Aptamers as therapeutics

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Abstract | Nucleic acid aptamers can be selected from pools of random-sequence oligonucleotides to bind a wide range of biomedically relevant proteins with affinities and specificities that are comparable to antibodies. Aptamers exhibit significant advantages relative to protein therapeutics in terms of size, synthetic accessibility and modification by medicinal chemistry. Despite these properties, aptamers have been slow to reach the marketplace, with only one aptamer-based drug receiving approval so far. A series of aptamers currently in development may change how nucleic acid therapeutics are perceived. It is likely that in the future, aptamers will increasingly find use in concert with other therapeutic molecules and modalities.

Oligonucleotide
A short nucleic acid; often implies generation by chemical synthesis.

SELEX
(Systematic evolution of ligands by exponential enrichment). SELEX is the process by which aptamers are discovered. A library of degenerate oligonucleotides are successively and iteratively partitioned for binding, and amplified enzymatically. Ultimately, individual members of the selected library are identified by being cloned and sequenced.

Aptamer
A folded single-stranded oligonucleotide that binds to a molecular target such as a protein.

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Nucleic acids can not only hybridize to one another based on a simple code, but can also form complex shapes that may act as scaffolds for molecular interactions and support complex formation with protein and small-molecule targets. Although this is true for biological nucleic acids[1–4], it was only recently that a series of technological advances allowed the development of *in vitro* evolutionary methods for the discovery of additional, non-biological oligonucleotides that can bind to protein targets. Advances in DNA synthetic methods have enabled the generation of large populations of degenerate oligodeoxynucleotides, while PCR allows small numbers of molecules to be amplified into amounts that can be readily manipulated by researchers. When these two advances were iteratively coupled with the ability to partition oligonucleotides on the basis of their binding or catalytic activities, *in vitro* selection of functional nucleic acids (termed SELEX) was born[5,6]. Nucleic acid ligands generated using SELEX have been termed aptamers, an invented Latin term that means 'to fit'. Aptamers are one of only a few classes of molecules that, like antibodies, can be crafted to bind to multiple different targets.

In a typical SELEX experiment a random sequence oligonucleotide library is synthesized that spans 20–100 residues in length, and each nucleotide is flanked by constant sequences required for enzymatic manipulation. The pool will frequently contain between 1×10^{13} to 1×10^{15} members, although it is claimed that even less diverse pools will yield useful aptamers[7,8]. In the case of RNA SELEX[5,6], the library of single-stranded RNA molecules is prepared by the *in vitro* transcription of double-stranded DNA templates, usually using recombinant T7 RNA polymerase. In the case of DNA SELEX, the library of single-stranded DNA molecules is often prepared by the strand separation of double-stranded PCR products[9].

Since the invention of the SELEX process around 1990 (REFS 5,6), researchers have identified high-affinity aptamers that target a broad cross-section of protein families including cytokines, proteases, kinases, cell-surface receptors and cell-adhesion molecules (TABLE 1). Although traditional SELEX methods require a soluble, pure form of the target, newer methods have been developed that target aptamers in a complex mixture such as cell-surface proteins[10] and human plasma[11]. Structurally constrained peptides and protein domains or fragments can also be used to generate aptamers in instances for which the full protein is not available or for when targeting of aptamers to a particular protein domain or site is desired.

For therapeutic applications aptamers are frequently in competition with small molecules and antibodies. Initially, aptamers targeted to VEGF found utility in the treatment of wet macular degeneration[12], in part because they had long half-lives in the ocular compartment. Aptamers that have properties more suitable for systemic administration are now being developed, primarily against targets in the bloodstream, such as thrombin, factor IXa and von Willebrand factor, or on cell surfaces such as epidermal growth factor receptor (EGFR). It may eventually be possible to use aptamers to access targets inside cells, either by delivering themselves or other drugs across membranes. In addition, the types of aptamers that can be discovered are beginning to expand, and now include agonists as well as antagonists. One aptamer has so far been marketed for therapeutic application, and a further eight aptamers are currently being evaluated in the clinic, including those comprising DNA, modified RNA and Spiegelmers. How the structural, chemical and pharmacokinetic characteristics of these and other aptamers influence their eventual clinical utility is a primary focus of this Review.

Table 1 | Aptamers to targets of therapeutic interest

Target (alternative name)	K_d (nM)	Therapeutic applications	Refs
α-thrombin	25	Prevent thrombosis	9
HIV-1 reverse transcriptase	1	Inhibit viral replication	134
HIV-1 Rev	<1	Inhibit viral replication	135
Fibroblast growth factor 2, basic	0.35	Prevent angiogenesis	136
Respiratory syncytial virus	40	Prevent infection	137
HIV-1 integrase	10	Inhibit viral replication	138
Vascular endothelial growth factor	0.14	Prevent neovascularization	139
Platelet-derived growth factor	0.1	Prevent tumour development	83
Immunoglobulin E	10	Prevent allergies	140
L-Selectin	3	Modulate inflammation	141
D-Adenosine	1,100	Unknown	39
Acetylcholine-specific auto-antibodies	60	Treat myasthenia gravis	142
Interferon-γ	68	Modulate inflammation and immune response	143
Keratinocyte growth factor	0.0002	Treat epithelial hyperproliferative disease	144
Neutrophil elastase	n/o	Modulate inflammation	145
P-selectin	0.04	Inhibit viral adhesion	146
Acetylcholine receptor	2	Control neurotransmission	147
Phospholipase A_2	118	Treat ARDS, septic shock	148
Protein tyrosine phosphatase	18	Inhibit oncogenesis, viral regulation	149
Activated protein C	110	Prevent thrombosis	150
CD4	0.5	Modulate immune response	151
Nuclear factor-κB	1	Treat chronic inflammatory disease	152
Lymphocyte function-associated antigen 1	500	Prevent tumour development, modulate inflammation	153
Cytohesin 1	5	Modulate cytoskeletal reorganization	154
αvβ3 integrin	2	Prevent tumour development	155, 156

VEGF
(Vascular endothelial growth factor). VEGF is an endogenous pro-angiogenic protein involved in some cancers and in macular degeneration.

Thrombin
An activated endogenous protein that catalyses reactions leading to coagulation.

Factor IXa
An activated endogenous protein that catalyses reactions leading to coagulation.

von Willebrand factor
An endogenous prothrombotic protein that recruits platelets to collagen exposed at sites of vascular injury.

Spiegelmer
An aptamer in which all of the sugars are the enantiomers (mirror image) of those found in biological nucleic acids.

Therapeutic relevance of SELEX

Aptamers as therapeutics. Aptamers can be used for therapeutic purposes in much the same way as monoclonal antibodies. However, unlike traditional methods for producing monoclonal antibodies, no organisms are required for the *in vitro* selection of oligonucleotides. This freedom from cellular biochemistry offers a huge advantage in manipulating the process of directed evolution. As we will see, chemistry, selection conditions and targets can be manipulated *in vitro* in ways that would be difficult or impossible if organisms were involved.

