RNA-based Drugs: Significant Developments and Challenges

In the last decade, the tremendous achievements in RNA technology, immunology (vaccines), and nanotechnology have paved the way to RNA-based drug discovery and development. The specific advantage of RNA-based drugs is that they delete or modify the information read from genes (DNA) at the RNA level *before* it is converted into proteins. RNA drugs do not change the genetic material itself and are therefore no gene therapies, but are causally effective therapies in hereditary diseases, as they prevent the expression of a genetic defect. RNA therapeutics have the advantage that they can also be directed against proteins that cannot be reached by classical small molecule drugs or biologics because they are localised *intracellularly* or do not offer specific targets.

However, the challenges for clinical development are considerable and compliance with Good Manufacturing Practice (GMP) is a prerequisite for first use in humans. The **GMP-area** of the Charité Research Organization enables us to serve also these exciting new therapeutic approaches of RNA technologies. Distinct from traditional small molecule drugs and biologics such as monoclonal antibodies, RNA-based products have their own unique challenges. For example, some mRNA vaccines and therapies utilize lipid formulations to deliver the mRNA into the cell. RNA manufacturing requires sophisticated technologies from transcription, purification, formulation to filling and storage. RNA-drugs are usually rapidly degraded and have to be stabilised with modifications to reach the cell nucleus and persist there long enough. The efficiency of RNA delivery into the cytoplasm through overcoming the extracellular and intracellular barriers remains critical for successful RNA therapy. Therefore, various chemical modifications and the engineering of delivery formulations have been explored to solve challenges related to pharmacodynamics and pharmacokinetics. Many of the hurdles have been meanwhile surmounted. Several RNA drugs are approved and many more are in clinical development to treat rare and common diseases. In this respect, this could mark the beginning of a new era in drug therapy.

Due to its research orientation and expertise, **Charité Research Organisation GmbH** is able to conduct and support innovative research projects with new RNA-based substances in the early phase of clinical development.

In this white paper, we provide an overview of the RNA-based drugs currently already available on the German market, their mechanisms of action, the challenges of RNA administration and current possible solutions.

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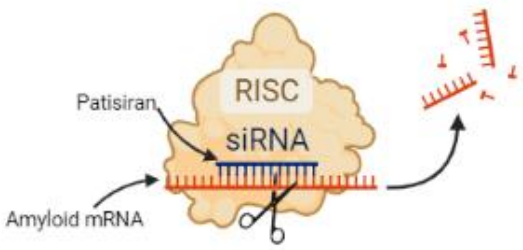
Key discoveries and developments in RNA-based therapies:

In 1953, James Watson and Francis Crick explained that base paring of nitrogenous bases in nucleotides stabilizes the double standard helices of DNA. The canonic model of base pairing was born. It took as many as 25 years to use the hybridisation of sense and antisense RNA for inhibition of a certain gene product and to the first proposal of antisense oligonucleotides (ASO) for inhibition of protein synthesis. It was not until 1998 that the first ASO based drug (Fomivirsen) targeting Cytomegalovirus (CMV) messenger RNA (mRNA) was approved for the treatment of CMV retinitis in HIV patients. In 2017, the splice-modulating ASO drug Nusinersen was introduced for spinal muscular atrophy. At the same time, RNA aptamers emerged which can function as agonists, antagonists or delivery agents and also block protein synthesis. In 2004, the VEGF-antagonist aptamer Pegaptanib was approved as therapy for macular degeneration [1].



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In the following decade, the spectrum of options for therapeutic targeting of RNA has been broadened with the introduction of RNA interference (RNAi) technology and the use of small interfering RNA (siRNA) for silencing of human genes. In 2018, the first approved siRNA drug (Patisiran, Onpattro®) targeting transthyretin (amyloid) mRNA was a breakthrough for the treatment of familial amyloid neuropathies.

Since 1993, microRNAs (miRNAs), tiny small non-coding single-strand RNAs that are conserved among all species, were discovered as essential molecules for post-transcriptional regulation of gene expression at mRNA level [2, 3]. The most advanced miRNA therapeutic is currently Miravirsen that showed phase 2 positive results for the treatment of hepatitis C virus (HCV) infection [4]. Taken together, RNA-based products such as ASOs, aptamers, siRNAs, and miRNAs can directly target mRNAs and noncoding RNAs (ncRNAs) through Watson–Crick base-pairing. Therefore, RNA-based drugs are versatile tools for modification of any interest gene by selecting the correct nucleotide sequence on the target RNA.

In 1990, a new class of drugs emerged with the in vitro transcribed (IVT) messenger RNA (mRNA) which can enter the cytoplasm and induce protein replacement or immunization, without causing irreversible genome changes [5]. Since then, mRNA vaccines for various infectious diseases, including influenza, Zika, and respiratory syncytial virus, have been developed [6]. During the ongoing COVID-19 pandemic, mRNA-based vaccines (Comirnaty® and Spikevax®) have proven clinically effective against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and are meanwhile worldwide approved and produced in large scales to meet the urgent demand. Further mRNA-based drugs are currently developed to combat cancer, infectious diseases and other diseases with high unmet medical need [7].

