The notorious R.N.A. in the spotlight - drug or target for the treatment of disease

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ABSTRACT

mRNA is an attractive drug target for therapeutic interventions. In this review we highlight the current state, clinical trials, and developments in antisense therapy, including the classical approaches like RNaseH-dependent oligomers, splice-switching oligomers, aptamers, and therapeutic RNA interference. Furthermore, we provide an overview on emerging concepts for using RNA in therapeutic settings including protein replacement by in-vitro-transcribed mRNAs, mRNA as vaccines and anti-allergic drugs. Finally, we give a brief outlook on early-stage RNA repair approaches that apply endogenous or engineered proteins in combination with short RNAs or chemically stabilized oligomers for the re-programming of point mutations, RNA modifications, and frame shift mutations directly on the endogenous mRNA.

Introduction

During the last 15 y the diverse roles of RNA in regular but also pathological cellular processes became increasingly clear. RNA is not only a short-lived messenger and part of the translational machinery but RNA contributes significantly to the regulation and diversification of the genetic information. There is now increasing insight into the mechanistic role of defective RNA processing, including (alternative) splicing, modification, translation, and decay for the etiology of various diseases. However, not only mis-regulation and defective processing cause disease, but even RNA species themselves can initiate disease processes independent of their protein-coding function. Nucleotide repeat diseases are typical examples. To employ this new mechanistic knowledge and to translate it into therapy requires drugs that reliably target nucleic acids in a sequence-specific manner. However, there are only few small molecule drugs that target nucleic acids and those are limited in their capacity of sequence addressing. In contrast, oligonucleotide analogs provide a basis for the rational design of highly sequence-specific drugs to target virtually any cellular nucleic acid in a specific manner. Classical drugs like small molecules target enzymes and receptors to block or alter their specific functions. In contrast, the interference at the nucleic acid level would allow to manipulate the transcriptome and the proteome itself. This is not limited to the simple up- or down-regulation of target gene expression. Most appealing is the possibility of actively creating new transcript and protein isoforms with altered properties and functions, for instance by re-programming a protein-coding stretch, or by altering splice sites, modification patterns, polyadenylation states, miRNA binding sites, etc. Affecting the cell by targeting its nucleic acids clearly enlarges the scope of currently available therapeutic interventions including the causal treatment of some genetic diseases.

However, already short oligonucleotides have unfavorable pharmacological properties. They are hydrophilic, polyionic macromolecules that can hardly overcome cellular membranes, are unstable against RNases, and suffer from rapid renal clearance. This leads to short half-life and low bioavailability. Furthermore, adverse toxic effects may appear that include immune-reactions and off-target binding to non-targeted cellular nucleic acids. Together, oligonucleotide drugs are often characterized by low efficacy and high toxicity which strongly limits their clinical application. During the last decades, medicinal chemists have put enormous effort into the development of new chemistries that improve lifetime, delivery, and immunogenicity. These new chemistries are now approaching clinical trials and will hopefully pave the way for...
the broad clinical application of oligonucleotide drugs. An overview on recent developments in oligonucleotide medicinal chemistry can be found elsewhere.6,7

In principle, interference with the genetic information could be achieved permanently at the DNA- or transiently at the RNA-level. In this review we will focus on the RNA-level. Even though novel approaches for genome engineering are currently keenly explored,9 we believe that it would be foolish to carelessly discard the RNA alternative. With respect to ethical issues and safety aspects, the transient and thus reversible nature of RNA manipulation could turn out as a blessing in disguise. Both, the therapeutic effects and the potential adverse effects, are likely to be tunable. Furthermore, manipulations are conceivable that are inaccessible or difficult to realize on the genome level per se. This includes amino acid changes or transcript level changes that would kill a cell if they are permanently enforced. Potentially lethal interventions on kinases, apoptosis factors, transcription or translation factors could be realized on the RNA-level suddenly, transiently or partially to obtain a therapeutic effect, for instance. Manipulation at the RNA-level might also be much more efficient compared to HDR-dependent genomic knock-in, which remained persistently inefficient in vivo, in particular in postmitotic tissues like the brain.9 For many genetic diseases, which are caused by loss-of-function mutations, a patient would benefit more from a drug that can restore a small fraction (like 5%) of functional gene product in a large fraction of the tissue than from a drug that can restore full gene function (100%) but only in a small fraction of the tissue. A typical example is cystic fibrosis.10

In this review we will first update on recent developments in the classical approaches, like RNaseH-dependent decay, chemically stabilized oligonucleotides that target m RNAs to induce splice-switching, aptamers, and the knock-down via RNAi (Fig. 1). After painful years of repeated relapse one seems to

Figure 1. Chemically stabilized, short oligonucleotides can employ various mechanisms for their therapeutic effects ranging from blocking ligand – receptor binding, RNA degradation via RISC or RNaseH(1) recruitment, and alteration of splicing. The classical modes of action are shown on the left panel, a small section of typically used chemical backbone modifications are depicted on the right.
have learned the lessons and have now substantially improved the effectiveness of such drugs. For instance, in 2015 therapeutic RNAi was demonstrated in a relevant monkey model by subcutaneous administration of a chemically stabilized siRNA that partially knocks down antithrombin in the monkey’s liver.\(^{11}\) The problem of delivery and toxicity seems to be solved, at least for simple oligonucleotide drugs and for some organs, and allows therapeutic intervention with an affordable amount of the drug under compliant administration routes. Consequently, the number of promising clinical phase II and III studies has increased during the last few years (see Table 1).

Every new discovery in RNA function and regulation offers a starting point to develop novel therapies. After its discovery in 1998 we now find numerous drug candidates in clinical studies that apply the RNAi mechanism (Table 1).\(^{12}\) In the second part of this review we highlight emerging concepts that are still in the pre-clinical or very early clinical exploration stage but that have the potential to become medicines of the future. This includes therapeutic mRNAs, mRNAs as vaccine, and RNA repair approaches. The latter apply endogenous or engineered enzymes to repair, re-program, or modify a target RNA at a specific site in order to provoke a therapeutically relevant effect (Fig. 2).