In addition, aptamers have a unique niche relative to other oligonucleotide therapeutics. For antisense oligonucleotides or siRNAs, the therapeutic target is intracellular, whereas aptamer therapeutics can be developed for intracellular, extracellular or cell-surface targets. Targeting proteins in these latter two classes alleviates the necessity for the therapeutic to cross the cell membrane. Much like monoclonal antibodies[13], aptamers can theoretically be used therapeutically in any disease for which extracellular blockade of protein–protein interactions is required. The focus on extracellular targets has so far not been a limiting factor for aptamer development, as aptamers are currently undergoing clinical evaluation for ocular

diseases, haematological diseases and cancer[14,15]. Some advantages and limitations of aptamers in comparison to antibodies are described in BOX 1.

The utility of SELEX for therapeutic applications. Many aptamers that are selected to bind to a specific protein also inhibit its function. This is possibly because protein active sites offer more exposed heteroatoms for hydrogen bonding and other interactions. Another explanation is that aptamers have a limited number of interactions that they can make with a protein target, and therefore aptamers that 'fit' into a crevice on a protein, such as an active site, are more likely to be selected (the so-called homing principle).

Most therapeutically useful aptamers tend to inhibit protein–protein interactions, such as receptor–ligand interactions, and thereby function as antagonists. However, at least some aptamers have been shown to have agonist-like activities. For example, aptamers isolated against the extracellular domain of the protein human epidermal growth factor receptor 3 (HER3; also known as ERBB3) can promote oligomerization (although this does not result in inhibition of downstream phosphorylation)[16]. By contrast, a DNA aptamer

Antisense oligonucleotide
An agent that inhibits gene expression by cleaving specific mRNA sequences using hybridization and RNase H.

siRNA
(Small interfering RNA). siRNA is an agent that inhibits gene expression by cleaving specific mRNA sequences using hybridization and the RNA-induced silencing complex.

Nuclease
An endogenous nucleic acid-cleaving enzyme.

Nucleotide
The phosphorylated monomeric unit of nucleic acids, comprising a base and a phosphorylated sugar.

isolated against an isoleucyl tRNA synthetase enhanced editing activity[17]. For antagonists, therapeutic effects are only observed as long as the aptamer can physically dock with the target. If the binding affinity of the aptamer is high, it is likely that the therapeutically relevant effect will be more prolonged. Therefore, as with many other drugs, the key features of aptamers that must be optimized for drug development include high affinity and specificity, and a long half-life in the relevant biological compartment. SELEX protocols have now been developed to increase target affinity (by decreasing off-rates) and to increase specificity[18]. For example, specificity can be selected for by using a specific subdomain of a protein as a target, or by immobilizing the target using a specific method or affinity tag[9]. Iteratively toggling or switching between multiple targets during selection (for example, between the full-length protein and a specific subdomain) can drive binding to a particular epitope[19]. Negative selection against closely related targets together with positive selection against the desired target can reduce cross-binding that might elicit toxicity[20].

Alternatively, sequential selection against different targets can be used to ensure that aptamers cross-bind to species homologues, yielding aptamers that can be used in both animal models as well as in humans[21].

In general, wild-type RNA and DNA molecules are too susceptible to nuclease-mediated degradation to be useful for most therapeutic applications. Accordingly, most SELEX experiments for therapeutics use oligonucleotides that are chemically modified in some manner. These approaches rely on DNA or RNA polymerases that can accept appropriately modified nucleotide triphosphates as substrates. Modified nucleotides can be introduced into libraries before initiation of SELEX by polymerases that can accept modified nucleotide triphosphates. This process can yield aptamers that are partially or completely substituted with one or more modifications, including 2′-amino pyrimidines[22–24], 2′-fluoro pyrimidines[25–27], and 2′-O-methyl ribose purines and pyrimidines[28,29] (FIG. 1a). Internucleotide linkages can also be modified (for example, phosphorothioate linkages)[30–32] (FIG. 1b) and high molecular mass

Table 1 (cont.) | **Aptamers to targets of therapeutic interest**

Target (alternative name)	K_d (nM)	Therapeutic applications	Refs
Tenascin C	4	Prevent tumour development	157
Prostate-specific membrane antigen	2.1	Treat progressive malignant prostate disease	115
U1A	4.5	Modulate gene regulation	90
Gonadotropin-releasing hormone 1	50	Prevent tumour development	41
E2F transcription factor	15	Prevent tumour development	158
Neurotensin 1	1.5	Prevent viral infection	159
Factor IXa	0.65	Prevent thrombosis	27
NS3 protease	10	Treat hepatitis C virus infection	160
Staphylococcus enterotoxin B	420	Treat *Staphylococcus* infection	43
Chemokine (C-C motif) ligand 2 (MCP1)	3	Treat lupus	40
Angiopoietin 2	0.06	Prevent angiogenesis	20
HIV gp120	5	Inhibit viral infectivity	161
Calcitonin gene-related peptide	3	Treat migraine	44
HER3 (ERBB3)	45	Prevent tumour development	16
Cytotoxic T-lymphocyte-associated protein 4	10	Prevent tumour development	162
Cytohesin 2	115	Prevent tumour development	163
Nociceptin	110	Manage pain	37
Ghrelin	35	Prevent tumour development	38
Chemokine (C-X-C motif) ligand 10 (IP-10)	1.5	Modulate inflammation	164
Receptor tyrosine kinase RETC634Y	35	Prevent tumour development	165
Substance P	40	Prevent tumour development	36
Mucin 1	0.135	Prevent tumour development	166
Amylin	3	Treat pancreatic cancer	34
Angiopoietin 1	2.8	Prevent angiogenesis	167
Bovine prion protein	6.8	Treat prion and Alzheimer's disease	168
Plasminogen activator inhibitor 1	n/o	Prevent metastasis	169
Epidermal growth factor receptor variant III	33	Prevent tumour development	170

ARDS, acute respiratory distress syndrome; n/o, not observed.

REVIEWS

polyethylene glycol (PEG) can be conjugated to the 5′-terminus. Finally, a wealth of chemistries are available by modifying nucleobases (for example, the 5-position of uridine[33]). The presence of these modified nucleotides stabilizes oligonucleotides against nuclease-mediated degradation, and imparts greater affinities to selected aptamers. So far, the highest affinity aptamers that have been reported in the literature contain

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Box 1 | Advantages and limitations of aptamers versus antibodies

Advantages of antibodies
- Pharmacokinetic and other systemic properties of antibodies are often sufficient to support product development
- Large size prevents renal filtration and together with binding to neonatal Fc receptors can give extended circulating half-lives
- Not susceptible to nuclease degradation
- Antibody technologies are widely distributed because the early intellectual property either never existed or has expired

Limitations of antibodies
- Antibodies are produced biologically in a process that is difficult to scale up without affecting product characteristics
- Viral or bacterial contamination of manufacturing process can affect product quality
- Often immunogenic
- Large size limits bioavailability or prevents access to many biological compartments
- Limited ability to utilize negative selection pressure or to select against cell-surface targets not available in functional recombinant form
- Susceptible to irreversible denaturation; limited shelf life
- Chemistries required for the attachment of conjugation partners are stochastic and lead to product mixtures and reduced activity

