British Journal of Clinical Pharmacology

Br J Clin Pharmacol (2016) **82** 659–672 659

REVIEW

Therapeutic nucleic acids: current clinical status

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Received 31 December 2015; **revised** 20 April 2016; **accepted** 21 April 2016

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Keywords antisense oligonucleotides, aptamers, gene therapy, ribozymes, RNA decoys

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are simple linear polymers that have been the subject of considerable research in the last two decades and have now moved into the realm of being stand-alone therapeutic agents. Much of this has stemmed from the appreciation that they carry out myriad functions that go beyond mere storage of genetic information and protein synthesis. Therapy with nucleic acids either uses unmodified DNA or RNA or closely related compounds. From both a development and regulatory perspective, they fall somewhere between small molecules and biologics. Several of these compounds are in clinical development and many have received regulatory approval for human use. This review addresses therapeutic uses of DNA based on antisense oligonucleotides, DNA aptamers and gene therapy; and therapeutic uses of RNA including micro RNAs, short interfering RNAs, ribozymes, RNA decoys and circular RNAs. With their specificity, functional diversity and limited toxicity, therapeutic nucleic acids hold enormous promise. However, challenges that need to be addressed include targeted delivery, mass production at low cost, sustaining efficacy and minimizing off-target toxicity. Technological developments will hold the key to this and help accelerate drug approvals in the years to come.

Introduction

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are simple linear polymers that consist of only four major subunits, yet these molecules carry out myriad functions both within the cell and in the laboratory [1]. Early assessment of nucleic acid function was rather narrow and restricted, but research in the past few decades has seen remarkable progress in developing nucleic acid-based therapeutics. The progress has covered diverse fields of research and a significant number of scientists and engineers are now involved in this area [2]. A series of pivotal discoveries in the last three decades has made this possible. First, a large body of work has obviously followed the decoding of the human genome that unlocked several molecular pathways that are important in disease. Secondly, several types of RNA with complex biological functions have been discovered in addition to messenger RNA (mRNA) and transfer RNA (tRNA) [1]. For example, two non-coding RNA types that were not considered essential but are now extremely relevant to therapeutics are the microRNA (miRNA) and the short interfering RNA (siRNA). Thirdly, the appreciation that RNAs can act as enzymes has led to the development of RNA analogues with useful or unusual properties. Of the analogues, the locked nucleic acids or LNAs have found therapeutic applications. In view of their polar nature, the cellular delivery of nucleic acids is poor relative to conventional low molecular weight drugs. The fourth major advance has been enhancing the bioavailability of nucleic acid-based drugs. None of these would have been possible without technological advances in DNA synthesis, including the *de novo* synthesis of increasingly longer DNA

DOI:10.1111/bcp.12987

constructs and use of DNA shuffling, bioprospecting, combinatorial chemistry, high throughput screening and genetic engineering of viruses. Advances in synthetic biology, systems biology, computational biology, bioinformatics and nanotechnology among others have greatly aided development in this area and set a new paradigm for the nucleic acids in therapy [3–5].

The fundamental basis of using nucleic acids except for gene therapy in therapeutics is either inhibition of DNA or RNA expression, thereby halting production of abnormal protein related to a disease while leaving all other proteins unaffected [6]. Therapeutic nucleic acids (TNAs) are nucleic acids themselves or closely related compounds used to treat disease. Although various types of TNAs exist, they share a common mechanism of action that is mediated by sequence-specific recognition of endogenous nucleic acids through Watson–Crick base pairing [7]. Their drug development has specific requirements that are unique as they fall somewhere between small molecules and biologics. TNA are charged, high molecular weight compounds with physicochemical properties different from small molecule drugs, and are unstable in a biological environment. In addition, TNA have to be delivered to the correct intracellular compartment. Because they are chemically synthesized, regulators considered that they are new chemical entities (NCEs). However, the above-mentioned characteristics make them closer to new biological entities (NBEs) [8]. Clinical trial regulations related to TNAs and subsequent approval for human use are more complex than for NCEs [9].

The present review divides nucleic acid therapeutics broadly into DNA therapeutics (antisense oligonucleotides, DNA aptamers and gene therapy) and RNA therapeutics (micro RNAs, short interfering RNAs, ribozymes, RNA decoys and circular RNAs). This review will restrict its focus to nucleic acids that are either in clinical development or already available for clinical use.

DNA Therapeutics

Anti-sense oligonucleotides (ASOs) and DNA aptamers

ASOs are single, short-stranded sequences, 8–50 base pairs in length, binding to the target mRNA by means of standard Watson–Crick base pairing. After an ASO binds with the mRNA, either the target complex will be degraded by endogenous cellular RNase H or a functional blockade of mRNA occurs due to steric hindrance [10, 11].

Aptamers (from the Latin *aptus*, to fit), also called 'chemical antibodies', are single-stranded synthetic DNA or RNA molecules, 56–120 nucleotides long, that can bind the nucleotide coding for proteins with high affinity and thus serve as decoys. DNA aptamers are short single-stranded oligonucleotide sequences similar to ASO with very high affinity for the target nucleic acids through structural recognition [12]. DNA aptamers that target coding nucleotides for lysozyme, thrombin, human immunodeficiency virus trans-acting responsive element, hemin, interferon γ, vascular endothelial growth factor, prostate specific antigen, dopamine and heat shock factor are still under development [13]. Aptamers are isolated from a large pool of nucleic acids by a process called Systematic Evolution of Ligands by EXponential Enrichment, or SELEX [14]. AptaBid is another selection process for generating appropriate aptamers [15]. They represent attractive alternatives to monoclonal antibodies because they are non-toxic, non-immunogenic, have good tissue penetration, are straightforward to make, can be modified easily *in vitro* and can be given either intravenously or subcutaneously [16–19].

