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Review

RNA-Based Therapeutics: Current Progress and Future Prospects

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Recent advances of biological drugs have broadened the scope of therapeutic targets for a variety of human diseases. This holds true for dozens of RNA-based therapeutics currently under clinical investigation for diseases ranging from genetic disorders to HIV infection to various cancers. These emerging drugs, which include therapeutic ribozymes, aptamers, and small interfering RNAs (siRNAs), demonstrate the unprecedented versatility of RNA. However, RNA is inherently unstable, potentially immunogenic, and typically requires a delivery vehicle for efficient transport to the targeted cells. These issues have hindered the clinical progress of some RNA-based drugs and have contributed to mixed results in clinical testing. Nevertheless, promising results from recent clinical trials suggest that these barriers may be overcome with improved synthetic delivery carriers and chemical modifications of the RNA therapeutics. This review focuses on the clinical results of siRNA, RNA aptamer, and ribozyme therapeutics and the prospects for future successes.

Introduction

Since the milestone discoveries of catalytic RNAs in the early 1980s and RNA interference in the late 1990s, the biological understanding of RNA has evolved from simply an intermediate between DNA and protein to a dynamic and versatile molecule that regulates the functions of genes and cells in all living organisms (Fire et al., 1998; Guerrier-Takada et al., 1983; Kruger et al., 1982). These and similar breakthroughs have led to the emergence of numerous types of RNA-based therapeutics that broaden the range of "drug-able" targets beyond the scope of existing pharmacological drugs (Melnikova, 2007). RNA-based therapeutics can be classified by the mechanism of activity, and include inhibitors of mRNA translation (antisense), the agents of RNA interference (RNAi), catalytically active RNA molecules (ribozymes), and RNAs that bind proteins and other molecular ligands (aptamers).

Despite a number of hurdles encountered along the way, more than 50 RNA or RNA-derived therapeutics have reached clinical testing. Challenges with the delivery, specificity, stability, and immune activation of RNA therapeutics have spawned improvements in synthetic and natural nucleic acid carriers and the development of chemically modified oligonucleotides (Peer and Lieberman, 2011). In this review, we will discuss many of these refinements and highlight several promising therapeutics currently in the clinic.

RNA is unstable in vivo due to the plethora of ribonucleases in serum and in cells, and chemical modifications can enhance desired properties without reducing activity. Chemical modifications to small interfering RNAs (siRNAs), aptamers, ribozymes, antisense (AS) oligonucleotides (ONs), and miRNAs may improve the pharmacokinetic (PK), pharmacodynamic (PD) properties and reduce immunogenicity. Such modifications in short synthetic ONs include changes in the sugar, base, or backbone and may increase target affinity and specificity, decrease susceptibility to nuclease degradation, improve PK, and improve RNAi silencing efficiency. Recent advances in the process of synthesizing modified RNA and DNA molecules have increased the efficiency and reliability of manufacturing while also reducing production costs (Sanghvi, 2011). While dozens of different sugar, base, and backbone modifications are available by ON synthesis, the variety of chemical modifications for RNA-derived ONs destined for the clinic include phosphorothioate (PS) backbone modification; 2′-*O*-methyl (2′-OMe), 2′-fluoro (2′-F), 2′-*O*-methoxyethyl (2′-MOE) sugar substitutions; 2′-*O*, 4′-*C*-methylene linked bicyclic ribonucleotides known as a locked nucleic acid (LNA); and L-RNA (enantiomer of natural RNA) ONs known as spiegelmers (Figure 1; Shukla et al., 2010).

The PS backbone modification, in which the nonbridging phosphate oxygen atom is replaced by a sulfur atom, was one of the earliest ON modifications and remains widely used in DNA antisense therapeutics, and to a lesser degree, in aptamers and siRNAs (Shukla et al., 2010). This simple and inexpensive chemical modification improves resistance to nucleolytic degradation, elicits RNase H-mediated cleavage of the target mRNA for antisense applications, and increases affinity for plasma proteins to hinder renal clearance of the ON (Bennett and Swayze, 2010; Sanghvi, 2011). Chemical substitutions at the 2′-hydroxyl group with 2′-OMe, 2′-F, or 2′-MOE groups often improve the ON potency, stability, and overall PK and PD properties. Even greater potency and stability improvements are observed with the LNA modification (Sanghvi, 2011). Collectively, modifications at the 2′ position of the sugar ring, including 2′-OMe, 2′-F, 2′-MOE, and LNA, confer the ON to adopt an RNA-like C3′-*endo* (*N*-type) sugar pucker, which is the most energy-favorable conformation of RNA. Thus, such modifications increase Watson-Crick binding affinity and, due to the proximity of the 2′-substituent and the 3′-phosphate, improve nuclease resistance (Bennett and Swayze, 2010). In contrast to the aforementioned modifications, spiegelmers do not contain any chemical substitutions, but rather are enantiomers of natural RNAs and thus are utilized as nuclease-resistant aptamers.

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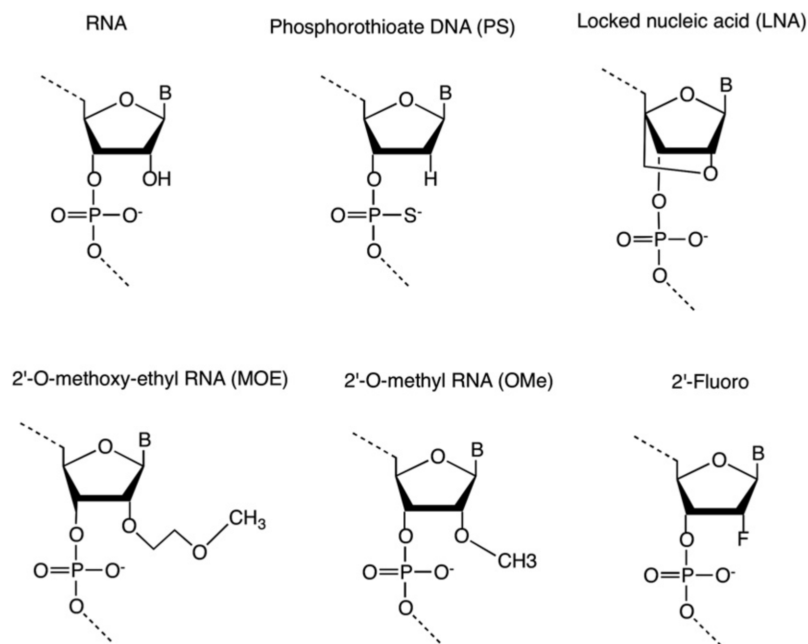

Figure 1. Common Chemical Modifications of Therapeutic Nucleic Acid Analogs
The unmodified RNA structure is shown next to backbone (5′-phosphorothioate), LNA, and 2′-substitutions (2′-O-methoxy-ethyl, 2′-O-methyl, and 2′-fluoro).