Since 2013, mRNA technology can also be used to deliver artificial programmable endonucleases for targeted gene editing to treat specific disorders. For these purposes, the clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein (CRISPR/Cas) nuclease system becomes more frequently used for gene editing [8].

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Characteristics and clinical applications of the main classes of RNA-therapeutics:

At current, there are only a few RNA therapeutics on the German market (see **Table 1**), but several new RNA molecules reached or completed already late phase clinical development (see GMP-area). In the following, the characteristics and clinical applications of the main classes of the current RNA-therapeutics and their mechanisms of action are described in more detail.

(1) **Anti-Sense-Oligonucleotides (ASOs)** are small (~15–25 nucleotides) *single-stranded* DNA-RNA gapmers (RNase H dependent ASOs) or pure RNAs (RNase H independent/steric block ASOs) that are designed to bind complementary to a target mRNA thereby inducing post-transcriptional gene silencing [Figure 1].

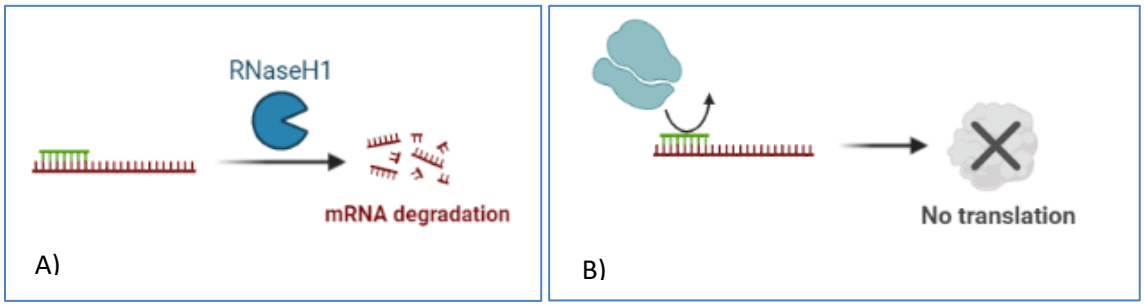

Figure 1: ASOs. (A) RNase H dependent ASOs and (B) RNase H independent/steric block ASOs.
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RNase H is an endogenous enzyme that recognizes DNA–RNA heteroduplexes bound to their complementary target mRNA strand and catalyses the degradation of the mRNA (mRNA downregulation). This approach can be used for the treatment of diseases caused by overexpression of certain genes. **Volanesorsen** (Waylivra®) has been approved by EMA (2019) for its clinical efficacy to reduce apolipoprotein C-III in patients with type I hyperlipoproteinemia.

In contrast, RNase H independent/steric block ASOs control the down-/up-regulation of target transcripts only by directly binding to them without the help of specific enzymes. The mostly used approaches are alternative splicing, promotion of mRNA translation, or alternative polyadenylation. **Nusinersen** (Spinraza®), an ASO that uses the alternative splicing approach, was approved by the EMA (2017) for the treatment of spinal muscular atrophy which is caused by deletions or mutations in the survival motor neuron 1 (SMN1) gene. These mutations lead to inadequate SMN protein expression, causing weakness and atrophy of skeletal and respiratory muscles [9, 10]. Children with a severe form of this disease usually die before the age of two. If the drug is administered early, such children not only survive, they can also develop largely normally. Nusinersen modulates splicing of SMN2, which varies only from SMN1 in that it undergoes alternative splicing and excludes exon 7 [11]. This exclusion results in a truncated protein that only has 5% to 10% functionality. However, Nusinersen regulates alternative splicing such that exon 7 is included, resulting in fully functional SMN leading to improved motor function in patients with SMA. Several ASOs are already used in the clinics (**Table 1**).

Further steric block ASOs such as Casimersen, Golodirsen and Vitolarsen, which are using alternative splicing to cause exon skipping to block translation of their target mRNA, are currently in late phase development for the treatment of Duchenne Muscular Dystrophy (GMP-area).

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(2) **Small interfering RNA (siRNA)** primarily causes translational repression of their target protein. They are similar to ASOs in size; however, they are *double-stranded* and more effective as ASOs in downregulating their target mRNA [12]. In RNA interference, gene expression is inhibited by destroying the targeted mRNA [Figure 2]. The siRNAs are either chemically synthesised and transfected into the cells or generated in cells themselves after transfection with siRNA-vectors. In the cells, siRNAs bind to enzyme complex RISC (RNA-induced silencing complex), where it is unwound: the RISC protein argonaute 2 (AGO2) carries out cleavage of the sense strand [13] allowing the antisense strand to bind its target mRNA. Once the target RNA is bound to the antisense strand, its phosphodiester backbone is cleaved by AGO2. This leads to sequence-specific knockdown of the target mRNA and therefore causes gene silencing.

