Published in final edited form as:
Curr Med Chem. 2011 ; 18(27): 4206–4214.

Nucleic acid aptamers: clinical applications and promising new horizons

Xiaohua Ni[1], **Mark Castanares**[1], **Amarnath Mukherjee**[1], and **Shawn E. Lupold**[1,2,3,*]

[1]James Buchanan Brady Urological Institute, Johns Hopkins University School of Medicine, Baltimore, MD

[2]Department of Radiation Oncology and Molecular Radiation Sciences, Johns Hopkins University School of Medicine, Baltimore, MD

[3]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD

Abstract

Aptamers are a special class of nucleic acid molecules that are beginning to be investigated for clinical use. These small RNA/DNA molecules can form secondary and tertiary structures capable of specifically binding proteins or other cellular targets; they are essentially a chemical equivalent of antibodies. Aptamers have the advantage of being highly specific, relatively small in size, and non-immunogenic. Since the discovery of aptamers in the early 1990s, great efforts have been made to make them clinically relevant for diseases like cancer, HIV, and macular degeneration. In the last two decades, many aptamers have been clinically developed as inhibitors for targets such as vascular endothelial growth factor (VEGF) and thrombin. The first aptamer based therapeutic was FDA approved in 2004 for the treatment of age-related macular degeneration and several other aptamers are currently being evaluated in clinical trials. With advances in targeted-therapy, imaging, and nanotechnology, aptamers are readily considered as potential targeting ligands because of their chemical synthesis and ease of modification for conjugation. Preclinical studies using aptamer-siRNA chimeras and aptamer targeted nanoparticle therapeutics have been very successful in mouse models of cancer and HIV. In summary aptamers are in several stages of development, from pre-clinical studies to clinical trials and even as FDA approved therapeutics. In this review, we will discuss the current state of aptamers in clinical trials as well as some promising aptamers in pre-clinical development.

Keywords

Aptamers; clinical trial; imaging; nanoparticle; oligonucleotides; SELEX; siRNA; target therapy

INTRODUCTION

Aptamers are short synthetic single-stranded oligonucleotides that specifically bind to various molecular targets such as small molecules, proteins, nucleic acids, and even cells and tissues. The term “Aptamer” is from the Latin word aptus, meaning “to fit”, which was chosen to describe the lock and key relationship between aptamers and their binding targets [1].

*Correspondence to S.E.L.: Johns Hopkins University School of Medicine 600 N Wolfe St., Park 209, Baltimore, MD 21287. Office: 410-502-4822 FAX: 410-502-7711 slupold@jhmi.edu .

Aptamer Selection

Aptamers are generally selected from a biopanning method known as SELEX (Systematic Evolution of Ligands by Exponential enrichment), which was first independently reported by two groups in 1990 [1,2]. The classic SELEX method starts with a random sequence library of ssDNA or ssRNA that spans 20–100 nucleotides (nt) in length. The randomization of nucleic acid sequences provides a diversity of 4^n, with *n* corresponding to the number of randomized bases. While seemingly infinite diversities can be achieved by this method, only diversities of ~10^{16} aptamers can be readily generated and screened. Each random sequence region is flanked by constant sequences required for capture or priming. The initial diverse pool of aptamers is then exposed to a target molecule, with the expectation that a portion of the aptamers can fold in such a way that they will specifically bind to the target molecule (Figure 1). Non-binding aptamers are then washed away, while candidate aptamers with high target binding affinity are enriched at each selection round by PCR amplification (DNA aptamers) or RT-PCR followed by *in vitro* transcription (RNA aptamers). The enriched pool of aptamers is then exposed to the target again, and the process repeats. During this iterative process, the aptamer pool can also be counter-selected: where the pool is incubated with unwanted targets in order to deplete it of non-specific binders. After multiple rounds of target selection and enrichment, aptamer pools will show increase binding affinity and begin to converge to one or more consensus sequences. Finally, individual aptamer clones can be generated and tested for target binding affinity and specificity.

New varieties of aptamer selection processes have developed over the years. One, called AptaBid, generates aptamers and finds novel biomarkers in parallel [3]. A series of counter- and positive-selections are designed to enrich for aptamers which bind biomarkers that are differentially expressed on cells in different states. The candidate aptamers are then used to isolate their binding partner, or biomarker, by mass spectrometry. This is just one example where the power of aptamer diversity and clever library screens can be used to generate new clinical tools or assays for disease detection, management, or therapy.

Taking Nucleic Acids to the Clinic

In the 1980s, it was discovered that HIV and adenovirus contain several small RNA sequences or regions which can specifically bind to viral or cellular proteins with high affinity. Functional studies indicated that these viral RNA-protein interactions could be exploited as competitive anti-viral therapeutics. In 1990, Sullenger and colleagues reported that decoys of a small HIV RNA region, called TAR, could be used to inhibit HIV virus replication in cellular models. This pioneering study introduced RNA-based therapies and indicated that other small structural RNAs could be exploited as a new approach for inhibiting proteins and enzymes [4]. With the concurrent development of SELEX in the 1990s, several aptamers were quickly developed against important clinical targets such as von Willebrand Factor(vWF), Platelet-derived growth factor (PDGF), E-selectin, Vascular endothelial growth factor (VEGF), Nuclear factor kB (NFkB), tenascin-C and Prostate Specific Membrane Antigen (PSMA), to name a few[5-11]. Some of these aptamers, and others, are now being tested in clinical trials and one has been approved by the US FDA (Table 1). However, as these were being developed for translation to the clinic, the issue of nucleic acid instability had to be considered.

Stabilization – Preparing Aptamers for Clinical Use

One obvious concern of RNA-based aptamers, when compared to antibodies, is their short half life due to serum degradation by nucleases**.** The two main regions of susceptibility in serum are the phosphodiester backbone, which is particularly vulnerable to serum ribonucleases at pyrimidine residues, and the 5′ and 3′-termini, which are susceptible to exonucleases. To overcome exonuclease degradation, aptamers can be chemically

synthesized and capped with modified or inverted nucleotides to prevent terminal degradation. Modified oligonucleotides can also be incorporated within the aptamer, either during or after selection, for enhanced endonuclease stability. Some modified nucleotide triphosphates, particularly 2′-O-modified pyrimidines, can be efficiently incorporated into aptamer transcripts by T7 RNA polymerases. Common chemical modifications included during selection are 2′-amino pyrimidines [12-14] and 2′-fluoro pyrimidines [15,16]. It is critical to include these modified nucleotides during the selection process, because they can influence aptamer folding and binding affinity. After selection, additional modifications such as 2′-O-methyl ribose purines and pyrimidines [6,17,18], can be chemically incorporated. However it should be noted that post-selection modifications can negatively affect aptamer activity, so additional modifications must be tested in a trial and error fashion. Other modifications, such as Locked-Nucleic Acids (LNAs), can be utilized to help stabilize aptamer structures [19]. In addition to modifications for nuclease stability, other chemical modifications such as polyethylene glycol (PEG) can be incorporated to prolong aptamer circulation times, resulting in a more favorable pharmacokinetic profile[20,21]. Most of the aptamers described below have been modified in some way, either before, after, or during selection, to improve stability.

CLINICAL APPLICATIONS OF APTAMERS

Clinical Trials of Anti-coagulant Aptamers

Anticoagulants are a major class of pharmaceutical agents used to prevent clotting during certain clinical procedures or to treat cardiovascular disease. Heparin, a highly-sulfated glycosaminoglycan, is one of the commonly used injectable anticoagulant in the clinic. One serious complication of heparin treatment is spontaneous hemorrhage and reduction of blood platelets. Heparin allergies can also preclude some patients from treatment. Due to these complications, much work has been invested into the discovery of novel anticoagulants. Below we review clinical trials of anti-coagulant aptamers (Table 1).

REG1—REG1 (Regado Biosciences Inc), is a reversible anticoagulant based on a factor IXa binding aptamer, RB006, and a complementary antidote, RB007. This regulatable aptamer system has been applied to clinical trials during percutaneous coronary intervention. RB006 is a 2′-fluoropyrimidine modified aptamer originally isolated in 2002[22], that was truncated to 34 nt, conjugated to 40 kDa PEG to reduce serum clearance, and capped at the 3′-terminus with an inverted nucleotide. RB006 specifically inhibits FX cleavage by the FIXa–FVIIIa enzyme complex and extends blood clotting times as assessed in activated partial thromboplastin time assays. RB007 is a fully 2′-O-methyl modified 17nt oligonucleotide antidote which is complementary to the 5′-terminus of RB006. Upon standard Watson-Crick binding of RB007, the conformation of RB006 is changed and its anticoagulation function is lost. REG1 is currently in Phase II clinical trials and shows rapid onset of anticoagulation *in vivo* after intravenous administration of RB006, which is followed by a rapid return to baseline after dosing of the RB007 antidote [23]. Recently, in a phase II study of REG1 for Acute coronary syndrome (ACS), results indicated a lower bleeding rate than heparin in an invasively managed ACS population [24].

