Aptamer Therapeutics: The 21st Century's Magic Bullet of Nanomedicine

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Abstract: Aptamers, also known as chemical antibodies, are short single-stranded DNA, RNA or peptide molecules. These molecules can fold into complex three-dimensional structures and bind to target molecules with high affinity and specificity. The nucleic acid aptamers are selected from combinatorial libraries by an iterative in vitro selection procedure known as systematic evolution of ligands by exponential enrichment (SELEX). As a new class of therapeutics and drug targeting entities, bivalent and multivalent aptamer-based molecules are emerging as highly attractive alternatives to monoclonal antibodies as targeted therapeutics.

Aptamers have several advantages, offering the possibility of overcoming limitations of antibodies: 1) they can be selected against toxic or non-immunogenic targets; 2) aptamers can be chemically modified by using modified nucleotides to enhance their stability in biological fluids or via incorporating reporter molecules, radioisotopes and functional groups for their detection and immobilization; 3) they have very low immunogenicity; 4) they display high stability at room temperature, in extreme pH, or solvent; 5) once selected, they can be chemically synthesized free from cell-culture-derived contaminants, and they can be manufactured at any time, in large amounts, at relatively low cost and reproducibly; 6) they are smaller and thus can diffuse more rapidly into tissues and organs, leading to faster targeting in drug delivery; 7) they have lower molecular weight that can lead to faster body clearance, resulting in a low background noise for imaging and minimizing the radiation dose to the patient in diagnostic imaging. Thus, the high selectivity and sensitivity, ease of screening and production, chemical versatility as well as stability make aptamers a class of highly attractive agents for the development of novel therapeutics, targeted drug delivery vehicles and molecular imaging.

In the review, we will discuss the latest technological advances in developing aptamers, its application as a novel class of drug on its own, as well as in surface functionalization of both polymer nanoparticles or nanoliposomes in the treatment of cancer, viral and autoimmune diseases.

Keywords: Aptamer, SELEX, cancer.

INTRODUCTION

The term 'aptamer' was first coined 20 years ago, and is derived from the Latin aptus, to fit, and the Greek meros, part or region [1]. These small molecules are short stranded DNA or RNA molecules that have the ability to fold into complex three dimensional structures which bind to target molecules with high affinity and specificity [2]. In 1990, the laboratories of both Gold and Ellington independently pioneered the selection procedures of nucleic acid aptamers from combinatorial libraries by an iterative in vitro selection procedure known as the 'systemic evolution of ligands by exponential enrichment' (SELEX) [1, 3].

SELEX is the method by which aptamers are selected to bind to a particular target from a combinatorial library of synthetic oligonucleotides consisting of a pool of single-stranded DNA fragments with enormous repertoire and functionality. The random oligonucleotide library consists of a randomized central region with fixed terminal regions to allow primer binding. Typical aptamer sequences are between 20 and 60 nucleotides in length, although aptamers have been selected at 220 nucleotides long. Randomization of the central sequence provides the diversity within the library, typically within the range of 1×10^{15} independent aptamers, which allows for the high probability of a binding sequence being presented within the library [1, 3, 4].

Aptamer selection is characterized by the completion of successive steps of binding between the random oligonucleotide library and a target molecule, separation of bound aptamers from unbound sequences, elution of bound aptamers from the target and amplification of bound aptamers via PCR to form a new, more refined aptamer library [4, 5]. Following a number of SELEX cycles (usually around 10-15 cycles) that continue to enrich the binding species, the library can be cloned and sequenced, followed by confirmation of the binding, resulting in an aptamer with high specificity and affinity for its target molecule.

Due to the evolutionary pressures placed on aptamer sequences during the selection process, aptamer sequences are optimized primarily towards the binding of ligands.

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These sequences, which are predominantly unstructured in solution, fold upon association with their ligands into complex shapes with the ligand becoming an integrated part of the nucleic acid structure. It has well been established that aptamers bind to their ligands via adaptive recognition involving conformational alteration of either the target or the aptamer, precise stacking of flat moieties, specific hydrogen bonding, and molecular shape complementarity, and these multiple, highly specific and spatially distinct contacts across the target/aptamer complex are formed without the involvement of covalent bonds. While RNA aptamers are more susceptible to hydrolysis and present greater problems during the selection process, they also provide a much higher level of structural diversity than DNA aptamers [6, 7]. One of the benefits of aptamers versus their natural counterparts is that natural nucleic acids require a biased co-optimization of different structural motifs, including ligand binding sites, to be part of the intricate network of biological processes. Conversely, aptamers generally only have one function, which is to bind to their target, thus having a much higher affinity for the single target that they have been optimized to recognize [6]. Aptamers also share some similarities with antibodies, in that they might not be translatable to other assays: functioning well in one assay does not necessarily mean it will adapt to new applications. They can also bind non-specifically to cells, in much the same way that most other reagents do. Thus, all cell binding experiments using aptamers should include proper negative controls [8].

