Mapping the future of DNA/RNA drug products: theory, therapy and testing

DNA and RNA, the building blocks of life, hold the key to our understanding of gene regulation and the development of gene editing tools and so there has been increased interest in their use in combating diseases, as exemplified during the COVID-19 pandemic – but what are some of the challenges in bringing these drugs to the clinic?

By John H Stockley at Eurofins BioPharma Product Testing

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) contain the genetic information coding for life. In the cell, RNA is transcribed from the sequence of DNA contained in genes to make messenger RNA (mRNA), which codes for the synthesis of proteins. Postulated by Francis Crick in 1957 during his historic lecture 'Protein Synthesis' at University College London, we now know this to be true and it is referred to as 'The Central Dogma'.[1]

DNA and RNA macromolecules consist of nucleotides connected by covalent phosphodiester bonds, forming polynucleotide chains. Within nucleotides, nitrogen-containing nucleobases – including adenine (A), guanine (G), cytosine (C), uracil (U) and thymine (T) – play crucial roles. The iconic Watson-Crick base pairing, with A-T and A-U linked by two hydrogen bonds and G-C held together by three hydrogen bonds, is central to their interaction. These ordered hydrogen bonds allow the double strand of DNA to be zipped and unzipped allowing genetic information to be preserved during cell division. Every nucleus in the 30 trillion-odd somatic cells in our body houses our DNA coding for over 23,000 genes, each one providing specific instructions for various proteins. Single-stranded mRNA is dynamically produced as transient templates from genes in our DNA. Initially, it takes the form of pre-mRNA, encompassing both protein-coding exons and non-coding introns. These non-coding introns are subsequently excised, ensuring that only mature mRNA, composed solely of exons, remains during the processing of pre-mRNA. A plethora of non-coding RNA (ncRNA) species exists in cells, like micro-RNA (miRNA), long non-coding RNA (lncRNA), ribosomal RNA (rRNA) and transfer RNA (tRNA), playing vital roles in regulating gene expression and protein synthesis.

The science of silence

Figuring out what a gene does is often accomplished by removing it, or by over-expressing it, in loss of function or gain-of-function experiments. As early as 1985, flipped gene expression generated an antisense RNA-impaired thymidine kinase synthesis, and in an attempt to make a deeper violet colour in petunias, over-expression of chalcone synthase (CHS) actually led to the flowers being unexpectedly white.[2,3] Both studies suggested a break from the 'Central Dogma', but it was work by Andrew Fire and Craig Mello that is credited with the discovery of RNA interference. They showed double-stranded RNA (dsRNA) activates RNA interference (RNAi) through the RNA-induced silencing complex (RISC) and a novel mechanism to supress gene expression was revealed.[4] This is consistent with the notion that dsRNA mimics viral infections inside cells, thereby activating a cellular response to shut down viral replication that may have escaped the extracellular viral surveillance systems. Using the correct sequence of nucleotides, different gene silencing strategies can be achieved using the RISC pathway, or through recruiting nucleases such as RNAse-H that recognise DNA/RNA duplexes or steric hindrance of DNA transcription or mRNA translation machinery for protein synthesis.[5,6] The faulty exons in pre-mRNA generated from disease-causing genes can be masked or skipped with pre-mRNA binding oligonucleotides to promote a healthier form of a protein.[5] The RNA-induced transcriptional activation (RITA) complex is a recent addition in our understanding of gene regulation.[7] Similar to RNAi but involving different protein partners, short RNA sequences can be used to increase gene expression and are referred to as RNA activation (RNAa).[7] This means that, by using the correct short sequences of oligonucleotides and delivering into cells, genes can be switched on or off. Such an elegant system is now being exploited as a therapeutic strategy to switch off disease-causing proteins or promote the expression of beneficial proteins.