ARC1779—The von Willebrand factor (vWF) plays an important role in the clotting cascade by recruiting platelets to damaged arteries. ARC1779 is a DNA aptamer selected to bind to the A1 region of vWF from a degenerate DNA library [5]. The resulting aptamer was further truncated to 39 nt and stabilized by backbone modifications, 20 kDa PEG conjugation, and 3′-capping. ARC1779 specifically binds to the A1 domain of vWF and blocks binding to platelet membrane glycoprotein Ib receptors, thus inducing antithrombotic effects [25]. By inhibiting vWF-dependent arterial thrombogenesis, ARC-1779 provides a

potential therapeutic benefit in acute coronary syndromes and von Willebrand disease. Additionally, the actions of ARC-1779 can be readily reversed by binding to a complementary sequence oligonucleotide, a feature unlike any other anti-platelet agents[26]. Currently ARC1779 is in Phase II clinical trials for thrombotic microangiopathies and in patients with carotid artery disease undergoing carotid endarterectomy.

NU172—Thrombin is a serine protease and a key activator of several proteins in the coagulation cascade. Nu172 is a DNA aptamer selected to bind and inhibit thrombin. Nu172 was discovered from a degenerate DNA library by SELEX and was subsequently truncated to 26 nt without additional chemical modification [27]. Nu172 is administered intravenously during acute cardiovascular surgical procedures to prevent formation of blood clots. This aptamer is currently being evaluated in Phase II clinical trials for anticoagulation in heart disease treatments by ARCA Biopharma.

Clinical Trials of Anti-angiogenic Aptamers

Angiogenesis is a vital process in growth and development, wound healing and granulation tissue. However, angiogenesis can also contribute to diseases such as tumor growth, cancer progression, and macular degeneration. Anti-angiogenesis agents are a class of inhibitors which prevent or delay such unwanted neovascularization.

Pegaptanib—Pegaptanib (brand name Macugen), is an anti-vascular endothelial growth factor (VEGF) RNA aptamer and is the first nucleic acid aptamer approved by the US FDA. It was approved in December 2004 as a anti-angiogenic therapeutic agent for neovascular (wet) age-related macular degeneration (AMD), a disease of the eye which does not cause total blindness, but a loss of central vision, leaving only peripheral, or side, vision intact. During selection of Pegaptanib, only pyrimidines were stabilized by 2′-fluoro modification. Once a candidate aptamer was discovered, purines were stabilized by 2′-O-methyl-modification resulting in all nucleotides being protected against nucleases [16]. To increase half-life and stability, a 40 kDa polyethylene glycol (PEG) was added to the 5′-terminus along with an inverted 3′-3′-deoxythymidine cap. The target of Pegaptanib is $VEGF_{165}$, the most abundant VEGF isoform that regulates vascular permeability. $VEGF_{165}$ is a homodimeric protein of two 165 amino acid polypeptide chains and it is critical in blood vessel formation and in causing the increased permeability involved in vision loss associated with neovascular AMD [28]. Pegaptanib binds to the heparin-binding domain of $VEGF_{165}$ and efficiently inhibits VEGF binding to its receptor, resulting in inhibition in the growth of blood vessels. Recently, a Phase 2/3 study indicated that Pegaptanib is effective in the treatment of Diabetic Macular Edema (DME) in intent-to-treat analyses of a total of 260 subjects who received 0.3 mg pegaptanib or sham injections every 6 weeks in years 1 and 2 [29]. Pegaptanib continues to be used clinically but also competes with antibody-based VEGF inhibitors such as Ranibizumab (trade name Lucentis, Genentech Inc, US FDA proved 2006), a recombinant humanized monoclonal antibody with high affinity for all isoforms of human VEGF [30].

ARC1905—ARC1905 is an RNA aptamer selected to bind complement component 5 (C5), an important protein in AMD. The complement system is part of the innate immune system which is based on a number of proteins produced by the liver. When the system is triggered, a cytokine cascade is started which recruits inflammatory cells and activates the membrane attack complex (MAC). Inhibition of C5 prevents the formation of the critical terminal fragments responsible for tissue pathology. C5 is pro-inflammatory and pro-angiogenic, as the MAC initiates cell lysis and releases molecules such as PDGF and VEGF. Thus ARC1905, as an inhibitor of C5, potentially inhibits the inflammation and angiogenic components of AMD. ARC1905 was selected from a modified RNA aptamer library and

was later truncated to 39 nt. It is further modified by 5′-PEG conjugation and 3′-capping [15]. ARC1905 is administered by intravitreal injection and is currently in Phase I clinical trials by Ophthotech where it is co-dosed with the VEGF-specific antibody fragment ranibizumab.

E10030—E10030 is a DNA aptamer selected to bind platelet-derived growth factor (PDGF), a critical protein of maturation and recruitment of pericytes [31]. E10030 was selected from a DNA aptamer library by SELEX and then subsequently truncated to 29 nt with further internal modifications for stability and terminal 5′- PEG and 3′-capping for exonuclease stability [7]. E10030 is currently in Phase II clinical trials for Age-Related Macular Degeneration.

Clinical Trials of Aptamers in Oncology

AS1411—AS1411 is a 26 nt G-rich DNA aptamer considered to be the first anticancer aptamer. AS1411 targets nucleolin, a nuclear matrix protein which can be found on the surface of cancer cells. AS1411 was not discovered by the classic SELEX method, but by a cell based screen of guanosine-rich oligonucleotides (GRO) that inhibited proliferation [32,33]. It was later found that AS1411 bound to nucleolin on the plasma membrane. AS1411 has been demonstrated to be effective in many different preclinical cancer and animal models such as human breast cancer, lung cancer, pancreatic cancer, and acute myelogenous leukemia [33-36]. The proposed mechanisms of cell death by AS1411 involves aptamer internalization via nucleolin and subcellular inhibition of nuclear factor-κB[34] as well as destabilization of the mRNA of the anti-apoptotic protein, B-cell lymphoma protein 2 (BCL-2) [36]. AS1411 is currently in Phase II clinical trials for acute myeloid leukemia by Antisoma Research.

NOX-A12—NOX-A12 is one of the aptamers in clinical development by NOXXON Pharma AG. NOX-A12 was developed by Spiegelmer technology (in German, “spiegel” means mirror), a chiral-twist of the classic SELEX process where D-RNA aptamers are screened against the chiral mirror image of the target [37]. Based on the principle of reciprocal chiral substrate specificity, the reciprocal L-RNA version of the aptamer then binds to the natural form of the target. The L-RNA aptamer is not recognized by nucleases and is therefore stable in human plasma for over 60 hours at 37 °C. NOX-A12 was selected to bind chemokine (C-X-C motif) ligand 12, a chemokine involved in tumor metastasis, angiogenesis, cell homing, and tissue regeneration [38]. The resulting NOX-A12 is a 45 nt L-RNA aptamer, conjugated with 3′-terminal PEG, which is currently in Phase I clinical trials for the treatment of lymphoma, multiple myeloma and Hematopoietic Stem Cell Transplantation.

Clinical Trials of Aptamers in Diabetes

NOX-E36—NOX-E36 is an L-RNA aptamer, also developed by Spiegelmer technology, to bind chemokine (C-C motif) ligand 2, a cytokine which is involved in recruiting monocytes, T-cells, and dendritic cells to sites of injury, infection, and inflammation [39]. NOX-E36 was further truncated to 40 nt and conjugated with 3′-terminal PEG [40]. It has been shown to significantly reduce glomerulosclerosis of type 2 diabetes in mouse models [41]. NOX-E36 is currently in a Phase I clinical trial for the treatment of Type 2 Diabetes Mellitus.