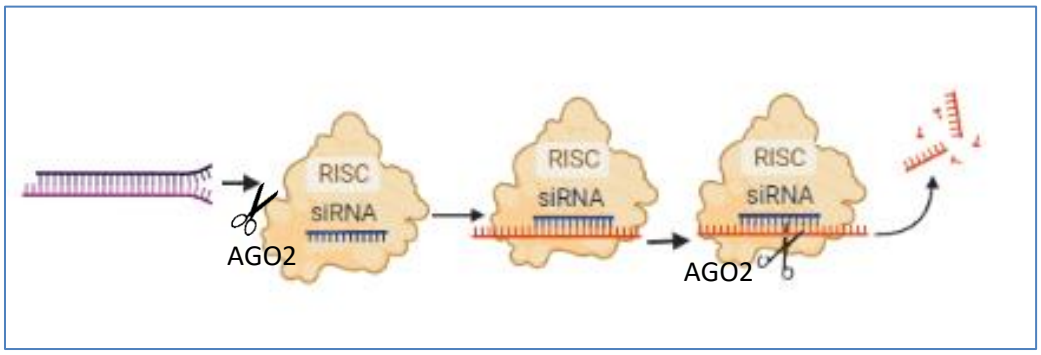

Figure 2: siRNA. siRNA associated with RISC (RNA-induced silencing complex) binds to its target mRNA and induces gene silencing by preventing translation of the mRNA.
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Since 2018 a total of five siRNA-drugs reached already the market (**Table 1**): **Patisiran** (Onpattro®) and **Vutrisiran** (Amvuttra®) which both downregulate transthyretin mRNA are used for the treatment of familial amyloid neuropathies, **Givosiran** (Givlaari®) which downregulates the mRNA of delta-aminolevulinic acid synthase 1 (ALAS1) is used to treat life-threatening attacks of hepatic porphyria. **Lumasiran** (Oxlumo®) degrades the mRNA that encodes hydroxyacid oxidase 1 thereby depleting the substrate for oxalate synthesis leading to a significant reduction of oxalate concentrations in patients with primary hyperoxaluria (HP1). **Inclisiran** (Leqvio®) lowers LDL-C levels by targeting the mRNA encoding for proprotein convertase subtilsin/kexin type 9 (PCSK9), which is involved in the regulation of cholesterol levels, is used to prevent cardiovascular events in patients with hypercholesterolemia.

Several more siRNA-products are currently in late phase clinical development (GMP-area): Fitusiran which downregulates antithrombin mRNA is developed for the treatment of hemophilia A/B. Nedosiran downregulates hepatic lactate dehydrogenase mRNA is intended for the treatment of PH1. Teprasiran is the first systemically administered siRNA product which temporary blocks the expression of p53 mRNA to reduce acute kidney injury in patients undergoing cardiac surgery. And, Tivanisiran that is designed to silence Transient Receptor Potential Vanilloid 1 (TRPV1) is developed as eye drops for the treatment of dry eye disease and Sjogren' Syndrome.

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(3) **Aptamers** are short DNA or RNA nucleotides that form secondary and tertiary structures and interact with specific peptides, proteins and other molecules and can promote or inhibit many different molecular pathways [Figure 3].

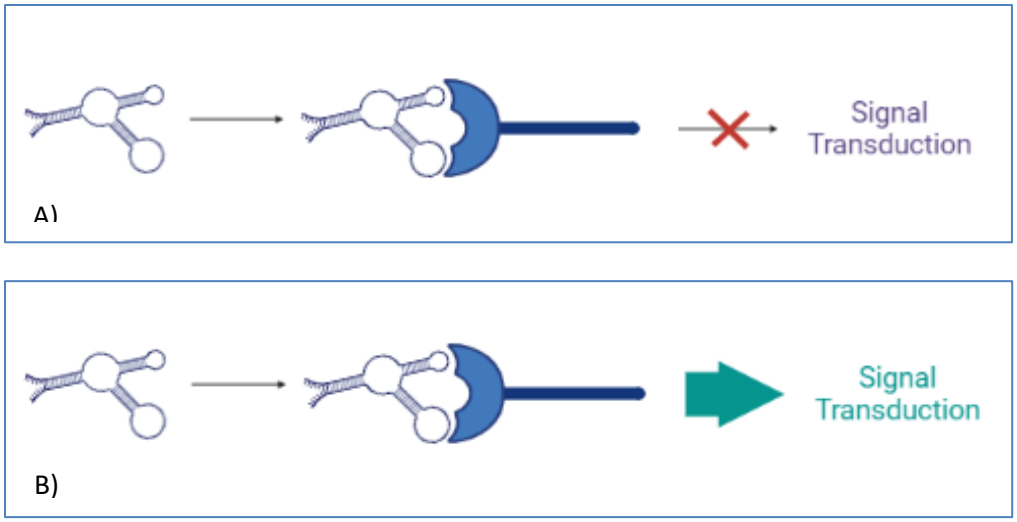

Figure 3: Aptamers. Aptamers are RNA, DNA, or RNA/DNA hybrids that form tertiary structures and bind to a target molecule, either (A) suppressing or (B) enhancing the signal transduction of the target molecule.
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Aptamers are generated using the systematic evolution of ligands by exponential enrichment (SELEX) method [14]. This large library allows fast high-throughput testing to obtain aptamers with high affinity and specificity for the target, which contributes to their favourable safety profile. Only two aptamers have reached clinical approval so far:

