The Potential Use of Aptamers in The Process of Drug Development

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Abstract
Single-stranded nucleic acids can fold and create unique 3-dimensional structures when interacting with other molecules. The unique structure can achieve high specificity and affinity for the particular target. Synthetic oligonucleotide binding agents, also known as aptamers, are generated through the rational process of Systematic Evolution of Ligands by Exponential Enrichment (SELEX). As this technology matures, it shows increasing promise for use in the field of therapeutic drugs, drug discovery, development, and delivery, and this report seeks to detail how this technology may be applied.

Introduction
The continuous development of new medications to meet rising medical demand is subject to a tightly regulated system to ensure that emergent drugs are rendered safe and effective. Some drugs might take several years to be developed and an investment of millions of dollars is required.1 The efficiency and efficacy of drugs could be improved while reducing the development time and investment costs.1 The implementation of synthetic oligonucleotides, or aptamers, offers new methods for advancing the identification, production, and delivery of drugs with high efficiency and efficacy. Aptamers are composed of single-stranded ribose nucleic acid (RNA) or deoxyribonucleic acid (DNA), which fold into target-specific unique 3D structures,2 where some of the nucleotide bases interact directly with the target while the remainder of the nucleotide bases associate with each other to stabilize the structure. Aptamers can bind to a target with high affinity and specificity, making them comparable in use to antibodies.3 This short communication endeavors to review how aptamers have been used as agents for drug discovery and drug development, as drug carriers, and as therapeutic drugs. We make emphasis on new approaches and novel technologies that use aptamers in therapeutics against cancer.

Evolution of Aptamers
Aptamer selection was first demonstrated in 1990 by Tuerk and Gold, and Ellington and Szostak, in two separate publications.4,5 The word aptamer was chosen as a combination of the Latin term 'aptus', meaning 'to fit', and the Greek term 'meros', meaning 'part'.4,5 These initial publications also described the in vitro process of generating aptamers, now commonly known as Systematic Evolution of Ligands by Exponential Enrichment (SELEX).4,5 SELEX begins with the target molecule being introduced to a synthetic custom-designed and randomized oligonucleotide library. Following an incubation period, oligonucleotides with no affinity to the target are removed from the reaction solution and the bound oligonucleotides are enriched through PCR. This cycle is repeated with the addition of various selection pressures in order to promote variants within the oligonucleotide library to attain improved affinity and specificity to the target molecule.6 Provided sufficient evolution and selection pressures are subjected, the resulting aptamers evolved can discriminate between similar molecules such as caffeine and theophylline which differ in only by a single methyl group at nitrogen atom N-7, or between enantiomers of the same molecule such as L-arginine and D-arginine,7,8 with the capability of binding the target in complex biological matrices.9 Since the SELEX technique was first published, it has been modified into several subtypes to develop aptamers that bind to a diversity of targets including small molecules such as toxins and drugs, proteins, and contaminants. Also, the modification and improvement of SELEX techniques allows the enhancement of the natural properties of the aptamers upon binding such as changes in their 3D structure, to implement aptamers in several...
The use of synthetic affinity reagents such as aptamers offer multiple advantages over biologically generated antibodies. The synthetic nature of aptamers also allows for greater ease in chemical modifications, allowing them to mimic amino acid side-chains increasing their chemical diversity and affinity to targets that unmodified aptamers show low-affinity. For example, Slow Off-rate Modified Aptamers (SOMAmers) are aptamers where the 5' position of the uridine bases has been replaced with naphthyl, tryptophan, benzyl, or isobutyl groups, adding to the potential functional groups that can bind to the target, and thus increasing both the dissociation time and the binding affinity for proteins.

The next-generation aptamers also known as X-aptamers are another technology where various functional groups are added to the bases of the oligonucleotide to improve binding and specificity. Aptamers with chemical modifications can be used in different detection platforms which enables the improvement of target-detection levels in complex matrices and permitting the measurement of target concentration.

The nucleic acid composition of aptamers makes them non-immunogenic, unlike antibodies. The non-immunogenic property contributes to their high rate of clearance rate by the kidneys and susceptible to degradation by exonucleases in the bloodstream.