PROMISING PRECLINICAL APPLICATIONS OF APTAMERS

The above clinical studies show the promise of aptamers as a new class of therapeutic agents. The majority of the clinically tested aptamers act as inhibitors, by binding and inhibiting enzyme activities or protein-protein interactions. However, aptamers also show

promise in therapeutic targeting or delivery. Below, we discuss some promising preclinical studies where aptamers have been utilized to target the delivery of nanoparticles, therapeutics, and imaging agents (Table 2).

Aptamer-targeted Nanoparticle Delivery

Nanoparticles (NPs) are engineered materials ranging in size from 5-200 nm and are being developed for multiple medical applications [42,43]. They have advantages over traditional therapeutics such being able to carry a large therapeutic "payload", accommodating multiple drug molecules simultaneously, and altering drug biodistribution, resistance, and pharmacokinetics [44]. NP therapeutics, as well as other therapeutics, can benefit from targeted delivery and retention within the diseased tissue. Most promising are targeted NPs which are internalized into the cell following ligand binding (Figure 2). NP targeting has been achieved with traditional targeting ligands, such as antibodies, antibody fragments, and peptides. However, aptamers are becoming an excellent choice due to their size, ease of conjugations, and their ability to evade the immune system. Below, we highlight some preclinical studies of aptamer-targeted NPs.

AS1411-Targeted Cobalt Ferrite NPs—The AS1411 aptamer, which target nucleolin, has been conjugated to a cobalt-ferrite NP, surrounded by fluorescent rhodamine. The resulting conjugate is called MF-AS1411 [45]**.** This product can also be labeled with ^{67}Ga to form MFR-AS1411, a multimodal NP probe with potential use in MRI, optical, and nuclear imaging. *In vivo* studies using ^{67}Ga SPECT imaging and MRI showed specific MFR-AS1411 uptake in C6 tumors in nude mice when compared to MFR-AS1411mt, a particle targeted with a control aptamer. Despite being coated with PEG moieties, high nonspecific uptake of MFR-AS1411 and MFR-AS1411mt was reported in the liver of injected animals, which may be due to the size of the particles. *Ex vivo* fluorescent imaging confirmed NP uptake in C6 tumors of MFR-AS1411 injected animals as well as the nonspecific uptake in the liver and intestines. One can foresee the promise of this technology once particle size can be optimized to minimize nonspecific uptake.

Muc1-Targeted Quantum Dots—Mucin 1 (Muc1) is a glycoprotein found on the surface of normal epithelial cells and functions as a barrier against microorganisms and degradative enzymes [46]. In cancer, Muc1 is an attractive target because its expression is increased by at least 10-fold, when compared to normal tissue, and its distribution pattern changes at the cell surface [47-50]. DNA aptamers, AptA and AptB, directed against Muc1 were discovered in 2006 [51] and have been implemented in *ex vivo* imaging and radiotherapy [52]. Pieve and colleagues radiolabelled both aptamers with ^{99m}Tc using the MAG_2 chelator and studied their use in animal models [53]. Imaging signal accumulated in the tumors, but the tumor/tissue ratios suggest that further development was necessary before these radiolabeled aptamers can be applied as clinical imaging agents. Savla and colleagues later attempted to use the Muc1 aptamers to target quantum dots (QDs) [54]. Because QD scaffolds allow one to label a single QD with multiple aptamers, targeting efficiency may be enhanced by avidity. In a mouse model bearing A2780/AD human ovarian cancer xenografts, more QD-Muc1 accumulated in the tumors when compared to non-modified QD. *Ex vivo* analysis of organs confirmed higher uptake in tumor and lower accumulation in other organs. This example supports that aptamer targeting may be improved by the avidity associated with NP decoration.

PSMA-Targeted NPs for Drug Delivery—Prostate Specific Membrane Antigen (PSMA) is a well established marker of prostate cancer with minimal expression in the kidney, small intestine, and normal prostate. Unlike PSA, PSMA expression is inversely related to androgen and its expression is increased after hormone ablative therapy [55,56].

PSMA is an attractive target since it is expressed in high abundance on the surface of prostate cancer cells, is known to internalize certain ligands, has enzymatic properties as a glutamate carboxypeptidase, and is a verified target of a clinically applied imaging agent for prostate cancer [57]. In 2002 we performed a 2′-fluorpyrimdine RNA aptamer screen and identified two stabilized RNA aptamers, A9 and A10, which are capable of binding PSMA with low nanomolar affinity [10]. These aptamers have been applied to several anti-cancer preclinical targeting studies of NPs, toxins, therapeutics, and small interfering RNAs.

Farokhzhad and colleagues have extensively studied the use of the anti-PSMA A10 aptamer, or its truncated version A10-3, to target NPs. In a pioneering NP-targeting study, they demonstrated that the A10-3 aptamer can be used to target poly (lactic acid)-block-polyethylene glycol (PEG) copolymer NPs to PSMA positive prostate cancer cells. The resulting NP-conjugate showed 77 fold higher binding to PSMA expressing LNCaP cells when compared to the untargeted NPs[58]. The A10-3 aptamer was again used to target modified poly(D,L-lactic-co-glycolic acid) (PLGA) NPs to deliver Docetaxol to prostate tumors *in vivo* where complete tumor regression was found in five out of seven mice after a single intratumoral injection [59]. Further, after systemic administration, A10-3 aptamer-targeting induced tumor accumulation of PLGA NPs by 3.7 fold when compared to the non-targeted particle [60]. A similar polymeric formulation has been used to deliver Cisplatin to PSMA expressing tumors where a dosage of 0.3 mg/kg of aptamer-targeted cisplatin NPs was more efficacious than a 1mg/kg dosage of free cisplatin [61]. These preclinical studies show the promise of aptamers as targeting ligands to alter the biodistribution, tumor uptake, and tumor retention of therapeutic and imaging NP reagents.

Aptamer-Targeted RNA interference

Small interfering RNAs (siRNAs) are 20-28 nt long RNA molecules that can specifically cause the cleavage of mRNA, through a cytoplasmic pathway known as RNA-interference (RNAi) [62]. siRNAs are attractive therapeutics because they theoretically have very high specificity and are applicable to an unlimited range of targets. For example, siRNAs can be derived for “undrugable” targets such as transcription factors, which have been difficult to inhibit with traditional small molecule therapeutics. Because of this, siRNA based therapeutics have been studied in almost every disease model. However, a critical challenge for RNAi based therapeutics is the efficient delivery of siRNAs to specific tissues or across the plasma membrane of cells. Aptamers which bind to cell-internalizing-ligands are now emerging as potential siRNA delivery vehicles. One appealing feature of aptamers is, as RNA molecules, they can be readily linked to siRNAs by transcription.

A10-Targeted siRNA Therapeutics—RNA interference is particularly attractive in cancer therapy because there are countless verified oncogene targets which have been difficult to inhibit by traditional approaches. PLK1 and BCl2 are two pro-survival oncogenes that are known to be over-expressed in a variety of cancers [63,64]. McNamara and colleagues, as well as Chu and colleagues, reported in 2006 that PSMA-aptamers could be used to deliver siRNAs to cancer cells [65,66]. By extending the anti-PSMA aptamer A10 to include siRNAs against PLK1 and BCl2, these oncogenes could be specifically targeted and down-regulated in PSMA positive LNCaP cells and tumors. After repeated intratumoral administration of the A10-PLK1 siRNA chimera, a significant reduction in tumor volume was observed in PSMA positive xenografts [65]. Several second generation A10-Plk1 chimeras were later derived to reduce the size of chimeras by 32nt, making those candidates for chemical synthesis [67 36]. These optimized aptamer-siRNA chimeras were found to be much more effective, achieving targeted gene knock-down with 100 fold lower doses. Importantly, these new configurations reveal that the PSMA aptamer can target and deliver siRNAs after systemic administration. With 10 consecutive intraperitoneal injections

of A-10-3.2-Plk1 chimera, PSMA positive tumors showed a statistically significant reduction in size when compared to controls. These results may lead to novel treatments of other diseases using aptamer-siRNA technology.

A10-3-Targeted Radiation-Sensitizing shRNAs—Other attractive targets for cancer therapy are cellular repair pathways, which are initiated for cancer cell survival after chemotherapy or radiation treatment. In order for these approaches to benefit cancer patients, targeting is necessary to selectively inhibit the repair pathways in cancer cells, but not in adjacent normal cells or non-target tissues. We recently reported that PSMA-targeted aptamer-shRNAs (short hairpin RNAs) could be used to attenuate DNA-repair pathways, leading to tissue-selective sensitization to Ionizing Radiation (IR) therapy [68]. In this study, A10-3-shRNAs targeting the catalytic subunit of the DNA Protein Kinase (DNAPK) caused a selective reduction of DNAPK RNA and protein in PSMA positive cells, xenografts, and human prostate tissues. Intratumorally administered aptamer-targeted DNAPK shRNAs, combined with IR, dramatically and specifically enhanced PSMA-positive tumor response to IR. These findings support aptamer-shRNA chimeras as selective sensitizing agents for the improved treatment of prostate cancer.