Advantages of aptamers
- Aptamers are produced chemically in a readily scalable process
- Chemical production process is not prone to viral or bacterial contamination
- Non-immunogenic
- Smaller size allows more efficient entry into biological compartments
- Able to select for and against specific targets and to select against cell-surface targets
- Can usually be reversibly denatured, and phosphodiester bond is extremely chemically stable
- Conjugation chemistries for the attachment of dyes or functional groups are orthogonal and can be readily introduced during synthesis
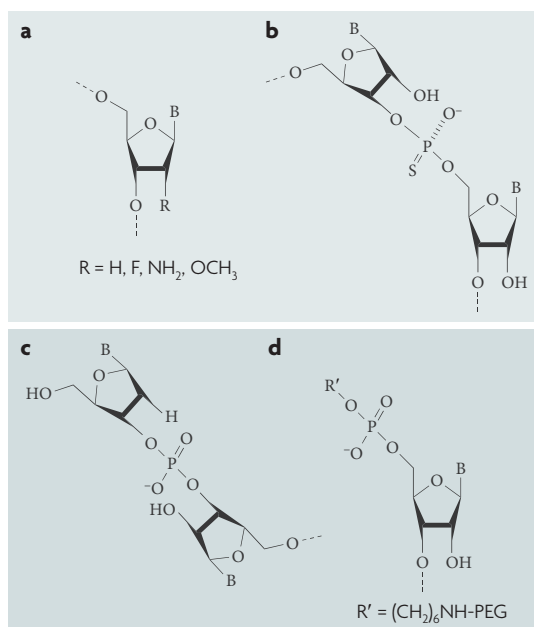
Limitations of aptamers
- Pharmacokinetic and other systemic properties are variable and often hard to predict
- Small size makes them susceptible to renal filtration and they therefore have a shorter half-life
- Unmodified aptamers are highly susceptible to serum degradation
- Aptamer technologies are currently largely covered by a single intellectual property portfolio

Strategies to overcome aptamer limitations
- Aptamers can be optimized for activity and persistence under physiological conditions during selection or during structure–activity relationship and medicinal chemistry studies conducted after discovery[133]
- Addition of conjugation partners such as polyethylene glycol or cholesterol can increase circulating half-life[46]
- Chemical modifications incorporated into the sugars or internucleotide phosphodiester linkages enhance nuclease resistance[29]
- Original intellectual property covering the SELEX (systematic evolution of ligands by exponential enrichment) technique will soon expire

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modified nucleotides and bind in the single-digit picomolar range (see REF. 22 for an example). The choice of RNA versus DNA, and sugar, phosphate and nucleobase modifications is largely driven by the need for stability; however, a finer appreciation of how these modifications affect function is for the most part still needed. For a fuller review of this topic see REF. 18.

Spiegelmers[34–44] are aptamers in which the sugars are the enantiomers (non-identical mirror images) of wild-type nucleic acid sugars. In the case of RNA Spiegelmers, the oligonucleotide backbone is composed entirely of L-riboses linked by phosphodiesters. Because nucleases are highly stereoselective these molecules are several orders of magnitude more nuclease-resistant than the corresponding wild-type RNA (D-ribose) sequences. SELEX cannot be performed with L-ribonucleotides directly because wild-type RNA polymerases will not accept L-ribonucleotide triphosphates as substrates and it is not yet technically feasible to prepare enantiomeric RNA polymerases (D-amino acids). Instead, wild-type RNA SELEX is performed using the enantiomer of the desired target. The sequences of wild-type RNA transcripts that bind to the enantiomer of the desired target, will, when prepared synthetically with L-ribose, bind to the desired target in the same manner. A significant limitation in the Spiegelmer approach is that enantiomeric targets need to be prepared synthetically, and so only relatively small protein domains or peptides can be used.

Aptamers as chemicals. Despite the fact that aptamers are discovered using an enzymatic process, aptamers are ultimately generated for therapeutic applications by chemical synthesis. Oligonucleotides are readily synthesized using solid-phase phosphoramidite chemistry in a process that has now been practiced for more than 25 years[45]. This process is automated and at small scale is considered routine even for oligonucleotides of up to 100 or more nucleotides or that contain a wide range of modifications. These modifications include phosphorothioate internucleotide linkages; non-deoxyribose sugars such as ribose, 2′-fluoro-ribose and 2′-*O*-methyl ribose; and oligonucleotide terminal caps, such as inverted nucleotides (FIG. 1c) or cholesterol. In addition, the process of chemical synthesis allows the site-specific introduction of non-nucleotide linkers (such as hexaethylene glycol), the introduction of chemical functionalities useful for conjugation (such as primary amines, thiol precursors and aldehyde precursors), and the addition of fluorescent or other reporter moieties (FIG. 1d). In particular, the site-specific placement of functional groups for conjugation means that the modification of aptamers after the solid-phase step (for example with high molecular mass PEG[46]) leads to products with discrete stoichiometries and defined chemical structures. These modifications avoid losses of activity that are often seen for stochastically modified therapeutic protein conjugates.

The cost of manufacturing aptamers has fallen over the past few years as the original patents in this area have begun to expire, and as the worldwide demand for oligonucleotides has driven the cost of nucleotide phosphoramidites down. At larger scales it is now possible

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Figure 1 | **Aptamer modifications. a** | Nucleotides can be modified by replacing the 2′ position with either a fluoro- (F), amino- (NH_2) or O-methyl (OCH_3) group for enhanced nuclease resistance. These modified nucleotides can be introduced either chemically or enzymatically. **b** | Bridging phosphorothioates can be incorporated enzymatically. **c** | End caps that involve reversing the polarity of the chain can be incorporated during chemical synthesis. **d** | Linkers are often inserted at the 5′-ends of aptamers by either chemical or enzymatic means to provide handles for conjugation or to alter pharmacokinetic properties. B, base; PEG, polyethylene glycol.

to pay less than US$3 per gram for DNA phosphoramidites, and less than $20 per gram for 2′-fluoro, 2′-ribo and 2′-O-methyl RNA phosphoramidites. It is relatively straightforward to acquire equipment to synthesize aptamers in the 10 g per batch range, and there are contract manufacturing organizations that are able to extend this to above 1 kg per batch. Purification is also straightforward because aptamers spontaneously refold after denaturation at elevated temperature or by chaotropic agents. These facts combine to make it much easier to advance the early stages of a therapeutic aptamer discovery project in comparison with a corresponding therapeutic antibody discovery project.

Factors affecting therapeutic potency *in vivo*

Although the selection of aptamers for therapeutic applications is relatively straightforward, the adaptation of aptamers for use *in vivo* has required extensive research. Relevant features of many protein therapeutics, such as those used in replacement therapies, can be extrapolated on the basis of characteristics of known, circulating proteins. For example, therapeutic antibodies tend to have long circulating half-lives because of their large molecular mass and FcRn-mediated recycling. However, there are few circulating nucleic acids from which similar comparisons can be drawn. Conversely, even though aptamers

FcRn
(Neonatal Fc receptor). FcRn recycles endosomal antibodies back into the extracellular space and thereby extends their circulating half-life.

Pegaptanib
Pegaptanib (trade name Macugen) is a vascular endothelial growth factor-specific aptamer approved in the United States and in Europe for the treatment of wet age-related macular degeneration by intravitreal injection.

are chemicals, as outlined above, they are large by the standards of traditional non-protein drugs and do not readily cross biological barriers such as cell membranes. Thus, many of the general rules that are typically applied by medicinal chemists during drug development do not apply to aptamers.

Most targets for therapeutic aptamers are either in solution in the blood plasma or displayed on the surface of cells that are accessible from the blood plasma, such as on the surface of the vasculature. Aptamers in this medium are subject to nuclease-mediated degradation by serum nucleases, renal filtration, uptake by the liver and other tissues such as the spleen.