Inhibitors of mRNA Translation: Antisense ONs

Sequence-specific antisense ONs inhibit gene expression by altering mRNA splicing, arresting mRNA translation, and inducing degradation of targeted mRNA by RNase H. Like other RNA- and DNA-derived therapeutics, antisense ONs often include chemical modifications to the backbone, base, or sugar to enhance the properties of the drug, such as PS backbones, 2′-O-Me, 2′-F, 2′-MOE, and LNA substitutions. A detailed survey of these technologies is beyond the scope of this review due to the wide variety of RNA and DNA-based antisense therapeutics. However, recent reviews have addressed the current status of clinical antisense drugs (Bennett and Swayze, 2010; Sanghvi, 2011).

Though most current antisense therapeutics target mRNA, one clinical drug employs antisense technology to inhibit an endogenous microRNA (miRNA). The dysregulation of endogenous miRNAs has been linked to numerous diseases, including many types of cancers (Garzon et al., 2010). Emerging therapeutic strategies to regulate miRNA activity include antisense-miRNA ONs (antagomirs) and RNA competitive inhibitors or decoys (miRNA sponges) (Ebert and Sharp, 2010). Santaris Pharma A/S developed miravirsen (SPC3649), a locked nucleic acid (LNA)-modified ON that specifically inhibits the endogenous microRNA-122 (miR-122), a liver specific miRNA required for the infection of Hepatitis C virus (HCV). Given the high mutation rate of HCV, miravirsen targets a critical host factor and thus may provide an elevated barrier for emergence of viral resistance (Lanford et al., 2010). Two completed Phase I trials (NCT00688012 and NCT00979927) have indicated that the drug is well tolerated and safe. Miravirsen has recently advanced into a Phase IIa clinical study (NCT01200420) to test safety, tolerability, and efficacy for treatment-naive patients with chronic HCV genotype 1 infection.

Agents of RNA Interference

The cellular process of RNA interference (RNAi) uses small RNAs to silence gene expression through posttranscriptional gene silencing (PTGS) or transcriptional gene silencing (TGS), though TGS pathway is not currently explored for clinical purposes (Castanotto and Rossi, 2009). PTGS is regulated by two distinct mechanisms: translational repression and degradation of mRNAs with imperfect complementarity, and sequence-specific cleavage of perfectly complementary mRNAs. Endogenous microRNAs (miRNAs) induce translational repression and mRNA degradation when the guide (antisense) strand has limited complementarity to the target mRNA. The sequence-specific cleavage mechanism is exploited by exogenous small interfering RNAs (siRNAs) or short hairpin RNAs (shRNAs) having perfect or near-perfect Watson-Crick base-pairing with the intended mRNA target. The production and processing of miRNAs requires an ensemble of host machinery that is ultimately guided by one of the two miRNA strands to the target mRNA (Figure 2). Likewise, siRNAs and shRNAs utilize many of the same endogenous factors, and siRNA/shRNA therapeutics may compete with the production/function of natural miRNAs.

In general, primary miRNAs (pri-miRNAs) are processed by a complex containing Drosha and DiGeorge syndrome critical region gene 8 (DGCR8) and incorporated into the pre-RISC complex with Dicer and TAR RNA-binding protein (TRBP) (Figure 2A; Han et al., 2006). Similarly, the Dicer/TRBP complex directs the processing of shRNA and double-stranded RNA (dsRNA) molecules into ~21–23 nt siRNAs (Figures 2B and 2C; Bernstein et al., 2001). One strand (guide or passenger) of the siRNA is loaded into RNA-Induced Silencing Complex (RISC) and may direct sequence-specific cleavage of mRNA by perfect or near-perfect Watson-Crick base-pairing (Martinez et al., 2002). The Argonaute 2 (AGO2) component of RISC contains an endonuclease activity that cleaves the target mRNA and the resulting mRNA fragments are destroyed by cellular exonucleases (Rand et al., 2005). While protected inside RISC, the guide siRNA strand can be repeatedly used to target other complementary mRNAs. These remarkable properties have prompted the widespread usage of synthetic siRNA molecules for the therapeutic knockdown of endogenous and viral mRNA (Davidson and McCray, 2011).

PTGS may be induced by delivering siRNA molecules to cells in dsRNA form or by shRNAs that are transcribed within the cell and processed into siRNAs (Figure 2). To mimic the Dicer cleavage products that are loaded into RISC, many RNAi applications in mammals commonly deliver synthetic siRNAs that are ~19–23 base pairs (bp) with 2 nt overhangs at both 3′ ends. However, this symmetric design often allows either strand (guide or passenger) to be selected into RISC. As an alternative strategy to bias the strand selection of