Aptamer-Targeted siRNAs for Treatment of HIV—The use of aptamer targeted RNA interference is not limited to cancer. The HIV envelope glycoprotein gp120 plays an important role in HIV-1 virus entry into CD4-positive cells. Cell fusion is also initiated by the interaction of gp120 and CD4. Modified RNA aptamers isolated to bind to gp120 are selectively internalized into cells expressing HIV-1 gp120 envelope protein [69-71]. Zhou and colleagues have covalently linked an anti-gp120 aptamer to anti-HIV tat/rev siRNAs and demonstrated siRNA-mediated gene knock-down and inhibition of retroviral replication. This strategy is attractive because the aptamer not-only delivers the siRNA, but it also inhibits gp120/CD4 interaction to block viral infection and spread [71]. In a humanized mouse model where HIV-1 replication and T cell depletion mimic the human situation, Neff and colleagues have found that either the anti-gp120 aptamer or the aptamer-siRNA chimera suppressed HIV-1 replication and prevented the viral-mediated T-cell reduction [72]. In another approach, Wheeler and colleagues constructed a human CD4 aptamer-siRNA chimera termed AsiCs, which inhibits HIV in two ways: either by blocking viral entry to CD4 positive cells or by knockdown of key HIV replication genes [73]. When administered intravaginally in a humanized mouse model, these constructs inhibit HIV infection in a dose dependent manner without triggering an immune response. These aptamer conjugates show promise both to prevent HIV transmission as well as a complement to drugs used in highly active antiretroviral therapy (HAART) regimens. Importantly, these studies confirm in different experimental preclinical models and different aptamer-targets, that aptamers can be utilized to target RNA interference.

Aptamer-Targeted Imaging Agents

Aptamers have properties which make them ideal imaging agents [74]. They are much smaller than antibodies yet still have similar binding affinities. Aptamers are also non-immunogenic and can rapidly diffuse from the circulation to interact with their binding partners. Importantly, they are also rapidly cleared from the bloodstream, resulting in high target to background ratios in short periods of time. Aptamers can be synthesized with specific functional groups on 5′- or 3′-termini for site-specific conjugation to imaging agents. Conjugation to the aptamer-termini generally does not significantly compromise binding affinity. Nonetheless, modification with chelators or other moieties can be optimized for better biodistribution and clearance. Preclinical studies of aptamer-targeted imaging agents have been most successful in cancer, but other disease processes have also been studied [75-77].

Elastase-Targeted Nuclear Imaging of Inflammation—The first report using aptamers for imaging was in 1997 to identify sites of inflammation. In inflammatory-related diseases, neutrophils invade the inflamed areas and release elastase, an enzyme that can degrade connective tissue proteins [78]. NX21909, a DNA aptamer which targets elastase, was fluorescently labeled and validated for specificity in flow cytometry experiments of neutrophils. Using a rat inflammation model, Charlton and colleagues compared the benefits of ^{99m}Tc-labeled NX21909 aptamer imaging with a clinically proven, broad-spectrum ^{99m}Tc-IgG-based imaging agent [75]. Radiolabelled IgG accumulated in the site of inflammation, yet clearance from circulation was slow, leading to high background. On the other hand, radiolabelled NX21909 was able to specifically accumulate in the site of inflammation while excess NX21909 rapidly cleared from circulation. NX21909 was therefore able to achieve a peak target-to-background ratio of 4-fold within two hours, as compared to the IgG agent which only reached a ratio of 3-fold within three to four hours. Interestingly the absolute intensity was three fold higher in the IgG agent, but this may be due to the different targeting mechanisms (IgG recruitment to inflammation versus elastase targeting) or nuclease degradation of the aptamer. Nonetheless, this preclinical study demonstrated the advantage of using aptamers as imaging agents to obtain high target to background ratios within hours as compared to antibodies which can take days to achieve favorable target to background ratios.

Tenascin-C Targeted Nuclear Imaging for Cancer—Tenascin-C is a hexameric protein found in the extracellular matrix. It is associated with tissue remodeling processes such as angiogenesis, embryonic development, wound healing, and tumor growth [79-82]. Hicke and colleagues performed a modified RNA-aptamer screen using purified Tenascin-C and U251 glioblastoma cells in a parallel SELEX strategy [8]. The resulting TTA1 aptamer was truncated and optimized by structure activity relationship for maximal stability *in vivo* and has entered clinical trials in Europe[19]. In preclinical studies, fully stabilized TTA1 aptamer-MAG_2 conjugates were used to image mice bearing U251 glioblastoma tumors or MDA-MB-435 breast tumors by ^{99m}Tc SPECT imaging. When compared to the control aptamer, TTA1 had specific binding which peaked at 10 minutes and then stabilized in the tumors at 3 hours [83]. A tumor-to-blood ratio of 50-fold was reached within 3 hours and 180-fold at 16 hours post injection of labeled TTA1. Different chelator modifications were tested for their effects on biodistribution and pharmacokinetics. Interestingly, different chelators significantly changed biodistribution and clearance of TTA1 without effecting tumor uptake. This suggests that aptamers can be combined with multiple chelators or linkers for imaging modalities other than SPECT.

Activatable Fluorescent Aptamer Imaging for Cancer—A recent report described an activatable aptamer imaging probe based on the sgc8 DNA aptamer which targets a cell membrane protein on CCRF-CEM cancer cells [84]. This strategy takes advantage of a unique property where the aptamer is able to adopt a new conformation upon binding of its target. The optical signal is quenched when in the unbound state, but once the aptamer binds its target a conformational change occurs which dissociates the quencher from the fluorophore. The probe sequence was optimized *in vitro* on target CCRF-CEM cells and found to have a dissociation constant in the nanomolar-to-picomolar range. When tested *in vivo* using mice bearing CCRF-CEM tumors, activation of the fluorophore occurred as early as 15 minutes post injection and persisted until 180 minutes. This is an attractive new application for aptamer imaging; however, it remains unclear whether or not this strategy can be generalized or applied to other aptamers.

CONCLUSIONS

Aptamers continue to grow as viable clinical agents. They represent a unique class of molecules that are larger than small molecule drugs, but smaller than antibodies. Importantly, they have unique pharmacokinetic properties which could be exploited for various purposes. One highly attractive feature of aptamers is they appear to be non-immunogenic. Moreover, they are amendable to chemical modification and bioconjugation to various moieties such as nanoparticles, imaging agents, siRNAs and therapeutic drugs. As discussed above, they are proven clinically as inhibitors with great promise as targeting ligands for treating or diagnosing cancer, HIV, and other diseases.

As with any pharmacologic agent, aptamer translation faces some obstacles. One initial decision is which nucleic acid sugar is best suited for the specific application. Both DNA and RNA aptamers are capable of binding a target with high specificity and selectivity. RNA aptamers are able to form more stable 3D structures due to the strong intrastrand RNA-RNA interactions, yet DNA aptamers have better chemical and thermodynamic stability due to its B-form helix structure. Therefore, RNA aptamer pools may yield more structurally distinct agents resulting in a quicker selection. However, this may comes at a cost since RNA aptamers require modified oligonucleotides. The cost to produce aptamers containing the commonly used modified oligonucleotides for stabilization is rather expensive, although the cost is decreasing. For example, the cost of 2′-fluoro, 2′-ribo and 2′-O-methyl RNA phosphoramidites are approximately $20 per gram[85]. Increased demand or new synthesis technology will likely further reduce these costs. Another current barrier is the difficulty to synthesize longer modified aptamers. Current oligonucleotide synthesis technology is limited to 50-60 nt making the larger aptamer siRNA chimeras difficult to translate into clinical practice as chemically produced agents. Advances in nucleotide synthesis technology will likely also overcome this limitation.

In summary, aptamers offer a very promising and unique technology with a bright future in clinical diagnostics and therapeutics. Their rapid development over the last two decades is notable and we anticipate that they will become a standard tool for clinicians in the near future.