The ASOs that were initially developed were unmodified and highly susceptible to nuclease enzyme [20, 21]. Chemical modifications to the backbone and ribose component of the nucleotides have improved their stability, binding strength and specificity to the target nucleic acid [22]. Second-generation ASO may have a phosphodiester or phosphoramidate or phosphorothioate modification [23]. Of these, phospohorothioate modification, wherein one of the non-bridging oxygen atoms in the backbone is replaced by a sulphur atom, was the most common type in second-generation ASO [24]. Phosphorothioate ASOs showed enhanced uptake by the target cells, more stability and so longer action, and high target specificity [25]. Modification of the ribose sugar at the 2′ position (most commonly either 2′-O-methyl or 2′-O-methoxy ethyl) further increases the resistance to exonucleases of ASOs modified by phosphorothioate replacement in the backbone [26]. ASO with a chemically modified non-sugar furanose ring show additional improvements in nuclease stability, target affinity and pharmacokinetic profiles. Locked nucleic acid (LNA), peptide nucleic acid (PNA) and morpholino phosphoroamidates (MF) are the three most commonly used third-generation ASOs [27]. The efficacy and safety of various ASOs is being explored in various currently incurable neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and other neurodegenerative diseases [28]. However, till now, only two ASOs have been approved by the United States Food and Drug Administration (US FDA) to be used on human beings, namely, fomivirsen, a first-generation ASO, and mipomersen, a second-generation ASO [29, 30].

Fomivirsen. The first ASO approved for treating humans is fomivirsen, developed by Isis pharmaceuticals for the management of cytomegalovirus (CMV) retinitis in 1998 [31]. Fomivirsen is a phosphorothioate ASO (first-generation) and binds to the IE2 fragment of mRNA of CMV that is involved in viral replication. Fomivirsen is administered as an intravitreal injection and has been found to have long duration of action; plasma concentrations are negligible [32]. Metabolism of fomivirsen is locally mediated by exonuclease enzyme in the eye. No significant drug interactions have been observed with fomivirsen except that it should not be administered within 4 weeks following administration of the antiretroviral agent cidofovir [33]. The drug is administered intravitreally once a week and the median time for progression of retinitis in patients was found to be 71–90 days [34]. No serious adverse drug reactions were observed during clinical trials for intravitreal fomivirsen [35]. Reports of occurrence of reversible bull's-eye maculopathy with fomivirsen administration have been documented without any effect on vision [36]. Following the advent of highly

active antiretroviral drugs for treating infections with human immunodeficiency virus, a decline in the rate of CMV retinitis in patients was observed. Hence, fomivirsen was withdrawn by the marketing authorization holder for commercial reasons.

Mipomersen. Mipomersen is an ASO that acts as an inhibitor of apolipoprotein B-100 synthesis, which is an essential component of both very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) [37]. It has been approved for patients diagnosed with homozygous familial hypercholesterolemia (HoFH). Familial hypercholesterolemia is an autosomal dominant genetic disorder resulting from mutations of the low-density lipoprotein-cholesterol (LDL-C) receptor, apolipoprotein B (apo B), or pro-protein convertase subtilisin/kexin 9 (PCSK9) [38]. The standard of care before approval of mipomersen for patients with HoFH was weekly or bi-weekly LDL apheresis. Mipomersen binds to the mRNA of the abnormal apo B in patients with HoFH and inhibits the synthesis of mutated apo B protein so that LDL-C is phagocytosed in the hepatocytes and cleared from the blood stream. Mipomersen is a long-acting ASO with an elimination half-life ranging between 21 and 33 days depending on the administered dose, and attributed largely to the fact that almost 90% of the compound is bound to plasma proteins [30]. Hence, mipomersen is administered once a week as subcutaneous injection. At 26 weeks a mean reduction of LDL was observed between 25% and 36% compared with placebo [39, 40]. The most commonly observed adverse drug reactions include local injection site reactions such as pruritis, erythema and pain and systemic reactions such as fatigue, pyrexia, chills, malaise, myalgia and arthralgia [39–41]. As liver enzymes were found to be elevated with the drug administration, mipomersen is contra-indicated in patients with moderate or severe hepatic impairment.

ASO in evaluation in clinical trials. ASOs are being evaluated for various cancer states and neurodegenerative diseases. Table 1 lists the clinical studies identified from clinicaltrials.gov using the key word 'Antisense oligonucleotides'. Of these agents a promising one is Factor XI ASO (FXI ASO) that has been proven to have potential in reducing the incidence of venous thromboembolism. Recently, Büller *et al.* [42] conducted a randomized, open-label study amongst 300 patients undergoing total knee arthroplasty receiving either FXI ASO or enoxaparin. The incidence of thromboembolism was observed to be 30% with enoxaparin while only 27% and 4% with a lower and higher dose of FXI ASO respectively. Additionally, the rate of bleeding was also significantly lower in the FXI ASO (3%) group as compared to enoxaparin (8%). The study results suggest that strategies to lower factor XI levels can be more effective in reducing postoperative thrombosis than conventional anticoagulants, without increasing the risk of bleeding.

Despite a large number of molecules being evaluated in the clinical trials, the clinical progress of ASO has been slow because of the challenges involved in intracellular delivery of the large, highly charged molecules within acceptable limits of toxicity. Progress is also limited by the need to synthesize the TNA at an affordable cost. Various strategies to deliver the nucleic acids successfully are being evaluated. They include the cationic polymer, poly(ethylene imine); a biodegradable polymer, poly(2-dimethylamino ethylamino) phosphazene; and dendritic α,ε-poly(L-lysine)s [43, 44]. Direct delivery of ASO to lung to avoid systemic exposure has also been promising in preclinical animal studies and is likely to be tested in humans [45]. Conjugated ASO is another arena for technological development. For instance, α-tocopherol-conjugated chimeric ASO was shown to have more tropism to hepatocytes and high efficacy in silencing the target gene *in vivo* [46].