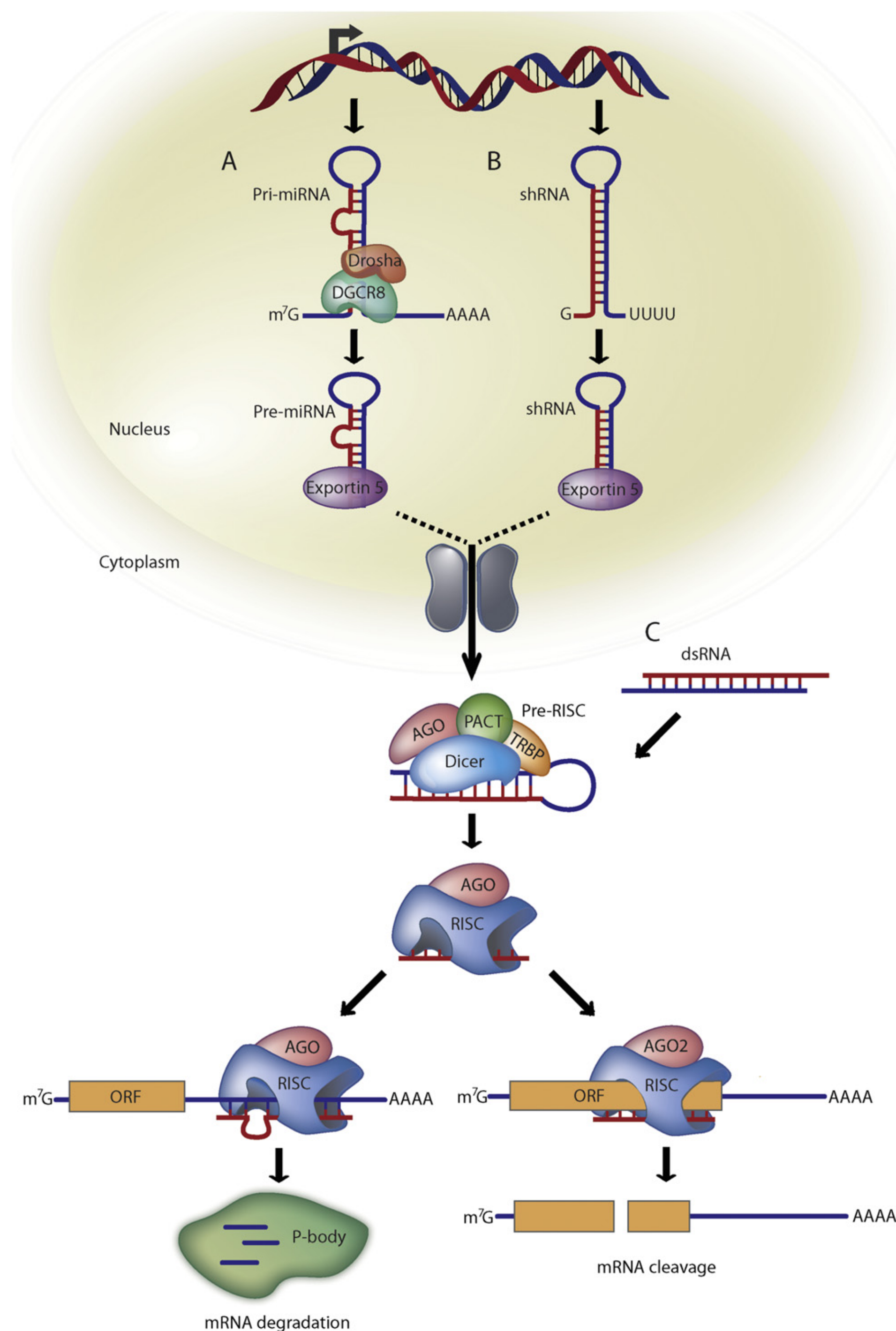

Figure 2. Mammalian PTGS Pathway for miRNAs, shRNAs, and siRNAs

(A) miRNAs are transcribed from DNA as primary miRNAs (pri-miRNAs) and processed into 70 nt stem-loop precursor miRNAs (pre-miRNAs) by Drosha and DGCR8. The pre-miRNAs are transported to the cytoplasm by dsRNA-binding protein exportin 5, where they are processed into ~22 nt miRNA duplexes by the Dicer/TRBP complex. The imperfectly complementary miRNA duplexes associate with an AGO protein and are loaded into RISC, where the passenger strand is removed and the guide strand remains to target mRNA for silencing. The resulting mature RISC complex may silence gene expression either by inhibiting the initiation of translation or by transporting the complex to cytoplasmic processing bodies (p-bodies) where the mRNA is deadenylated and destroyed.

(B) Like miRNAs, shRNAs are transcribed from DNA and undergo similar processing. However, the perfect Watson-Crick base-pairing between the guide strand and the target mRNA triggers AGO2-mediated cleavage of the mRNA target.

(C) In contrast to shRNAs, siRNAs are artificially introduced into the cytoplasm. All steps of siRNA and shRNA are the same after processing by Dicer/TRBP.

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RISC, the dsRNA may be designed asymmetrically with a 2 nt 3′ overhang at one end and a blunt end at the other (Kim et al., 2005). The resulting Dicer substrate (D-siRNA), with a 25 nt passenger strand and a 27 nt guide strand, directs the preferential biogenesis of the guide strand, thereby increasing the potency of the siRNA and decreasing off-target effects (Amarzguioui and Rossi, 2008).

In contrast to siRNAs, which transiently knock down gene expression after each drug treatment, shRNAs that are constitutively expressed from promoters can induce long-term gene silencing for the duration of their transcription and biogenesis. Although elevated levels of shRNAs may be desired to achieve maximum knockdown of the mRNA target, expression from strong RNA Pol III promoters can saturate the natural miRNA machinery resulting in severe toxicity (Castanotto et al., 2007; Grimm et al., 2010). To avoid saturation of the natural RNAi machinery, multiple shRNAs can be expressed as a multicistronic transcript from a RNA Pol II promoter or combined with other non-RNAi therapies like ribozymes and RNA decoys (Li et al., 2005; Zhang and Rossi, 2010).

Over the past decade, interest in siRNA/shRNA technologies has surpassed many of the antisense strategies due to variety of reasons, such as ability of siRNAs to elicit potent target-specific knockdown of any mRNA, ease of siRNA design and screening against the mRNA target, and achievement of long-lasting silencing as the siRNA can retain its catalytic activity in RISC for long periods (Castanotto and Rossi, 2009). Additionally, reduction of off-target toxicity and increased potency can be accomplished by designing siRNA as asymmetric Dicer substrates to bias the loading of the guide strand (Kim et al., 2005). Finally, unlike some antisense therapies, which act stoichiometrically on the mRNA target, siRNAs are constantly recycled after inducing mRNA cleavage. Moreover, with the expectation that siRNA-directed cleavage of the mRNA target occurs between nucleotides 10 and 11 relative to the 5′ end of the guide strand, 5′-rapid amplification of cDNA ends (5′-RACE) PCR is used to precisely confirm sequence-specific cleavage by AGO2 (Soutschek et al., 2004), an important consideration in validating that the siRNA is operating through the RNAi pathway (Alvarez et al., 2009; Davis et al., 2010; Neff et al., 2011).

Approximately 22 different siRNA/shRNA therapeutics have reached clinical testing for the treatment of at least 16 diseases (Table 1; Burnett et al., 2011). Similar to other RNA-based therapeutics, the efficacy of siRNA/shRNA drugs relies on maximizing targeted delivery while minimizing off-target toxicity and degradation. Delivery methods can be categorized as ex vivo, local or systemic, and systemic methods can be further classified as active (targeted) or passive (Peer and Lieberman, 2011). The current state of clinical trials using different siRNA/shRNA delivery methods are discussed in more detail below.

Local Delivery of siRNAs

Local delivery of siRNA is advantageous for tissues that are external and/or locally restricted including ocular, epidermal, pulmonary, colonic, and pancreatic tissue. Additionally, local delivery may be suitable for noninvasive therapies that require patient administration, such as eye drops and nasal sprays.

The treatment of vision loss in age-related macular degeneration (AMD) and diabetic macular edema (DME) using intravitreal (IVT) injections were some of the first clinical applications for siRNAs as these drugs can be delivered directly to ocular tissue to target well-characterized gene targets for these diseases (Lares et al., 2010). However, despite some initial successes, many of these approaches ended with disappointing results. These siRNAs were designed against the VEGF pathway to inhibit neovascularization leading to retinal edema and damage. Opko Health Inc. developed Bevasiranib, a 21 mer siRNA containing two deoxythymidine (dT) residues on both 3′ ends, that was designed to knockdown the mRNA of VEGF A. Although the therapy demonstrated some biological activity in Phase I and II trials (NCT00722384 and NCT00259753, respectively), the Phase III trial (NCT00499590) of Bevasiranib for AMD was terminated as a result of poor efficacy in reducing vision loss (Dejneka et al., 2008). Despite the completion of a Phase II trial (NCT00306904) using Bevasiranib in DME, no Phase III trial has been announced. Similarly, after the completion of a Phase I/II trial (NCT00363714), the Allergan siRNA AGN-745 against the VEGF receptor was discontinued in the Phase II trial (NCT00395057) due to an off-target effect (Cho et al., 2009; Kleinman et al., 2008). Quark Pharmaceuticals and Pfizer have tested the PF-655 siRNA therapeutic, which targets proangiogenic factor RTP801, in Phase I and II trials for AMD (NCT00725686 and NCT00713518, respectively) and a now-terminated Phase II trial for DME (NCT00701181). These siRNAs inadvertently activated Toll-like receptors (TLRs), leading to the suggestion that modifications to the backbone chemistry, the terminal nucleotides, and the particular siRNA sequences may reduce immunostimulatory responses (Samuel-Abraham and Leonard, 2010).