Nuclease resistance. Aptamers composed of unmodified nucleotides have half-lives in the blood that can be as short as 2 minutes[47]. As we have previously stated, chemical modifications can be incorporated into the nucleotide sugars or internucleotide phosphodiester linkages to increase serum half-life. Endogenous serum nucleases have higher degradation rates when cleaving at pyrimidine residues[48] and accordingly the earliest modified composition aptamers showed considerable increases in serum stabilities even though their purines remained unsubstituted during SELEX[24,26]. More recently, SELEX with fully modified oligonucleotide compositions has also been reported[28]. Modifications can also be introduced after discovery, in a manner that is conceptually similar to small-molecule drug medicinal chemistry. Medicinal chemistry approaches can also be utilized to stabilize aptamers against nuclease-mediated degradation. Although it is laborious, aptamers can be scanned for the site-specific introduction of nuclease-resistant modifications[26,49,50]. The capping of oligonucleotide termini, especially at the 3′-terminus, also increases stability to endogenous serum nucleases[51]. This is often accomplished by inverting the nucleotide at the 3′-terminus so that the oligonucleotide has two 5′-termini and no 3′-termini, as 3′-exonuclease activity in serum is much higher than 5′-exonuclease activity.

Overall, such strategies can greatly increase serum half-lives[28]. Together with mechanisms to avoid renal filtration (see below) highly 2′-modified aptamers that are conjugated to 40 kDa PEG show half-lives as long as 1 day in rodents[28] and 10 days in humans (see prescribing information for pegaptanib (Macugen; Pfizer/Eyetech); Further information).

Renal filtration. Most aptamers fall into the 5–15 kDa molecular mass range (15–50 nucleotides long) and are therefore susceptible to renal filtration regardless of how well they resist nuclease-mediated degradation. As the molecular mass cutoff for the renal glomerulus is 30–50 kDa, aptamers that are conjugated to polymers in this size range show significant reduction in renal filtration rates. The most commonly used polymer for preventing exclusion by renal filtration is high molecular mass PEG[46,52,53].
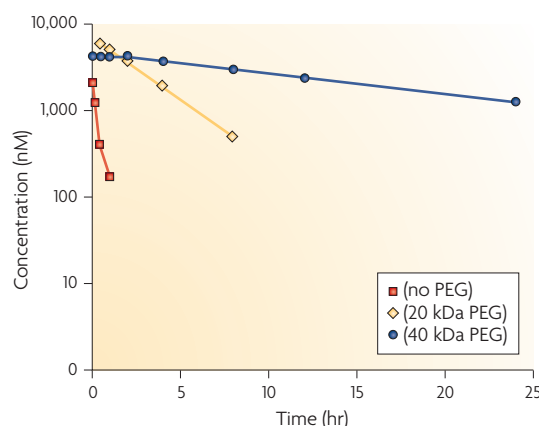
Unconjugated aptamers clear from the mouse circulatory system with a half-life of 5–10 minutes, whereas 40 kDa PEG–aptamer conjugates can have circulating

Figure 2 | **The pharmacokinetics of aptamers conjugated to different molecular mass PEGs.** Pharmacokinetic profiles of 39-mer 2′-deoxy purine, 2′-O-methyl pyrimidine composition aptamers. These aptamers were unconjugated or conjugated to either 20 kDa polyethylene glycol (PEG) or 40 kDa PEG and administered intravenously to CD-1 mice (*n* = 3 per time point) at 10 mg per kg. Data redrawn from REF. 54.

half-lives as long as 1 day[28]. The pharmacokinetic behaviours of unconjugated, 20 kDa PEG-conjugated and 40 kDa PEG-conjugated aptamers in the mouse after intravenous administration[54] are shown in FIG. 2.

Cholesterol conjugation has also been reported as an alternative strategy to reduce renal filtration rates, although the extent of this effect seems to be less than for PEG conjugation[55]. Conjugation of a 40 kDa PEG to a 2′ fluoro/2′-O-methyl modified aptamer increased its circulating half-life from 0.5 hours to 12 hours when administered to Sprague-Dawley rats. Moreover, a 40 kDa PEG conjugated to a fully 2′-O-methyl aptamer persisted in circulation with a half-life of 23 hours in mice[28]. In another example, Rusconi *et al.*[56] report the conjugation of cholesterol to a factor IXa-specific aptamer that resulted in an increase in half-life from 5–10 minutes to 1–1.5 hours in swine. Aptamer circulating half-lives are expected to increase as the clearance rate decreases, which is in turn proportional to the mass of the animal raised to the power of 0.75 (REF. 57). Consequently, longer half-lives are expected to be observed for animals larger than rodents.

Aptamers may be particularly amenable to modifications that lead to improved pharmacokinetic properties. Protein–PEG conjugation is generally performed stochastically through lysine side chains and this often results in a mixture of products and a loss of activity. By contrast, as aptamers are chemically synthesized and lack many of the functional groups commonly present in proteins, a single functional group (such as an alkyl amine) can be site-specifically introduced and used as a unique site for conjugation of other molecules to the aptamer without disrupting structure or function. Such site-specific handles for conjugation can be scanned through a number of positions to ensure that conjugation to PEG does not affect the activity of the conjugate.

Toxicology. As with other therapeutics, aptamer toxicity can manifest itself through on-target or off-target mechanisms. Off-target effects of oligonucleotides have previously been extensively studied as part of the development of antisense oligonucleotide therapeutics, and include anticoagulation, complement activation and innate immune stimulation. Antibodies to synthetic oligonucleotides are not generally observed, but have been seen for some oligonucleotide conjugation partners such as PEG[58]. There is limited published information concerning the toxicological properties of aptamer therapeutics because so far there is only one approved aptamer drug (pegaptanib), which is administered in small doses by intravitreal injection. However, there is literature concerning the toxicology of antisense therapeutics because this modality is a more mature research topic; reviews include REFS 59–61. Antisense oligonucleotide therapeutics and aptamer therapeutics differ in several respects including chemical substitution, size, secondary structure and conjugation to PEG; however, some parallels can be drawn.

Innate immune activation occurs as a consequence of the activation of Toll-like receptor 3 (TLR3), TLR7, TLR8 or TLR9. TLR3 responds to double-stranded RNA, TLR7 and TLR8 respond to single-stranded RNA, and TLR9 responds to unmethylated CG motifs in DNA (CpG motifs). Because unmodified RNA is highly sensitive to endogenous nucleases and because 2′-modification abrogates the TLR response[62], it is probably TLR9 that is most relevant to the study of potential adverse effects of therapeutic aptamers, although other TLRs may be relevant to the interpretation of *in vivo* responses to siRNA. Innate immune responses mediated by TLR9 include the activation of antigen-specific B cells and the secretion of cytokines including interleukin-6 and interferon[63,64]. Nevertheless, TLR9-mediated immune responses to oligonucleotides are being considered for therapeutic applications in which immune stimulation is desirable such as in oncology[65].

Anticoagulation has been observed in primates after administration of oligonucleotides. It is thought that this is a consequence of low-affinity interactions between the oligonucleotide and protein components of the clotting cascade. Many of these proteins bind to heparin and therefore might be expected to bind to other polyanions such as oligonucleotides. Measurable effects on clotting times have been observed using activated partial pro-thromboplastin time assays[66]. This effect is probably not significant enough to be of concern to patients as increases in bleeding times have not been reported.

