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REVIEW ARTICLE

APTAMERS IN THE CLINIC

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ABSTRACT

In 1990, an RNA molecule was designed that bound to a nucleic acid binding protein to act as a decoy, thereby preventing HIV replication. That same year, two research groups identified a high-throughput method to select for nucleic acids to protein targets and the field of aptamer therapeutics was born. Over the last 20 years, numerous aptamers to therapeutic targets have been isolated and undergone in vitro *and ex vivo analysis before in vivo testing. Aptamer therapeutics represents a promising new class of agents to treat disease. The capability to extensively modify these compounds provides a potential broader spectrum of clinical applications than antibodies and small molecules. A brief historical review of aptamers is presented and the therapeutic aptamers currently in clinical development are described.*

INTRODUCTION

Aptamers are single-stranded nucleic acid molecules that bind to their targets by folding into a three-dimensional conformation similar to antibodies or small molecules. The name aptamer is derived from *aptus*, a Latin word meaning to fit and *meros*, a Greek word meaning a shape or repeating structure. The development of aptamers as a class of therapeutic molecules came from two main bodies of research: studies that showed that aptamers could inhibit the activity of target proteins and a high-throughput method of selecting nucleic acid ligands to virtually any protein.

Research into human immunodeficiency virus (HIV) 30 years ago revealed that small, structured RNA molecules had evolved that bound to viral or cellular proteins, regulating their activity (1, 2). In 1990, an RNA aptamer was designed to mimic the activity of endogenous TAR (*trans*-activating response) on the viral protein Tat, which plays a role in viral gene replication. This TAR aptamer bound to Tat instead of the viral TAR RNA and inhibited HIV replication (3, 4).

While RNA-mediated protein inhibition was being elucidated, another group of nucleic acid scientists demonstrated that large RNA libraries could be screened for ligands that bound to a target protein with high affinity and specificity (5, 6). Screening these large libraries of RNA was called systematic evolution of ligands by exponential enrichment or SELEX (6). The resulting nucleic acid ligands were called aptamers (5).

Aptamers have a number of advantages over antibodies or peptides, which include:

1. Binding to their target with high affinity and specificity. While antibodies bind with high affinity, they have variable specificity, aptamers on the other hand, can discriminate between targets that share a high degree of structural homology (7, 8). Small molecules, while having a high degree of specificity, generally do not achieve aptamer binding affinities, which are in the low picomolar to high nanomolar range.
2. Aptamers, rather than being "raised" like antibodies, have a system of selection that isolates relevant compounds in an efficient manner.
3. Aptamers can be chemically modified to tailor their bioavailability.
4. Aptamers have an unlimited shelf-life.
5. Antidotes can be designed to aptamers that tightly regulate their activity.

Since their initial discovery as potential candidates for protein inhibition, aptamers have been generated to a number of compounds (9-11). This review focuses on aptamers that are currently approved or are in clinical trials (Table I).

CLINICAL APTAMERS

Anticoagulation

Developing aptamers against coagulation factors is ideal, as they are largely extracellular proteins circulating in the bloodstream. There are several anticoagulant aptamers that are currently undergoing clinical trials. The first is an aptamer and matched antidote directed against factor IXa named REG1, a fluoro-modified and *O*-methylated RNA molecule with a polyethylene glycol (PEG) moiety. This is the only aptamer–antidote pair to date that has been tested in humans and represents a significant step forward in RNA-based therapies, as it is the first antidote-controlled anticoagulation drug

Table I. *Aptamers in clinical trials or approved for use.*

Target	Aptamer	Type	Indication	Clinical data
Antithrombotic				
FIIa	NU-172	DNA	PCI CABG	Phase I: increased coagulation parameters in healthy volunteers with return to baseline after discontinuation of i.v. infusion.
FIXa	REG1	2'-Fluoro RNA + PEG	PCI CABG	Phase Ia: increased aPTT in healthy volunteers, with return to baseline within 5 min after antidote administration. Phase Ib: increased aPTT in dose-dependent manner in patients with coronary artery disease. No hemorrhage or other major side effects.
Antiplatelet				
vWF	ARC-1779	DNA/RNA + PEG	CEA TMA/TTP	Phase I: bolus and continuous i.v. infusion in healthy volunteers resulted in > 95% of active vWF. PFA was increased dose-dependently No hemorrhage or other major side effects.
Antineoplastic				
nucleolin/NF-κB	AS-1411	DNA	Solid tumors	Phase I: dose-dependent studies in patients with solid tumors showed partial response in 1 patient and stable disease after 2-6 months in 67% of patients.
Ocular disease				
VEGF	Pegaptanib sodium	2'-Fluoro RNA	AMD	Phase I: of patients who received pegaptanib, 80% had stable or improved vision at 3 months and showed a three-line or greater improvement in visual testing. No major side effects of therapy. Phase II: compared with patients who received PDT only, 88% of patients receiving pegaptanib + PDT had stable or improved vision and 25% had three-line or greater improvement in vision testing. No major side effects of therapy. Phase III: double-blind, placebo-controlled study showed improvement in pegaptanib-treated patients compared with sham-treated patients. Currently approved to treat wet-AMD.
C5	ARC-1905	2'-Fluoro RNA	AMD	Currently in phase I.
PDGF	E-10030	DNA	AMD	Currently in phase I.