Quark Pharmaceuticals has developed the siRNA QPI-1007 with proprietary modifications to the siRNA structure and chemistry that maintains drug efficacy while reducing off-target effects. QPI-1007 is specifically designed to down modulate caspase-2, a key activator in the apoptotic cascade, as a treatment for optic nerve-related visual loss (NAION) (Guo et al., 2002). Preclinical studies suggest that QPI-1007 exhibits neuroprotective effects in animal models of NAION and glaucoma. A dose-escalated Phase I trial is currently in development (NCT01064505).

TransDerm, along with the International Pachyonychia Congenita Consortium (IPCC), has designed the first mutation-specific siRNA to be used for human therapy. The TD101 siRNA is directed at the mRNA sequence encompassing the dominant mutation (N171K) in the *keratin 6a* gene (KRT6A). This mutation causes pachyonychia congenita, a rare skin disorder characterized by painful calluses on weight-bearing areas and hypertrophic nails among other epidermal defects. The siRNA therapy was administered by intralesional injection in a single patient using a split body control. Since the Phase Ib therapy (NCT00716014) was well tolerated and efficacious in reducing the callus, TransDerm is developing less painful alternatives for delivering the drug (Leachman et al., 2010), such as an ointment with lipid-based carriers (GeneCreme) and a dissolvable microneedle array (Protrusion Array Device).

Sylentis developed an siRNA drug (SYL040012) against the beta-2 adrenergic receptor (ADRB2) to inhibit the production of aqueous humor and thereby relieve intraocular hypertension (Vaishnaw et al., 2010). The eye drop mode of delivery helps

Table 1. Anti-miRNA and siRNA/shRNA Therapeutics in Clinical Trials

Company	Drug	Delivery Route	Target	Vehicle	Disease	Phase	Status
Santaris	SPC3649 (LNA)	SC	miR-122	Naked LNA	HCV	IIa	Ongoing
Opko Health	Bevasiranib	IVT	VEGF	Naked siRNA	AMD/DME	III	Terminated
Allergan/ Sirna	AGN-745	IVT	VEGF-R1	Naked siRNA	AMD	II	Terminated
Quark/Pfizer	PF-655	IVT	RTP801	Naked siRNA	AMD/DME	II	Completed
Quark Pharma	QPI- 1007	IVT	Caspase 2	Naked siRNA	NAION	I	Ongoing
TransDerm/ IPCC	TD101	Intralesional injection	KRT6A(N171K)	Naked siRNA	Pachyonychia Congenita	Ib	Completed
Sylentis	SYL040012	Ophthalmic drops	ADRB2	Naked siRNA	Intraocular Pressure	II	Ongoing
Sylentis	SYL1001	Ophthalmic drops	TRPV1	Naked siRNA	Dry eye syndrome	I	Ongoing
ZaBeCor	Excellair	Inhalation	Syk kinase	unknown	Asthma	II	Ongoing
Alnylam/ Cubist	ALN-RSV01	Nebulization or intransal	RSV Nucleocapsid	Naked siRNA	RSV	IIb	Ongoing
Marina Biotech	CEQ508	Oral	Beta catenin	tkRNAi in E. Coli	FAP/ colon cancer	I	Ongoing
Silenseed Ltd	siG12D LODER	EUS biopsy needle	KRASG12D	LODER polymer	PDAC	I	Ongoing
Tekmira	TKM-ApoB	IV	Apo B	SNALP	Hypercholesterolemia	I	Terminated
Tekmira	TKM-PLK1	IV	PLK1	SNALP	Solid tumors	I	Ongoing
Alnylam/ Tekmira	ALN-VSP02	IV	KSP and VEGF	SNALP	Solid tumors	I	Completed
Alnylam	ALN-TTR01	IV	TTR	SNALP	TTR-mediated amyloidosis (ATTR)	I	Ongoing
University Duisburg	Bcr-Abl siRNA	IV	Bcr-Abl	Anionic liposome	CML	I	Completed
Silence Therapeutics	Atu027	IV	PKN3	siRNA-lipoplex	Advanced solid cancer	I	Ongoing
Quark Pharma	I5NP	IV	P53	Naked siRNA	AKI and DGF	II	Ongoing
Calando Pharma	CALAA-01	IV	RRM2	Cyclodextrin nanoparticle, TF, and PEG	Solid tumors	I	Ongoing
Gradalis Inc.	FANG vaccine	Ex vivo IV	Furin and GM-CSF	Electroporation	Solid tumors	II	Ongoing
Duke University	iPsiRNA	Ex vivo intradermal injection	LMP2, LMP7, MECL1	Transfection	Metastatic melanoma	I	Ongoing
City of Hope/ Benitec	Tat/Rev shRNA	Ex vivo transplant	HIV Tat and Rev	Lentivirus	HIV	0	Ongoing

to direct SYL040012 to the affected ciliary epithelium. An initial Phase I trial (NCT00990743) evaluated the safety of SYL040012 in patients with ocular hypertension and glaucoma, while a scheduled Phase I/II study (NCT01227291) will continue to evaluate the tolerance and efficacy of this drug. In September 2011, Sylentis announced a Phase I trial (NCT01438281) for their second siRNA drug (SYL1001) for the treatment of ocular pain associated with "dry eye" syndrome. SYL1001 triggers the knockdown of transient receptor potential cation channel subfamily V member 1 (TRPV1), also known as the capsaicin receptor, on the ocular surface, which alleviates ocular surface irritation, inflammation, and pain in animal models.

ZaBeCor Pharmaceuticals reported improvements in asthmatic symptoms for patients treated with Excellair in a Phase I study (Burnett et al., 2011). This siRNA drug inhibits spleen tyrosine kinase (Syk), which is involved in activating proinflammatory transcription factors. These benefits have helped usher Excellair into a Phase II trial.

Alnylam has developed a siRNA therapeutic (ALN-RSV01) against the respiratory syncytial virus (RSV) nucleocapsid (N) protein for prophylaxis against RSV infections in healthy patients (NCT00496821) and for treating RSV infections in lung transplant patients (NCT00658086 and NCT01065935). The drug is composed of a double-stranded RNA duplex with 19 base pairs of complementarity and 2 nt dT overhangs at both 3′ ends (Alvarez et al., 2009). Nasal spray and electronic nebulizer aid the delivery of the therapeutics to healthy or lung transplant patients, respectively. The antiviral effect of ALN-RSV01 was demonstrated by a reduced infection rate in healthy patients and by alleviating the daily symptoms in transplant patients (DeVincenzo et al., 2010; Zamora et al., 2011). Although the clinical trials have not yet shown direct evidence for a human

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antiviral RNAi mechanism, such mechanism is supported by animal studies (Alvarez et al., 2009).

Marina Biotech has launched a Phase I trial of the first orally administered shRNA drug for treating familial adenomatous polyposis (FAP), a rare hereditary disease that often leads to colon cancer. The CEQ508 shRNA therapy downregulates β-catenin to slow the polyp growth in intestinal cells (Xiang et al., 2009). The drug is encapsulated by the company's TransKingdom RNA interference (*tk*RNAi) technology, which utilizes nonpathogenic *Escherichia coli* to produce and deliver the shRNAs to target cells (Nguyen and Fruehauf, 2009). The bacterial vector is coated with Invasin protein from *Yersinia pseudotuberculosis* to facilitate the entry of the bacterial carrier into intestinal cells expressing the β-1 integrin receptor. Finally, the vector encodes lysteriolysin O pore forming protein (from *Listeria monocytogenes*) to permit the shRNA to escape the bacterial vehicle and enter the cytoplasm.