Gene therapy

Gene therapy replaces the function of an abnormal or non-functional gene with a functioning variant. Although the concept was proposed three decades ago, advances in the methods of delivery were made only over the past decade. Treatment now covers genetic disorders such as adenosine deaminase deficiency, α-1-antitrypsin deficiency, cystic fibrosis, familial hypercholesterolemia, Gaucher's disease and haemophilia B [47]. The desired gene is delivered with the aid of a vector, which is usually a virus – most commonly a retrovirus or rarely adenovirus – as a therapeutic gene expression cassette. A therapeutic gene expression cassette is typically composed of a promoter that drives gene transcription, the transgene of interest, and a termination signal to end gene transcription [48]. Also, there exists two approaches for gene delivery, namely germ line and somatic gene therapy. The former involves the introduction of the desirable gene into germ cells which are transmissible to the next generations while the latter involves the transfer of the desired gene to the somatic cells that are not heri Table [49, 50]. Although several case reports for gene therapy were published in the 1970s, a major setback was seen in the year 1999 when an 18-year-old patient with ornithine transcarboxylase deficiency died during a clinical trial of gene therapy [51]. A review of clinical trials with gene therapy from 1989 to 2015 revealed a total of 2210 clinical trials with the maximum number of trials (n = 130) conducted in the year 2014 and only 4% entering phase 3 clinical trial [52]. Also the authors found that 64% of the trials are being carried out in the field of oncology. Despite so many ongoing clinical trials, only two gene therapies are approved for human use: gendicine and alipogene tiparvovec.

Gendicine. Gendicine is a recombinant adenovirus engineered to express the tumour suppressor gene p53, for patients with head and neck squamous cell carcinoma and was approved by the Chinese Food and Drug Administration in 2003 [53]. When combined with chemotherapy and radiotherapy, gendicine can improve treatment efficacy by 3.4-fold (complete regression of tumour in 64% when gendicine is combined with radiotherapy as against 19% with radiotherapy alone) in addition to amelioration of the associated toxicity [54]. Furthermore, gendicine has been shown to improve the therapeutic effect of radiation in pancreatic cancer cells [55]. However, clinical trials in pancreatic cancer are yet to follow.

Alipgene. Alipogene tiparvovec, an adeno-associated virus with lipoprotein lipase gene, is a gene therapy approved by the European Union for patients with lipoprotein lipase

Table 1

Summary of registered clinical trials with ASO in clinical trials.gov

Study id; year of registration; country of conduct of study	Sponsors	Disease condition	Phase of clinical trial; target mRNA for ASO
NCT02627820; 2015; United States	GlaxoSmithKline Isis Pharmaceuticals	Senile cardiac amyloidosis	Transthyretin
NCT02406833; 2015; Germany	Isarna Therapeutics GmbH	Glaucoma patients undergoing trabeculectomy	Transforming growth factor-β.
NCT00780052; 2008; United States of America	University of Pennsylvania	Advanced haematological malignancies (acute myeloid or lymphoid leukaemia; or myeloproliferative disorder (MPD) including chronic myelogenous leukaemia (CML); or myelodysplastic syndrome (MDS); or non-Hodgkin's lymphoma (including CLL); or multiple myeloma)	Phase 1; c-myb ASO
NCT00159250; 2005; United Kingdom	Imperial College, London	Duchenne muscular dystrophy	Phase 1/2 studies against AVI-4658 PMO
NCT02417753; 2015; United States of America	National Cancer Institute	Malignant ascites	Phase 2; STAT3
NCT02507583; 2015; United States of America	Thomas Jefferson University	Malignant glioma	Phase 1; Insulin like growth factor-1
NCT00466583; 2007; United States of America	Enzon Pharmaceuticals, Inc.	Advanced solid tumours or lymphoma	Phase 1; Hypoxia-inducible factor-1α
NCT01550523; 2012; United States of America	Thomas Jefferson University	Recurrent malignant glioma	Phase 1; Insulin like growth factor receptor
NCT00100672; 2005; United States of America	INSYS Therapeutics Inc	Advanced cancer	Phase 1; c-raf
NCT01120288; 2010; United States of America	National Cancer Institute	Advanced solid tumours with metastasis	Phase 1; Hypoxia inducible factor-1α
NCT01159028; 2010; United States of America	Bio-Path Holdings, Inc.	Recurrent adult acute myeloid leukaemia or acute lymphoblastic leukaemia or myelodysplastic syndrome or Ph1 Positive chronic myeloid leukaemia	Phase 1; Growth factor receptor bound protein-2
NCT00959868; 2009; Canada	Vancouver Coastal Health	Superficial bladder tumour	Phase 1; Heat shock protein 27
NCT00048321; 2002; United States of America	Isis Pharmaceuticals	Rheumatoid arthritis	Phase 2; Tumor necrosis factor
NCT02423590; 2014; United Kingdom	Queen Mary University of London	Advanced squamous cell lung cancer	Phase 2; Heat shock protein 27
NCT00445913; 2007; United States of America	University of Pittsburgh	Type 1 diabetes mellitus	Phase 1; CD40, CD80 and CD86
NCT01470911; 2011; Germany NCT01554319; 2012; Germany NCT01577953; 2012; Germany	Sterna Biologicals GmbH & Co. KG	Bronchial asthma	Phase 1; GATA-3
NCT01743768; 2012; Germany	Sterna Biologicals GmbH & Co. KG	Bronchial asthma	Phase 2; GATA-3
NCT02564354; 2015; United States of America	ProQR Therapeutics	Cystic fibrosis	Phase 1; ΔF508
NCT02532764; 2015; United States of America	ProQR Therapeutics	Cystic fibrosis	Phase 1/2; ΔF508
NCT00021749; United States of America	Genta Incorporated	Chronic lymphocytic leukaemia	Phase 1/2; Bcl-2
NCT00002592; 1999; United States of America	Abramson Cancer Center of the University of Pennsylvania	Chronic myelogenous leukaemia	Phase 2; c-myb
NCT00487786; 2007; United States of America	OncoGenex Technologies	Prostate cancer, ovarian cancer, non-small cell lung cancer, breast cancer, bladder cancer	Phase 1; Heat shock protein 27