Harnessing the whisper: mRNA vaccines

Our immune system, a marvel of complexity, continuously monitors our bodies for invaders. Antigen-presenting cells (APCs), like macrophages, patrol tissues and blood, and consume bacteria, viruses and even tumour cells. These APCs digest their prey, generating protein fragments called antigens, which they present on their surface using major histocompatibility complexes (MHCs). This presentation signals T-cells to activate, orchestrating B-cell selection and antibody production, forming our humoral immune response. Antibodies specifically target antigens, marking them for destruction by

our immune system. Researchers sought to trigger immune responses through protein fragments from target cells or viruses expressed in APCs, which could lead to a potential vaccine. Early attempts using mRNA coding for protein fragments sparked an inflammatory response releasing TNFα. However, Katalin Karikó and Drew Weissman's discovery that modified RNA nucleotides could circumvent this immune response paved the way for mRNA therapeutics.[8] The promise of mRNA therapeutics was further validated during the COVID-19 pandemic by Moderna and Pfizer/BioNTech. mRNA-based drugs are revolutionising disease treatment, offering the unique advantage of rapid design and manufacturing for personalised medicine. For example, BioNTech's Individualised NeoAntigen-Specific Therapy (iNest) rapidly designs personalised mRNA vaccines for cancer patients. This remarkable achievement demonstrates the potential of mRNA therapeutics in reshaping medicine.

"Elementary, my dear Watson"

Next-generation genome sequencing (NGS) has transformed personalised disease investigation, particularly for rare and congenital disorders. Genome mutations have been linked to the elusive origins of these conditions, establishing NGS as a new tool for medical testing and diagnosis. A degree of gene correction can be accomplished using tools like zinc finger nucleases and TALENS but the simplicity of CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats), is changing biology. CRISPR works with a guide RNA (gRNA) complementary to the target DNA directing the enzyme Cas9 to precisely nick DNA, allowing mutations to be eliminated or edited to a correct state.

Despite concerns about off-target effects, permanent genetic modifications, delivery challenges and the introduction of bacterial-derived proteins, CRISPR therapeutics are advancing in clinical trials for diseases such as hereditary transthyretin amyloidosis by Intellia-Regeneron NTLA-2001.[9] RNA editing, often overshadowed by DNA editing, holds huge potential. RNA editing is anticipated to be safer than CRISPR as the gRNA is all that is necessary, as the editing machinery is already present in cells and is reversible.[10] It is estimated that precise A to G


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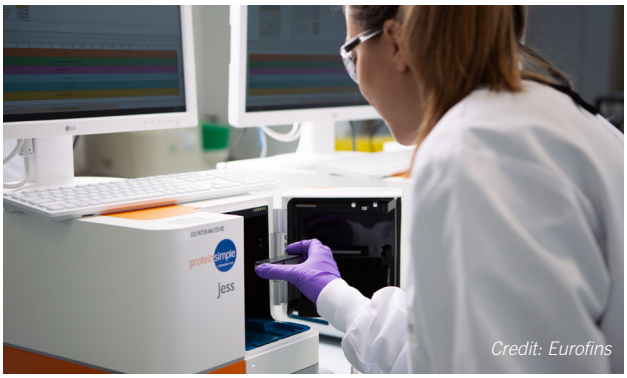

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editing could fix nearly half of all genetic diseases and ADAR (adenosine deaminase acting on RNA)-based editing gRNA are progressing to clinical trials, such as Wave Life Sciences-GSK WVE-006 for alpha-1 antitrypsin deficiency (AATD). Both DNA and RNA editing systems allow unparalleled interventions for diseases previously deemed untreatable.

Compliance compass

New DNA/RNA-based therapies must address several vital concerns. Foremost, they must ensure safety by avoiding immune system activation, notably through Toll-Like Receptors (TLRs) that recognise DNA and dsRNA. This can often be mitigated by using specialised delivery vectors or incorporating modified nucleotides. Given the genomic differences between humans and animals, off-target hybridisation might go unnoticed, even when evaluating these therapies across species. Addressing this concern necessitates comprehensive in silico analysis and in vitro testing across various human cell lines. Additionally, there's a need to prevent the breakdown and potential incorporation of modified nucleotides into the genome, with a focus on targeting only somatic cells. The principles outlined in ICH S6 for the safety evaluation of biotech-derived pharmaceuticals should guide non-clinical safety testing in this context.