Silenseed Ltd is launching a Phase 0/I trial (NCT01188785) to evaluate an siRNA drug (siG12D) that targets somatic mutations in the KRAS oncogene (KRASG12D) for pancreatic ductal adenocarcinoma (PDAC). An endoscopic ultrasound (EUS) needle is used to inject the siRNA drug directly into the tumor. The siRNA is encapsulated in the Local Drug EluteR (LODER) biodegradable polymer, which enables slow release of the siRNA therapeutic over an 8 week period (Burnett et al., 2011).

Systemic Delivery of siRNAs

Upon intravenous (IV) injection, unmodified siRNAs tend to accumulate in the kidneys, whereas siRNAs encapsulated in liposomes and nanoparticles often become trapped in the liver. Thus, siRNA therapeutics designed for these tissues can be delivered by nontargeted (passive) systemic delivery, in which uptake of the therapeutics relies on the filtering organs of the reticuloendothelial system (Peer and Lieberman, 2011). For applications that require targeted systemic delivery, synthetic carriers may be decorated with cell-specific ligands or aptamers that allow receptor-mediated uptake (Bartlett et al., 2007), and biodegradable nanoparticle carriers allow for slow drug release once inside the cell (Li et al., 2010).

Tekmira Pharmaceuticals has developed two distinct siRNA drugs that are encapsulated in the stable nucleic acid lipid particle (SNALP). SNALP is a first generation lipid nanoparticle developed by Tekmira that is designed to deliver the siRNA to the targeted tissue by IV injection. The first drug (TKM-ApoB or PRO-040201) is an siRNA that targets the mRNA of ApoB and is designed to indirectly reduce the uptake of cholesterol in cells. A total of 17 patients received TKM-ApoB and one of the two that received the highest dosage of the drug exhibited flu-like symptoms that were consistent with siRNA induced immune stimulation (Burnett et al., 2011). Although the drug did not show evidence of toxicity in the liver, the Phase I clinical study (NCT00927459) was terminated because patients exhibited only transient reductions in cholesterol levels (Watts and Corey, 2010). Nonetheless, the company is working on improvements to the nanoparticle carriers and siRNA design. The second drug known as TKM-PLK1, which targets polo-like kinase 1 (PLK1), will be tested in two Phase I trials (NCT01262235 and NCT01437007) for patients with advanced solid tumors that are resistant to current therapies since downmodulation of PLK1 levels prevents cell cycle progression into mitosis and induces apoptosis in tumor cells (Reagan-Shaw and Ahmad, 2005).

Alnylam Pharmaceuticals has partnered with Tekmira for the use of SNALP carriers to package siRNA therapies for two diseases, liver cancer and transthyretin (TTR)-mediated amyloidosis (ATTR). To treat hepatocellular carcinoma, Alnylam generated a therapeutic (ALN-VSP02) with two distinct siRNAs against vascular endothelial growth factor (VEGF) and kinesin spindle protein (KSP). The therapy was generally well tolerated in a Phase I trial (NCT00882180), and an anti-VEGF effect was observed in most patients and confirmed (Vaishnaw et al., 2011). Long-term follow up of patients treated with ALN-VSP02 continues in a second Phase I trial (NCT01158079). Additionally, Alnylam has initiated Phase I study (NCT01148953) to determine the safety and tolerability of siRNA treatment for ATTR, ALN-TTR01. The ALN-TTR01 siRNA targets the *ttr* mRNA to reduce the accumulation of amyloid deposits in surrounding tissues (Vaishnaw et al., 2010). Since TTR is mainly expressed in the liver, Alnylam has teamed with Tekmira to design SNALP carriers with high affinity for hepatocytes. In November 2011, Alnylam announced that ATTR patients receiving ALN-TTR01 exhibited a statistically significant reduction in serum TTR protein levels that was dose dependent and durable after a single dose.

Taking a similar approach to the Tekmira-designed cationic SNALP carriers, multiple clinical trials utilize other design strategies for encapsulating the siRNA therapeutics. A Phase I trial sponsored by the University of Duisburg-Essen (Germany) incorporated an siRNA drug against *bcr-abl* with anionic liposomes. *BCR-ABL* is a fusion oncogene uniquely expressed in chronic myeloid leukemia (CML) resulting from a chromosomal defect. Despite transient knockdown of the *bcr-abl* fusion, the inhibition of the oncogene mRNA was not maintained in patients (Koldehoff et al., 2007). Silence Therapeutics is conducting a Phase I trial (NCT00938574) for the treatment of advanced solid cancers. The Atu027 siRNA is designed to inhibit protein kinase N3 (PKN3), a downstream target of the phosphoinositide 3-kinase (PI3K) signaling pathway that mediates metastasis of cancer cells (Santel et al., 2010). The Atu027 therapeutic is formulated as an siRNA-lipoplex, a complex with negatively charged nucleic acids and cationic lipids, known as AtuPLEX.

Quark Pharmaceuticals has opted to deliver uncoated siRNA therapeutics to the kidney for preventing acute kidney injury (AKI) and delayed graft function (DGF). The natural pathway of excretion by the kidneys allows internalization of the siRNAs making this tissue a tractable target for siRNA therapies. A Phase I trial indicated that the siRNA I5NP (or QPI-1002) temporarily suppresses the proapoptotic p53 protein as prophylaxis for AKI postcardiovascular surgeries (NCT00554359). This strategy is currently in a Phase I/II trial for DGF after kidney transplants (NCT00802347).

Calando Pharmaceuticals tested the first receptor-mediated delivery of siRNA nanoparticles as treatment for relapsed/refractory cancers. In this Phase I trial (NCT00689065), the siRNA is complexed in cyclodextrin nanoparticles that are coated with polyethylene glycol (PEG) for stability and the human transferrin (TF) protein for receptor mediated uptake via the transferrin receptor, which is often highly expressed in tumor cells. The siRNA CALAA-01 is directed against the M2 subunit of ribonucleotide reductase (RRM2), which is essential in providing the

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ribonucleotide pool for DNA synthesis and repair. Interestingly, this landmark study also demonstrated first evidence for RNAi mechanism in humans as the investigators verified the occurrence of siRNA-induced cleavage of the target RRM2 mRNA (Davis et al., 2010).

Ex Vivo Delivery of siRNA/shRNA

Delivery of siRNA/shRNA via bacterial or viral carriers is often performed ex vivo, as the targeted cells are collected, modified, and reinfused back into the patient. This delivery method is often preferred when simultaneous delivery and expression of multiple therapeutic genes (mRNAs, ribozymes, aptamers, etc.) are required and/or when a specific cell type (generally leukocytic lineages) is targeted for therapeutic gene applications.