(*continues*)

Table 1

(Continued)

Study id; year of registration; country of conduct of study	Sponsors	Disease condition	Phase of clinical trial; target mRNA for ASO
NCT00005032; 2000; United States of America	University of Chicago	Recurrent small cell lung cancer	Phase 1/2; Bcl-2
NCT00059813; 2003; United States of America	National Cancer Institute (NCI)	Metastatic renal cell cancer	Phase 2; Bcl-2
NCT00078234; 2004; United States of America	Genta Incorporated	Chronic lymphocytic leukaemia	Phase 1/2; Bcl-2
NCT00017251; 2001; United States of America	National Cancer Institute (NCI)	Extensive-Stage Small Cell Lung Cancer	Phase 1; Bcl-2
NCT00471432: 2007; United States of America	NCIC Clinical Trials Group	Bladder cancer, breast cancer, kidney cancer, lung cancer, ovarian cancer, prostate cancer	Phase 1; Clusterin
NCT00063934; 2003; United States of America	National Cancer Institute (NCI)	Breast cancer	Phase 1/2; Bcl-2
NCT01563302; 2012; United States of America	Isis Pharmaceuticals	Lymphoma	Phase 1/2; STAT 3
NCT00016263; 2001; United States of America	Genta Incorporated	Malignant melanoma	Phase 3; Bcl-2
NCT00258375; 2005; United States of America	NCIC Clinical Trials Group	Metastatic breast cancer	Phase 2; Clusterin
NCT00070083; 2003; United Kingdom	British Columbia Cancer Agency	Diffuse large B cell lymphoma	Phase 1; Bcl-2
NCT00017602; 2001; United States of America	Genta Incorporated	Multiple myeloma	Phase 3; Bcl-2
NCT00365781; 2006; United States of America	Isis Pharmaceuticals	Type 2 diabetes mellitus	Phase 1; Tyrosine phosphatase 1B
NCT00030641; 2002; United States of America	Genta Incorporated	Non small cell lung cancer	Phase 2/3; Bcl-2
NCT00070343; 2003; United States of America	Jonsson Comprehensive Cancer Center	Malignant melanoma	Bcl-2
NCT00024440; 2001; United States of America	Genta Incorporated	Chronic lymphocytic leukaemia	Phase 3; Bcl-2
NCT00085228; 2004; Belgium	European Organisation for Research and Treatment of Cancer	Adenocarcinoma prostate	Phase 2; Bcl-2
NCT00543205; 2007; United States of America	Genta Incorporated	Melanoma	Phase 2/3; Bcl-2
NCT00054106; 2003; United States of America	NCIC Clinical Trials Group	Prostate cancer	Phase 1; Bcl-2
NCT01710852; 2012; United States of America	Isis Pharmaceuticals	Paroxysmal atrial fibrillation	Phase 2; CRP
NCT00004870; 2000; United States of America	The University of Texas Health Science Center at San Antonio	Colorectal cancer	Phase 1/2; Bcl-2
NCT00003236; 1999; United States of America	Eastern cancer cooperative agency	Metastatic breast cancer	Phase 2; Protein kinase-alpha
NCT01120470; 2010; United Kingdom	British Columbia Cancer Agency	Castration resistant prostate cancer	Phase 2; Heat shock protein-27
NCT00543231; 2007; United States of America	Genta Incorporated	Solid tumours	Phase 1; Bcl-2
NCT00636545; 2007; United States of America	Genta Incorporated	Solid tumours	Phase 1; Bcl-2
NCT00896857; 2009; United States of America	Comprehensive Cancer Center of Wake Forest University	Breast cancer	Bcl-2

(*continues*)

Table 1

(Continued)

Study id; year of registration; country of conduct of study	Sponsors	Disease condition	Phase of clinical trial; target mRNA for ASO
NCT00024648; 2001; United States of America	INSYS Therapeutics Inc	Advanced malignancies	Phase 1; Raf-1
NCT00024661; 2001; United States of America	INSYS Therapeutics Inc	Advanced solid tumours	Phase 1; Raf-1
NCT02079688; 2014; United States of America	Sterna Biologicals GmbH & Co. KG	Atopic eczema	Phase 2; GATA-3
NCT01839604; 2013; Hong Kong	AstraZeneca	Metastatic hepatocellular carcinoma	Phase 1; STAT-3
NCT00264966; 2005; Canada	Pharmaxis	Asthma	Phase ½; CCR-3
NCT00056173; 2003; United States of America	Aptose Biosciences Inc.	Renal cell carcinoma	Phase 1/2; ribonucleotide reductase
NCT00048113; 2002; United States of America	Isis Pharmaceuticals	Crohn's disease	Phase 3; intercellular adhesion molecule
NCT00048295; 2002; United States of America	Isis Pharmaceuticals	Crohn's disease	Phase 3; intercellular adhesion molecule
NCT01780545; 2013; United States of America	OncoGenex Technologies	Metastatic bladder cancer	Phase 2; Heat shock protein 27
NCT01598948; 2012; Germany	Ludwig-Maximilians - University of Munich	Atherosclerosis	Phase 3; apolipoprotein B
NCT01083615; 2010; United States of America	OncoGenex Technologies	Prostate cancer	Phase 3; Clusterin
NCT02549651; 2015; United States of America	MedImmune LLC	Diffuse large B-cell lymphoma	Phase 1; STAT 3
NCT02243124; 2014; United States of America	Eleos, Inc.	Myelodysplastic syndrome	Phase 1; P 53
NCT02144051; 2014; United States of America	AstraZeneca	Advanced solid tumours with androgen receptor pathway as a potential factor	Phase 1; Androgen receptor
NCT00363974; 2006; United States of America	Aegera Therapeutics	Acute myelomonocytic leukaemia	Phase 1; X-linked inhibitor of apoptosis protein
NCT00054548; 2003; United States of America	National Cancer Institute	Advanced solid tumours	Phase 1; Bcl-2
NCT00557596; 2007; United States of America	Aegera Therapeutics	Advance pancreatic cancer	Phase 1/2; X-linked inhibitor of apoptosis protein
NCT00558922; 2007; United States of America	Aegera Therapeutics	Non-small cell lung cancer	Phase 1/2; X-linked inhibitor of apoptosis protein
NCT00080847; 2004; United States of America	National Cancer Institute	Advanced diffuse large B-cell non-Hodgkin's lymphoma	Phase 2; Bcl-2
NCT00385775; 2006; United States of America	Aegera Therapeutics	Advanced tumours	Phase 1; X-linked inhibitor of apoptosis protein
NCT00967512; 2009; United States of America	Eleos, Inc.	Acute myelogenous leukaemia	Phase 2; P 53
NCT00558545; 2007; United States of America	Aegera Therapeutics	Advanced breast cancer	Phase 1/2; X-linked inhibitor of apoptosis protein