Complement activation has been attributed to the interaction of oligonucleotides with complement factor H, which is a control protein for the alternative complement pathway. Complement activation has been observed in studies conducted in nonhuman primates in which effects are predominantly acute and seem to manifest themselves only after a high oligonucleotide concentration has been reached. Observations include hypotension and elevated heart rate, and can include cardiovascular collapse when severe[60].

Table 2 | **Aptamers in the clinic**

Name (company)	Composition	Target	Indication	Current phase	Refs
Pegaptanib sodium/Macugen (Pfizer/Eyetech)	2′-O-methyl purine/2′-fluoro pyrimidine with two 2′-ribo purines conjugated to 40 kDa PEG, 3′ inverted dT	Vascular endothelial growth factor	Age-related macular degeneration	Approved in the US and the EU	12,69,70
AS1411/ AGRO001 (Antisoma)	G-rich DNA	Nucleolin	Acute myeloid leukaemia	Phase II	71,72,74
REG1/RB006 plus RB007 (Regado Biosciences)	2′-ribo purine/2′-fluoro pyrimidine (RB006)/40 kDa PEG plus 2′-O-methyl antidote (RB007)	Coagulation factor IXa	Percutaneous coronary intervention	Phase II	62,63
ARC1779 (Archemix)	DNA and 2′-O-methyl with a single phosphorothioate linkage conjugated to 20 kDa PEG, 3′ inverted dT	A1 domain of von Willebrand factor	Thrombotic microangiopathies and carotid artery disease	Phase II	64,65
NU172 (ARCA biopharma)	Unmodified DNA aptamer	Thrombin	Cardiopulmonary bypass to maintain steady state of anticoagulation	Phase II	66
ARC1905 (Ophthotech)	2′-ribo purine/2′-fluoro pyrimidine conjugated to 40 kDa PEG, 3′ inverted dT	Complement component 5	Age-related macular degeneration*	Phase I	67
E10030 (Ophthotech)	DNA and 2′-O-methyl 5′-conjugated to 40 kDa PEG, 3′ inverted dT	Platelet-derived growth factor	Age-related macular degeneration*	Phase I	68
NOX-A12 (NOXXON Pharma)	L-RNA with 3′-PEG	CXCL12	Multiple myeloma and non-Hodgkin's lymphoma‡	Phase I	84
NOX-E36 (NOXXON Pharma)	L-RNA with 3′-PEG	CCL2	Type 2 diabetes, diabetic nephropathy	Phase I	40,85,86

CCL2, chemokine (C-C motif) ligand 2 (also known as MCP1); CXCL12, chemokine (C-X-C motif) ligand 12 (also known as SDF-1α); EU, European Union; PEG, polyethylene glycol; US, United States. *Co-dosed with a vascular endothelial growth factor-specific antibody fragment. ‡With autologous stem cell transplants.

Oligonucleotides have also been observed to accumulate in cells. This can be observed qualitatively by immunohistochemistry, *in situ* hybridization or the histopathology of stained tissue[59,67]. The accumulated material is generally seen in phagocytes such as macrophages or in renal proximal tubule epithelial cells. Degenerative effects have also been observed at high doses[68]. It is also possible that particular mechanisms of accumulation could be exploited for therapeutic applications, as described below.

Aptamers in the clinic

There are now several aptamers that have undergone clinical trials (TABLE 2), and a consideration of the observations that have been made in these trials will provide a better understanding of both the possibilities and limitations of aptamers as therapeutics. These will be discussed below.

Pegaptanib. Pegaptanib[69] was approved for therapeutic use by the US Food and Drug Administration in December 2004 and is currently marketed by Pfizer and Eyetech as Macugen. Pegaptanib is a VEGF-specific aptamer that binds to all isoforms of human VEGFA except for the smallest (VEGF121). Once pegaptanib is bound to VEGF it inhibits the interaction of VEGF with its receptors VEGFR1 (also known as FLT1) and VEGFR2 (also known as KDR/FLK1), with an IC_{50} value of 49 pM[26]. Pegaptanib was originally discovered in a 2′-ribo purine/2′-fluoro pyrimidine (rRfY) transcript library using SELEX. Once selected, the aptamer was truncated to 27 nucleotides to decrease synthesis costs, and then 12 out of the 14 ribopurines were replaced with 2′-O-methyl purines to increase nuclease resistance. The oligonucleotide was then conjugated to 40 kDa PEG to reduce the rate with which it distributes into tissues and it also was capped at the 3′-terminus with an inverted nucleotide to reduce 3′-exonuclease-mediated degradation. Pegaptanib is administered by intravitreal injection of 0.3 mg per eye once every 6 weeks, and is used to ameliorate the loss of visual acuity that is caused by the aberrant angiogenesis that is characteristic of age-related macular degeneration (AMD)[70]. Although pegaptanib sold well after regulatory approval, it has more recently lost significant market share to the antibody fragment ranibizumab (Lucentis; Genentech), which binds to all isoforms of human VEGFA including VEGF121.

AS1411. AS1411, formerly AGRO001, is a G-rich 26-mer oligodeoxynucleotide that contains only guanines and thymines and exists in solution as a guanine-quartet-mediated dimer. AS1411 is thought to elicit its therapeutic effects through its interaction with nucleolin[71]. AS1411 was discovered as part of a screen of antisense oligonucleotides for antiproliferative activity[72]. In common with other therapeutics discovered by cell-based screening (as opposed to screening or selection against individual protein targets) it is less certain what its exact mechanism of action is. Various biochemical effects have been demonstrated for this molecule including the destabilization of B-cell lymphoma protein 2 (BCL-2) mRNA[73], the inhibition of nucleolin-containing complexes[74] and the inhibition of nuclear factor-κB[75]. AS1411 inhibits the proliferation of cells in a wide range of cancer cell lines. The suggested mechanism of action for the antiproliferative activity of AS1411 includes binding to, and subsequent internalization by, cell-surface nucleolin followed by binding to cytoplasmic nucleolin. AS1411 is being developed by Antisoma and is currently in Phase II clinical trials for acute myeloid leukaemia, but it has recently been decided not to continue clinical evaluation of AS1411 for renal cancer[76].

REG1. REG1 is an anticoagulation system that includes RB006, a coagulation factor IXa-specific aptamer, and its oligonucleotide antidote RB007. REG1 is currently being evaluated in the clinic as a reversible anticoagulant for use during percutaneous coronary intervention[77]. RB006 was discovered in a 2′-ribo purine/2′-fluoro pyrimidine transcript library using SELEX[27]. The aptamer was then truncated to 34 nucleotides, conjugated to 40 kDa PEG to reduce the rate with which it is cleared from the serum by renal filtration and capped at the 3′-terminus with an inverted nucleotide to reduce 3′-exonuclease-mediated degradation. RB006 binds to coagulation factor IXa with an equilibrium dissociation constant (K_d) of 2.8 nM and prolongs clotting times as assessed in activated partial thromboplastin time assays. It does not prolong the clotting time of plasma when measured in prothrombin time assays, which is as expected for a specific coagulation factor IXa inhibitor. RB007 is a fully 2′-O-methyl substituted 17-mer oligonucleotide that is complementary to the 5′-terminal region of RB006. RB007 rapidly disrupts the structure of RB006 and inhibits its anticoagulation function. REG1 is being developed by Regado Biosciences. It is currently in Phase II clinical trials and shows rapid onset of anticoagulation *in vivo* after intravenous administration, followed by a rapid return to baseline levels after dosing of the RB007 antidote[78].