**Update on established approaches**

**RNaseH-dependent antisense oligonucleotides**

Oligonucleotides working through an RNaseH-dependent cleavage mechanism are the oldest class of antisense oligonucleotides (ASO). They are extensively explored and represent the largest class of nucleic acid analog drugs in clinical trials. RNaseH-dependent ASOs are short DNA oligomers targeting mRNA. Once the DNA-oligo/mRNA heteroduplex is formed, human RNaseH1 binds to it and catalyzes RNA cleavage under release of the intact DNA oligomer.\(^{13}\)

Medicinal chemists have undertaken great efforts to improve ASO design regarding nuclease resistance, circulation half-life, target affinity (potency), and tissue specificity. The first ASOs tested in clinical trials, also referred to as 1\(^{st}\) generation ASOs, have been modified by oxygen-to-sulfur substitutions in the phosphate backbone. ASOs with such a phosphothioate (PS) backbone show enhanced nuclease resistance and prolonged plasma half-life due to non-specific binding to plasma proteins preventing them from rapid renal filtration. However, numerous toxicities were also associated with that type of modification.\(^{6}\) In 1998, fomivirsen was the first FDA-approved ASO and was applied for the treatment of human cytomegalovirus-induced retinitis in HIV patients.\(^{14-16}\) Marketed as Vitravene, the 21 nt PS-oligonucleotide was administered by intravitreal injection to target the immediate early region 2 of the viral mRNA. Since the approval of fomivirsen, several ASOs belonging to the 1\(^{st}\) generation are under clinical review. For instance, targeting the mRNA of intercellular adhesion molecule 1 and the insulin receptor substrate 1 are advanced in the treatment of pockutis\(^{7,18}\) and vascular disorders in the eye,\(^{19-22}\) respectively. The RNaseH-mediated degradation of Akt-1 mRNA to impede tumor proliferation\(^{23}\) is currently tested for clinical application.\(^{24-26}\)

Due to the early success with 1\(^{st}\) generation ASO, further medicinal chemistry was explored to improve half-life and potency of the drugs in order to reduce the administered dose, the application frequency, the costs, and to minimize adverse effects.\(^{27}\) This resulted in the 2\(^{nd}\) generation ASOs, also referred to as gapmers. A typical gapmer is a 20 nt oligonucleotide comprising a PS backbone and 5 flanking 2’O-methoxyethyl (MOE) groups at both termini. Due to the unmodified internal DNA gap, such ASOs remain good substrates for RNaseH, whereas the terminal MOE modifications increase nuclease resistance and enhances the binding of the ASO to the target mRNA.\(^{28}\) 2\(^{nd}\) generation ASOs entered clinical trials for various therapeutic applications. The most prominent representative of the 2\(^{nd}\) generation is the MOE gapmer mipomersen as the second FDA-approved RNaseH-dependent ASO. The compound targets apolipoprotein B-100 mRNA and is subcutaneously administered to treat familiar hypercholesterolemia. The genetic disorder is caused by the loss of low-density lipoprotein (LDL) receptor function leading to high LDL cholesterol plasma concentration and early cardiovascular disease. Phase III trials had demonstrated an efficient decrease of LDL cholesterol by lowering ApoB-100 amount in patients obtaining mipomersen.\(^{29-31}\) The treatment obviously profited from the general pharmacokinetics of systemically administered ASOs which preferably accumulate in the liver where ApoB-100 synthesis takes place.\(^{8}\) Recently, an RNase-dependent ASO\(^{32}\) has reached clinical phase III to reduce transthyretin expression in patients suffering from familial amyloid polyneuropathy.\(^{33-35}\) Chemotherapy combined with RNaseH-mediated degradation of clusterin mRNA is a potential therapeutic option in the treatment of prostate\(^{36-38}\) and lung cancer.\(^{39,40}\)

Generation 2.5 ASO are derived from the traditional gapmer design. For this, the MOE modifications are replaced by 2’,4’-constrained ethyl (cEt) bridges in the flanking nucleotides. It was found that cEt-modified oligonucleotides provide the same superior target affinity, but increased nuclease resistance as compared to locked nucleic acid (LNA)-containing oligonucleotides.\(^{41}\) One of the generation 2.5 ASOs targets the mRNA of signal transducer and activator of transcription 3\(^{42}\) and is currently tested for the treatment of various cancer types.\(^{43-46}\)

Most recently, a new chemistry has been developed that strongly increases the liver-specific uptake of oligonucleotide drugs, including ASO and siRNA therapeutics. For this, ASO\(^{47}\) and siRNAs\(^{48}\) are conjugated with triantennary N-acetyl galactosamine (GalNAc\(_3\)). GalNAc\(_3\) mediates liver-specific uptake through the asialoglycoprotein receptor (ASGPR) that is exclusively expressed on hepatocytes. Marketed as ligand-conjugated antisense (LICA) technology (Ionis Pharmaceuticals), it could be shown that the conjugation increases the potency of MOE gapmers up to 10-fold for inhibiting the expression of hepatic genes in mice.\(^{49}\) When using a GalNAc\(_3\)-conjugated cEt gapmer, the RNaseH-mediated mRNA degradation was enhanced around 60-fold as compared to the corresponding 2\(^{nd}\) generation MOE ASO. Additionally, Ionis Pharmaceuticals announced that its LICA drug targeting apolipoprotein(a) was 30-fold more potent in a phase I study than the unconjugated MOE gapmer.\(^{50,51}\)
Table 1. Overview on the most recent and advanced clinical trials in the corresponding fields not claiming completeness. For RNAi, failed early trials are also listed that have been terminated in 2009 or before.

| Compound | Application Route | Target | Indication | Company / Initiator | Phase / Status | Trial ID / Reference |
|----------|------------------|--------|------------|---------------------|----------------|---------------------|
| Fomivirsen (Vitravene®, ISIS 2922) | IVT | IE2 | CMV retinitis | Ionis Pharmaceuticals / Ciba Vision | FDA Approved | 14-16 |
| Mipomersen (Kynamro®, ISIS 301012) | SC | ApoB-100 | HoFH | Ionis Pharmaceuticals / Genzyme | FDA Approved | 29-31 |
| Custirsen (OGX-011) | IV | Clusterin | Prostate cancer | Ionis Pharmaceuticals / OncoGenex Technologies | III - Completed | NCT01188187 |
| Custirsen (OGX-011) | IV | Clusterin | Prostate cancer | Ionis Pharmaceuticals / OncoGenex Technologies | III - Active | NCT01578655 |
| Custirsen (OGX-011) | IV | Clusterin | Lung cancer | Ionis Pharmaceuticals / OncoGenex Technologies | III - Recruiting | NCT01630733 |
| Aganirsen (GS-101) | Eye drops | IRS-1 | Corneal neovascularization in graft patients | Gene Signal | III - Completed | EudraCT 2008-005388-33 |
| Aganirsen (GS-101) | Eye drops | IRS-1 | Ischemic central retinal vein occlusion | Gene Signal | II/II - Recruiting | EudraCT 2014-000239-19 |
| Alicaforsen (ISIS 2302) | Enema | ICAM-1 | Pouchitis | Ionis Pharmaceuticals / Atlantic Healthcare | III - Recruiting | NCT02525523 |
| IONIS-TTRRx (ISIS 420915) | SC | TTR | FAP, TRR Amyloidosis | Ionis Pharmaceuticals / GlaxoSmithKline | II - Recruiting | NCT01737398 |
| IONIS-TTRRx (ISIS 420915) | SC | TTR | FAP, TRR Amyloidosis | Ionis Pharmaceuticals / GlaxoSmithKline | III - Enrolling by invitation | NCT02175004 |
| RX-0210 (Archexin®) | IV | Akt-1 | Pancreatic cancer | Rexahn Pharmaceuticals | II - Completed | NCT0128495 |
| RX-0210 (Archexin®) | IV | Akt-1 | Renal cell cancer | Rexahn Pharmaceuticals | IB/II - Recruiting | NCT02089334 |
| IONIS-STAT3-2.5Rx (AZD9150, ISIS 481464) | IV | STAT3 | Gastrointestinal or ovarian cancer | Ionis Pharmaceuticals / AstraZeneca | II - Recruiting | NCT0217753 |
| IONIS-STAT3-2.5Rx (AZD9150, ISIS 481464) | IV | STAT3 | Metastatic Squamous Cell Carcinoma of the Head and Neck | Ionis Pharmaceuticals / AstraZeneca | I/II - Recruting | NCT01899328 |
| IONIS-STAT3-2.5Rx (AZD9150, ISIS 481464) | IV | STAT3 | Lymphoma | Ionis Pharmaceuticals / AstraZeneca | I/II - Active | NCT01563302 |
| IONIS-APO(a)-LRx (ISIS 681257) | SC | ApoA | Elevated Lipoprotein(a) | Ionis Pharmaceutical / Akcea Therapeutics | I - Completed | NCT02415494 |