Acknowledgments

We would like to thank the following funding sources: NIH/NCI 5P50CA058236 (XN, SEL), NIGMS F31GM79838 (MC); 5R01CA143299-02 (SEL), the Patrick C Walsh Prostate Cancer Research Fund, the Prostate Cancer Foundation, and Safeway (AM, SEL).

REFERENCES

[1]. Ellington AD, Szostak JW. In vitro selection of RNA molecules that bind specific ligands. Nature. 1990; 346(6287):818–822. [PubMed: 1697402]

[2]. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science. 1990; 249(4968):505–510. [PubMed: 2200121]

[3]. Berezovski MV, Lechmann M, Musheev MU, Mak TW, Krylov SN. Aptamer-facilitated biomarker discovery (AptaBiD). J. Am. Chem. Soc. 2008; 130(28):9137–9143. [PubMed: 18558676]

[4]. Sullenger BA, Gallardo HF, Ungers GE, Gilboa E. Overexpression of TAR sequences renders cells resistant to human immunodeficiency virus replication. Cell. 1990; 63(3):601–608. [PubMed: 2225067]

[5]. Gilbert JC, DeFeo-Fraulini T, Hutabarat RM, Horvath CJ, Merlino PG, Marsh HN, Healy JM, Boufakhreddine S, Holohan TV, Schaub RG. First-in-human evaluation of anti von Willebrand

factor therapeutic aptamer ARC1779 in healthy volunteers. Circulation. 2007; 116(23):2678–2686. [PubMed: 18025536]

[6]. Green LS, Jellinek D, Bell C, Beebe LA, Feistner BD, Gill SC, Jucker FM, Janjic N. Nuclease-resistant nucleic acid ligands to vascular permeability factor/vascular endothelial growth factor. Chem. Biol. 1995; 2(10):683–695. [PubMed: 9383475]

[7]. Green LS, Jellinek D, Jenison R, Ostman A, Heldin CH, Janjic N. Inhibitory DNA ligands to platelet-derived growth factor B-chain. Biochemistry. 1996; 35(45):14413–14424. [PubMed: 8916928]

[8]. Hicke BJ, Marion C, Chang YF, Gould T, Lynott CK, Parma D, Schmidt PG, Warren S. Tenascin-C aptamers are generated using tumor cells and purified protein. J. Biol. Chem. 2001; 276(52): 48644–48654. [PubMed: 11590140]

[9]. Lebruska LL, Maher LJ 3rd. Selection and characterization of an RNA decoy for transcription factor NF-kappa B. Biochemistry. 1999; 38(10):3168–3174. [PubMed: 10074372]

[10]. Lupold SE, Hicke BJ, Lin Y, Coffey DS. Identification and characterization of nuclease-stabilized RNA molecules that bind human prostate cancer cells via the prostate-specific membrane antigen. Cancer Res. 2002; 62(14):4029–4033. [PubMed: 12124337]

[11]. Mann AP, Somasunderam A, Nieves-Alicea R, Li X, Hu A, Sood AK, Ferrari M, Gorenstein DG, Tanaka T. Identification of thioaptamer ligand against E-selectin: potential application for inflamed vasculature targeting. PLoS One. 2010; 5(9):el13050.

[12]. Jellinek D, Green LS, Bell C, Lynott CK, Gill N, Vargeese C, Kirschenheuter G, McGee DP, Abesinghe P, Pieken WA, et al. Potent 2′-amino-2′-deoxypyrimidine RNA inhibitors of basic fibroblast growth factor. Biochemistry. 1995; 34(36):11363–11372. [PubMed: 7547864]

[13]. Lin Y, Nieuwlandt D, Magallanez A, Feistner B, Jayasena SD. High-affinity and specific recognition of human thyroid stimulating hormone (hTSH) by in vitro-selected 2′-amino-modified RNA. Nucleic Acids Res. 1996; 24(17):3407–3414. [PubMed: 8811096]

[14]. Lin Y, Qiu Q, Gill SC, Jayasena SD. Modified RNA sequence pools for in vitro selection. Nucleic Acids Res. 1994; 22(24):5229–5234. [PubMed: 7529404]

[15]. Biesecker G, Dihel L, Enney K, Bendele RA. Derivation of RNA aptamer inhibitors of human complement C5. Immunopharmacology. 1999; 42(1-3):219–230. [PubMed: 10408383]

[16]. Ruckman J, Green LS, Beeson J, Waugh S, Gillette WL, Henninger DD, Claesson-Welsh L, Janjic N. 2′-Fluoropyrimidine RNA-based aptamers to the 165-amino acid form of vascular endothelial growth factor (VEGF165). Inhibition of receptor binding and VEGF-induced vascular permeability through interactions requiring the exon 7-encoded domain. J. Biol. Chem. 1998; 273(32):20556–20567. [PubMed: 9685413]

[17]. Burmeister PE, Wang C, Killough JR, Lewis SD, Horwitz LR, Ferguson A, Thompson KM, Pendergrast PS, McCauley TG, Kurz M, Diener J, Cload ST, Wilson C, Keefe AD. 2′-Deoxy purine, 2′-O-methyl pyrimidine (dRmY) aptamers as candidate therapeutics. Oligonucleotides. 2006; 16(4):337–351. [PubMed: 17155909]

[18]. Eaton BE, Gold L, Hicke BJ, Janjic N, Jucker FM, Sebesta DP, Tarasow TM, Willis MC, Zichi DA. Post-SELEX combinatorial optimization of aptamers. Bioorg. Med. Chem. 1997; 5(6): 1087–1096. [PubMed: 9222502]

[19]. Schmidt KS, Borkowski S, Kurreck J, Stephens AW, Bald R, Hecht M, Friebe M, Dinkelborg L, Erdmann VA. Application of locked nucleic acids to improve aptamer in vivo stability and targeting function. Nucleic Acids Res. 2004; 32(19):5757–5765. [PubMed: 15509871]

[20]. Healy JM, Lewis SD, Kurz M, Boomer RM, Thompson KM, Wilson C, McCauley TG. Pharmacokinetics and biodistribution of novel aptamer compositions. Pharm. Res. 2004; 21(12): 2234–2246. [PubMed: 15648255]

[21]. Watson SR, Chang YF, O'Connell D, Weigand L, Ringquist S, Parma DH. Anti-L-selectin aptamers: binding characteristics, pharmacokinetic parameters, and activity against an intravascular target in vivo. Antisense Nucleic Acid Drug Dev. 2000; 10(2):63–75. [PubMed: 10805157]

[22]. Rusconi CP, Scardino E, Layzer J, Pitoc GA, Ortel TL, Monroe D, Sullenger BA. RNA aptamers as reversible antagonists of coagulation factor IXa. Nature. 2002; 419(6902):90–94. [PubMed: 12214238]

[23]. Chan MY, Cohen MG, Dyke CK, Myles SK, Aberle LG, Lin M, Walder J, Steinhubl SR, Gilchrist IC, Kleiman NS, Vorchheimer DA, Chronos N, Melloni C, Alexander JH, Harrington RA, Tonkens RM, Becker RC, Rusconi CP. Phase 1b randomized study of antidote-controlled modulation of factor IXa activity in patients with stable coronary artery disease. Circulation. 2008; 117(22):2865–2874. [PubMed: 18506005]

[24]. Povsic TJ, Cohen MG, Mehran R, Buller CE, Bode C, Cornel JH, Kasprzak JD, Montalescot G, Joseph D, Wargin WA, Rusconi CP, Zelenkofske SL, Becker RC, Alexander JH. A randomized, partially blinded, multicenter, active-controlled, dose-ranging study assessing the safety, efficacy, and pharmacodynamics of the REG1 anticoagulation system in patients with acute coronary syndromes: design and rationale of the RADAR Phase IIb trial. Am. Heart J. 2011; 161(2):261–268. e261–262. [PubMed: 21315207]

[25]. Diener JL, Lagasse H.A. Daniel, Duerschmied D, Merhi Y, Tanguay JF, Hutabarat R, Gilbert J, Wagner DD, Schaub R. Inhibition of von Willebrand factor-mediated platelet activation and thrombosis by the anti-von Willebrand factor A1-domain aptamer ARC1779. J. Thromb. Haemost. 2009; 7(7):1155–1162. [PubMed: 19422452]

[26]. Cosmi B. ARC-1779, a PEGylated aptamer antagonist of von Willebrand factor for potential use as an anticoagulant or antithrombotic agent. Curr. Opin. Mol. Ther. 2009; 11(3):322–328. [PubMed: 19479665]

[27]. Waters EK, R.J. Schaub RG, Kurz JC. Effect of NU172 and bivalirudin on ecarin clotting time in human plasma and whole blood. J. Thromb. Haemost. 2009; (Supplement 2) Abstract PP-WE-168.