Two autologous immune cell therapies for cancer combine an siRNA/shRNA therapeutic with the expression of a recombinant gene or codelivery of therapeutic mRNA. Gradalis Inc. is treating advanced solid cancers, including stage IIIc ovarian cancer, in Phase I (NCT01061840) and Phase II trials (NCT01309230) by expressing recombinant granulocyte-macrophage colony stimulating factor (GM-CSF) and a bifunctional shRNA against Furin (bi-shRNA^{furin}) in the FANG Vaccine (Maples et al., 2010). Both stem loops in the bi-shRNA^{furin} target the *furin* mRNA, but one contains a perfectly complementary guide strand that induces mRNA cleavage whereas the other has a guide strand that is mismatched to the target 3′ UTR and therefore functions as an miRNA. Downregulation of Furin indirectly reduces the transforming growth factor (TGF)-β1 and TGF-β2 isoforms that contribute to diminished T cell responsiveness in tumor cells. GM-CSF overexpression induces differentiation of dendritic cells (DCs) antigen presentation. Therefore, the combined downregulation of TGF-β isoforms and overexpression of GM-CSF is designed to mobilize the patient's immune cells to eradicate malignant cells.

A similar Phase I study (NCT00672542) conducted by Duke University is administering the siRNA/mRNA therapy for metastatic melanoma. Autologous DCs harvested from patients are transfected with siRNAs against immunoproteasome subunits LMP2, LMP7, and MECL1 (iPsiRNA) and subsequently transfected with melanoma antigens MART, MAGE-3, gp100, and tyrosinase (Dannull et al., 2007). The downregulation of immunoproteasomes is believed to enhance presentation of melanoma antigens by DCs. By boosting proteasome-mediated antigen presentation in autologous DCs, this strategy enhances the immune response toward melanoma cells.

The City of Hope, in partnership with Benitec, Inc., has conducted an all RNA based gene therapy human pilot feasibility (Phase 0) study for patients with AIDS-related non-Hodgkin's lymphoma (NHL) (DiGiusto et al., 2010). The four patient cohort consisted of patients requiring transplantation of autologous CD34+ hematopoietic progenitor cells (HPCs) for the treatment of NHL. However, to protect the transplanted progenitor cells, and particularly the eventual subset of differentiated CD4+ T cells, from HIV infection, a fraction of the cells were transduced ex vivo with a replication incompetent lentiviral vector that encoded three anti-HIV small RNAs (pHIV7-shI-TAR-CCR5RZ). The three small RNAs are each expressed from separate RNA polymerase III promoters and are designed to inhibit infection and/or replication of HIV-1 by a distinct mechanism: (1) an anti-CCR5 ribozyme intended to block viral entry, (2) an shRNA designed to destroy viral mRNA, and (3) an RNA hairpin known as the transactivating region (TAR) decoy that antagonizes viral transactivation (Li et al., 2005). The shRNA component is designed to induce RISC-mediated cleavage of viral mRNA at a site with overlapping, frame-shifted reading frames of Tat and Rev, which mediate transactivation of viral gene expression and nuclear export of viral mRNA transcripts, respectively. The therapy was well tolerated and genetic marking of the siRNA was detected in primary blood mononuclear cells (PBMCs) and/or primary blood granulocytic cells (PBGCs) of all patients up to 6 months after treatment, including at least 24 months in one patient (DiGiusto et al., 2010). A second clinical study with the same AIDS-related NHL cohort is scheduled to begin in early 2012 (Burnett et al., 2011).

Ligand RNAs: Aptamers and Decoys

Aptamers are single-stranded nucleic acids that bind to molecular targets with high affinity and specificity due to their stable-three dimensional shapes (Bouchard et al., 2010). Many aptamers exist as hairpin-like monomers that bind targets via unpaired nucleotides, but some aptamers function as duplexes (Huang et al., 2003), triplexes (Sussman and Wilson, 2000), or quadruplexes (Mashima et al., 2009; Phan et al., 2011). RNA and DNA aptamers are typically identified for a particular function through multiple rounds of in vitro or cell-based selection in a process known as systematic evolution of ligands by exponential enrichment (SELEX) (Ellington and Szostak, 1990; Tuerk and Gold, 1990). Using SELEX, aptamers of 20–100 nucleotides (nt) can be selected from libraries (up to $10^{\sim15}$ unique sequences) to bind with high affinity to a wide array of protein families to modulate the protein function similar to antibodies (Keefe et al., 2010). Like other RNA therapeutics, RNA aptamers are often modified during chemical synthesis to increase their resistance to nucleases and improve pharmacological properties. These modifications include 2′-F, 2′-OMe, and LNA sugar substitutions or the spiegelmer form of the aptamer. Additionally, aptamers may be conjugated with cholesterol or polyethylene glycol (PEG) to reduce renal filtration (Keefe et al., 2010).

While the affinity and specificity of RNA aptamers for their target ligands rival the properties of antibodies, aptamers offer several advantages over their protein counterparts. Aptamers are evolved and identified in vitro using SELEX, and can be reproducibly and economically synthesized in large scale for clinical applications. Using chemical substitutions and other modifications (including L-RNA), aptamers elicit minimal immunogenicity relative to antibodies. The small size of aptamers allows for improved transport and tissue penetration compared to antibodies. Finally, aptamers are amenable to applications that require engineering, such as the conjugation of aptamers to ribozymes (riboswitches) and aptamer-siRNA chimeras.

There are at least 6 RNA-based aptamers or decoys that have been clinically tested (Table 2), including a VEGF-specific modified RNA aptamer (Macugen by Pfizer/Eyetech) that is now an FDA approved drug for the treatment of AMD (Keefe et al., 2010; Sanghvi, 2011; Thiel and Giangrande, 2009). In addition to their antibody-like abilities to inhibit or activate the functions of protein targets, aptamers also offer novel functions as therapeutic and diagnostic agents. By utilizing Watson-Crick pairing of nucleic acids, RNA aptamers can be engineered to undergo conformational changes in the presence and/or

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Table 2. Aptamers and Decoys in Clinical Trials

Company	Drug	Route	Target	Modification(s)	Disease	Phase	Status
Eyetech / Pfizer	Pegaptanib sodium (Macugen)	IVT	VEGF	2′-OMe purine/2′-F pyrimidine with two 2′-ribo purines conjugated to 40 kDa PEG, 3′ inverted dT	AMD	FDA approved	Approved
Archemix Corp.	ARC19499 (BAX499)	IV and SC	Tissue factor pathway inhibitor (TFPI)	Unknown	Hemophilia	II	Not yet recruiting
Regado Biosciences	REG1 (RB006 & RB007)	IV	Factor IXa	2′-Ribo purine/2′-F pyrimidine (RB006); PEG and 2′-Ome antidote (RB007)	ACS	II	Completed
Ophthotech	ARC1905	IVT	Complement component 5 (C5)	2′-Ribo purine/2′-F pyrimidine conjugated to 40 kDa PEG, 3′ inverted dT	AMD	I	Ongoing
City of Hope / Benitec	TAR decoy	Ex vivo transplant	HIV Tat protein	U6 snoRNA domain	HIV	0	Ongoing
Childrens Hospital Los Angeles	RRE decoy	Ex vivo transplant	HIV Rev protein	Expressed by HIV promoter	HIV	0	Ongoing