SELEX is, however, a complex process, comprising several steps within each cycle, with each step requiring optimization for successful SELEX to occur. The most critical step of the cycle is the separation of the bound species from non-binding species, and given the nature of the SELEX cycle, it is not possible to determine success until several cycles have been completed, making it a time-consuming and laborious process. For a laboratory entering this area of development, trial and error is necessary to optimize buffer conditions, partitioning of bound versus unbound species, number of PCR cycles and the total number of SELEX cycles to complete the selection. Since the first publication in 1990 detailing the selection of the first aptamer, numerous methodologies have been described to promote efficient partitioning, and increase aptamer affinity and specificity [4, 9-15].

APTAMERS AS THERAPEUTICS AND THERANO-STICS

Aptamers, as therapeutic molecules, possess numerous advantages and disadvantages over other therapeutic entities. As small molecules with non-modified nucleic acids, they have a half-life of minutes to hours due to nuclease degradation and can be cleared rapidly by the kidneys. This had led to the use of these unmodified aptamers for the treatment of transient conditions, like blood clotting, or for the use of treating organs where local delivery is possible, such as the eye. It has also led to the suggestion that these compounds can be used in vivo diagnostic imaging [16]. However, these benefits become disadvantages when considering their use as in vivo therapeutic agents, due to their inherent instability. It is possible to improve their half-life through the use of base modifications and the linkage of polyethylene glycol (PEG) [17]. Base modifications,

including 2'-O-methyl pyrimidines, 2'-deoxy purines, and 2'-fluorine-pyrimidines, have been shown to extend their in vivo stability to 86 hours [18]. The first aptamer to be approved by the US Food and Drug Administration (FDA) in 2004, Pegaptanib sodium (Macugen), for the treatment of age-related macular degeneration, contains not only 2'-O-methylated purines, and 2'-fluorine-pyrimidines to protect against endonucleases, but also a PEG moiety and a 3'-dT to enhance its pharmacokinetic properties and protect it against exonuclease attack [19]. These modifications led to Macugen having an initial half-life of 1.4h, which was extended to 131h following all these modifications [20]. In this way, it is possible to tailor the various chemical modifications to achieve a desired pharmacokinetic and biodistribution profile of a specific aptamer for a specific clinical application. These are the properties that make aptamers such promising therapeutic agents.

While aptamers can be selected against virtually any target, their mode of action is dependent on their target. In most cases, the actual binding of the aptamer to the target inhibits its biological activity, with this being due to interference with the enzymatic catalytic site, or ligand receptor recognition sites, or possibly inducing allosteric effects, such as conformational changes resulting in loss of function, or through interaction with its natural ligand [17, 18]. Due to loss of function, aptamers have found a niche in virtually every area of pathology, including vaccine production [21, 22], virology [23], parasitology [24, 25], and oncology [26, 27].

IMMUNOMODULATION

Virology is one area that can be greatly benefited from aptamer research. Vaccination and immunomodulation has proven immensely beneficial to human health, as evidenced by the great success of the smallpox, measles, polio and rabies vaccines, though there are several inherent problems associated with vaccine production, including both the genetic instability and heat sensitivity of the vaccines [28]. Aptamers can at least overcome the heat sensitivity, being highly stable at room temperature, and given the ease of selection and production, these nucleic acids can be quickly selected against new emerging strains [20, 29]. Nucleic acids can elicit a broad spectrum activity against a wide range of viruses, or be designed against a specific viral strain. These aptamer based drugs can stimulate the host's immune response and offer protection against viruses regardless of origin or genetic mutations. Indeed, studies have already been successfully completed using aptamers that inhibit viral enzymes, including proteolytic processing, reverse transcription and chromosomal integration, as well as viral expression, packaging and entry. Several aptamers have been shown to interact with viral coats that cause pandemic zoonotic viral respiratory infections [21, 22].