Pegaptanib (the first FDA-approved aptamer, 2004) has high affinity for the heparin binding domain of VEGF and prevents VEGF binding to its receptor [1]. Pegaptanib is used as solution for intravitreal injection to reduce vision loss in patients with neovascular age-related macular degeneration and currently tested as a new treatment for diabetic macular oedema (**Table 1**).

Defibrotide that is acting as an adenosine receptor agonist is used for the treatment of hepatic veno-occlusive disease/sinusoidal obstruction syndrome in patients with renal or pulmonary dysfunction following chemotherapy and a stem-cell transplant [15]. Defibrotide stabilizes endothelial cells and protects them from further damage in this severe condition (**Table 1**).

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(4) **Micro-RNAs (miRNAs)** are tiny small non-coding single stranded RNAs of *endogenous origin* with low potential for immunogenic reactions. Mature microRNAs (~22 nucleotides) are formed from precursor transcripts by the enzyme DICER (a type III ribonuclease). Normal function of miRNAs is to suppress translation of the target mRNA with an open reading frame (ORF), or cause mRNA degradation, by guiding RNA-induced silencing complex (RISC) to the 3' untranslated region (3'UTR) of the mRNA. There is increasing evidence that many diseases like cancer, diabetes mellitus, heart failure, infections, and immunologic disorders are associated with false expression of miRNAs.

Therapeutic miRNAs are used to either inhibit or restore protein synthesis (Figure 4): miRNA mimics can block protein synthesis through association with AGO proteins leading to activation of the enzyme complex RISC and subsequent imperfect binding to the mRNA target leading to gene silencing. In contrast, miRNA inhibitors block gene silencing activity of an endogenous miRNA by complimentary binding. Thus, miRNA-inhibitors restore protein synthesis by complementary binding to the target miRNA that represses the translation of a particular mRNA.