Table 1: Clinical Trials using RNA-Therapeutics
RNaseH-dependent Antisense Oligonucleotides

| Compound | Application Route | Target | Indication | Company / Initiator | Phase / Status | Trial ID / Reference |
|----------|------------------|--------|------------|---------------------|----------------|---------------------|
| PRO001/GSK2402968 (drisapersen/ Kyndrisa®) | SC | Dystrophin exon 51 (skipping) | DMD | BioMarin Pharmaceuticals (developed by Prosensa Therapeutics/GlaxoSmithKline) | III - Recruiting | NCT01803412 |
| PRO001/GSK2402968 (drisapersen/ Kyndrisa®) | SC | Dystrophin exon 51 (skipping) | DMD | BioMarin Pharmaceuticals (developed by Prosensa Therapeutics/GlaxoSmithKline) | IIIB - Enrolling by invitation | NCT02636686 |
| PRO004/BMN 044 | SC/IV | Dystrophin exon 44 (skipping) | DMD | BioMarin Pharmaceuticals (developed by Prosensa Therapeutics/GlaxoSmithKline) | II - Recruiting | NCT02329769 |
| PRO005/BMN 045 | SC | Dystrophin exon 45 (skipping) | DMD | BioMarin Pharmaceuticals (developed by Prosensa Therapeutics) | IIB - Active | NCT01826474 |
| PRO005/BMN 053 | SC/IV | Dystrophin exon 53 (skipping) | DMD | BioMarin Pharmaceuticals (developed by Prosensa Therapeutics) | I/II - Active | NCT01957059 |

Splice-switching Oligonucleotides

| Compound | Application Route | Target | Indication | Company / Initiator | Phase / Status | Trial ID / Reference |
|----------|------------------|--------|------------|---------------------|----------------|---------------------|
| PRO001/GSK2402968 (drisapersen/ Kyndrisa®) | SC | Dystrophin exon 51 (skipping) | DMD | BioMarin Pharmaceuticals (developed by Prosensa Therapeutics/GlaxoSmithKline) | III - Recruiting | NCT01803412 |
| PRO001/GSK2402968 (drisapersen/ Kyndrisa®) | SC | Dystrophin exon 51 (skipping) | DMD | BioMarin Pharmaceuticals (developed by Prosensa Therapeutics/GlaxoSmithKline) | IIIB - Enrolling by invitation | NCT02636686 |
| PRO004/BMN 044 | SC/IV | Dystrophin exon 44 (skipping) | DMD | BioMarin Pharmaceuticals (developed by Prosensa Therapeutics/GlaxoSmithKline) | II - Recruiting | NCT02329769 |
| PRO005/BMN 045 | SC | Dystrophin exon 45 (skipping) | DMD | BioMarin Pharmaceuticals (developed by Prosensa Therapeutics) | IIB - Active | NCT01826474 |
| PRO005/BMN 053 | SC/IV | Dystrophin exon 53 (skipping) | DMD | BioMarin Pharmaceuticals (developed by Prosensa Therapeutics) | I/II - Active | NCT01957059 |
| Compound | Application Route | Target | Indication | Company / Initiator | Phase / Status | Trial ID / Reference |
|----------|-------------------|--------|------------|---------------------|----------------|---------------------|
| Pegaptanib sodium (Macugen<sup>®</sup>) | IVT | VEGF | AMD | Macular Degeneration | OPKO Health | III - Terminated | NCT00499590<sup>01</sup> |
| RB006 (pegivacogin) as component of Regi1 | IV | Coagulation factor IXa | CAD | REGADO Biocience | III - Terminated | NCT01848106<sup>28</sup> |
| E10030 (Fosfiva<sup>®</sup>) | IVT | PDGF | AMD | Hypercholesterolemia | Allergan | II - Terminated | NCT00295067<sup>04</sup> |
| NOX-A12 (olaptesed pegol) | IV | CXCL12/SDF-1 | MM | Allograft Rejection | Noxxon Pharma | IIA - Completed | NCT01521533<sup>88</sup> |
| NOX-A12 (olaptesed pegol) | IV | CXCL12/SDF-1 | MM | Allograft Rejection | Noxxon Pharma | IIA - Completed | NCT01521533<sup>88</sup> |
| NOX-E36 (emapticap pegol) | IV | CCL2/MCP-1 | DM2, Albuminuria | Noxxon Pharma | II - Terminated | NCT0154789<sup>86</sup> |
| NOX-H94 (lexaptepid pegol) | IV | Hepcidin | Anemia | Noxxon Pharma | IIA - Completed | NCT01691040<sup>82</sup> |