absence of other effector RNAs both in vitro and in vivo. Such a strategy has been tested in two Phase II trials (NCT00932100 and NCT00113997) with the REG1 (by Regado Biosciences) dual-aptamer therapy for acute coronary syndrome (ACS). Using this therapy, the first aptamer RNA aptamer (RB006 or pegnivacogin) is administered to bind coagulation factor IXa, but after the subsequent injection of the second "antidote" aptamer (RB007 or anivamersen), RB006 binds to RB007 thereby releasing it from its factor IXa target (Cohen et al., 2010). Also, aptamers can be combined with other types of therapeutic agents to serve as a delivery vehicle for siRNAs (McNamara et al., 2006; Neff et al., 2011; Pastor et al., 2010), enzymes (Chen et al., 2008), and anticancer drugs (Hicke et al., 2006; Hwang et al., 2010). Finally, although no clinical applications have yet been demonstrated, RNA aptamers can be engineered to form self-organizing RNA scaffolds (Delebecque et al., 2011), combined with ribozymes to create "riboswitches" (Serganov and Patel, 2007), or conjugated to miRNAs to create ligand-responsive miRNAs (Beisel et al., 2011). While many of these therapeutic aptamers consist of RNA-based molecules with 2′-OMe and/or 2′-F substitutions and PEG conjugation, numerous examples of DNA and spiegelmer aptamers are also in the clinic (Keefe et al., 2010).

While the SELEX technique enables the discovery of novel RNA and DNA aptamers, mimics of protein-binding RNAs can be used as therapeutic decoys. Like RNA aptamers, RNA decoys bind target proteins due to their three-dimensional structure. Two examples of clinically tested RNA decoys have both been used to inhibit HIV-1 replication (Haasnoot and Berkhout, 2009). The Rev response element (RRE) decoy is composed of the 41 nt RRE portion of HIV-1 transcripts, which is a hairpin-like structure that binds to the viral Rev protein (Kohn et al., 1999). This therapeutic RNA is expressed from a retroviral vector following ex vivo transduction and reinfusion of CD34+ HPCs. A similar anti-HIV decoy is composed of the transactivating region (TAR) hairpin at the 5′ end of viral mRNA transcripts that recruits and binds the viral Tat protein (DiGiusto et al., 2010). While the natural TAR transcript is located in the nucleus at the site of viral transcription, the TAR decoy is designed to translocate to the nucleolus, where it binds and sequesters the Tat protein from its natural target (Li et al., 2005). As described earlier, this therapy also includes an shRNA and a ribozyme and is expressed from a lentiviral vector (pHIV7-shI-TAR-CCR5RZ) in autologous CD34+ HPCs.

Catalytic RNAs: Ribozymes

Ribozymes are catalytic RNAs that function as enzymes and do not require proteins for catalysis. Most known natural ribozymes are self-processing RNAs that catalyze RNA cleavage and ligation reactions. However, the substrate recognition domain of ribozymes can be artificially engineered to stimulate site-specific cleavage in *cis* (the same nucleic acid strand) or *trans* (a noncovalently linked nucleic acid) (Scherer and Rossi, 2003). Moreover, ribozymes are amenable to in vitro selection and directed evolution to generate improved properties and new functions for therapeutic and diagnostic reagents. Ribozymes can be engineered to be allosterically activated by effector molecules, which has led to the development of artificial "riboswitches" as biosensors and synthetic biological tools (Liang et al., 2011; Wieland et al., 2010). There are numerous types of ribozymes in biology, but the most common ribozyme therapeutics are derived from either "hammerhead" or "hairpin/paperclip" motifs.

Like siRNA/shRNA therapeutics, ribozymes can either be delivered to the target cells in RNA form or can be transcribed from therapeutic genes (Table 3). Due to poor stability of fully-RNA ribozymes, therapies that rely on direct delivery often require chemically stabilized ribozymes, including the following modifications: 5′-PS backbone linkage, 2′-*O*-Me, 2′-deoxy-2′-*C*-allyl uridine, and terminal inverted 3′-3′ deoxyabasic nucleotides. All of these modifications were incorporated for Angiozyme (RPI.4610), the first synthetic ribozyme tested in clinical trials (Kobayashi et al., 2005). Currently licensed by Merck-Sirna, Angiozyme is a ribozyme that targets the mRNA of the vascular endothelial growth factor receptor-1 (VEGFR-1)

Table 3. Ribozymes in Clinical Trials

Company	Drug	Delivery Route	Target	Modification(s)	Disease	Phase	Status
Merck-Sirna	Angiozyme	SC	VEGFR-1	5′-PS, 2′-O-Me, 2′-deoxy-2′-C-allyl uridine, inveted 3′-3′ dT	renal cancer	II	Completed
Merck-Sirna	Heptazyme	SC	HCV IRES	5′-PS, 2′-O-Me, 2′-deoxy-2′-C-allyl uridine, inveted 3′-3′ dT	HCV	II	Terminated
UCSD	MY-2	Ex vivo, autologous CD4+ T cells	HIV U5 and pol	Expressed in MMLV vector	HIV	I	Completed
Johnson & Johnson, St. Vincent's Hospital	RRz1	Ex vivo, syngeneic CD4+ T cells	HIV Tat and Vpr	Expressed in MMLV vector	HIV	I	Completed
Janssen-Cilag Pty Ltd, UCLA	OZ1 (RRz1)	Ex vivo, autologous HPCs	HIV Tat and Vpr	Expressed in MMLV vector	HIV	II	Ongoing
City of Hope, Benitec	CCR5 ribozyme	Ex vivo, autologous HPCs	CCR5	Expressed in lentiviral vector	HIV	0	Ongoing
Ribozyme, City of Hope	L-TR/Tat-neo	Ex vivo, autologous HPCs	HIV Tat and Rev	Expressed in MMLV vector	HIV	II	Completed

to block angiogenesis and tumor growth. A Phase I trial successfully demonstrated no pharmacokinetic interactions between Angiozyme and chemotherapeutic agents carboplatin and paclitaxel for 12 patients with advanced solid tumors (Kobayashi et al., 2005). Another Phase I study evaluated the maximum tolerated dose, pharmacokinetic variables, pharmacodynamic markers, clinical response, and safety of daily subcutaneous (SC) injection of Angiozyme for 28 patients with refractory solid tumors (Weng et al., 2005). A Phase II trial (NCT00021021) for patients with metastatic renal cancer was completed in 2009, though details of this trial have not been published. The same company also developed Heptazyme, a synthetic ribozyme against hepatitis C virus (HCV). However, despite encouraging results in Phase I and II trials (Sandberg et al., 2001; Tong et al., 2002), this drug was discontinued after the observation of vision loss in one animal during simultaneous testing in nonhuman primates (Berk, 2006).

