURITY RATIOS IN HUMAN GENE THERAPY PRODUCTS

In this case study, slope spectroscopy, namely SoloVPE©, was utilized to determine the pDNA purity ratios in human gene therapy products in place of traditional nucleic acid analytics. The aim of the study was to evaluate the impact of dilution on the measurement process and compare the reliability and accuracy of the SoloVPE method with traditional spectrophotometry techniques.

Figure 1. Traditional UV spectroscopy versus variable pathlength spectroscopy.

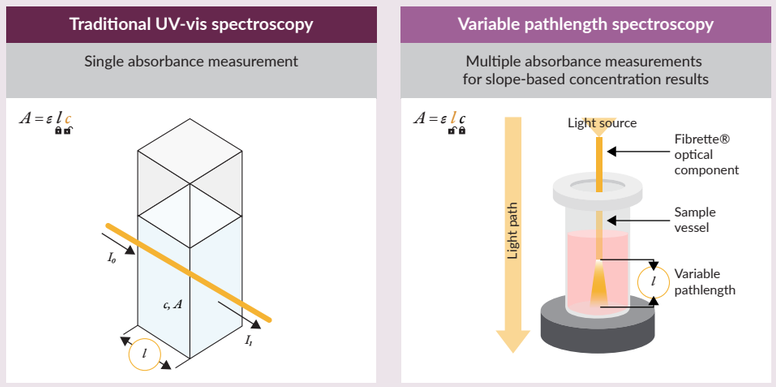

Table 1. pDNA purity study results.

Level	Theoretical purity ratio	Observed purity ratio	% Difference
1	0.62590	0.62723	0.21
2	0.87087	0.90315	−0.22
3	1.05311	1.06122	0.74
4	1.18483	1.17076	−1.14
5	1.28451	1.28847	0.30
6	1.42528	1.42358	−0.11
7	1.51996	1.52481	0.31
8	1.58798	1.58959	0.10
9	1.63927	1.65568	0.96
10	1.67930	1.67489	−0.25
11	1.71134	1.69203	−1.09
12	1.73770	1.71515	−1.25
13	1.75964	1.73533	−1.33
14	1.77821	1.77064	−0.41
15	1.79418	1.81956	1.36
16	1.80804	1.77874	−1.56
17	1.82026	1.80414	−0.85
18	1.83094	1.81184	−1.00
19	1.84046	1.82975	−0.56
20	1.84902	1.85457	0.29
21	1.85672	1.84667	−0.52
22	1.86028	1.88378	1.22
23	1.86365	1.85282	−0.56
24	1.86692	1.84941	−0.90
25	1.87000	1.87147	0.08

Firstly, 25 different purity levels for a mixture of DNA and protein were defined, and corresponding solutions for each level were prepared. These ratios ranged from 100% protein and 0% DNA to 100% DNA and 0% impurities. Triplicate measurements were taken at each purity level to obtain the 260/280 nm absorbance ratio and slope values. The SoloVPE software automatically calculated the R-values for each measurement. To compare the experimental R-values with theoretical values, theoretical purity ratios were calculated for each solution and compared with the R-values obtained from SoloVPE. Based on the measurements, the observed purity ratio results closely matched the theoretical values (**Table 1**).

Overall, the case study demonstrated that SoloVPE can be a critical analytical tool for determining plasmid DNA purity, expediting sample testing, providing immediate feedback, and expanding possibilities for meeting medical needs. The study also showed that the SoloVPE system provides significantly improved sensitivity compared to traditional spectrophotometers.

OTHER GENE THERAPY APPLICATIONS USING VPT

In addition to measuring the pDNA purity ratio in human gene therapy products, there are various other areas where VPT could be applied, including pDNA downstream processing monitoring, fermentation analysis, chromatography, mRNA purity ratio measurements, and AAV capsid and genome titer analysis.

SUMMARY

Adopting a unified analytical platform with VPT can offer a number of benefits, such as a broad concentration range without the need for baseline correction, robust and accurate measurements, and no requirement for dilution. Each result produced by VPT systems is based on multiple measurements, providing an R^2 value for every outcome, ensuring reliable and precise data in gene therapy analytics.

Watch the webinar here

Read the full transcript here

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