ARC1779. ARC1779 binds to the A1 domain of von Willebrand factor[19] and inhibits the capacity of this domain to bind to platelet membrane glycoprotein Ib receptors, thereby eliciting an antithrombotic effect without significant anticoagulation[79] (FIG. 3). ARC1779 was discovered in a degenerate DNA library using SELEX and then truncated to 39 nucleotides. It was also substituted with a single phosphorothioate linkage to increase target affinity and 5′-conjugated to 20 kDa PEG to reduce the rate with which it is subject to renal filtration. Additional alterations included capping at the 3′-terminus with an inverted nucleotide, and 26 2′-O-methyl modifications were introduced to increase nuclease resistance and thermal stability. ARC1779 binds to von Willebrand factor with a K_d value of 2 nM and inhibits platelet function with an EC_{90} value of 196 nM[80]. ARC1779 is being developed by Archemix and is currently in Phase II clinical trials for thrombotic microangiopathies and in patients with carotid artery disease undergoing carotid endarterectomy.

NU172. NU172 is a 26-mer oligodeoxynucleotide that is not modified, capped or conjugated and binds to and inhibits thrombin by an interaction with exosite 1. NU172 was discovered within a degenerate DNA oligonucleotide library using SELEX and was subsequently truncated to 26 nucleotides. It has an IC_{50} value of 5–10 μg per ml in plasma in an ecarin clotting time assay as measured by thromboelastography[81]. This aptamer is currently being evaluated in Phase II clinical trials by ARCA Biopharma. Because NU172 is not capped, substituted or conjugated to PEG, it has a short duration of action *in vivo*. NU172 is intended to be given by continuous infusion during cardiopulmonary bypass or other surgical procedures to maintain a state of anticoagulation with a rapid return to haemostasis once the infusion ceases.

ARC1905. ARC1905 binds to complement component 5 (C5), which is thought to play a role in AMD because it is pro-inflammatory and has been found in retinal deposits of patients with AMD[82]. ARC1905 was discovered in a 2′-ribo purine/2′-fluoro pyrimidine transcript library using SELEX. It was truncated to 39 nucleotides, 5′-conjugated to 40 kDa PEG to reduce the rate with which it is subject to renal filtration and capped at the 3′-terminus with an inverted nucleotide[25]. ARC1905 binds to C5 with a K_d value of 2–5 nM. ARC1905 is being developed by Ophthotech and is currently in Phase I clinical trials in which it is co-dosed with the VEGF-specific antibody fragment ranibizumab by intravitreal injection.

E10030. E10030 binds to platelet-derived growth factor (PDGF), which is known to play a role in the recruitment and maturation of pericytes that can increase resistance to the anti-VEGF treatment of AMD. E10030 was discovered in a degenerate DNA library using SELEX and then truncated to 29 nucleotides. Further engineering efforts included 5′-conjugation to 40 kDa PEG to reduce the rate with which it is subject to renal filtration and capping at the 3′-terminus with an inverted nucleotide. In addition, it was substituted with seven 2′-fluoro nucleotides and five 2′-O-methyl nucleotides to reduce nuclease degradation rates, and it has hexa ethylene glycol linkers in place of loops within the oligonucleotide structure[83]. E10030 is being developed by Ophthotech and is currently in Phase I clinical trials for AMD in which it is co-dosed with the VEGF-specific antibody fragment ranibizumab by intravitreal injection.
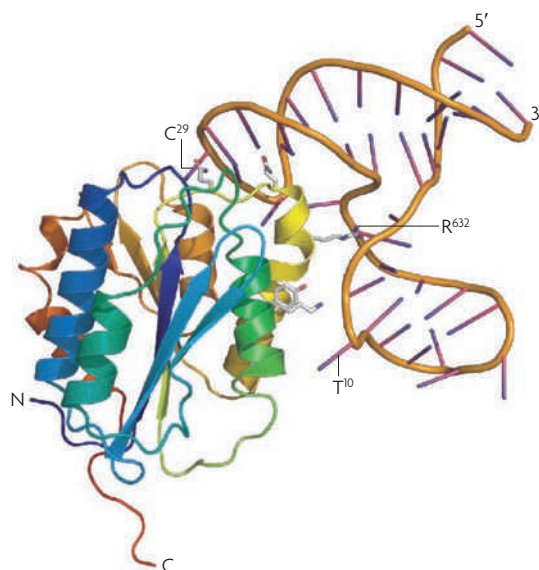
Figure 3 | **Crystal structure of the all-DNA parent of ARC1779 bound to the A1 domain of von Willebrand factor.**

NOX-A12. NOX-A12 binds to chemokine (C-X-C motif) ligand 12 (CXCL12; also known as SDF-1α), which is a chemokine thought to have multiple roles in cell homing, tumour metastasis, angiogenesis and tissue regeneration. NOX-A12 binds its target with an affinity of less than 1 nM, has an IC_{50} value of 300 pM in a Jurkat cell migration assay, and significantly reduces glomerulosclerosis in a mouse model of type 2 diabetes[84]. NOX-A12 is an L-RNA aptamer (Spiegelmer) that is 45 nucleotides long and has no chemical modifications beyond the reversed stereochemistry of the sugars and a high molecular mass PEG at the 3′-terminus. NOX-A12 was selected from a wild-type (D-RNA) library using the mirror-image target (D-amino acids), but was ultimately synthesized as an L-RNA in order to bind the wild-type target (L-amino acids). NOX-A12 is being developed for use with autologous haematopoietic stem cell transplants, as inhibition of CXCL12 affects the homing, mobilization and retention of these cells. It is intended to be used in the treatment of multiple myeloma or non-Hodgkin's lymphoma. NOX-A12 is being developed by NOXXON Pharma and is currently being evaluated in a Phase I clinical trial in which it is administered intravenously.

NOX-E36. NOX-E36 binds to chemokine (C-C motif) ligand 2 (CCL2; also known as MCP1), which is a protein that recruits monocytes and T cells from the vascular compartment to the extravascular space at sites of inflammation. NOX-E36 binds its target with an affinity of 150 pM and has an IC_{50} value of 3 nM in a mCCL2-stimulated migration assay. It has shown activity in a mouse model of lupus nephritis[85]. Like NOX-A12, NOX-E36 is an L-RNA aptamer (Spiegelmer) that is 40 nucleotides long and has no chemical modifications beyond the reversed stereochemistry of the sugars and

Base
A nucleic acid side-chain that displays specific hydrogen-bonding patterns utilized for selective interactions with other bases and binding partners for complex formation.

a high molecular mass PEG at the 3′-terminus[40,85–87]. NOX-E36 is currently being developed for the treatment of complications of type 2 diabetes, including diabetic nephropathy, by NOXXON Pharma. It recently completed evaluation in a Phase I clinical trial in which it was administered both intravenously and by subcutaneous injection.