AMD = age-related macular degeneration, aPTT = activated partial thromboplastin time, C5 = complement factor 5, CABG = coronary artery bypass graft, CEA = carotid endarterectomy, F = factor, i.v. = intravenous, NF-κB = nuclear factor-κB, PCI = percutaneous coronary intervention, PDGF = platelet-derived growth factor, PDT = photodynamic therapy, PEG = polyethylene glycol, PFA = platelet function analyzer, TMA = thrombotic microangiopathies, TTP = thrombocytopenic purpura, VEGF = vascular endothelial growth factor, vWF = von Willebrand factor.

since heparin-protamine. Heparin has been the principal anticoagulant used, in part because protamine can reverse its activity. Unfortunately, heparin-protamine therapy has been hampered by adverse side effects from both drugs. Heparin molecules range from 15 to 30 kDa and the different sizes of heparin in each batch bind to different targets that can result in variability in anticoagulation potency (12, 13). In addition, 3-5% of patients develop heparin-induced thrombocytopenia, which can result in dangerously low platelet levels (14). Of these patients, 30% go on to develop life-threatening heparin-induced thrombocytopenia with thrombosis. Protamine use can result in pulmonary hypertension, systemic hypotension and depressed myocontractility (15-17). The goal of antidote-controlled aptamers in this clinical scenario is to provide tight control of coagulation without the side effect profile of heparin-protamine. There was a dose-dependent increase in activated partial thromboplastin time (aPTT) following administration of the aptamer REG1 in a phase Ia trial of healthy volunteers (18). Moreover, a return of the clotting parameter to baseline was demonstrated within 5 min of antidote administration. A phase Ib study was completed that tested the drug in patients with stable coronary artery disease who were either taking aspirin or clopidogrel. These patients were randomized to receive REG1 at one of four doses. The results revealed a dose-dependent increase in aPTT, with return to baseline following administration of the antidote (19). There were no reported incidences of hemorrhage or major side effects from drug or antidote administration. A subsequent trial tested the aptamer and antidote in repeat doses in healthy volunteers. The aPTT increased with administration of the drug and returned to baseline with antidote

administration. There was very little interdose variability and no adverse effects from repeat administration (20). REG1 is currently undergoing phase II trials to assess its potential in coronary artery bypass graft surgery (CABG) and percutaneous coronary intervention (PCI).

Thrombin is a natural target for anticoagulation and numerous aptamers have been generated with variable effect to inhibit thrombin activity in vitro (21, 22). Archemix Corp. is a biopharmaceutical company with a main focus on nucleic acid therapeutics. In collaboration with ARCA Biopharma Inc. (formerly Nuvelo Inc.), researchers at Archemix have tested NU-172, a DNA aptamer against thrombin. In phase I clinical trials, NU-172 was administered intravenously as a continuous infusion to healthy volunteers. Results from the phase Ib trial showed an increase in activated clotting time with return to baseline when administration ceased (23). They are currently pursuing a phase II clinical study with the goal of using NU-172 in CABG and PCI (ClinicalTrials.gov identifier NCT00808964).

Antiplatelet

At the initial site of vascular insult, tissue factor and factor VII generate a surge of thrombin, which in turn, stimulates platelets. The resulting platelet stimulation induces a cascade of events that facilitates platelet adhesion to the endothelial surface and aggregation between platelet molecules. Central to this adhesion is von Willebrand factor (vWF) (24). A DNA/RNA aptamer conjugated to PEG named ARC-1779 has been generated against vWF and has undergone a phase I trial in healthy volunteers. The endpoints were: (a) effect in a platelet function analyzer (PFA), a whole-blood assay that is sensitive to vWF-mediated platelet inhibition; and (b) the amount of "free" or active vWF with a functional A1 domain in plasma (25). The plasma half-life of the compound was also determined and the safety profile in terms of bleeding risk was evaluated. The results demonstrated that the aptamer increased PFA in a dose-dependent manner. Moreover, a slow intravenous bolus followed by a 4-h continuous infusion inhibited > 95% of vWF function, which returned to baseline over 12-16 h. There were no major bleeding events in the study.