Defining DNA And RNA therapeutics and quality attributes

The choice of compendia for nucleic acid therapies is influenced by regulatory requirements and the geographic region where the products will be developed or marketed. There are currently no specific guidelines that address quality expectations and standards for oligonucleotide products but mRNA-based vaccine guidelines from the USP and PhEur are expected soon, and industry-driven recommendations exist for mRNA and oligonucleotides.[11,12]

From a European legal perspective, mRNA drugs are classified as Biological Medicinal Products (BMPs), falling under the subcategory of Advanced Therapy Medicinal Products (ATMPs) due to their nucleic acid content (DNA or RNA) being derived at some point recombinantly (from genetically modified bacteria). This classification predates the widespread use of oligonucleotide-based drugs. According to European Medicines Agency (EMA) 2001 Annex 1, Part IV, mRNA drugs

are intended to 'regulate, repair, replace, add, or delete genetic sequences'.[13] In contrast, non-coding nucleic acids, like antisense oligonucleotides are chemically synthesised and are not regarded as ATMPs, despite having similar intended uses as mRNA drugs. As active pharmaceutical ingredients (APIs), these non-coding nucleic acids adhere to the same chemistry, manufacturing and control (CMC) standards as other APIs, in accordance with ICH Q11. Critical attributes include impurity control, quality risk management, analytical methods and stability testing. ICH Q7 ensures compliance with good manufacturing practice for APIs. For mRNA vaccines classified as biologically derived, ICH Q5 and ICH Q6 apply, as they fall under the categories of biotechnological and biological products, along with ICH Q7 and ICH Q11.

However, chemical synthesis of oligonucleotides has limitations. A 20-base oligonucleotide can generate over half a million stereoisomers, and stereospecific synthesis has reported improvements in pharmacokinetics and pharmacodynamics (PKPD).[14] Notably, manufacturing 1kg of a 20-base oligonucleotide involves an average process mass index of 3980kg, which equates to nearly four metric tons of raw materials for every kilogram of synthesised drug.[15] In contrast, cells excel at efficient and error-free DNA and RNA synthesis, making enzymatic synthesis a greener alternative. Enzymatic methods can include modified nucleotides, offering enhanced resistance to degradation, target binding and increased patentability in the US. Enzymatic synthesis of oligonucleotides classifies them as biologics, similar to mRNA-based vaccines, and hence necessitates similar qualitative attributes, especially concerning safety profiles. This includes sterility and endotoxin levels, given that most enzymes originate from bacterial recombinant sources.

Delivery of DNA and RNA drugs

Effective delivery is paramount in DNA/RNA-based drug products. Lipid nanoparticles enhance delivery and in vivo stability for mRNA and non-coding nucleotides, while ligand-directed methods, like trimeric GalNAc, facilitate liver uptake. Conjugation to antibodies will aid oligonucleotide delivery but introduces additional quality requirements, akin to antibody-drug conjugates (as per ICH S9 and M12). In CRISPR-based therapies, combining lipid nanoparticles with mRNA and gRNA necessitates adherence to multiple ICH guidelines, as it involves APIs and biotechnological substances. Other nucleotide-based therapies – such as aptamers, which bind to epitopes like antibodies, and ribozymes, which are RNA-folded structures with nuclease activities – share similar CMC and safety attributes. However, their applications are still being developed, so it is difficult to assign specific quality attributes. Nevertheless, it is likely that more DNA and RNA-based tools will emerge as this exciting field evolves.

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Dr John Stockley, **Eurofins**' 2022 recruit, serves as principal scientist, specialising in oligonucleotide testing. With 13 years' experience in Cambridge, UK, collaborating with distinguished neuroscientists at the Cambridge Stem Cell Institute, MedImmune, and a university spin-out, he led studies spanning small and large molecules, as well as CGTs. John holds degrees in Biochemistry from University College Cork, Ireland, brings a diverse skill set encompassing biochemistry, chemistry, neurobiology, spatial-transcriptomics, stem cell biology and assay development to advance oligonucleotide analytical testing. His current focus lies in assessing the impact of oligonucleotide structure in method development by HPLC and HRMS.