Some of the registered studies did not have any mention of the phase of clinical trials and so the details are not captured in this table.

deficiency in 2012 [56]. As the condition is very rare (one in a million individuals), the decision was based on the results obtained from a study of just 27 patients.

The major concern related to gene therapy is the uncertain long-term risk. The Office of Cellular, Tissue, and Gene Therapies (OCTGT) in the Centre for Biologics Evaluation and Research regulates the clinical trials that are evaluating gene therapy. The long-term risk of gene therapy is uncertain but the US FDA has drafted a framework that is based on the integration of the vector with the target human genome and the potential for latency and reactivation. The US FDA recommends the sponsors to have a 15-year follow-up for study subjects with gene therapy, with a minimum of five years of annual examination and ten years of annual queries either in person or by questionnaire [57]. A major challenge resides in the successful delivery of target genes. Modified human immunodeficiency virus, lentivirus, adeno-virus, adeno-associated virus and herpes simplex virus are the

most widely used viral vectors. Non-viral vectors for gene therapy are promising especially for the non-invasive administration through skin, eyes and lungs [58]. Novel layer-by-layer based nanoparticles [59] and liposomes [60] have been engineered that might play a useful role in successful delivery of genes into the human cells. Also, cell-penetrating peptides have been shown in mouse models to deliver hepatitis B DNA that has potential applications in the management of chronic hepatitis B infections [61].

RNA Therapeutics

RNA interference [RNAi] and short interfering RNAs [siRNAs]

The understanding of the role of RNA in cell biology underwent a sea change in the early 1980s, when RNA moved from being perceived as a passive player in the transfer of information between DNA and protein synthesis to playing significant structural, enzymatic and information-decoding roles during protein synthesis. Walter Gilbert first proposed the concept of 'The RNA World' in 1986 [62, 63] and the pathway of RNA interference [RNAi] was discovered by Fire *et al.* and colleagues in the nematode *Caenorhabditis elegans* in 1998 [64]. The natural function of RNAi appears to be protection of the genome against invasion by mobile genetic material such as from viruses that produce aberrant RNA or dsRNA when they become active [65] and thus RNA interference serves as a powerful defence mechanism. Briefly, RNAi is a process by which RNA molecules with sequences complementary to a gene's coding sequence induce degradation of the corresponding messenger RNAs [mRNAs] thus blocking the translation of mRNA into protein. Computational models that can quantify the important parameters of the RNAi process in living mammalian cells have recently been developed [66]. Therapy with siRNA thus has great potential application for diseases caused by abnormal expression or mutation such as cancers, viral infections and genetic disorders [67] as RNA interference can be experimentally triggered. The fact that the process of interference occurs in the cytoplasm and does not require nuclear penetration makes it even more attractive. In 2001, Elbashir and colleagues used synthetic siRNA to knock down a gene in several mammalian cell lines [65]. Overexpression of Fas protein has been associated with death of hepatocytes and is implicated in a broad variety of liver disorders [68]. Song *et al.* [69] injected Fas siRNAs that protected mice from autoimmune hepatitis which marked the more rapid development of RNA therapeutics.

siRNAs are 'short' double-stranded molecules in that they are only 21–23 nucleotides long and generally can be chemically synthesized. siRNAs have the advantage over DNA oligonucleotides in that they are always delivered as duplexes, which are more stabile [70]. Two major issues with siRNAs relate to their off-target effects and delivery into the cell. Off-target effects occur by suppression of genes other than the target gene, which can have deleterious consequences. This results primarily from partial sequence homology that exists with other mRNAs that are not the target mRNAs. siRNA is also recognized by immunoreceptors, like the Toll-like receptors, that can lead to cytokine release and alteration in gene expression.

The first report of the application of the RNAi technology was to demonstrate the safety and efficacy of an siRNA ALN-RSV01 in 101 healthy participants with experimentally induced respiratory syncytial virus (RSV) infection [71]. ALN-RSV01 targets the N protein of the RSV and was subsequently evaluated in a cohort of. Encouraging results were observed with the ALN-RSV01 in 16 lung transplant recipients [72]. The latter study also raised the possibility that siRNA can silence genes involved in allograft rejection [73]. According to the database of clinical trials [clinicaltrials.gov], a total of 40 siRNAs are in various stages of development and one or more of them could receive approval in the next few years (Table 2).