HIV has become a popular target for aptamer research, with one aptamer entering clinical development which inhibits HIV replication [23]. HIV binds to the CD4 receptor on T cells to enter the cell through the use of its gp120 envelope glycoprotein. The extensive glycosylation of gp120 allows HIV to evade the immune response. However, the small size of aptamers allows them to bind to the small conserved domains of gp120, whereas large molecules, such

as antibodies, can't. In vitro studies have shown that these aptamers were capable of blocking cellular infection with HIV by blocking the interaction between HIV and its receptor [26]. Hepatitis C has also received attention, with two aptamers having been developed against it, achieving a 70% inhibition of the maltose-binding protein-NS3 (MBP-NS3) protease activity [2].

As well as targeting viruses, aptamers can also be selected against bacteria, thus acting as antibiotics if selected to inhibit a crucial bacterial protein or to disrupt cell membrane formation. In addition, by linking the aptamer to an antibiotic agent, these molecules could be used as a 'targeting system' directed against specific pathogens [9].

PARASITOLOGY

One of the most promising areas of aptamer research is in the treatment of endemic diseases in developing countries. These diseases are in need of novel, effective and rapid treatments. Parasitic infections, the causative agent of the majority tropical diseases, have proven interesting targets. Human African trypanosomiasis, caused by a protozoan parasite of the genus Trypanosoma, is known to cause sleeping sickness and Chagas' disease. Again, in vitro studies have proven therapeutic value in the RNA aptamers that have been selected against the trypanosome cell surface [24, 25]. Trypanosoma cruzi is the causative agent of Chagas' disease. RNA aptamers have been developed which bind to the parasite receptors for host-cell matrix molecules on the trypomastigote cell surface. The biological activity in vitro blocked cell invasion by 50-70%, though the results also indicate that there are other mechanisms by which T. cruzi invades the cell [18].

Another parasite that has been the focus of aptamer research is the Plasmodium parasite, the causative agent of malaria. This disease is a devastating and frequently fatal disease. Molecular tools for gene disruption have been developed which has led to the identification of several parasite-derived proteins. Aptamers have been developed against these proteins of P. falciparum and have been postulated to be promising candidates as adjunct therapy in severe malaria [18, 30].

PRIONS

Prion diseases have also been benefited from this area of research, with aptamers raised against the insoluble amyloid conformer of the prion protein in transmissible spongiform encephalopathies, such as bovine spongiform encephalopathy and Creutzfeldt-Jakob disease. Aptamers have been developed against the abnormal prion protein and can distinguish between the abnormal and its normal counterpart even though they differ only in secondary and tertiary structure. Cell based assays have shown that these aptamers can prevent the accumulation of the insoluble abnormal form or the prion protein [9, 22, 31].

COAGULATION

The pathological formation of a clot in response to injury, stasis, or hypercoagulability follows abnormal coagulation. Aptamers with moderate affinity have been developed against thrombin to prolong the clotting time of human plasma. A DNA aptamer with a half-life of approximately 1-2 min was developed against thrombin for use in surgical indications requiring regional anticoagulation of an extracorporeal circuit. There are a number of factors and cofactors involved in the clotting cascade, with the enzymatic cascade initiated by the protease FVIIa with the aid of its cofactor, tissue factor. An RNA aptamer with 2′-fluoropyrimidines has been generated against FVIIa, with a half-life of about 15h, and has been shown to inhibit the factor X activation by 95% by preventing formation of an active FVIIa-tissue factor complex [32]. The aptamer targeting factor IXa (RB006) has now entered phase 2b clinical trials, aimed at enrolling 800 patients with acute coronary syndrome undergoing cardiac catheterization. This aptamer has had an 'antidote' designed against it which has shown a $51 \pm 14\%$ to $68 \pm 15\%$ successful partial reversal of RB006 activity, and a $93 \pm 11\%$ to $103 \pm 13\%$ complete reversal of RB006 activity in phase 2a clinical trials [33].

INFLAMMATORY DISORDERS

Inflammatory bowel disease (IBD) is another area of medicine benefiting from the area of aptamer development. Mucosal biopsies in patients suffering from IBD are characterized by an increased expression of the inducible form of nitric oxide synthase (iNOS) and increased levels of the radical nitric oxide. Inhibition of iNOS expression with aptamers has been proven in animal studies in areas of encephalomyelitis, sepsis, cerebral and renal ischemia, showing a potential therapeutic application in the treatment of IBD [34].