**RNA Interference**

| Compound | Application Route | Target | Indication | Company / Initiator | Phase / Status | Trial ID / Reference |
|----------|-------------------|--------|------------|---------------------|----------------|---------------------|
| Bevasiranib | IV | VEGF | Macular Degeneration | OPKO Health | III - Terminated | NCT00499590<sup>01</sup> |
| AGN-211746 | IV | VEGFR-1 | CNV, AMD | Allergan | II - Terminated | NCT00925057<sup>04</sup> |
| ISNP | IV | p53 | AKI | Quark Pharmaceuticals | I - Terminated | NCT00683553<sup>00</sup> |
| PF-04523655 | IVT | DDT4 | Diabetic Retinopathy, Diabetes Complications | Quark Pharmaceuticals | II - Terminated | NCT00701181<sup>02</sup> |
| PRO-040201 | IV | ApoB | Hypercholesterolemia | Arbutus Biopharma | I - Terminated | NCT00927459<sup>03</sup> |
| ND-L02-2020I | IV | HSP47 | Hepatic Fibrosis | Nitto Denko | I - Ongoing | NCT02227459<sup>14</sup> |
| ALN-AS1 | SC | ALAS1 | AIP | Alnylam Pharmaceuticals | IIA - Terminated | NCT02452372<sup>16</sup> |
| pbi-shRNA STN11 LP | IT | STN1 | Advanced / Metastatic Cancer, Solid Tumors | Gradalis | I - Ongoing | NCT01505153<sup>19</sup> |
| Bamatinib | Eye drops | ADRB2 | OAG, Ocular Hypertension | Sylenis | II - Completed | NCT02250612<sup>21</sup> |
| ARB-001-467 | IV | Three sites of the HBV genome | Chronic Hepatitis B | Arbutus Biopharma | II - Recruiting | NCT02631906<sup>16</sup> |
| ALN-TTR-ss/ revusiran | SC | TTR | FAC | Alnylam Pharmaceuticals | III - Recruiting | NCT02319005<sup>17</sup> |
| ALN-AT3 | SC | AT | Hemophilia | Alnylam Pharmaceuticals | I - Recruiting | NCT0035605<sup>018</sup> |
| sRNA-transfected PBMCs (APN401) | IV | Cbl-b | Several types of cancer | Comprehensive Cancer Center of Wake Forest University | I - Recruiting | NCT02166255<sup>20</sup> |
| QPI-1007 | IV | CASP2 | NAION | Quark Pharmaceuticals | II/III - Recruiting | NCT02341560<sup>22</sup> |
| MRX34 | IV | miRNA34a | Several types of cancer | Mina Therapeutics | I - Recruiting | NCT01829971<sup>28</sup> |
| Tarxirins | IV | miRNA16 | Malignant Pleural Mesothelioma NSCLC | University of Sydney | I - Recruiting | NCT02369198<sup>27</sup> |
| AZD4076 | SC | miRNA103/107 | NASH | AstraZeneca | I - Recruiting | NCT02612662<sup>24</sup> |
| Miravirsen | SC | miRNA122 | Hepatitis C | Santaris Pharma A/S | II - Ongoing | NCT02031133<sup>25</sup> |

(continued)
| Compound          | Application Route | Target                  | mRNA Therapy | Company / Initiator | Phase / Status | Trial ID / Reference |
|-------------------|-------------------|-------------------------|--------------|---------------------|----------------|---------------------|
| CV9104            | ID                | —                       | Prostate Cancer | CureVac             | II - Ongoing   | NCT01817738        |
| CV9202            | ID                | —                       | Stage IV NSCLC | CureVac             | I - Ongoing    | NCT01915524        |
| CV7201            | IM                | Rabies                  | CureVac      | CureVac             | I - Ongoing    | NCT02340449        |
| CV9104            | ID                | —                       | Prostate Carcinoma | CureVac         | II - Ongoing   | NCT02140138        |
| IVAC_W_bre1_uID   | Not available     | —                       | TNBC         | BioNTech            | I - Not yet Recruiting | NCT02316457     |
| IVAC_MUTANOME RB001/RB002 | IN         | —                       | Melanoma     | BioNTech            | I - Ongoing    | NCT02035956        |
| Lipo-MERIT        | IV                | —                       | Melanoma     | BioNTech            | I - Recruiting | NCT02410733        |
| AGS-003           | ID                | —                       | mRCC         | Argos Therapeutics  | III - Ongoing  | NCT01582672        |
| Dendritic cell vaccine (plus temozolomide chemotherapy) | IV | — | MPM | University Hospital, Antwerp | II - Recruiting | NCT02649829 |
| Dendritic cell vaccine (plus chemotherapy) | IV | — | MPM | University Hospital, Antwerp | II - Recruiting | NCT02649829 |
| pp65 Dendritic cell vaccine | SC | — | GBM | University of Florida | II - Not yet Recruiting | NCT02465268 |
| ZFN Modified CD4+ T Cells | Infusion | CCR5 | HIV | University of Pennsylvania | I - Recruiting | NCT02388594 |

Abbreviations of targets, indications and administration routes: ADRB2: Adrenergic receptor β2, AIP: Acute intermittent porphyria, AKI: Acute kidney injury, ALAS: ALA-synthase, AMD: Age-related macular degeneration, ApoA/B: Apolipoprotein A/B, ApoB-100: Apolipoprotein B-100, AT: Antithrombin, CAD: Coronary artery disease, CASP2: Caspase 2, Cbl-b: Casitas B-lineage lymphoma proto-oncogene B, CCL2/MCP-1: CC-chemokine ligand 2/monocyte chemotactic protein 1, CMV: Cytomegalovirus, CNV: Choroid neovascularization, CCL: Chronic lymphocytic leukemia, CXCL-12: C-X-C motif chemokine ligand 12, DDIT4: DNA damage-inducible transcript 4, DMD: Duchenne muscular dystrophy, FAP: Familial amyloid polyneuropathy, FACS: Familial amyloidotic cardiomyopathy, GBM: Glioblastoma multiforme, HSP47: Heat shock protein 47, HoFH: Homozygous familial hypercholesterolemia, HIV: Human immunodeficiency virus, ICAM-1: Intercellular adhesion molecule 1, IE2: Immediate early 2 protein, IL: intra-dermal, IN: intra-nodal, IRS-1: Insulin receptor substrate 1, IT: intra-tumoral, IV: intra-venous, IVT: intra-vitreal, MPM: Malignant pleural mesothelioma, mRCC: Metastatic renal cell carcinoma, MM: Multiple myeloma, Akt-1: Murine thymoma viral oncogene homolog 1, NASH: Non-alcoholic steatohepatitis, NAION: Nonarteritic anterior ischemic optic neuropathy, NSCLC: Non-small cell lung cancer, OAG: Open angle glaucoma, PDGF: Platelet-derived growth factor, SDF-1: Stromal cell-derived factor 1, STAT3: Signal transducer and activator of transcription 3, SMA: Spinal muscular atrophy, STKN1: Stathmin-1, SC: Sub-Cutaneous, TTR: Transthyretin, DM2: Type 2 diabetes mellitus, VEGF: Vascular endothelial growth factor, VEGFR-1: Vascular endothelial growth factor receptor 1.
**Splice-switching oligonucleotides**