[28]. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat. Med. 2003; 9(6): 669–676. [PubMed: 12778165]

[29]. Sultan MB, Zhou D, Loftus J, Dombi T, Ice KS. A Phase 2/3, Multicenter, Randomized, Double-Masked, 2-Year Trial of Pegaptanib Sodium for the Treatment of Diabetic Macular Edema. Ophthalmology. 2011; 118(6):1107–1118. [PubMed: 21529957]

[30]. van Wijngaarden P, Coster DJ, Williams KA. Inhibitors of ocular neovascularization: promises and potential problems. JAMA. 2005; 293(12):1509–1513. [PubMed: 15784876]

[31]. Bradley J, Ju M, Robinson GS. Combination therapy for the treatment of ocular neovascularization. Angiogenesis. 2007; 10(2):141–148. [PubMed: 17372853]

[32]. Bates PJ, Kahlon JB, Thomas SD, Trent JO, Miller DM. Antiproliferative activity of G-rich oligonucleotides correlates with protein binding. J. Biol. Chem. 1999; 274(37):26369–26377. [PubMed: 10473594]

[33]. Bates PJ, Laber DA, Miller DM, Thomas SD, Trent JO. Discovery and development of the G-rich oligonucleotide AS1411 as a novel treatment for cancer. Exp. Mol. Pathol. 2009; 86(3):151–164. [PubMed: 19454272]

[34]. Girvan AC, Teng Y, Casson LK, Thomas SD, Juliger S, Ball MW, Klein JB, Pierce WM Jr. Barve SS, Bates PJ. AGRO100 inhibits activation of nuclear factor-kappaB (NF-kappaB) by forming a complex with NF-kappaB essential modulator (NEMO) and nucleolin. Mol. Cancer Ther. 2006; 5(7):1790–1799. [PubMed: 16891465]

[35]. Ritchie C, Doran B, Shah K, Rowlinson-Busza G, Courtenay-Luck N, Jones D. Combination of the aptamer AS1411 with paclitaxel or Ara-C produces synergistic inhibition of cancer cell growth. AACR. 2007

[36]. Soundararajan S, Chen W, Spicer EK, Courtenay-Luck N, Fernandes DJ. The nucleolin targeting aptamer AS1411 destabilizes Bcl-2 messenger RNA in human breast cancer cells. Cancer Res. 2008; 68(7):2358–2365. [PubMed: 18381443]

[37]. Nolte A, Klussmann S, Bald R, Erdmann VA, Furste JP. Mirror-design of L-oligonucleotide ligands binding to L-arginine. Nat. Biotechnol. 1996; 14(9):1116–1119. [PubMed: 9631062]

[38]. Sayyed SG, Hagele H, Kulkarni OP, Endlich K, Segerer S, Eulberg D, Klussmann S, Anders HJ. Podocytes produce homeostatic chemokine stromal cell-derived factor-1/CXCL12, which contributes to glomerulosclerosis, podocyte loss and albuminuria in a mouse model of type 2 diabetes. Diabetologia. 2009; 52(11):2445–2454. [PubMed: 19707743]

[39]. Carr MW, Roth SJ, Luther E, Rose SS, Springer TA. Monocyte chemoattractant protein 1 acts as a T-lymphocyte chemoattractant. Proc. Natl. Acad. Sci. U S A. 1994; 91(9):3652–3656. [PubMed: 8170963]

[40]. Kulkarni O, Pawar RD, Purschke W, Eulberg D, Selve N, Buchner K, Ninichuk V, Segerer S, Vielhauer V, Klussmann S, Anders HJ. Spiegelmer inhibition of CCL2/MCP-1 ameliorates lupus nephritis in MRL-(Fas)lpr mice. J. Am. Soc. Nephrol. 2007; 18(8):2350–2358. [PubMed: 17625118]

[41]. Ninichuk V, Clauss S, Kulkarni O, Schmid H, Segerer S, Radomska E, Eulberg D, Buchner K, Selve N, Klussmann S, Anders HJ. Late onset of Ccl2 blockade with the Spiegelmer mNOX-E36-3′PEG prevents glomerulosclerosis and improves glomerular filtration rate in db/db mice. Am. J. Pathol. 2008; 172(3):628–637. [PubMed: 18258851]

[42]. Jain RK, Stylianopoulos T. Delivering nanomedicine to solid tumors. Nat. Rev. Clin. Oncol. 2010; 7(11):653–664. [PubMed: 20838415]

[43]. Mamo T, Moseman EA, Kolishetti N, Salvador-Morales C, Shi J, Kuritzkes DR, Langer R, von Andrian U, Farokhzad OC. Emerging nanotechnology approaches for HIV/AIDS treatment and prevention. Nanomedicine (Lond). 2010; 5(2):269–285. [PubMed: 20148638]

[44]. Heath JR, Davis ME. Nanotechnology and cancer. Annu. Rev. Med. 2008; 59:251–265. [PubMed: 17937588]

[45]. Hwang do W, Ko HY, Lee JH, Kang H, Ryu SH, Song IC, Lee DS, Kim S. A nucleolin-targeted multimodal nanoparticle imaging probe for tracking cancer cells using an aptamer. J. Nucl. Med. 2010; 51(1):98–105. [PubMed: 20008986]

[46]. Gendler SJ. MUC1, the renaissance molecule. J. Mammary Gland Biol. Neoplasia. 2001; 6(3): 339–353. [PubMed: 11547902]

[47]. Brockhausen I, Yang JM, Burchell J, Whitehouse C, Taylor-Papadimitriou J. Mechanisms underlying aberrant glycosylation of MUC1 mucin in breast cancer cells. Eur. J. Biochem. 1995; 233(2):607–617. [PubMed: 7588808]

[48]. Girling A, Bartkova J, Burchell J, Gendler S, Gillett C, Taylor-Papadimitriou J. A core protein epitope of the polymorphic epithelial mucin detected by the monoclonal antibody SM-3 is selectively exposed in a range of primary carcinomas. Int. J. Cancer. 1989; 43(6):1072–1076. [PubMed: 2471698]

[49]. Reis CA, David L, Seixas M, Burchell J, Sobrinho-Simoes M. Expression of fully and under-glycosylated forms of MUC1 mucin in gastric carcinoma. Int. J. Cancer. 1998; 79(4):402–410. [PubMed: 9699534]

[50]. Yang JM, Byrd JC, Siddiki BB, Chung YS, Okuno M, Sowa M, Kim YS, Matta KL, Brockhausen I. Alterations of O-glycan biosynthesis in human colon cancer tissues. Glycobiology. 1994; 4(6):873–884. [PubMed: 7734850]

[51]. Ferreira CS, Matthews CS, Missailidis S. DNA aptamers that bind to MUC1 tumour marker: design and characterization of MUC1-binding single-stranded DNA aptamers. Tumour Biol. 2006; 27(6):289–301. [PubMed: 17033199]

[52]. Borbas KE, Ferreira CS, Perkins A, Bruce JI, Missailidis S. Design and synthesis of mono- and multimeric targeted radiopharmaceuticals based on novel cyclen ligands coupled to anti-MUC1 aptamers for the diagnostic imaging and targeted radiotherapy of cancer. Bioconjug. Chem. 2007; 18(4):1205–1212. [PubMed: 17583928]

[53]. Pieve CD, Perkins AC, Missailidis S. Anti-MUC1 aptamers: radiolabelling with (99m)Tc and biodistribution in MCF-7 tumour-bearing mice. Nucl. Med. Biol. 2009; 36(6):703–710. [PubMed: 19647177]

[54]. Savla R, Taratula O, Garbuzenko O, Minko T. Tumor targeted quantum dot-mucin 1 aptamer-doxorubicin conjugate for imaging and treatment of cancer. J. Control. Release. 2011 [Epub ahead of print].