Several ribozymes against HIV have been clinically tested using a gene therapy-based approach in CD4+ T cells or CD34+ hematopoietic stem cells (HSCs), which differentiate into various hematopoietic lineages including CD4+ T cells (Amado et al., 2004; Macpherson et al., 2005; Michienzi et al., 2003; Wong-Staal et al., 1998). Since HIV-1 preferentially infects CD4+ T cells, the therapeutically modified CD4+ cells would be protected from producing functional HIV-1 virus. Each of these trials used either autologous (patient's own cells) or syngeneic (cells from identical twin) cell therapy, in which the cells are harvested from the patient or healthy twin, treated with the ribozyme-embedded gene therapy, and reinfused back into the patient. Retroviral or lentiviral gene vectors were utilized for these trials, which facilitates integration of the therapeutic genes into the host genome and long-term gene expression after integration. While all of these trials have demonstrated the safety and feasibility of gene-delivered ribozyme therapy, none has proven a clear survival advantage for the protected cells versus the empty vector (control) transduced cells. This might be due to the poor engraftment of transduced cells, limited efficacy of the therapeutic ribozyme, chromatin silencing of the integrated ribozyme, suboptimal ribozyme kinetics, or other possible factors (Burnett and Rossi, 2009).

As mentioned earlier in the siRNA/shRNA section, a Phase 0 clinical study at the City of Hope uses gene-modified autologous CD34+ HPCs to deliver three RNA-based for the treatment of HIV-1 (DiGiusto et al., 2010). In addition to the shRNA and the TAR decoy encoded in the lentiviral gene vector, this therapeutic expresses a hammerhead ribozyme that cleaves the mRNA of the chemokine receptor 5 (CCR5) protein (Li et al., 2005). The CCR5 receptor is expressed on a subset of CD4+ T cells and serves as a coreceptor for HIV-1 infection. However, CCR5 is not essential for normal T cell function and offers an attractive target for anti-HIV therapeutics since, unlike many viral targets, it is not prone to mutational escape.

Design and Delivery

In the development of RNA-based therapeutics, in vitro and animal studies have specifically and efficiently treated infectious diseases, gene disorders, and cancers by using siRNA/shRNAs to induce PTGS, ribozymes to cleave mRNA transcripts, and aptamers to bind and block targeted proteins. However, these therapies have encountered obstacles in clinical testing, including the efficiency and specificity of delivery, the stability of the RNA drug, the minimization of immune stimulation, and the prolonged duration of the drug. These issues have raised serious concerns for several RNA-based drugs and turmoil within the RNAi industry. In particular, Bevasiranib and AGN-745, two intravitreally injected naked siRNAs for the treatment of AMD and DME, were terminated due to the lack of patient improvement and TLR-mediated inflammation (Dejneka et al., 2008; Kleinman et al., 2008). Other RNA-based drugs, including the Bcr-Abl and TKM-ApoB siRNAs and several anti-HIV ribozymes, have been discontinued due to insufficient in vivo drug efficacy (Burnett and Rossi, 2009; Koldehoff et al., 2007; Tiemann and Rossi, 2009). Therefore, the future progress of RNA therapeutics relies heavily on improvements in the design of RNA drugs and delivery technologies that can improve drug efficacy and minimize off-target effects.

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As discussed, local delivery of naked siRNAs or aptamers, typically preferred for lung, eye, and skin applications, may generate a proinflammatory response due to activation of TLRs (Cho et al., 2009; Kleinman et al., 2008) and suffer from poor cellular uptake and nuclease sensitivity of naked siRNAs. In some cases, these problems can be alleviated encapsulating the nucleic acid with a synthetic carrier, or introducing chemical modifications to ONs, including clinically promising LNA and spiegelmer conversions, which are expected to improve the specificity, stability, and immunoresistance of RNA-based drugs.

A major benefit of synthetic RNA drug carriers is the ability to engineer tissue specificity in local or systemic delivery applications, thereby preventing nonspecific delivery and degradation of the drug during transport. Biodegradable polymers, such as the LODER delivery system and the Calando cyclodextrin carrier, can be engineered to release the RNA drug over a localized tissue area for a controllable duration. Carriers designed from liposomes tend to accumulate in the liver, which is the intended target for the SNALP-encapsulated siRNAs ALN-TTR01 and ALN-VSP02.

Ex vivo delivery is ideal for bacterial or lentiviral vectors that express shRNAs, ribozymes, and/or aptamer-like RNA decoys. This delivery method is the most direct method of introducing therapeutic genes, though it is limited to certain cell types and patient cohorts. However, as observed with the cell-based gene therapy strategies of anti-HIV ribozymes, the efficacy of the therapy is dependent upon the success of the transplant. As an alternative, synthetic carriers can be modified to specifically deliver the RNA drug to desired cells or tissues. In particular, ligand-decorated nanoparticles (Davis et al., 2010) and aptamer-mediated siRNAs (Neff et al., 2011; Pastor et al., 2010) may increase drug efficiency while avoiding the effects of off-target toxicity.

In addition to improvements in drug delivery for RNA therapeutics, advances in drug design may also improve drug efficacy and reduce off-target toxicity. An example mentioned earlier, an asymmetric 25/27-R Dicer substrates that bias incorporation of the guide ssRNA stand into RISC, increases the drug potency and mitigate the off-target effects from loading of the passenger RNA strand (Kim et al., 2005). Additionally, dual-targeting siRNAs are designed so that both strands will be incorporated into RISC and separately target different mRNA transcripts with complete complementarity (Tiemann et al., 2010). In contrast to siRNAs, which tend to saturate the natural RNAi machinery and become toxic at high concentrations, ribozymes can be combined with each other or with another siRNA therapy to provide a multipronged approach. Other combinations of multiple RNA-based drugs, as with siRNA-aptamer chimeras, riboswitches, and gene therapy vectors, may offer the advantages of improving drug specificity, reducing the required drug dosage, and preventing disease resistance.

Future Prospects

While RNA-based therapeutics must overcome barriers in clinical testing for future success, results from previous trials have revealed important lessons. In general, siRNAs will require some chemical modifications to minimize nonspecific inflammation, whereas natural or synthetic carriers should be employed for efficient and tissue-specific delivery. Many of these considerations have contributed to encouraging clinical results for several siRNA drugs including CALAA-01, TD101, ALN-VSP02, and ALN-RSV01. While these examples hint at the potential of siRNA therapeutics, they also affirm the need for tailored carriers that specifically target the intended cells.

Like siRNAs, ribozymes and aptamers face similar challenges of delivery and off-target toxicity. These RNA drugs are amenable to chemical modification and, for some applications, delivery via gene therapy. The chemically modified and highly specific aptamer Macugen has gained FDA approval for the treatment of AMD, signifying the most notable success in RNA-based therapeutics to date. The REG1 dual-aptamer therapeutic highlights the versatility of RNA therapeutics, as one aptamer serves as a controlling mechanism for the therapeutic aptamer.

Collectively, these and other examples of RNA-based therapeutics have demonstrated early promise in the treatment of cancers, viruses, and genetic disorders. However, advanced delivery strategies are critical to fully harness the power of RNAi and the flexibility of RNA-based therapeutics. Engineered designs, such as aptamer-siRNA chimeras and transferrin-decorated nanoparticles, will continue to dramatically improve the precision of delivery for RNA drugs. Therefore, the future prospects of RNA-based drugs will require biochemical refinements to maximize drug potency while minimizing off target toxicity and immunogenicity.

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