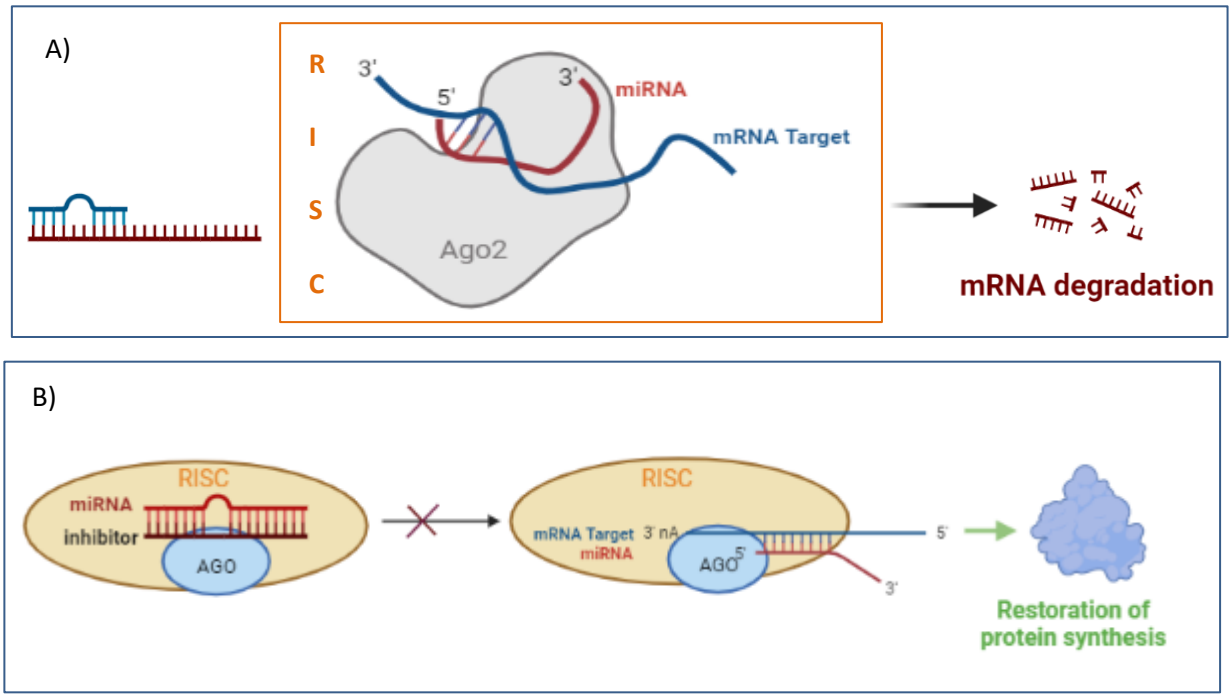

Figure 4: miRNA mimics and miRNA inhibitors. (A) miRNA mimics are small double-stranded RNA molecules that associate with and guide the RISC complex **to its target mRNA.** The mimic will bind with imperfect complementarity to its target mRNA, and translation will be blocked or the mRNA will be degraded leading to gene silencing. (B) miRNA inhibitors are antisense miRNA oligonucleotides (AMOs), including 2'-O-methyl modified AMO, antagomir, locked nucleic acid (LNA), phosphorodiamidate morpholino oligonucleotide (PMO) and peptide nucleic acid (PNA), that bind to and suppress the endogenous (mature) target miRNA. This results in restored mRNA translation and protein synthesis.
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Miravirsen is a short locked nucleic acid (LNA) that functions as miRNA-inhibitor of endogenous miR-122 that is critical for HCV replication. Miravirsen has shown a significant reduction of HCV viral load in HCV patients phase 2 trials (GMP-area) [4]. Several miRNA mimics and inhibitors are currently in phase 1 clinical trials or at preclinical stage for the treatment of diseases with high unmet medical need [16].

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(5) **mRNA-based drugs** are in vitro transcribed RNAs (IVT-RNAs) that serve to encode proteins (antigens). mRNAs are around 2 kb long and contain a 5' cap, 5' untranslated region (UTR), coding region, 3' UTR, and poly(A) tail (Figure 5). They are increasingly used for the treatment of diseases with a known genetic component [17]. Injected mRNA encoding immuno-stimulants such as cytokines can promote antigen-presenting cell (APC) activation and induce a T-cell-mediated response against tumor cells [18].

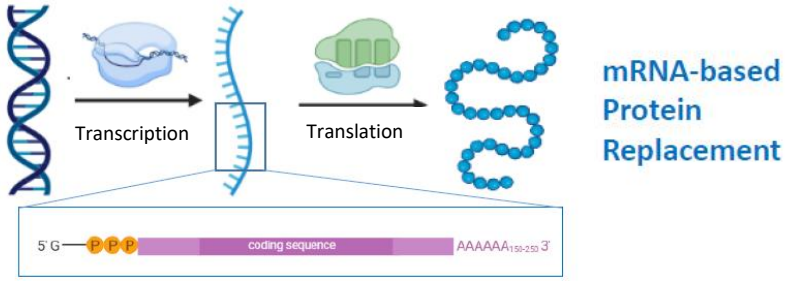

Figure 5: mRNA-based protein replacement. DNA in vitro transcription into target mRNA and ribosomal translation of mRNA into desired protein.
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In recent years, especially since the COVID-19 pandemic, mRNAs are also used as vaccines. Injected mRNA vaccines are delivered into the cytoplasm of APCs where they are translated into the targeted antigens. Subsequently, the major histocompatibility complexes present the expressed antigens to the surface of APCs to activate B cell/antibody-mediated humoral immunity and CD4+ T/CD8+ cytotoxic T-cell-mediated immunity [19]. Currently there are two approved mRNA COVID-19 vaccines on the German market: **BNT162b2** (Comirnaty®) developed by BioNTech and Pfizer and **mRNA-1273** (Spikevax®) developed by Moderna Biotech (Figure 6, **Table 1**).

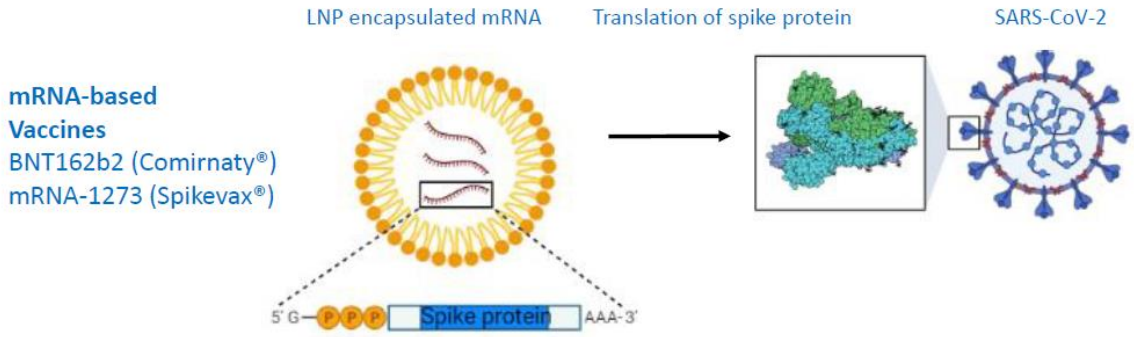

Figure 6: Example of mRNA-based vaccine technology.
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Both COVID-19 vaccines include 1-methylpseudouridine to impede innate immune sensing, while also increasing the translational ability of the mRNA [20]. Both mRNA vaccines are very safe because (unlike live attenuated vaccines) they cannot cause infections and cannot integrate into the human genome (human cells have no reverse transcriptase). In addition, unlike viral vector vaccines (e.g. adenovector vaccines), they do not contain any viral proteins, and therefore no antivector-immunity can develop. Several mRNA-based vaccines are currently in late phase development for other viral infections with high medical need (GMP-area).