[55]. Troyer JK, Beckett ML, Wright GL Jr. Location of prostate-specific membrane antigen in the LNCaP prostate carcinoma cell line. Prostate. 1997; 30(4):232–242. [PubMed: 9111600]

[56]. Wright GL Jr. Haley C, Beckett ML, Schellhammer PF. Expression of prostate-specific membrane antigen in normal, benign, and malignant prostate tissues. Urol. Oncol. 1995; 1(1):18–28. [PubMed: 21224086]

[57]. Bander NH. Technology insight: monoclonal antibody imaging of prostate cancer. Nat. Clin. Pract. Urol. 2006; 3(4):216–225. [PubMed: 16607370]

[58]. Farokhzad OC, Jon S, Khademhosseini A, Tran TN, Lavan DA, Langer R. Nanoparticle-aptamer bioconjugates: a new approach for targeting prostate cancer cells. Cancer Res. 2004; 64(21): 7668–7672. [PubMed: 15520166]

[59]. Farokhzad OC, Cheng J, Teply BA, Sherifi I, Jon S, Kantoff PW, Richie JP, Langer R. Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo. Proc. Natl. Acad. Sci. U S A. 2006; 103(16):6315–6320. [PubMed: 16606824]

[60]. Cheng J, Teply BA, Sherifi I, Sung J, Luther G, Gu FX, Levy-Nissenbaum E, Radovic-Moreno AF, Langer R, Farokhzad OC. Formulation of functionalized PLGA-PEG nanoparticles for in vivo targeted drug delivery. Biomaterials. 2007; 28(5):869–876. [PubMed: 17055572]

[61]. Dhar S, Kolishetti N, Lippard SJ, Farokhzad OC. Targeted delivery of a cisplatin prodrug for safer and more effective prostate cancer therapy in vivo. Proc. Natl. Acad. Sci. U S A. 2011; 108(5):1850–1855. [PubMed: 21233423]

[62]. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature. 1998; 391(6669):806–811. [PubMed: 9486653]

[63]. Di Cresce C, Koropatnick J. Antisense treatment in human prostate cancer and melanoma. Curr. Cancer Drug Targets. 2010; 10(6):555–565. [PubMed: 20482488]

[64]. Reagan-Shaw S, Ahmad N. Silencing of polo-like kinase (Plk) 1 via siRNA causes induction of apoptosis and impairment of mitosis machinery in human prostate cancer cells: implications for the treatment of prostate cancer. FASEB J. 2005; 19(6):611–613. [PubMed: 15661849]

[65]. McNamara JO 2nd, Andrechek ER, Wang Y, Viles KD, Rempel RE, Gilboa E, Sullenger BA, Giangrande PH. Cell type-specific delivery of siRNAs with aptamer-siRNA chimeras. Nat. Biotechnol. 2006; 24(8):1005–1015. [PubMed: 16823371]

[66]. Chu TC, Twu KY, Ellington AD, Levy M. Aptamer mediated siRNA delivery. Nucleic Acids Res. 2006; 34(10):e73. [PubMed: 16740739]

[67]. Dassie JP, Liu XY, Thomas GS, Whitaker RM, Thiel KW, Stockdale KR, Meyerholz DK, McCaffrey AP, McNamara JO 2nd, Giangrande PH. Systemic administration of optimized aptamer-siRNA chimeras promotes regression of PSMA-expressing tumors. Nat. Biotechnol. 2009; 27(9):839–849. [PubMed: 19701187]

[68]. Ni X, Zhang Y, Ribas J, Chowdhury WH, Castanares M, Zhang Z, Laiho M, Deweese TL, Lupold SE. Prostate-targeted radiosensitization via aptamer-shRNA chimeras in human tumor xenografts. J. Clin. Invest. 2011; 121(6):2383–2390. [PubMed: 21555850]

[69]. Dey AK, Griffiths C, Lea SM, James W. Structural characterization of an anti-gp120 RNA aptamer that neutralizes R5 strains of HIV-1. RNA. 2005; 11(6):873–884. [PubMed: 15923374]

[70]. Khati M, Schuman M, Ibrahim J, Sattentau Q, Gordon S, James W. Neutralization of infectivity of diverse R5 clinical isolates of human immunodeficiency virus type 1 by gp120-binding 2′F-RNA aptamers. J. Virol. 2003; 77(23):12692–12698. [PubMed: 14610191]

[71]. Zhou J, Li H, Li S, Zaia J, Rossi JJ. Novel dual inhibitory function aptamer-siRNA delivery system for HIV-1 therapy. Mol. Ther. 2008; 16(8):1481–1489. [PubMed: 18461053]

[72]. Neff CP, Zhou J, Remling L, Kuruvilla J, Zhang J, Li H, Smith DD, Swiderski P, Rossi JJ, Akkina R. An aptamer-siRNA chimera suppresses HIV-1 viral loads and protects from helper CD4(+) T cell decline in humanized mice. Sci. Transl. Med. 2011; 3(66):66ra66.

[73]. Wheeler LA, Trifonova R, Vrbanac V, Basar E, McKernan S, Xu Z, Seung E, Deruaz M, Dudek T, Einarsson JI, Yang L, Allen TM, Luster AD, Tager AM, Dykxhoorn DM, Lieberman J. Inhibition of HIV transmission in human cervicovaginal explants and humanized mice using CD4 aptamer-siRNA chimeras. J. Clin. Invest. 2011; 121(6):2401–2412. [PubMed: 21576818]

[74]. Hicke BJ, Stephens AW. Escort aptamers: a delivery service for diagnosis and therapy. J. Clin. Invest. 2000; 106(8):923–928. [PubMed: 11032850]

[75]. Charlton J, Sennello J, Smith D. In vivo imaging of inflammation using an aptamer inhibitor of human neutrophil elastase. Chem. Biol. 1997; 4(11):809–816. [PubMed: 9384527]

[76]. Cui ZQ, Ren Q, Wei HP, Chen Z, Deng JY, Zhang ZP, Zhang XE. Quantum dot-aptamer nanoprobes for recognizing and labeling influenza A virus particles. Nanoscale. 2011; 3(6): 2454–2457. [PubMed: 21509395]

[77]. Yigit MV, Mazumdar D, Lu Y. MRI detection of thrombin with aptamer functionalized superparamagnetic iron oxide nanoparticles. Bioconjug. Chem. 2008; 19(2):412–417. [PubMed: 18173225]

[78]. Watorek W, Farley D, Salvesen G, Travis J. Neutrophil elastase and cathepsin G: structure, function, and biological control. Adv. Exp. Med. Biol. 1988; 240:23–31. [PubMed: 3266707]

[79]. Erickson HP, Bourdon MA. Tenascin: an extracellular matrix protein prominent in specialized embryonic tissues and tumors. Annu. Rev. Cell Biol. 1989; 5:71–92. [PubMed: 2480799]

[80]. Koukoulis GK, Gould VE, Bhattacharyya A, Gould JE, Howeedy AA, Virtanen I. Tenascin in normal, reactive, hyperplastic, and neoplastic tissues: biologic and pathologic implications. Hum. Pathol. 1991; 22(7):636–643. [PubMed: 1712747]

[81]. Mackie EJ, Halfter W, Liverani D. Induction of tenascin in healing wounds. J. Cell Biol. 1988; 107(6 Pt 2):2757–2767. [PubMed: 2462568]

[82]. Zagzag D, Friedlander DR, Miller DC, Dosik J, Cangiarella J, Kostianovsky M, Cohen H, Grumet M, Greco MA. Tenascin expression in astrocytomas correlates with angiogenesis. Cancer Res. 1995; 55(4):907–914. [PubMed: 7531617]

[83]. Hicke BJ, Stephens AW, Gould T, Chang YF, Lynott CK, Heil J, Borkowski S, Hilger CS, Cook G, Warren S, Schmidt PG. Tumor targeting by an aptamer. J. Nucl. Med. 2006; 47(4):668–678. [PubMed: 16595502]

[84]. Shi H, He X, Wang K, Wu X, Ye X, Guo Q, Tan W, Qing Z, Yang X, Zhou B. Activatable aptamer probe for contrast-enhanced in vivo cancer imaging based on cell membrane protein-triggered conformation alteration. Proc. Natl. Acad. Sci. U S A. 2011; 108(10):3900–3905. [PubMed: 21368158]

[85]. Keefe AD, Pai S, Ellington A. Aptamers as therapeutics. Nat. Rev. Drug Discov. 2010; 9(7):537–550. [PubMed: 20592747]