Autoimmune diseases occur when the patient develops antibodies against their own cells or tissues. One example is Myasthenia gravis, a neuromuscular disorder characterized by muscular weakness and fatigue resulting from an antibody-mediated autoimmune response to the nicotinic acetycholine receptor (AChR). Animal models have shown that a modified RNA aptamer against these autoantibodies can inhibit the autoimmune response [9, 26].

CANCER

The development of novel approaches for early cancer detection and effective therapy will significantly contribute to the improvement of patient survival [35]. Cancer is a disease that affects all age groups and places an extreme burden on patients, families and societies and it's the leading cause of death in the Western industrialized world [36, 37]. It is pointless to select an aptamer against any target, and for an aptamer to have an effect, it must be directed against a target of importance [32]. The four basic targeting criteria of monoclonal antibodies for cancer therapeutic applications are that the antigen of interest is overexpressed by tumor cells, the antigen participates as a principle component in the progression of the disease, the antigen is stable in its present form on the tumor cell surface, and that the antigen is expressed by a large percentage of tumor cells and a large variety of tumors, and these same criteria can be applied to aptamer research [38]. Cancerous cells have numerous biologically important targets and the majority of these are already being targeted by immunotherapy. Conventional cancer treatment includes surgery, radiotherapy and chemotherapy. However, the small therapeutic window of killing cancerous cells while preserving normal cells is a major limitation. Tumor–specific monoclonal antibodies

allow for the specific targeting of tumors, thus reducing the maximum tolerated dose and minimizing side effects, with current technology focusing on antibodies and antibody fragments against almost any tumor antigen [36, 37]. This technology isn't new, and aptamers have again found their niche in this area of research, having many advantages over antibodies, including low immunogenicity and a much smaller size, allowing for rapid diffusion [37]. The most advanced aptamer, in terms of clinical trials, in the field of cancer is AS-1411, which appears to bind to cell surface nucleolin and internalizes, leading to an inhibition of DNA replication. This has led to multiple cases of stable disease and one near complete response in a patient with renal cancer. In addition these promising signs were observed with no significant adverse effects [16, 17]. Aptamers that are currently in pre-clinical stages include a chemosensitizer for the treatment of cancer and retinopathy (ARC-127), an inhibitor of the isoform b of transcription factor HMGA1 (NOX-A50), and the anti-MUC1 aptamer designed against the Muc-1 protein in epithelial malignant cells [17].

Aptamers have also been selected against cytotoxic T-cell antigen-4 (CTLA-4), with inhibition thought to enhance anti-tumor immunity. This inhibition of CTLA-4 by aptamers induces the antigen-dependent expansion of T cells by blocking inhibitory signals delivered by CTLA-4. The efficacy of CTLA-4 aptamer in tumor suppression has been demonstrated in murine cancer models in which several aptamers have been shown to have similar, or superior functionality, to the anti-CTLA-4 antibody [26, 27].

APTAMER CONJUGATION

One of the benefits of targeted cancer treatments with antibodies or aptamers is the ability to directly couple therapeutic agents or package these therapeutic agents into particles that can then be delivered to specific cells [7]. Gene silencing siRNAs have been linked to aptamers to specifically deliver siRNAs to target cells, thus removing one of the limitations of using siRNA. This has been successfully demonstrated with a prostate specific membrane antigen (PSMA) aptamer delivering siRNA against pro-survival genes [16, 39, 40]. PSMA has also been directly conjugated to a chemotherapeutic drug, doxorubicin. Doxorubicin can directly intercalate into DNA, preferentially binding to double stranded 5'-GC-3' or 5'-CG-3' sequences. The cytotoxicity of the aptamer-doxorubicin conjugate was significantly enhanced against PSMA-positive cells as compared to PSMA-negative cells [41].

In addition to the anthracycline drug, doxorubicin, other drugs can be conjugated to aptamers through solid-phase synthesis or post-synthesis by incorporating an amino or thiol group at one end of the oligonucleotide during their assembly. Doxorubicin has also been attached by the addition of an acid-labile hydrazone linker to a 41-nucleotide long tyrosine kinase 7 PTK-7-specific DNA aptamer which then selectively internalizes into CCFR-CEM cells (T-cell acute lymphoblastic leukemia cells) with no apparent reduction in aptamer affinity for its target, and no non-specific internalization of the drug [7].