The future of SELEX and aptamers

Advanced methods for aptamer discovery. Although high-affinity aptamers can be selected against numerous targets, the selection method itself is often labour intensive and time consuming. To further advance the number and types of aptamers available for therapeutic applications, it will be necessary to develop selection methods that are more high-throughput and to ensure that selected aptamers have exceedingly high affinities and are, or can be engineered to be, resistant to degradation and clearance. There have been several approaches to these goals.

One option is to automate the selection process so that aptamers can be reproducibly generated. In an early study, the adaptation of aptamer selection protocols to liquid-handling robots was attempted[88]. At a rate of 12 rounds of selection every 2 days, successful selections were achieved in just under 1 week[89]. Aptamers have also been robotically selected against a human protein, U1A, coordinately produced *in vitro*[90]. The Klussmann group at NOXXON Pharma took automation a step further by directly monitoring PCR and quantifying the amount of nucleic acid bound to the target[36]. Although these modifications decreased the speed of automated selection, there were also improvements in the separation of RNA from salts, buffers and urea, and a concomitant decrease in PCR artefacts.

Affinity capture onto magnetic beads is also amenable to miniaturization[91,92], and using picomolar amounts of targets aptamers with dissociation constants as low as 25 nM were selected. However, the most extensive work that has been done with high-throughput discovery methods for aptamers is that by the company Somalogic, where investigators recently reported the selection of 498 aptamers and their use to simultaneously interrogate blood samples[93]. Somalogic has pioneered the use of modified bases for SELEX, as opposed to just modified sugars (for example, 2′-*O*-methyl) or modified internucleotide linkages (for example, phosphorothioate). It is likely that their success in identifying multiple, high-affinity aptamers against many different targets is in part due to the fact that they have capitalized on the dizzying range of modifications at the 5-position of uridine originally developed and reported by Eaton's group[33,94–96].

Most reported initiatives that try to streamline the discovery process of therapeutic aptamers focus only on the selection and amplification steps. Although it is possible that microelectromechanical systems[91], deep sequencing[97] or computational prediction[98] will accelerate the identification of aptamers, it seems likely that 'analogue' predictions (selection itself) will greatly outperform such efforts for some time to come. In any event, once selection is complete the library is cloned
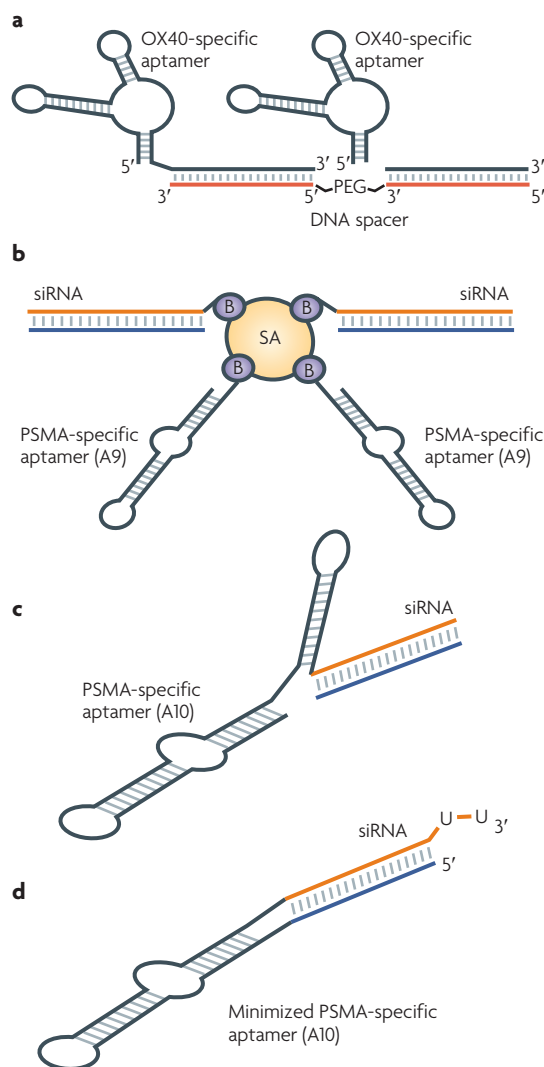
Figure 4 | **Aptamer architectures for therapy.**
a | OX40-specific aptamers were hybridized to an 'organizer' consisting of two antisense oligonucleotides separated by a polyethylene glycol (PEG) spacer[108,109]. **b** | Prostate-specific membrane antigen (PSMA)-specific aptamer A9 and 27-mer small interfering RNAs (siRNAs) were biotinylated (B) and conjugated to streptavidin (SA)[124]. **c** | PSMA-specific aptamer A10 was extended with a sequence that promoted hybridization to the guide strand of a siRNA[125]. The processed extension of the aptamer can then participate in gene silencing. **d** | PSMA-specific aptamer A10 was extended with a short hairpin RNA-like sequence. Processing again leads to gene silencing[126].

and sequenced, and multiple individual clones are assessed for binding to, and inhibition of, the desired target protein. Clones are ranked and this is followed by the truncation of the best performing aptamers, often dispensing with flanking sequence elements or internal loops that are not required for function. This is in turn followed by structure–activity relationship analyses in which the introduction of individual sequence or chemical substitutions yields variants that may have

improved nuclease resistance and inhibitory activity. Finally, conjugation of the aptamer to PEG or cholesterol leads to reduced renal filtration rates in animal models in which therapeutic potency is ultimately assessed. However, there has been very little reported work done to try to streamline these latter steps and any further attempts to improve SELEX will, even if successful, still mean that the entire discovery process is protracted and laborious.

One exception to this generalization may be the advent of complex target SELEX, in which aptamers are directly selected against cells or even in organisms[99–101]. Although cell-based selection can result in cross-reacting aptamers[102], many of the cell-specific aptamers generated so far have served well as internalizing escorts, neutralizing ligands or real-time imaging probes[99,102–104]. Particularly impressive examples of selections against organismal targets include the isolation of potent aptamers against infection-causing African trypanosomes[105], and a recent selection against intrahepatic carcinomas in a mouse model by the Clary group at the Duke University Medical Center, North Carolina, USA[106]. In the work done by the Clary group, modified RNA pools were intravenously injected into tumour-bearing mice and aptamers that were localized to the tumours were extracted and amplified. Two selected aptamers were found to target a biomarker (P68) that was overexpressed in the tumour.

Exploiting aptamer hybridization. One of the unique aspects of working with nucleic acid therapeutics is that novel interactions can be readily engineered via Watson–Crick base-pairing. Sullenger and co-workers originally selected a factor IXa-specific aptamer that could efficiently inhibit clot formation, then showed that the function of the aptamer could be reversed by an antisense 'antidote' both *in vitro* and *in vivo*[27,56]. The aptamer–antidote pair was tested in mouse and porcine model systems[107], and showed both robust anticoagulation activity and surprisingly rapid reversal of this activity. As the antidote was known to be cleared from the system within minutes, these experiments demonstrate an extraordinarily efficient hybridization of aptamer and antisense in serum. Oligonucleotides have also been used to assemble, rather than inhibit, aptamers. The Sullenger group at Duke University has dimerized an OX40-specific aptamer by hybridization to an oligonucleotide 'organizer' and showed that it behaves as a receptor agonist[108,109] (FIG. 4a). It may also be possible to generate multimeric aptamer constructs with corresponding avidity effects[110]. When melanoma cancer cells were co-injected into mice together with the aptamer agonist, tumour formation was significantly reduced or delayed. In the future, it is possible that aptamers will use methods associated with nucleic acid computation to act as circulating circuitry networks with combined diagnostic and therapeutic capabilities[111].