MicroRNAs – their targeting and the use of antagonists and mimics of microRNA as therapeutic agents

Components of the genome that had hitherto been considered non-functional were discovered to have gene regulatory capacity approximately 20 years ago. MicroRNAs (miRNAs) were first discovered in 1993 [74] and have today made the transition from worm genetics to human disease. Victor Ambros and Gary Ruvkun discovered the first miRNA gene in *C. elegans* when a small RNA encoded by the lin-4 locus was found to be associated with the developmental timing of the nematode. Since their discovery, miRNAs have been shown to be an essential part of the non-coding genome. miRNAs are short single-stranded non-coding RNA molecules of 15–22 nucloetides, which function as key negative regulators of post-transcriptional modulation in almost all biological processes. Today, miRNA dysregulation is frequently shown to be associated with pathogenesis of human disease thus making them attractive therapeutic targets [75, 76]. Their advantage as therapeutic agents lies in their ability to target multiple molecules unlike the traditional approach of targeting a single gene thus making them extremely efficient in regulating distinct biological cell processes relevant to both normal and cancer cell biology [77].

Early evidence of the involvement of miRNAs in human cancer came from studies on chronic lymphocytic leukaemia (CLL). The human miRNA genes mir-15a and mir-16-1 were shown to be deleted or downregulated in over two-thirds of the cases of CLL [78]. The first oncogenic miRNAs described were the miR-17-92 cluster, which is amplified in B cell lymphomas among other cancers, and miR-155, which is overexpressed in haematological cancers [79, 80]. Subsequently, miRNAs have been identified for several cancers, including lung, ovarian and breast cancers, and glioblastomas [81]. In addition, there are many diseases where it has been shown that there is significantly greater expression of miRNA in diseased tissue than in normal tissue. These include diabetes, obesity, Alzheimer's and Parkinson's disease and cardiovascular and autoimmune disorder [82]. MicroRNA therapeutics can be broadly classified into two types – the use of miRNA antagonists and miRNA mimics, with the latter also being called 'miRNA replacement therapy'. In cancer, they can be targeted in two ways. Direct approaches involve the use of oligonucleotides or virus-based constructs to block

Table 2

Registered clinical studies with siRNA

Study id; year of registration; country of conduct of study	Sponsors	Disease condition	Phase of clinical trial; target mRNA for ASO
NCT00716014; 2008; United States of America	Pachyonychia congenita project	Pachyonychia congenital (PC)	Phase 1; PC keratins - K6a
NCT00363714; 2006; United States of America	Allergan	Age-related macular degeneration	Phase 1/2; Vascular endothelial growth factor
NCT00672542; 2008; United States of America	Scott Pruitt	Metastatic melanoma	Phase 1; immunoproteasome beta subunits LMP2, LMP7, and MECL1
NCT02166255; 2014; United States of America	Comprehensive Cancer Center of Wake Forest University	Melanoma, renal or pancreatic cancer	Phase 1; E3 ubiquitin ligase Cbl-b
NCT00395057; 2006; United States of America	Allergan	Age-related macular degeneration	Phase 2; Vascular endothelial growth factor
NCT00938574; 2009; Germany	Silence Therapeutics GmbH	Advanced solid tumours	Phase 1; Protein kinase 3
NCT00689065; 2008; United States of America	Calando Pharmaceuticals	Solid tumours	Phase 1; ribonucleotide reductase
NCT01188785; 2010; United States of America	Silenseed Ltd	Pancreatic adenocarcinoma	Phase 1; KRAS (G12D) oncogene
NCT01591356; 2012; United States of America	M.D. Anderson Cancer Center	Advanced cancers	Phase 1; EphA2
NCT01064505; 2010; United States of America	Quark Pharmaceuticals	Optic atrophy	Phase 1; pro-apoptotic protein, Caspase 2
NCT00927459; 2009; United States of America	Arbutus Biopharma Corporation	Hypercholesterolemia	Phase 1; polo-like kinase I, kinesin spindle protein
NCT01437007; 2011; United States of America	National Cancer Institute	Hepatocellular carcinoma	Phase 1; polo-like kinase 1
NCT01858935; 2013; United States of America	Nitto Denko Corporation	Healthy individuals	Phase 1; Heat shock protein 47
NCT02314052; 2014; United States of America	Dicerna Pharmaceuticals, Inc.	Hepatocellular carcinoma	Phase 1/2; MYC oncoprotein
NCT02227459; 2014; United States of America	Nitto Denko Corporation	Hepatic fibrosis	Phase 1; Heat shock proteins
NCT02110563; 2014; United States of America	Dicerna Pharmaceuticals, Inc.	Multiple Myeloma Non-Hodgkins Lymphoma	Phase 1; MYC oncoprotein
NCT00306904; 2006; United States of America	OPKO Health, Inc.	Diabetic macular oedema	Phase 2; Vascular endothelial growth factor
NCT02250612; 2014; United States of America	Sylentis, S.A.	Open angle glaucoma	Phase 1; Beta-2 adrenergic receptor
NCT01776658; 2012; United States of America	Sylentis, S.A.	Dry eye syndrome	Phase 1/2; Vascular endothelial growth factor
NCT01739244; 2012; United States of America	Sylentis, S.A.	Open angle glaucoma	Phase 2; Vascular endothelial growth factor
NCT01676259; 2012; Israel	Silenseed Ltd	Advanced pancreatic cancer	Phase 2; KRAS (G12D) oncogene
NCT01438281; 2011; Spain	Sylentis, S.A.	Dry eye syndrome	Phase 1; transient receptor potential cation channel subfamily V member 1 (TRPV1)
NCT00557791; 2007; United States of America	OPKO Health, Inc.	Age related macular degeneration	Phase 3; Vascular endothelial growth factor
NCT00802347; 2008; Canada	Quark Pharmaceuticals	Renal transplantation	Phase 1/2; P 53
NCT01808638; 2013; Germany	Silence Therapeutics GmbH	Pancreatic ductal carcinoma	Phase 1/2; Protein kinase 3
NCT02596347; 2015; United States of America	National Jewish Health	Chronic beryllium disease	JAM2
NCT00154934; 2005; Taiwan	National Taiwan University Hospital	Pre-eclampsia	IL-10