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CRISPR/Cas-based genome therapy: The discovery of certain endonucleases as precision tools for DNA or RNA double strand breaking was fundamental for the development of gene editing. Besides the Zinc Finger Nucleases (ZFN) and Transcription–activator like Effector Nucleases (TALENs), the prokaryote-derived CRISPR-associated protein Cas nuclease have been widely used in mammalians to precisely edit genome sequence, resulting in irreversible "knockout" or "knockin" of a target gene [21]. The technique has already been successfully used in isolated human cells for correction of the mutated cystic fibrosis transmembrane conductance regulator (CFTR)-gen in stem cells form patients with cystic fibrosis [22] as well as in vivo in primates for PCSK9 base editing to lower cholesterol levels [23].

Challenges in RNA delivery: Distinct from traditional small molecule drugs and biologics such as monoclonal antibodies, RNA-based products have their own unique challenges for manufacturing and drug delivery. RNA manufacturing requires sophisticated technologies from transcription, purification, formulation to filling and storage. The RNA therapeutics offer promising opportunities of being more specific towards molecular structures than small molecules and combine it with the advantage of being more convenient than protein-based drugs. RNA drugs impress by the biochemical mechanisms of action used to manipulate genes and gene expression. However the transfer from the hypothetical cellular mechanism to the in vivo realization, both the transition first to animals and finally to humans, is demanding and subject to development work.

Naked RNA presents itself with prominent drawbacks [24]:

- Short half-life due to ribonuclease degradation and clearance by the reticuloendothelial system
- Negative charge and molecular weight reduce the cellular uptake efficiency
- Structural instability during the uptake process
- Immunogenicity
- Possibility of exercising off-target effects in normal cells with follow-up consequences
- Disease specific obstacles, endocytotic and endosomal escape mechanisms avoiding sufficient RNA concentrations at the target cellular structure

For the efficient and safe transport of RNA into cells, the material needs to be packaged in appropriate carriers so that the active substance is taken up by the target cells and exerts the respective effect. The function of these drug delivery vehicles depends as well on the RNA payload, the amount and the molecular size of the RNA moiety. Nevertheless most of the therapeutic oligonucleotides still need to be maintained at high concentrations over time to exert the effect on the specific target. Targeted carriers such as nanoparticles towards specific structures could increase the RNA concentration at the target.

Principle approaches for RNA drug delivery [24]: RNA delivery can be mediated by viral and non-viral vectors/carriers. These vectors have been developed to provide better protection against RNA degradation in the blood circulation and neutralize RNA-negative charge to allow an efficient endocytosis, enabled to allow active targeting and more accurate action on target genes. For the viral RNA delivery, adeno-associated viruses have been engineered to carry nucleic acid cargo, however organisms develop immunoreactions and neutralize the effects after a few repeated doses and ineffectiveness has to be anticipated. In the group of non-viral vectors the nanoparticles have been intensively studied and developed. Those structures encapsulate the RNA protecting it against

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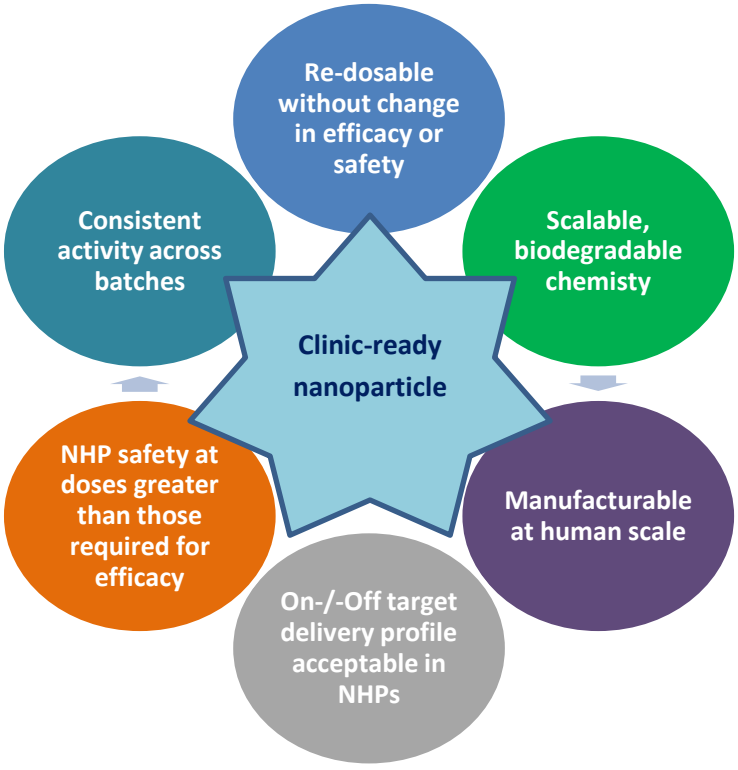
degradation and facilitate cellular uptake and reduce endosomal escape. Cationic lipids and lipid-like compounds electrostatically cover the nucleic acid for example in acidic environment only, influencing endosomal escape and reducing toxicity. Lipids are also capable of self-assembly into well-ordered nanoparticles, known as lipoplexes, driven by a combination of electrostatic and hydrophobic interactions. By addition of other hydrophobic structures, such as cholesterol or polyethylen-glycol (PEG)-lipids the liponanoparticles (LNPs) can enhance stability and enhance the delivery of RNA complexes. Developments of further structures such as cationic polymers, liposomes, exosomes, protein and peptide structures pursue the same goal and try to identify the appropriate delivery carrier for each RNA task.