Pre-mRNA is matured during a complex nuclear process called splicing that removes the introns (non-coding sequences) and joins the exons (coding sequences). By applying alternative splice sites and by occasional inclusion or exclusion of exons and introns, multiple protein variants are derived from one gene (alternative splicing). Several diseases are related to aberrant RNA-splicing leading to non-functional proteins, and great efforts have been undertaken to develop antisense oligonucleotides, referred to as splice-switching oligonucleotides (SSOs) that manipulate splicing. Therapeutic SSOs promoting exon skipping and exon retention for the treatment for Duchenne muscular dystrophy (DMD) and spinal muscular atrophy (SMA) are currently evaluated in clinical trials.7

Dystrophin, the protein encoded by the DMD gene, is crucial for the integrity of muscle tissue.52 In rare cases, newborn males harbor a defect dystrophin gene on their X chromosome. The patients suffer from successive muscle wasting resulting in a premature death due to respiratory or cardiac failure. In most cases, the loss-of-protein-function results from exonic out-of-frame deletions. In many cases the reading frame can be restored by skipping the aberrant exon by addressing a SSO to an internal exonic splicing enhancer.53 The resulting truncated dystrophin protein retains partial function and gives the less severe Becker muscular dystrophy phenotype.54 Several SSOs have been developed that are clinically evaluated for the skipping of exons 44, 45, 51, and 53, including drisapersen and eteplirsen (Table 1). Recently, both companies submitted new drug applications for their lead compounds drisapersen55-57 and eteplirsen,58,59 both amenable to exon 51 skipping. In case of drisapersen, the FDA rejected the application due to major concerns about the efficacy and safety of the drug.60 The high dosage required led to severe adverse effects including renal and vascular injury. To improve efficacy and safety other SSO chemistries might be more successful. Whereas drisapersen is a 20 nt 2’-O-methoxy phosphorothioate RNA analog, eteplirsen is a 30 nt phosphorodiamidate oligomer, a so-called morpholin. The final decision on the efficacy and safety evaluation by the FDA is still pending for eteplirsen. Additionally, a new, morpholino-based SSO for exon 53 skipping is currently under clinical evaluation (NS-065/NCNP-01).61,62 For the future, we can hope in new chemistries. A SSO that relies on 2’O,4’C-ethylene-bridged nucleosides (ENA oligonucleotides)63 which mediate nuclease resistance and improved binding affinity to RNA has now entered a clinical phase I/II trial for the treatment of DMD (DS-4151b).64,65

Spinal muscular atrophy (SMA) is a rare genetic disorder caused by survival of motor neuron 1 (SMN1) gene mutations.66 Infant patients affected by this disease suffer from the loss of motor neurons and associated muscle wasting. However, there is a therapeutic approach by activating the SMN2 gene, which is almost identical to SMN1, but a single mutation in a splicing enhancer strongly prevents the inclusion of exon 7 resulting in an unstable protein unable to replace the lost SMN1 function.67 In a mouse model, a highly potent 2’O-methoxethyl PS SSO for exon 7 retention in SMN2 was identified (IONIS-SMNRx).68 The drug is injected in the spinal cord ensuring the direct delivery to the affected motor neurons without the need to cross the blood-brain barrier. After promising clinical phase II results regarding efficacy and safety of the drug candidate,69 two phase III trials were recently initiated for evaluating IONIS-SMNRx.70,71

Although the SSO design remains challenging, several new therapeutic applications were successfully validated in preclinical studies.72 Possible drug approvals of eteplirsen or IONIS-SMNRx in the near future could eventually proof the feasibility of the splice-modulating antisense oligonucleotide approach.

**Aptamers**

Aptamers are 20 – 100 nt long oligomers that adopt complex three dimensional structures that allow them to interact potently and specifically with various proteins typically achieving nM- to pM binding affinities.73 They are readily obtained in an iterative laboratory evolution procedure called SELEX (systematic evolution of ligands by exponential enrichment).74 Currently, aptamers are mainly targeting extracellular structures such as plasma proteins and cell surface receptors thus avoiding the problem of intracellular delivery. Hence, aptamers are comparable in many aspects to antibodies, however, aptamers are much smaller, can penetrate tissues deeper, are chemically synthesized to highest purity and homogeneity and differ in their toxicity and immunogenicity profile. To improve their plasma life-time and to adjust their toxicity, aptamers are typically chemically stabilized (2’-OMe, 2’-F, 3’ inverted dT) and PEGylated.

In 2004, the first (and until today the only) aptamer, Macugen, was approved by the FDA for clinical therapy of AMD (age-related macular degeneration). The 27-nt chemically stabilized RNA oligomer is directed against the vascular endothelial growth factor (isoform 165) and blocks VEGF-receptor-induced neovascularization.75,76 After achieving its highest sales in 2010, it has now almost entirely been displaced by antibodies (Ranibizumab and Bevacizumab, for instance) which can bind additional VEGF isoforms besides VEGF-165 and thus benefit for their poorer specificity compared to the aptamer. After this early breakthrough with Macugen, numerous aptamers have been explored in clinical settings. However, some programs suffered very unfortunate setbacks at late clinical trial states, like the aptamer-containing anticoagulation system REG1 which was terminated in 2014 in a phase III study due to unexpected toxicity / immunogenicity issues (Table 1).77,78

Currently, several aptamers for the local treatment of eye diseases are in late clinical trials (II and III), for instance the aptamers Fovista79-81 and Zimura,82,83 which target PDFG (it is a growth factor) and C5, respectively. In combination with VEGF inhibitors they might find application in the treatment of AMD in the near future. To overcome the prevalent problems with toxicity and immunogenicity, NOXXON Pharma develops so-called Spiegelmer therapeutics.84 These drugs apply stereochemically inverted nucleotides based on L-ribose instead of the natural D-ribose, can be evolved via SELEX, and are suggested to be resistant against nucleases85 and invisible for the immune system.86 Currently, 3 Spiegelmer aptamers86-92 are in clinical phase II studies (Table 1).