(*continues*)

Table 2

(Continued)

Study id; year of registration; country of conduct of study	Sponsors	Disease condition	Phase of clinical trial; target mRNA for ASO
NCT01075360; 2010; Taiwan	National Taiwan University Hospital	Neuroblastoma	E2F1
NCT01834105; 2013; China	Fujian Institute Of Traditional Chinese Medicine	Post menopausal osteoporosis	Phase 2; cardiotrophin-like cytokine factor 1
NCT01227291; 2010; Spain	Sylentis, S.A.	Glaucoma	Phase 1/2; Beta-2 adrenergic receptor
NCT01382511; 2011; Israel	Tel-Aviv Sourasky Medical Center	Pachyonychia congenita	STAT 1
NCT02528682; 2015; Netherlands	Radboud University	Hematological malignancies	Phase 1/2; PD-1/PD-L1 co-inhibitory pathway
NCT02055846; 2012; France	Institut Paoli-Calmettes	Prostate cancer	Heat shock protein 27
NCT00554359; 2007; United States of America	Quark Pharmaceuticals	Acute renal failure	Phase 1; P53
NCT01058798; 2010; Taiwan	National Taiwan University Hospital	Neuroblastoma	β1,4-N-acetylgalactosaminyltransferase III
NCT01445899; 2011; Israel	Quark Pharmaceuticals	Diabetic macular oedema	Phase 2; hypoxia-inductible gene
NCT00259753; 2005; United States of America	OPKO Health, Inc.	Age related macular degeneration	Phase 2; Vascular endothelial growth factor

Some of the registered studies did not have any mention of the phase of clinical trials and so the details are not captured in this table

their expression or introduce a tumour suppressor miRNA that has been lost due to the disease process. Indirect approaches involve use of drug therapy that modulates miRNA expression by targeting their transcription and their processing [83].

One miRNA-based agent that is in advanced clinical development is actually for use in an infectious disease and not cancer. miRNA-122 is expressed in hepatocytes and interacts with hepatitis C virus (HCV) leading to proliferation of HCV [84]. This seminal discovery paved the way for the discovery of the first anti-viral agent that targets a miRNA, called miraversen (formerly SPC3649) [85].

Miravirsen. Miravirsen is a β-D-oxy-locked nucleic acid-modified phosphorothioate antisense oligonucleotide that targets the liver-specific microRNA-122 (miR-122) and is one of a class of miRNA inhibitors or antagonists that has undergone clinical trials in patients with hepatitis C. Studies with the drug have been conducted in healthy participants (n = 82) and patients (n = 37) with chronic HCV infection in studies lasting 12 weeks. No dose-limiting toxicities or participant discontinuations were seen [86]. Miravirsen has also demonstrated broad spectrum antiviral activity and a relatively high genetic barrier to resistance which is slow to emerge [76]. The drug should prove to be a useful therapeutic option and also as an addition alongside agents such as pegylated interferon, telepravir or bocepravir and ribavarin. The clinical trials registry clinicaltrials.gov lists seven studies with miravirsen of which two have been completed and the remaining five are active. The latter studies include long-term safety, pharmacokinetics and evaluation of drug interactions. Another miRNA anatagonist RG-101 has entered Phase II trials for the same indication. An miRNA mimic that targets miRNA-34 is likely to be the first candidate mimic to reach the clinic for the treatment of hepatocellular carcinoma [87]. Other potential roles of miRNA include targeting malignant plasma cells in multiple myeloma and modifying insulin release and resistance in diabetes mellitus [88, 89].

RNA aptamers and RNA decoys

A facet of RNAs that makes them promising as therapeutic agents is the ability of some small RNAs to fold into three-dimensional structures which can then bind to proteins and thereby inhibit them in a manner similar to protein antagonists. This is the logic behind the use of RNA 'decoys' or RNA aptamers. They can bind viral proteins and thus can also be used as vehicles to transport siRNAs. RNA aptamers are developed by methods similar to those described above for DNA aptamers. RNA aptamers are highly susceptible to exonuclease degradation and so require capping with modified or inverted nucleotides such as 2′-O-modified pyrimidines, 2′-amino pyrimidines or 2′-fluoro pyrimidines to prevent terminal degradation [90]. The advantage of RNA aptamers lies in both their ability to reach intracellular targets and also to bind directly to extracellular targets, unlike other RNA-based therapeutics that must first enter the cell to carry out their functions [91].

An early clinical trial was conducted in patients with HIV using the RNA decoy strategy [92] against the HIV Tat protein. The approach was shown to be safe but disappointing in four children with HIV infection. It is likely that the RNA decoy was insufficiently expressed for effective anti-viral