Targeting therapeutics with aptamers. Aptamers that bind to the cell surface can specifically cause therapeutics (such as drugs, toxins or siRNA) to persist in the vicinity of a specific cell or tissue type. This can also potentially

LNCaP
A prostate cancer-derived cell line with cell-surface prostate-specific membrane antigen.

PC3
A prostate cancer-derived cell line without cell-surface prostate-specific membrane antigen.

PLGA
(Poly(lactic-co-glycolic) acid). PLGA is a controlled-release, spontaneously degradable matrix for drugs.

cause an increase in the rate of internalization into cells by receptor-mediated endocytosis; for example, by acting as escort molecules to deliver intracellular therapeutics[112]. One popular epitope that has been targeted for therapy has been prostate-specific membrane antigen (PSMA), a protein that is widely expressed but rarely found on the cell surface. PSMA is observed on the surface of some prostate cancer cells[113,114]. The Coffey group at Johns Hopkins University School of Medicine, Maryland, USA, selected two aptamers against this receptor, termed A9 and A10, that demonstrate low nanomolar IC$_{50}$ values for this target[115]. As PSMA is constitutively internalized, the PSMA-specific aptamer was also an excellent candidate as an escort aptamer that could mediate delivery via endocytosis.

Conventional small-molecule therapeutics have been delivered by aptamers. Doxorubicin, an anthracycline drug, is widely used for anticancer treatment and is well known to interact with the double helix of DNA. Doxorubicin has been directly bound to the PSMA-specific aptamer A10 and delivered to cells[116]. Other aptamers, including the PTK7-specific sgc8c, have also been conjugated to doxorubicin and have proved their utility as vectors for drug delivery[117].

A more innovative application involves the generation of phototoxic aptamers for the targeted therapy of specific cancer cells[118]. DNA aptamers selected against short *O*-glycan peptides specifically expressed only on the surface of cancer cells were modified at their 5′-ends with chlorin e6, a photodynamic agent, and internalized into epithelial tumour cells. Light-activated cytotoxicity relative to the drug alone was found to be enhanced 500-fold, and resulted in tissue-specific killing of cancer cells.

The delivery of biopolymer therapeutics has also been examined. Gelonin is a toxin that has been conjugated to antibodies or other proteins for delivery to tumour cells, and as it lacks a translocation domain in the absence of conjugation it has little inherent cytotoxicity[119]. Gelonin conjugated to the PSMA-specific aptamer A9 can target and specifically destroy PSMA-overexpressing prostate cancer cells. The conjugates have an increased potency of at least 600-fold relative to cells that do not express PSMA[120]. Interestingly, studies with both gelonin and drugs have shown that conjugation can reduce the spontaneous uptake of the free therapeutic into non-cancerous cells, and hence potentially reduce side effects.

An interesting recent innovation is the use of aptamers to deliver other oligonucleotide therapeutics such as siRNAs[121]. One of the key difficulties facing the development of siRNA and other RNA therapeutics is their delivery, both systemically and to specific cell or tissue types[122,123]. Several approaches to link PSMA-specific aptamers to siRNA have been reported (FIG. 4b–d). Biotin-labelled aptamers were conjugated to biotin-labelled siRNAs via streptavidin[124], or aptamers were hybridized to siRNAs[125]. In both instances, specific targeting of siRNAs to LNCaP cells relative to PC3 cells was observed. The Sullenger group showed that siRNA conjugates could silence two survival genes that are commonly overexpressed in human tumours, polo-like kinase 1 (*PLK1*)

and *BCL-2*. More importantly, intratumoral injections of aptamer–siRNA chimeras into LNCaP xenografts reduced tumour volume, whereas a scrambled aptamer (still bearing the correct siRNA) did not. No interferon response was elicited with aptamer–siRNA constructs, despite the fact that the aptamers contained significant duplex structures. The Giangrande group at the University of Iowa, USA, followed up on these results by further optimizing the aptamer–siRNA chimera[126]. Several standard options for improving siRNA processing were introduced into the original construct, and the identification of a cell line amenable to uptake enabled the demonstration of efficacy after systemic administration[126].

Aptamer–siRNA chimeras have also been generated by fusing the HIV gp120-specific aptamer and a tat/rev-specific siRNA. This construct targets cells infected with HIV-1, and can inhibit HIV replication through both the aptamer and the siRNA components[127]. More aptamers generated against gp120 as well as other HIV proteins such as gp160 may allow for combination aptamer–siRNA delivery therapies[128].

Finally, aptamers can be used to direct the delivery of supramolecular structures. Using the PSMA-specific aptamer A10, Farokhzad, Langer and their co-workers directed the delivery of nanoparticles to tumour cells[129]. Farokhzad's group further delivered an encapsulated cancer drug, docetaxel, to LNCaP xenografts in nude mice. All seven of the mice treated by intratumoral injection survived and showed reductions in tumour volume, compared with the survival of only four out of seven mice in a control group that was treated with drug-encapsulated nanoparticles lacking the PSMA-specific aptamer. Some efficacy was also demonstrated with the chemotherapeutic drug cisplatin delivered to tumour cells via aptamer functionalized PLGA–PEG nanoparticle conjugates[130,131]. Similarly, a nucleolin-specific aptamer was conjugated to a liposome that encased the potent chemotherapeutic cisplatin and led to delivery of the drug to tumours[132]. Furthermore, controlled release of the conjugate to tumours was achieved by using an antisense oligonucleotide. These targeted encapsulation strategies may decrease the systemic toxicity normally associated with chemotherapies.

Summary

Aptamers represent an interesting class of pharmaceuticals that are intermediate in size, complexity and synthetic accessibility between traditional organic pharmacophores and protein drugs. Aptamers routinely achieve the same affinities and specificities as therapeutic antibodies, avoid the immunogenicity concerns of protein drugs, and can be generated to a range of targets more efficiently than is the case for high-throughput screening methods associated with small molecules.

However, aptamers have not yet demonstrated their place in the therapeutic arena as their high molecular mass and complex syntheses make them more expensive to manufacture than small molecules. Moreover, their still largely unknown pharmacokinetic properties make

them harder to develop than any given therapeutic antibody. It is hoped that further efforts to reduce the cost of synthesis, to rationally improve pharmacokinetic properties, and to develop approaches to non-antagonist modes of action will improve therapeutic opportunities. It is also true that at some level the relatively small number of therapeutic advances and clinical trials that have been undertaken is a result of the relatively small number of companies that can participate in aptamer development. As the earliest aptamer intellectual property begins to expire, it is anticipated that more companies will join the throng, and among the more promising future possibilities is the emergence of escort aptamers for therapeutic siRNAs to which it is conjugated. In addition, bivalent aptamers that can engage more than one target are emerging, including, for example, the recruitment of a specific cell type to a tumour-associated target, as well as *in vivo* selections to simultaneously optimize pharmacokinetic properties along with other contributions to drug potency.

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Competing interests statement
The authors declare competing financial interests: see web version for details.

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Laboratory of Andrew Ellington: http://ellingtonlab.org
Prescribing information for pegaptanib sodium (Macugen): http://www.macugen.com/macugenUSPI.pdf

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