**Therapeutic RNAi**

RNA interference (RNAi) is a mechanism of posttranscriptional gene regulation that was discovered in 1998.12 RNAi can
interfere with gene expression in various ways including the degradation of a specific mRNA target via endonucleolytic cleavage, or via recruitment of deadenylation / decapping enzymes, but it can also positively affect the stability and translation of a specific mRNA. The mechanistic details that lead to the respective responses are still under exploration. In principle, a dsRNA that is introduced into the cytoplasm is processed by the RNase dicer into ~22 bp RNA duplexes and loaded onto the endonuclease Argonaut-2 (Ago-2). Ago-2 slices the passenger strand of the RNA-duplex and applies the remaining guide strand for sequence-specific mRNA-targeting.93 While short interfering RNAs (siRNAs) are fully complementary to their target mRNA and promote cleavage (knock-down), micro RNAs (miRNAs) contain bulges and loops that prohibit slicing by Ago-2, but alter the stability and translational activity of the target.94

Allowing the selective knock-down of genes in cell culture and animal-models, RNAi quickly became a valuable tool in basic biology.95-97 In parallel a race started to exploit the RNAi mechanism for therapeutic purposes and several big pharma companies, like Merck, Roche, and Pfizer made large investments that resulted in the first clinical trials in 2004, already 6 y after the discovery of RNAi.98,99 However, in the aftermath those early trials mostly failed due to strong innate immune reactions and/or lack of patients' benefit, and in the consequence big pharma left RNAi again.100-104 In the 18 y since its discovery the field of therapeutic RNAi went from enthusiastic interest over despondence and back again, resulting in a reassessment of the technological obstacles and more realistic expectations for clinical trials. This has been accompanied by commentary elsewhere.105,106

However, after recent successes in clinical trials, showing the efficacy of RNAi therapeutics to reduce transthyretin107 and PCSK9108 in patients, the interest in RNAi is currently growing and immune-related toxicity.109 Off-target effects include immune-reactions induced by the siRNA/miRNA precursors, and up- and downregulation of non-target mRNAs due to saturation of the RNAi machinery and off-target binding of the siRNA.110 There is now increasing success in tackling all those issues. Current innovations include chemical modification / sequence optimization of siRNAs and its precursors, and new solutions to the delivery problem. The latter include various forms of (lipid) nanoparticles and bioconjugates. The details of this progress are comprehensively reviewed elsewhere.110-113 Briefly, clinical trials seem more successful when they are confined to readily accessible organs like the liver, cancer, and immune-privileged areas like the eye.114-122 Whereas the eye is a good target for naked siRNAs, treatment of the liver benefited from lipid-based nanoparticles and the above-mentioned GalNAc3 conjugates.16 In particular the GalNAc3 approach has significantly improved the efficacy of siRNA-conjugates, allowing now the weekly administration of liver-targeting siRNA via subcutaneous injection in non-human primates to knock-down antithrombin to clinically relevant levels.11 Notable in this approach is that it allows to knockdown an essential protein (like antithrombin) in a tunable and reversible manner, whereas the permanent knock-out of antithrombin (for instance at the DNA-level) is lethal.11 Overall, more than 20 siRNA drugs in various formulations are in clinical trials now (up to phase III, Table 1).123 RNAi-therapy clearly has the potential to tackle currently undruggable diseases and to appear in the clinics soon.

The therapeutic use of the miRNA-related mechanism (not applying the slicing activity of Ago2) is still in its infancy. Attractive is the possibility of manipulating larger networks of genes simultaneously in both, a negative and positive manner.124 This might become interesting for the treatment of complex diseases like cancer. On the other hand, endogenous miRNAs are involved in many cellular processes and their manipulation could also be disease-relevant. The knockdown of miRNA 122 with antisense oligonucleotides was shown to interfere with hepatitis C virus progression and is currently in phase II clinical studies.125 As the hepatitis virus seems to require the endogenous miRNA for its functioning the knockdown of this host-specific factor is particularly promising as the virus cannot adapt easily by evolution.126 Other miRNAs that are linked to cancer like miRNA 16 and 34a are also targeted with ASOs and are currently in clinical trials phase I.127,128

**Emerging concepts for therapy**

**Therapeutic mRNA**

For a long time it has been believed that only short, chemically stabilized oligonucleotides are suitable as drugs. However, long (protein-encoding) mRNAs haven recently proven their enormous therapeutic potential. Protein replacement experiments were first performed in the early 1990ties with naked mRNA in mice and rats.129,130 Even though replacement experiments were successful to some degree, there have been massive problems related to the well-known RNA-dependent immune-stimulation through interferon-I (IFN-I) and a generally low translation efficiency.131,132

However, during the last 15 years, our mechanistic understanding of the immune-stimulatory effect of RNA has substantially improved. This was due to the discovery of RNA sensors including the Toll-like receptors (TLR) 3, 7, 8, Melanoma differentiation-associated protein 5 (MDA-5), Retinoic acid inducible gene 1 (RIG-I), as well as various RNA helicases.133 Besides the activation of the innate immune response under release of the respective signaling molecules we have also learned how these RNA-sensing events are directly linked to the general repression of mRNA translation in the affected cells. Among others, general translation repression is mediated by phosphorylation of translation initiation factor 2α via protein kinase R activation.134,135 In the worst case, IFN-I activates 2′-5′-adenylate synthase and RNaseL and leads to apoptosis.136 RNA replacement strategies aim to achieve high translation levels under minimal immune stimulation. Both can be achieved by designing mRNAs that evade RNA-sensing. The following strategies turned out as particularly successful.

a) Chemically modified pyrimide nucleotides like pseudouridine (ψ), 2-thiouridine (s2U), and 5-methylcytidine (m5C) are incorporated into mRNAs during in-vitro transcription to minimize recognition by RNA
sensors. Substitution of uridine by pseudouridine was shown to diminish recognition by TLR-3, -7, -8, and RIG-I. To fine-tune effects on translation efficiency, nucleotide analogs are often mixed with their natural counterparts. The extent to which these modifications may induce mistranslation is yet unknown.

b) Rigorous purification of the mRNA product from unincorporated nucleoside triphosphates, small abortive transcripts, remaining DNA templates, and in particular dsRNA via HPLC (High performance liquid chromatography) was shown to dramatically reduce immunogenicity of the transcripts and can increase the translation 10- to 1000-fold.

c) Synthetic cap analog structures like ARCA (anti-reverse-cap-analog) can further decrease immune response and improve translation. In contrast to older cap analogs, ARCA is always incorporated in correct orientation. A new ARCA variant contains a phosphothioate that resists enzymatic decapping and can increase the half-life of the mRNA.

d) Computational sequence design allows to reduce the number of particularly immune-stimulatory nucleotides and combinations (like UW, with W = A or U). Furthermore, transcript stability can be optimized by the introduction of 3’-UTRs (or some elements) taken from other mammalian or viral genes as well as addition of Poly(A)-tails.

The RNA replacement strategy is particularly advantageous when a transient, burst-like expression of a protein is desired. Typical examples for the latter are the epigenetic reprogramming (induced pluripotency), wound healing, and genome editing. In this sense, in-vitro transcribed mRNA (IVT-mRNAs) has been used to deliver a) human bone morphogenetic protein 2 (hBMP-2) to support bone regeneration in rats; to deliver b) the transcription factor mix that induces pluripotency; and to deliver c) vascular endothelial growth factor-A (VEGF-A) into a mouse model for myocardial infarction resulting in an improved heart function and enhanced survival. Furthermore, IVT-mRNAs have been successful in the delivery of surfactant protein B in deficient mice, and in the delivery of murine erythropoietin to increase the hematocrit.