activity. Just a few years later, in December 2004, pegaptanib (Macugen, Eyetech Pharmaceutics/Pfizer), an aptamer against vascular endothelial growth factor (VEGF), become the first RNA aptamer to receive approval from the US FDA for use in age-related macular degeneration (AMD) [93]. NOX-A12 (olaptesed pegol) is a 45-nucleotide-long RNA aptamer that specifically antagonizes the CXC chemokine ligand 12/stromal cell derived factor-1 (CXCL 12/SDF-1) which is a key regulatory chemokine for the migration of leukaemic stem cells into the bone marrow [94]. This inhibition of binding of SDF-1 to its receptors can lead the leukaemic stem cells to re-enter the cell cycle and become vulnerable to chemotherapeutic attack. A Phase 1 study with NOX-A12 proved its safety in 48 normal, healthy participants. A Phase II study with the aptamer in combination with bendamustine and rituximab (NCT01486797) in relapsed chronic lymphocytic leukaemia is ongoing. Another aptamer NOX-E36 (ematicap pegol) has undergone studies in both normal, healthy participants and patients with Type-2 diabetes. NOX-E36 interacts with the chemokine ligand 2 (CCL2), a protein involved in the recruitment of monocytes and T cells to sites of injury and inflammation. CCL2 has recently been identified as an 'adipokine' that plays an important role in obesity and complications of Type 2 diabetes [95]. Both the NOX aptamers belong to a special class of aptamers called spiegelmers (from the German *Spiegel*, a mirror) as they are composed of L-ribose units, not the naturally occurring D-enantiomers, and are highly resistant to degradation by nucleases [96]. Another distinguishing feature of spiegelmers is that they are not synthesized by SELEX technology but by mirrored target molecules [97]. Olaptesed pegol is tried in clinical trials as an add-on drug with other chemotherapeutic agents, while ematicap pegol is used alone for patients with diabetic nephropathy. REG-1 (RB006/RB007, pegnivacogin/anivamirsen) is an RNA aptamer along with a single-stranded RNA nucleotide that is given subcutaneously and which targets the coagulation factor IXa. A Phase 1 study in 36 healthy participants (NCT01872572) has been completed without serious adverse effects emerging. It has also been studied in patients with stable cardiovascular disease taking dual antiplatelet therapy with aspirin and clopidogrel [98, 99] and in patients with acute coronary syndromes undergoing percutaneous coronary intervention with no catheter or guide wire thrombosis.

Ribozymes

A subset of RNAs called catalytic RNAs or ribozymes can act as enzymes in the complete absence of protein [100]. Because of their high specificity, wide range of target selection, and action before protein translation, both naturally occurring and artificially synthesized ribozymes can be used to specifically suppress gene function. They can also be used to validate disease-related genes as potential targets for new therapeutic interventions. Their ability to cleave mRNA to prevent protein synthesis enables them to find applications in cancer and virology. Of the several different types of ribozymes, the hammerhead and hairpin ribozyme have been studied extensively. The former is a small ribozyme that has both specificity and catalytic efficacy and thus likely the most widely studied [101].

The first synthesized ribozyme to be evaluated in clinical trials was ANGIOZYME^{TM}. It was designed to combat angiogenesis by cleaving the mRNA that produces VEGF. The anti-Flt-1 ribozyme was designed specifically to cleave the mRNAs for the primary VEGF receptors FLt- and KDR [102]. This ribozyme was well tolerated after intravenous infusion or subcutaneous bolus administration to healthy individuals. A significant increase in serum von Willebrand factor antigen, a marker for endothelial cell dysfunction, was also seen after use of angiozyme [102]. A nuclease-resistant hammerhead ribozyme, Heptazyme (LY466700), completed a Phase I/II clinical trial in 28 patients with chronic HCV and was reported safe and well tolerated. Similarly, in Phase II studies, it was found safe and well tolerated. Clinical evaluation of this agent was eventually discontinued due to safety concerns in primates [103].

Circular RNAs

Normally, RNA is linear with both 3′ and 5′ ends open. But circular RNAs (circRNA) have a covalent bond between the ends so that they are closed. This confers on them resistance to degradation by exonucleases so they tend to be more stable; as a result there may be ten times as much circRNA as linear RNA in the cytoplasm [104]. Another property of circRNA distinguishing it from linear RNA is that it is mostly non-coding [105]. The transcripts of linear mRNA typically are capped and polyadenylated and so can be detected by reverse transcriptase-polymerase chain reaction, unlike circRNAs [106]. CircRNAs are detected by either scrambled exon enrichment in an RNAse-treated sample or by 'TRAP electrophoresis' or 2D electrophoresis [107, 108]. Recently, circRNAs have been found to bind to RNA-binding proteins or ribonucleprotein complexes acting as 'sponges', as so have also been called competing endogenous RNAs [109]. Although no therapeutic molecule related to circRNA has been produced or approved by any regulatory agency, it has been implicated to play a crucial role in some human diseases. For example, cANRIL, a circRNA for a long non-coding RNA, has been found to be a risk factor for atherosclerosis [110]. CiRS-7 is a circRNA that can act as a miRNA sponge to adsorb and quench normal miRNA-7 which has been implicated in the pathophysiology of Parkinson's disease, Alzheimer's disease and cancers including colorectal cancer and pancreatic ductal adenocarcinoma [111]. Additionally, reports indicate that circRNAs can also act as potential biomarkers for aging and gastric cancer [112]. Studies are underway to explore the varied physiological and pathological roles of circRNAs and their therapeutic potential.

Conclusions

Though nucleic acids have been recognized as potential therapeutic agents for more than 40 years, it was the decoding of the human genome coupled with technological advances that has led to the realization of their therapeutic applications. Numerous studies, both in the laboratory and

clinic, in the last two decades have demonstrated the enormous potential of nucleic acids as therapeutic agents, particularly with their promise of specificity, functional diversity and limited toxicity. Of the various nucleic acid therapies, gene therapy has been widely investigated clinically followed by ASOs but only few agents have been approved by the regulatory agencies so far. Among the several hundred studies that have been carried out, there have been two deaths [51] and four instances of malignancy. Overall, however, the agents appear acceptably safe. The spectrum of diseases that can potentially be addressed by nucleic acids is broad and ranges from infectious diseases on one end to those like diabetes and cancer at the other. Key challenges that need to be addressed for the successful translation of nucleic acids will be their delivery to the site of action, choice between direct delivery and the use of a vehicle, mass production at low cost, more clearly defined pharmacokinetics, ability to produce sustained long-term effects, immunogenicity and toxicity (including inappropriate or excessive expression). Studies addressing these areas are needed to improve success in clinical trials. There is no doubt that an improved understanding of the biology of the various nucleic acid pathways will lead to novel therapies in future.

Competing Interest

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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