As well as conjugating drugs to the aptamer, radionuclides can be attached, allowing for both imaging and killing cancerous cells. Tenascin-C is a large hexameric glycoprotein associated with the extracellular matrix and expressed during tissue remodeling events occurring during angiogenesis and tumor growth. The aptamer directed against it, TTA1 has had a metal chelator, mercaptol-acetyl diglycine, incorporated into the 5' end which allows it to chelate Technetium-99m and has been used to successfully determine its biodistribution in vivo in a human glioblastoma nude mouse model [7]. Tenascin-C is not only a marker of glioblastomas, however, and this radionuclide conjugate has been shown to be taken up by a variety of solid tumors, including breast, lung and colon. Tumor uptake was rapid and blood, kidney and non-target tissue clearance was fast, providing a clear tumor imaging modality [16].

Aptamers are the ideal small molecule for conjugation to nanoparticles. Larger molecules, such as antibodies or proteins, together with their conjugated nanoparticles, can get 'caught up' by the reticular endothelial system and/or mononuclear phagocyte system. In contrast aptamer-nanoparticle conjugates are able to travel through the microvasculature as well as the tumor interstitium, a function that depends strongly and inversely on size [42]. Aptamers show superiority over other targeting ligands due to their smaller size which allows for a superior tissue penetration. Nanoparticle systems offer major improvements in therapeutics with aptamers having been designed to deliver toxins, radioisotopes and chemotherapeutic agents encapsulated in nanoparticles [7, 38]. Nanoparticles that are currently in use for cancer therapeutic applications include carbon nanotubes, micelles, dendrimers, liposomes, and metallic/polymeric/protein/ceramic/viral nanoparticles, with the material properties of each nanoparticle system having been specifically developed to enhance their delivery to tumors. Surface functionalization with the use of PEG can reduced the opsonization effects from the adherence of serum proteins, thus increasing circulation times. Current US FDA and European Medicines Agency approved nanoparticles, however consist of liposomes and PEGylated liposmes, and recently albumin-bound nanoparticles, containing either doxorubicin, daunorubicin or paclitaxel [38]. However, liposome formulations are the most successful drug delivery system. Liposome-aptamer conjugates have been shown to have increased circulation times and the aptamers have aided the delivery of the conjugate to their desired site of action [7]. One of the main problems associated with the use of chemotherapeutic drugs, especially anthracyclines such as doxorubicin, is cardiotoxicity. Through the use of targeted drug delivery such as intercalating it directly into the aptamer, or placing it within the liposome, avoid damage to healthy tissue, thus reducing the cardiotoxicity effects [39].

Of the four most common cancer types in the US (breast, lung, colorectal and prostate cancer), only breast (MUC-1) and prostate cancer (PSMA) have so far had aptamers successfully designed against a suitable target [7, 38]. One group has attached their PSMA aptamer to a functionalized nanoparticle containing docetaxel and showed negligible cytotoxicity and complete tumor regression in 70% of their study animals. In a similar study by the same group, more than four times greater accumulation was observed in subcutaneously injected human prostate cancer xenografts in rats with aptamer-targeted nanoparticles versus non-targeted nanoparticles [7, 43].

MULTIVALENT APTAMERS

One of the main problems associated with drug targeting is that the terminology would seem to imply a 'smart missile' type of activity. These drug carriers do not actively seek out target cells, but rather stumble on to their targets. In this way, the aptamer-drug conjugate must combine both 'passive' and 'active' targeting. Firstly, the conjugate must be able to pass through the fenestraes on the tumor blood vessels by chance through convectional flow and random diffusional processes. This passive targeting must, however, overcome the increased hydrostatic forces associated with the tumor extracellular space. Once the conjugate has successfully evaded these mechanisms, it must still be viable enough to attach to its specific target. If the drug is released prematurely, there may be insufficient accumulation at the tumor site [44]. These problems have been overcome due to the various modifications and conjugation to liposomes. However, once the drugs reach the tumor and retards growth, it has been postulated that the rapid blood flow, once very attractive initially to promote tumor targeting, will decrease due to a decreasing size and a normal supply of blood flow being sufficient to maintain its size. In addition, tumor cells are very heterogeneous and the remaining cancer cells may not possess the correct marker on its surface [44]. One method to overcome this very positive size reduction is to conjugate aptamers against different target. Previous work has formed multivalent aptamers against B52 in Drosophila and the cytotoxic T-cell antigen-4 (CTLA-4). The multivalent antagonist aptamer directed against B52 demonstrated increased avidity and potency. The CTLA-4 was postulated to promote T-cell activation and generate an anti-tumor immune response through inhibition. Through the formation of a tetrameric CTLA-4 aptamer, the immune response was enhanced both in cell culture and in vivo [7, 39].