IVT-mRNA could turn out as a valuable tool for genome editing. Genome editing holds great promise for the treatment of various diseases by a permanent repair of a gene via a site-directed knock-in or knockout. However, the respective nucleases that induce the required double-strand DNA breaks including ZFNs, Talens, and CRISPR/Cas, should not be persistently expressed as this would dramatically increase the chance of off-target genome editing. Consequently, its delivery as an mRNA is beneficial compared to a DNA vector and also circumvents the typical safety risks of viral and non-viral DNA-based methods like genomic insertion and antivector immunogenicity. Encoding of genome editing tools via IVT-mRNAs has already been widely used to generate transgenic animals. In a proof-of-concept study, gene function was restored via homology-directed promoter exchange in a surfactant-B-deficient mouse model by in-vivo-delivery of the ZFN in form of an IVT-mRNA. However, this required the additional delivery of the repair template (with the promotor) in form of an AAV6 (Adeno-associated-virus serotype 6).

Successful promoter exchange was demonstrated and resulted in a prolonged life of the treated mice. IVT-mRNA encoded Talen has been used successfully to disrupt the CCR5 (CC chemokine receptor type 5) gene via non-homologous-end-joining in the T-cell line PM1. As the loss of CCR5 function confers resistance toward R5-tropic HIV-1 infection, side-directed nucleases are promising to target this infectious disease. An initial clinical phase I study is currently starting. As IVT-mRNA is a young field, this study represents the first clinical study that uses IVT-mRNAs, but more are likely to follow soon.

mRNA can have many advantages over DNA vectors to deliver therapeutically proteins. Besides its transient nature, we want note that mRNA is very well and quickly translated in postmitotic cells that are difficult to transfected with DNA vectors. mRNA also works independent of a promoter, but this can potentially limit its application if tissue-specificity is required. However, we know from various studies that there is a large number of regulatory elements, typically in the 3’-UTR, including mRNA binding sites, stabilizing and destabilizing elements that could allow to manipulate the expression of an IVT-mRNA in a tissue-specific manner in the future.

Oligonucleotides for vaccination and desensitization

As indicated above, very successful strategies haven’t been developed to evade the RNA-sensing event and to trick the innate immune system. However, inducing a specific immune response can be highly desired. Thus the recent knowledge on the immune stimulation by RNA can be used for the latter. Currently, the classical vaccination is based on the delivery of inactivated or living viruses, virus-like particles, or antigenic peptides. While the antigenic peptides require additional vaccination adjuvants like alum salts, the other entities contain sufficient pathogen-associated-molecular-patterns (PAMPs) in form of proteins, nucleic-acids, and lipopolysaccharides. These PAMPs are detected by pattern-recognition-receptors (including the above-mentioned RNA sensors) and induce the release of type-I interferons, pro-inflammatory cytokines, and chemokines. This is reviewed in-depth elsewhere. Short peptide fragments are then presented to the immune system via MHC-complexes on dendritic cells and other antigen presenting cells. This process finally induces a humoral as well as cellular immune response of the adaptive immune system.

The presented antigens are mainly protein-derived peptides. This opens the intriguing possibility to deliver antigens for MHC-presentation encoded as IVT-mRNAs under simultaneous induction of the necessary innate and adaptive immune stimulation as the IVT-mRNA itself can function as PAMP. By doing so, it is well conceivable to create specific immune responses not only against viruses and bacteria, but also against cancer cells or for allergy treatment. The design of such mRNA-based vaccines would be highly rational, fast, cheap, and could be done in a personalized manner, for instance against the specific transcriptome of a patient-specific cancer. IVT-mRNA vaccines would be faster available as the generation of virus-particles (and similar entities) would be circumvented. Lyophilized mRNA vaccines can be stored at 37 °C for several weeks. This allows the transport of vaccines into
regions that cannot provide an uninterrupted cold chain. The safety-profile could also be better compared to DNA-based methods (insertion mutagenesis, low efficiency) or virus-like entities (therapy-induced virus-specific humoral immune response).184-186 Again, also for vaccination, the transient nature of RNA expression is beneficial, as a low-level, long-term expression of an antigen might induce tolerance.187

Two major IVT-mRNA-based vaccination strategies are currently explored: the ex-vivo and the in-vivo approach. The first, which was earlier developed, is based on the ex-vivo pulsing of allogenic (= patient-derived) dendritic cells with antigen-encoding mRNA, which allows the redirection of the adaptive immune system to target cancer or virus-infected cells. The feasibility and safety of this method was proven in pre- and clinical trials focused on HIV and various cancer types. However, personalized ex-vivo therapies require time-consuming and expensive individualized manufacturing processes which currently limit their broad clinical application.188-199 Nevertheless, further clinical trials up to phase III are currently running.196-202

Even though cumbersome, the ex-vivo strategy allows to optimize and control mRNA transfection and immune stimulation more carefully. The in-vivo approach, however, is potentially more simple and elegant, but encounters additional problems. Whereas all IVT-mRNA strategies require stable and highly translatable transcripts, the in-vivo strategy requires additionally the immune-stimulatory effect that counteracts translation. It was found that complexation of IVT-mRNA with protamine enhances immunogenicity via TLR-7 activation and simultaneously improves stability, however, with the downside of low antigen expression.203 Anyway, a combination of protamine-complexed IVT-mRNA together with naked IVT-mRNA of the same sequence turned out to satisfy both needs at the same time: high translation efficiency and immune stimulation. Those self-adjuvanting mRNAs are currently in phase I and II clinical trials against prostate cancer, late stage lung cancer, and rabies; pre-clinical trials against influenza have been performed.183,186,204-209 We wish to mention that also other approaches that apply naked or formulated IVT-mRNAs are in clinical trials, for instance for targeting other cancer entities.172,210 Furthermore, non-coding RNA can also be used as a vaccination adjuvant replacing the classical alum salts as adjuvant of protein- or peptide-based vaccines.211

Currently, IVT-mRNA are expensive therapies. On one hand, the GMP (Good manufacturing practice) production of IVT-mRNA in large scale is not yet fully established, but CureVac has announced significant progress here.211 On the other hand the potency of IVT-mRNA could be further improved by assisted delivery via lipid-nanoparticles, polymeric nanoparticles, gold nanoparticles, among others, as reviewed elsewhere.212 Furthermore, there are promising attempts to develop self-replicating RNA-vaccines that apply viral RNA-dependent RNA-polymerases (from α-virus) to produce the RNA vaccine from a dilute IVT-mRNA template.214-216 However, there are safety concerns related to the control of the replication process and the tolerance against the viral RNA-polymerase, but the strategy is still in the pre-clinical exploration phase.217