Characteristics of a clinical relevant RNA-Delivery system: The requirements for a RNA delivery system adequate for the purpose and easily to be used in the clinics should be studied early in the development process; and should be compliant with current Good Manufacturing Practice (GMP). The following six characteristics should be considered (Figure 7) [25]: A chemistry that is scalable and biodegradable (e.g. adding ester bonds, which can degrade in water, to ionisable lipids improves LNP safety) is of high relevance to reduce the risks for the environment. The drug delivery system should be chemically simple enough to be manufactured at human scale. A lipid:RNA mass ratio of 20:1 is common and the lipid loss during the synthesis and formulation process must be taken into account.



GalNAc complexes can be conjugated to siRNAs or ASOs at human scale with large-batch manufacturing capability compliant with GMP. On-target and off- target delivery should be measured both as biodistribution (that is, where does the delivery system go?) and as function (that is, where does the payload affect cell function?). As most of RNA can be retained in endosomes, biodistribution is necessary, but may be not sufficient, for functional cytoplasmic RNA delivery. Of importance, the dose required for RNA efficacy must be substantially lower than the dose at which toxicity occurs in non-human primates (NHP).

Figure 7: Requirements for clinical relevant RNA drug delivery

The activity of the RNA drug should be consistent across many batches, even after shipping. Re-dosing of the RNA drug is often required in the clinics and should be possible without losing efficacy or safety. siRNA drugs such as Patisiran (Onpattro®) have been safely re-dosed in patients with familial amyloidosis when doses have been given 3 weeks apart. mRNA vaccines such as BNT162b2 (Comirnaty®) and mRNA-1273 (Spikevax®) have been safely dosed several times, with doses at least 3 weeks apart.

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The GMP-area of the Charité Research Organisation GmbH has qualified clean rooms and enables a subject-specific preparation of the investigational RNA-based drug product under the requested standard conditions to the ready-to-use form. Our GMP-facility is supervised by a Qualified Person (QP = responsible for compliance testing with the current GMP-rules), who decides the release of a clients' investigational RNA-based drug for application in early phase clinical trials. In addition, our QP has the up-to-date compliance expectations to share with our clients as needed.

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***Table 1*: Approved RNA therapeutics with marketing authorisation in Germany**

Product	Dosage form/Route of administration/target organ	Mechanism of Action	Indication	Marketing Authorisation Holder	Approval Status[1,2]
Antisense Oligonucleotides					
Nusinersen (Spinraza®)	12 mg solution for injection/ Intrathecal	Splicing modulation of SMN2 pre-mRNA	5q associated spinal muscular atrophy	Ionis Pharmaceuticals; Biogen Netherlands B.V	FDA (2016) EMA (2017)
Inotersen (Tegsedi®)	284 mg (as sodium) solution for injection /SC	Downregulation of transthyretin mRNA	Amyloid Neuropathies, Familial	Akcea Therapeutics Ireland Limited; Ionis Pharmaceuticals	EMA (2018) FDA (2018)
Volanesorsen (Waylivra®)	285 mg solution for injection/SC	Downregulation of apolipoprotein C-III	Hyperlipoproteinemia Type I	Akcea Therapeutics Ireland **LIMITED**	EMA (2019)
siRNA					
Patisiran (Onpattro®)	2 mg/mL concentrate for solution for infusion/IV	Downregulation of transthyretin mRNA	Amyloid Neuropathies, Familial	Alnylam Netherlands B.V.	EMA (2018) FDA (2018)
Givosiran (Givlaari®)	189 mg/mL solution for injection/SC	Downregulation of ALAS1	Porphyrias, Hepatic	Alnylam Netherlands B.V.	EMA (2020) FDA (2020)
Lumasiran (Oxlumo®)	94.5 mg/0.5 mL solution for injection/SC	Downregulation Hydroxyacid oxidase 1 mRNA	Hyperoxaluria, Primary	Alnylam Netherlands B.V.	EMA (2020) FDA (2020)
Inclisiran (Leqvio®)	284 mg (as sodium) solution for injection/SC	Downregulation of PCSK9	Hypercholesterolemia, Dyslipidemias	Novartis, Europharm Limited, Ireland	EMA (2020) FDA (2021)
Vutrisiran (Amvuttra®)	25 mg solution for injection/SC	Downregulation of transthyretin mRNA	Amyloid Neuropathies, Familial	Alnylam Netherlands B.V.	EMA (SEP2022)
Aptamer					
Pegaptanib (Macugen®)	0,3 mg solution for injection/Intravitreal	Blocking the heparin-binding domain of VEGF-165	Neovascular age-related macular degeneration	OSI Pharmaceuticals	FDA (2004) EMA (2006)
Defibrotide (Defitelio®)	80 mg/mL concentrate for solution for infusion/IV	Activation of Adenosine A1 / A2 receptor	Hepatic Veno-Occlusive Disease	Gentium S.r.l., Italy; Jazz Pharmaceuticals, Ireland	EMA (2013) FDA (2016)
mRNA					
BNT162b2 (Comirnaty®)	30 µg per dose/IM	Expression of SARS-CoV-2 S antigens	Covid-19	BioNTech Manufactoring GmbH, Germany; Pfizer, USA	EMA (2020) FDA (2020)
mRNA-1273 (Spikevax®)	50 µg or 100 µg per dose/IM	Expression of SARS-CoV-2 S antigens	Covid-19	Moderna Biotech Spain, S.L.	EMA (2021) FDA (2020)