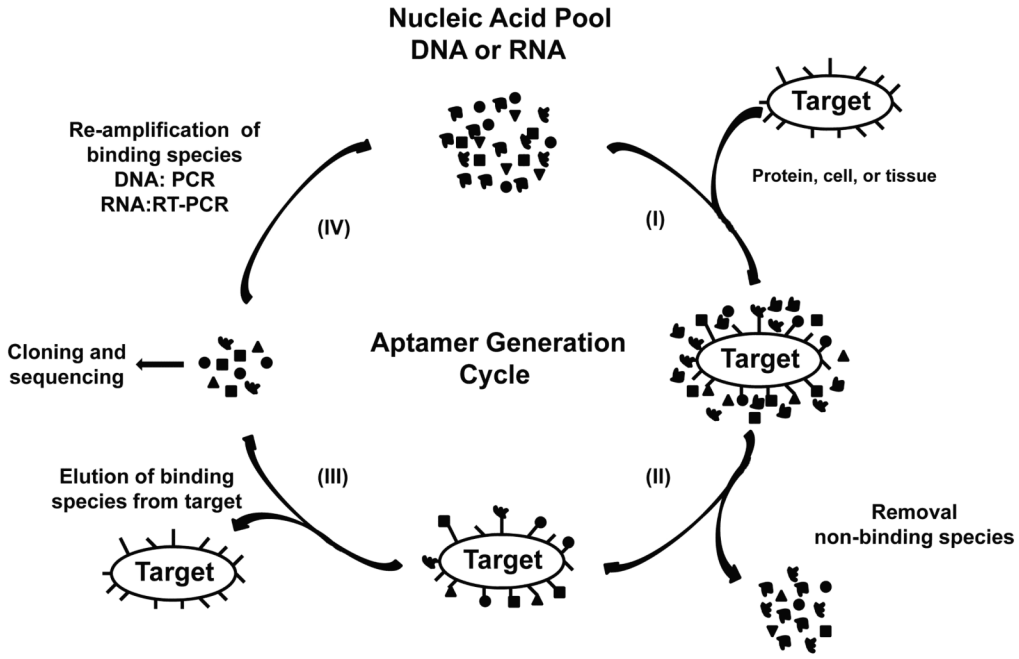

Figure 1.

Generation of aptamers by SELEX. I) Aptamer pools are incubated with target for binding. II) Targets are thoroughly washed to remove non-binding species. III) Target-bound aptamers are eluted from the target. IV) Amplification of candidate aptamers by PCR (DNA) or RT-PCR and transcription (RNA). Steps I-IV is repeated for multiple rounds to deplete non-binders and enrich for target-binders. Following the final round of selection, binding nucleotides are cloned and sequenced to determine the specific nucleotide composition.

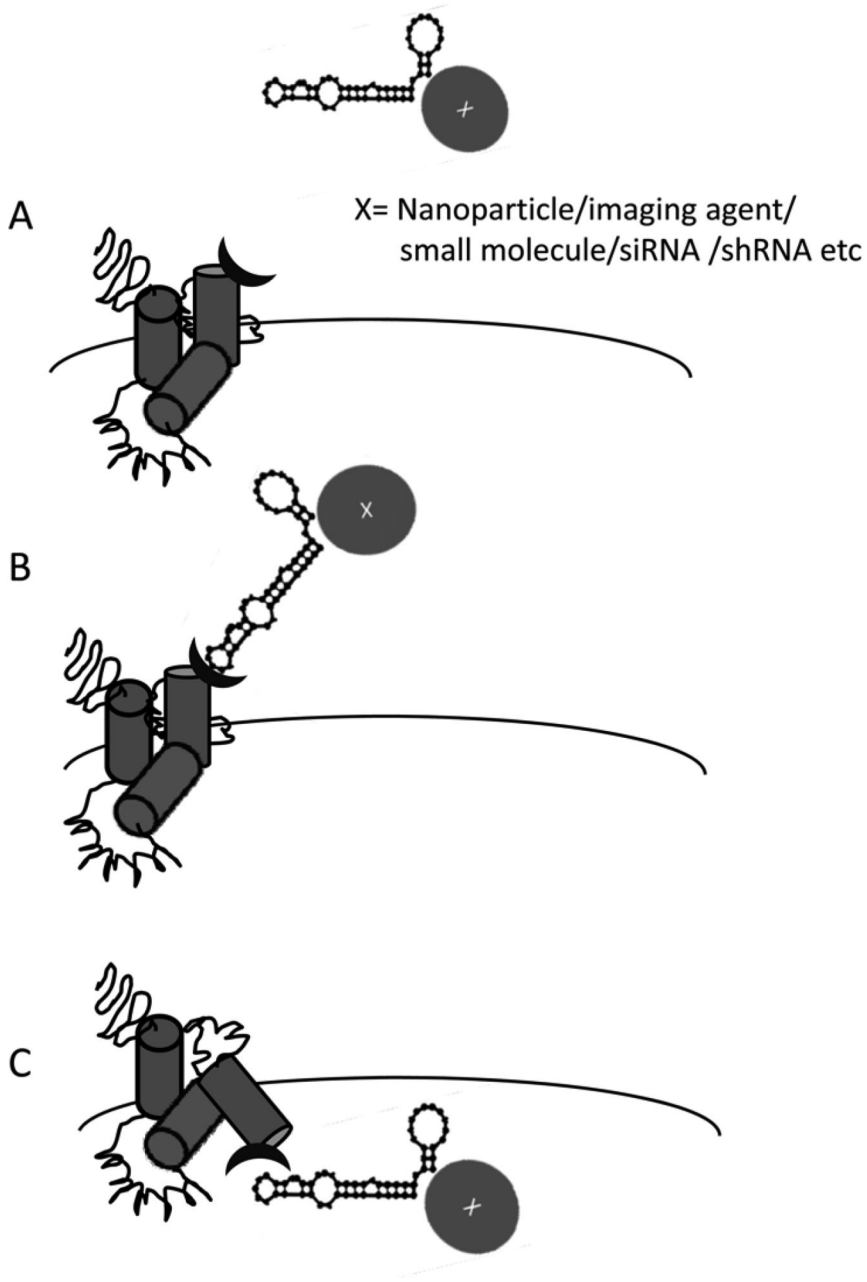

Figure 2.

Use of aptamers to target cell surface ligands. A) Aptamers can be conjugated to various agents such as nanoparticles, imaging agents, or siRNAs in a site-specific manner. B) Aptamer-targeted therapeutics or imaging agents can be captured on the surface of cells or in specific tissues. C) Receptor-mediated internalization of aptamer conjugates can result in intracellular delivery and accumulation.

Table 1

Current aptamers in various stages of clinical development.

Name	Nucleotide	Target	Condition	Phase	Company
Pegaptanib	RNA	VEGF	Macular Degeneration	FDA approved	Pfizer/Eyetech
AS1411	DNA	Nucleolin	Acute Myeloid Leukemia	Phase II	Antisoma Research
REG1 (RB006/ RB007)	RNA	Coagulation Factor IX	Coronary Artery Disease	Phase II	Regado Biosciences
ARC1779	DNA	vWF	Purpura, Thrombotic Thrombocytopenic	Phase II	Archemix
NU172	DNA	Thrombin	Heart Disease	Phase II	ARCA biopharma
NOX-A12	RNA	CXCL12	Hematopoietic Stem Cell Transplantation	Phase I	NOXXON Pharma AG
NOX-E36	RNA	CCL2	Type 2 Diabetes Mellitus	Phase I	NOXXON Pharma AG
ARC1905	RNA	C5	Age-Related Macular Degeneration	Phase I	Ophthotech
E10030	DNA	PDGF	Age-Related Macular Degeneration	Phase II	Ophthotech

Table 2

Promising aptamers evaluated in preclinical models for treatment and diagnosis of various diseases

Name	Nucleotide	Target	Payload	Clinical Application	Reference
NX21909	DNA	Elastase	^{99m}Tc	Imaging (SPECT)	75
TTA1	RNA	TenascinC	^{99m}Tc	Imaging (SPECT)	83
sgc8	DNA	Unknown	Fluorophore	Imaging (Optical)	84
AptA, AptB	DNA	Mucin1	^{99m}Tc, QDot	Imaging (SPECT/Optical)	53, 54
AS1411	DNA	Nucleolin	Cobalt-ferrite NP, ^{67}Ga, Rhodamine	Imaging (MRI/SPECT/Optical)	45
A10, A10-3, A10-3.2	RNA	PSMA	Docetaxol, Cisplatin, siRNAs	Prostate cancer therapy	59, 60, 61, 65- 68
gp120 aptamer	RNA	gp120	siRNAs	HIV therapy	71, 72
CD4 aptamer	RNA	CD4	siRNAs	HIV therapy	73