Finally, mRNA vaccines could also be used in allergy treatment to desensitize the immune system against a specific antigen. Desensitization against type-I allergies is typically accomplished through repeated intra-dermal, intra-nodal, or sub-lingual application of allergens. Whereas a strong Immunglobuline E and CD8+ T-cell responses is intended during vaccination, desensitization aims to change the T_{H1}/T_{R1} to T_{H2}2 cell ratio toward T_{H1}/T_{R1} to fine-tune the immune response and to induce tolerance.179 Application of low-dose IVT-mRNA could be used for that purpose, and there is pre-clinical data that prove efficacy and suggest a long-term protective effect.218 One can expect first clinical trials to start within the next few years. Applying mRNA as an anti-allergic vaccine has several advantages compared to the classical allergen extract (like standardized cat extract) or DNA-based vaccines.219,220 IVT-mRNA is

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**Figure 2.** Overview on selected enzymatic processes that could be harnessed to restore gene function by repairing or re-programming mRNA site-specifically. Site-directed A-to-I editing, 2′-O-methylation, pseudouridylation, and frameshift correction via expression or administration of short guideRNAs has already been demonstrated. Many other processes are conceivable and currently under exploration.
obtained in a defined and highly pure state thus avoiding unintended antigens that can be included in allergen extracts.\textsuperscript{211,222} DNA-based allergy treatment on the other hand suffers from the above mentioned safety concerns and thus harbors disproportional risk in the context of a preventative therapy.

RNA repair

Besides the manipulation of splicing, most interventions on the RNA-level aim to destroy or block their endogenous targets. Strategies to restore the function of an RNA that is corrupted by missense, nonsense or frameshift mutation, or by defective processing are rare. In case of loss-of-function mutations, the administration of a therapeutic mRNA to replace the non-functional variant might solve the problem, as discussed above. However, this is only feasible with a small number of therapeutically important mRNAs that can be translated under low control of translation level and tissues specificity. Indeed, many transcripts are tightly regulated with respect to their dose and tissue specificity and come as a mixture of various isoforms due to alternative promotor usage, alternative splicing, alternative polyadenylation, and alternative posttranscriptional modification. Such transcript variants may differ in their function, localization, stability, etc. To address this variety in an mRNA replacement strategy seems impractical. A better alternative would be the repair of the endogenously expressed but defective RNA transcript, a strategy, we call RNA repair.

Very recently, we and others have engineered artificial RNA-guided editing machineries that allow to re-program genetic information at the RNA level.\textsuperscript{223–225} For this, adenosine-to-inosine (A-to-I) RNA editing enzymes\textsuperscript{226,227} are directed toward specific sites on selected transcripts and allow for the precise posttranscriptional manipulation of the genetic information. The manipulation results from the fact that inosine is biochemically interpreted as guanosine. Thus, formal A-to-G conversions become accessible, in a highly site-specific manner. The specificity comes from the guide-RNA that addresses the editing enzymes and can be readily programmed in rational way, simply by applying Watson-Crick pairing rules.\textsuperscript{228} Even though only A-to-G mutations are accessible, the scope of manipulations is large. Twelve out of the 20 canonical amino acids can be manipulated, comprising almost all of the polar ones which are essential for protein function.\textsuperscript{223} Furthermore, START and STOP codon, splice elements, polyadenylation signals, and viral RNA are potential targets.\textsuperscript{226,227} We and others have shown that such strategies work inside mammalian cell culture\textsuperscript{229} and even in a simple organism\textsuperscript{230} and allow the repair of disease-relevant genes, like the CFTR mRNA.\textsuperscript{225}

Other people have recently shown the possibility of re-directing snoRNA-guided RNA modification machineries, like the 2′-O-methylation\textsuperscript{231} and the pseudouridylation machinery.\textsuperscript{232} The first modification allows interference with splicing, the second allows the read-through of premature STOP codons. Mammalian cells harbor a plethora of RNA modifying and processing enzymes. There is no need to restrict ourselves to the usage of nuclease, like RISC, RNaseH, and RNaseP.\textsuperscript{232} Just to give a few examples, there are RNA editing and modifying enzymes inside the cell that can change nucleotides (A-to-I, C-to-U\textsuperscript{233}, U-to-ψ, A-to-m6A,\textsuperscript{234} and many more for the tRNAs\textsuperscript{235}), that add the cap\textsuperscript{236} and the poly(A)-tail,\textsuperscript{237} RNAs can be precisely processed, for instance by the CCA-adding enzymes,\textsuperscript{238,239} TUTases,\textsuperscript{240} etc.\textsuperscript{241} Thus, even complex repair processes are conceivable, including the repair of insertion and deletion mutations at the RNA-level. In this respect, we want to recall a largely overseen work from 2004, done by Paul Zamecnik, the pioneer of antisense therapy, in his early nineties shortly before he passed away. He demonstrated the possibility of repairing the terrible Δ508 deletion mutation in the CFTR gene, the main cause of cystic fibrosis, simply by administration of 2 chemically stabilized RNA oligomers.\textsuperscript{242} In cell culture, the efficiency of mRNA repair was sufficient to restore the chloride channel function. Unfortunately, he was unable to elucidate the mechanism, but he could clearly demonstrate the repair to take place at the mRNA. Such a complex repair requires a concerted nuclease, ligase (and polymerase) activity at a specific site on an mRNA molecule. In summary, it seems that numerous endogenous enzymes stand ready inside the cell for RNA repair processes. We just have to learn how to make use of them.\textsuperscript{243,244} If successful, one can establish novel platforms for therapeutic intervention.

Conclusions

While splice-switching oligomers and aptamers are still struggling on their ways to the clinic, major progress has been made for RNaseH-dependent ASOs and for therapeutic RNAi with chemically stabilized siRNAs. This is due to the development of new chemistries that improve efficacy and delivery of the drugs to some specific organs. An impressive example is the development of the GalNAc\textsubscript{3} conjugation that clearly improves liver targeting and might allow for the administration of siRNA and ASO by subcutaneous administration in the future. However, overcoming problems with delivery and efficacy remains elusive for many organs and will require massive basic research in the future.

Among the emerging approaches, the usage of in-vitro-transcribed mRNA for protein replacement and vaccination has made impressive progress. This was mainly due to the tailored suppression or harnessing of the RNA-induced immune response by chemical modification and formulation. The approach has the potential to find wide application in the clinics whenever a transient, burst-like expression is advantageous. The RNA repair approach is still in its infancy, but we believe that the harnessing of artificial and in particular endogenous RNA repair proteins might enable new therapies, complementing the above-mentioned classical RNA-based and the approaching genome editing methods, and being superior to the latter with respect to safety and ethical issues.

Overall, the progress during last years is impressive. The increasing number of clinical trials for various approaches makes us feel optimistic that numerous nucleic-acid-based drugs will soon find their ways to the patients to enable novel therapies.

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