Enrollment has commenced to study the drug in patients who have carotid artery disease and require a carotid endarterectomy, in addition to patients who suffer from thrombotic microangiopathies, which is a group of thrombotic disorders characterized by increased levels of vWF (23).

Antineoplastic

While the large majority of aptamers have been isolated by SELEX technology (11), the anti-proliferative DNA aptamer AS-1411 was developed based on observations that guanosine-rich oligonucleotides have antiproliferative effects in tumor cells (26). Molecular studies revealed that this aptamer binds to the cell surface protein nucleolin and inhibits the activity of nuclear factor-κB (NF-κB), a ubiquitous transcription factor, through intracellular complex formation (27). Clinical studies of AS-1411 have focused on patients with renal, pancreatic and other solid tumors. The aptamer was administered to patients as a continuous infusion for 4 or 7 days. If no toxicity was observed up to 28 days post-treatment, the dose was increased and the treatment was repeated. Doses of 1, 2, 4, 8 and 10 mg/kg/day have been studied. One patient with renal cancer had a partial response, while almost 67% of patients had stable disease for at least 2 months (28).

Ophthalmological disease

Vascular endothelial growth factor (VEGF) is a growth factor well characterized as being involved in both benign and neoplastic angiogenesis (29), and patients with macular degeneration have elevated levels of VEGF (30). Pegaptanib sodium is a 2′-fluoropyrimidine-modified RNA aptamer to a 165-amino acid subtype of VEGF that is clinically used to treat age-related macular degeneration (AMD) (31, 32). Preclinical and clinical studies of pegaptanib sodium to treat AMD have been carried out (31, 33, 34). A phase I trial revealed that 80% of patients who received pegaptanib sodium had stable or improved vision after 3 months and that 27% had three-line or greater improvement in vision by conventional reading test (33). There were no major side effects associated with the therapy. The phase II study was conducted on patients with and without photodynamic therapy (PDT) – a standard treatment for patients with AMD. Similarly to the phase I results, 88% of patients treated with pegaptanib sodium had stable or improved vision after 3 months, and 25% had three-line or greater improvement in vision (34). Completion of phase III studies, the VEGF inhibition study in ocular neovascularization (VISION) trial, demonstrated similar data, which led to approval of the aptamer to treat AMD (31). The significance of this research was that it involved the first aptamer to be approved for clinical use, thereby establishing this class of molecules as a viable therapeutic agent.

Other targets to treat AMD include complement factor C5 and platelet-derived growth factor (PDGF) (35). C5 is a serum glycoprotein that cleaves to form vasoactive molecules C5a and C5b that mediate inflammatory reactions and injury (36, 37). A fluoro-modified RNA aptamer against C5, ARC-1905, has been co-developed by Archemix and Ophthotech to treat dry AMD. While wet AMD is primarily the result of neovascularization interfering with the function of the photoreceptors of the eye, dry AMD is a gradual degeneration of the photoreceptor itself, which results in permanent blindness. Ophthotech's hypothesis is that dry AMD is primarily an inflammatory process and therefore, by inhibiting C5-mediated inflammation, its progression will be slowed. Currently the compound is in phase II clinical trials as an adjuvant treatment with an approved VEGF inhibitor (35).

PDGF is another target in the treatment of wet AMD. It is a ubiquitous mitogen composed of three dimers made of two homologous chains (38). PDGF is linked to disease processes, including glomerulonephritis and atherosclerosis, and is secreted by numerous tumor cell lines (38-40). A DNA aptamer named E1-0030 that targets the PDGF-B subtype is currently undergoing phase I clinical trials to be tested with an anti-VEGF compound (35).

CONCLUSIONS

Since the description of the first aptamer and the development of a system to isolate oligonucleotide ligands, there has been rapid

growth in nucleic acid therapeutics. There have been a number of technological advancements in the field of SELEX to further enhance drug discovery. These include automated SELEX systems (41) and complex selection, either to multiple targets (42) or cell-based SELEX, in which nucleic acids are exposed to normal or diseased cell populations (43).

For aptamer therapeutics to firmly establish itself as a superior class of drug compounds, innovation leading to expanded use will be essential. Advances, such as modulating pharmacokinetic activity and antidote development, are further creative applications for aptamer-based therapy. More research will need to be carried out to make aptamers available as oral agents, which, once successful, will exponentially increase its clinical utility. With the focus of pharmaceutical companies on bringing drugs to market, this innovation will largely fall on academic centers. Hopefully, new paradigms of drug development will emerge that foster continued innovation between academic centers and the pharmaceutical industry even after the initial discovery of a compound at the bench top, thereby leading to more effective therapies for patients.

DISCLOSURE

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