References for Table 1 and Table 2:

(1) EMA/ EPAR Website accessed in December 2022: https://www.ema.europa.eu/en/medicines/field_ema_web_categories%253Aname_field/Human/ema_group_types/ema_medicine/field_ema_med_status/authorised-36?search_api_views_fulltext

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(3) Yiran Z, Liyuan Z, Xian W and Hongchuan J. RNA-based therapeutics: an overview and prospectus. Cell death and disease (2022) 13:644.

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Table 2: RNA drugs currently in late phase clinical development

Product	Route of administration/ target organ	Indication	Mechanism of Action	Sponsor	NCT and/or Eudra-CT
Antisense Oligonucleotides					
Casimersen (Amondys 45™) and Golodirsen (Vivondys 53™)	Intravenous infusion/Muscle	Duchenne Muscular Dystrophy (DMD)	DMD pre-mRNA splicing (skipping exons 45 and 53)	Sarepta Therapeutics	NCT02500381 – Phase 3
Vitolarsen (Viltepso®)	Intravenous infusion/Muscle	Duchenne Muscular Dystrophy (DMD)	DMD pre-mRNA splicing (exon 53 skipping)	NS Pharma	NCT04768062 – Phase 3 NCT04687020 – Phase 4 NCT04060199 – Phase 3
siRNA					
Fitusiran	Subcutaneous/ Blood	Hemophilia A / B	Antithrombin mRNA	Alnylam Pharmaceuticals and Sanofi Genzyme	NCT03974113 – Phase 3 ongoing NCT03417102 – Phase 3 completed
Nedosiran	Subcutaneous/ Liver	Hyperoxaluria, Primary	Hepatic lactate dehydrogenase mRNA	Dicerna Pharmaceuticals	NCT04042402 – Phase 3 ongoing
Teprasiran	Intravenous/ Kidney	Acute kidney injury in patients undergoing cardiac surgery	Temporary inhibition of p53 mRNA	Quark Pharmaceuticals	NCT02610296 – Phase 3 completed
Tivanisiran	Topical eye drop	Dry eye disease, Sjögren's Syndrome	Transient receptor potential cation channel subfamily V member 1 mRNA	Sylentis, S.A.	NCT03108664 – Phase 3 NCT04819269 – Phase 3 ongoing
Aptamer					
Pegaptanib (Macugen®)	Intravitreal injection	Diabetic Macular Edema	VEGF (165 isoform)	Pfizer	Phase 4 completed: NCT01486238, NCT01486238, NCT00406107, NCT00324116
miRNA					
Miravirsen (SPC3649)	Subcutaneous	Hepatitis C virus infection	Binding and inhibition of miR-122	Santaris Pharma	NCT01200420, NCT01727934, NCT01872936, Phase 2 positive results, upcoming phase 3 (?)
mRNA					
mRNA-1647 CMV Vaccine	Intramuscular	Cytomegalovirus Infection	mRNA-1647	ModernaTX, Inc.	NCT05085366 – Phase 3 ongoing
mRNA-1010 Influenza Vaccine	Intramuscular	Influenza Virus Infection	mRNA-1010	ModernaTX, Inc.	NCT05566639 – Phase 3 ongoing
mRNA-1345 RSV Vaccine	Intramuscular	Respiratory Syncytial Virus Infection	mRNA-1345	ModernaTX, Inc.	NCT05330975, NCT05127434 – Phase 3 ongoing

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