Tumor targeting is not the only condition benefiting from multivalent aptamers. A bivalent aptamer consisting of two separate RNA aptamers binding two distinct stem-loop structures within the 5'-UTR of HIV has shown a more stable interaction with the target sequences in vitro compared to the individual aptamers. Bivalent aptamers targeting thrombin have also shown increased avidity and, therefore, enhanced anticoagulation effects [39].

CELL SELEX

While multivalent aptamers can solve some of the issues surrounding heterogeneity and increase avidity and potency, a relatively new method of selecting aptamers is gaining increased interest. So far, the aptamers mentioned have been developed against known targets. Through the use of whole cells, aptamers can be designed against targets on the cell surface without prior knowledge of the known target. In this way, it is possible to develop aptamers against specific cell types, such as cancer cells, that don't bind to normal cells. This method has successfully been used to recognize human receptor tyrosine kinase (RET) [45], whereas Tan used this method to design aptamers against the cultured precursor T cell acute lymphoblastic leukemia cell line (CCRF-CEM) [46]. In addition, this method can also be used to differentiate between different cancer cell types or biomarkers differentially expressed under varying physiological conditions. It also makes it possible to select aptamers against proteins with cancer-derived post-translational modifications. A benefit of using SELEX is that a pool of aptamers is selected following the SELEX cycle. Therefore, it is highly probable that following cell-SELEX, several aptamers directed against different cell surface targets are produced. This can be greatly beneficial when targeting a disorder, such as cancer, that has a heterogeneous composition [18, 46, 47].

Table 1. Summary of Therapeutically-Targeted Aptamers

Anti-Viral	TAR decoy/Tat aptamers
	HIV-1 Rev response element
	HIV reverse transcriptase
	HIV gp120
	Hepatisis C virus NS3
	Trypanosoma cruzi
	PrP^{Sc}
Anti-coagulation	Thrombin
	Factor VIIa
	Factor IXa
	Activated protein C
Anti-angiogenesis	Vascular endothelial growth factor (VEGF)
	Angiopoietin-2
	Angiogenin
Anti-inflammatory	Human neutrophil elastase
	Platelet-derived growth factor
	P-Selectin/ L-Selectin/ Sialyl Lewis X
	Human non-pancreatic secretory phospholipase A2
	Nuclear factor κ-B (NF- κ-B)
	Human complement C5
Anti-proliferation	Transcription factor E2F
	Prostate specific membrane antigen
	VEGF
	Tenacin-C
Immune modulation	Insulin receptor
	Acetylcholine receptors
	Immunoglobulin E
	Human interferon-γ
	Cytotoxic T cell antigen-4
	CD4
Cancer targeting	MUC-1
	EGFR-3
	Nucleolin
	Pigpen
	ERK2

CONCLUSIONS

While Macugen is the only FDA approved aptamer currently on the market, Aptanomics (Lyon, France) are working on therapeutic targets involved in signaling

pathways that are deregulated in a broad range of cancers, including a Bcl antagonist and a unique kinase antagonist, whereas Archemix (Cambridge, MA, USA) have 9 therapeutic aptamers currently in either phase I or phase II clinical trials spanning hematology, cardiovascular disease, oncology and age-related macular degeneration. While the therapeutic aptamers reviewed here are nowhere near a complete list of all the aptamers currently being investigated as therapeutic molecules (see Table **1** [2, 48, 49] for additional aptamer therapeutics), it does provide an overview and provides a basis for further reading into this interesting area of therapeutic medicine. There are many more potential areas of research that can be explored further, and hopefully soon, some of the more promising candidates will be approved by the US FDA for treatment of these diseases/disorders.

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ABBREVIATIONS

AChR =Acetylcholine receptor

CTLA-4 = Cytotoxic T-cell antigen-4

FDA = Food and Drug Administration

IBD = Inflammatory bowel disease

MBP-NS3 = Maltose-binding protein-NS3

PEG = polyethylene glycol

PSMA = prostate specific membrane antigen

SELEX = Systemic evolution of ligands by exponential enrichment

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