### Table 1

| Apatmers | Antibodies |
|----------|------------|
| Synthetic origin | Biological origin |
| Size is ~12 – 30 kDa | Size is ~150 – 170 kDa |
| Binding affinity down to pM | Binding affinity down to pM |
| In vitro and in vivo generation | In vivo generation |
| Adaptable to a variety of conditions | Restricted to function in biological conditions |
| Wide range of targets | Limited to targets that can be altered to provoke immune response |
| Enantiomer specific | Not enantiomer specific |
| Uniform batch performance | Non-uniform batch performance |
| Can be modified to improve pharmacokinetics, or how it moves into, through and out of the body. | Limited modification to improve pharmacokinetics |
| Selection time variable | Selection time long and affects specificity |
| Can regain function after denaturation | Loss of function after denaturation |
| Possibility of rational antidote design | No rational method of antidote design |
| Unlimited shelf life | Limited shelf life |
| Usually not immunogenic | Frequently immunogenic |
| Binding site can be modified to change specificity | Binding to target only |
| Targeted by exonucleases | Not targeted by exonucleases |
| High renal filtration | Low renal filtration |

Aptamers

**Chemical and Biological Properties**

The oligonucleotide structure of aptamers has substantial impact on their properties and can be advantageous. Since their synthesis is carried out using well-established DNA synthesis laboratories, aptamers synthesis quality is maintained without the batch to batch variations expected from antibody synthesis. The synthetic nature of aptamers also allows for greater ease in chemical modifications, allowing them to mimic amino acid side-chains increasing their chemical diversity and affinity to targets that unmodified aptamers show low-affinity.

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The use of non-natural bases with un-natural forms. The use of spiegelmers, which are constructed from D-ribose instead of the L-ribose enantiomer recognised by exonucleases, can greatly extend the half-life of administered aptamers. Spiegelmers are synthesized by creating a 'mirror image' of an existing aptamer out of D-ribose. The use of non-natural bases can also decrease the degradation and renal clearance of administered aptamers. A common method is to substitute the 2'-hydroxyl group of the pyrimidine bases with fluoro or amino groups, reducing the ability of exonucleases to recognize the nucleobase and thus slowing degradation.

The nucleic acid structure of aptamers allows for the rational design of antidotes. Antibodies and conventional drugs have no systematic method of antidote design, but aptamers can be disabled by the introduction of the antisense strand to the original nucleic acid sequence. The antisense oligonucleotide performs Watson-Crick base pairing and disables the aptamer's shape, so the aptamer is no longer capable of binding to the target. This has been a key advantage of aptamers in the design of detection and quantification sensor platforms.

The pharmacokinetics of aptamers or how aptamers move into, through, and out of the body, can be improved by modifying the natural oligonucleotide with un-natural forms. The use of spiegelmers, which are constructed from D-ribose instead of the L-ribose enantiomer recognised by exonucleases, can greatly extend the half-life of administered aptamers. Spiegelmers are synthesized by creating a 'mirror image' of an existing aptamer out of D-ribose. The use of non-natural bases can also decrease the degradation and renal clearance of administered aptamers. A common method is to substitute the 2'-hydroxyl group of the pyrimidine bases with fluoro or amino groups, reducing the ability of exonucleases to recognize the nucleobase and thus slowing degradation.

### Table 1

The functional and chemical differences between antibodies and aptamers are comparable due to their ability to bind target molecules ranging from ions to proteins, all the way up to whole cells, and do so with high specificity and affinity. As shown here, aptamers have several key advantages over antibodies.
Aptamers Use in Drug Development

Table 2. The list of companies that are currently developing aptamers as drugs, their year of founding, and the field they work in.

| Company                             | Established | Field                                      |
|-------------------------------------|-------------|--------------------------------------------|
| Antisoma Plc                         | 2001        | Aptamer-based cancer therapeutics          |
| Apta Biosciences                    | 2013        | Aptamer-based therapeutics, diagnostics    |
| AptaMatrix                          | 2003        | Aptamer discovery                          |
| Apta Sciences Inc                   | 2011        | Aptamer discovery                          |
| AptaTargets                         | 2014        | Aptamer-based therapeutics                 |
| Arterna                             | 2011        | Aptamers to assist drug delivery           |
| Apptitude Medical Systems Inc       | 2011        | Aptamer-based therapeutics, diagnostics    |
| Archemix                            | 2001        | Thrombin-inhibiting aptamers               |
| Centauri Therapeutics               | 2014        | Aptamer-based immunogenic therapeutics     |
| NOXXON Pharma                       | 1997        | Aptamer-based therapeutics                 |
| IVERIC Bio (formerly Ophthotech)    | 2007        | Aptamer-based eye therapeutics             |
| Ribomic                             | 2003        | Aptamer-based therapeutics                 |
| Somalogic                           | 2000        | Aptamer optimisation, biosensors           |
| Veraptus                            | 2011        | Aptamer-based therapeutics for bacteria and viruses |

Anticoagulation drugs.\(^{35,36}\) An aptamer can be introduced before or during surgery to stop blood clotting, and the antidote can be introduced as part of the recovery process, resulting in a faster resumption of the clotting process than a conventional drug and a better health outcome for the patient.\(^{37}\) Anti-coagulation aptamers pegnivacogin and NU172 are currently undergoing drug trialing.\(^{15,38}\)

**Context of Aptamer Drugs**

The potential use of aptamers as drugs was first discussed in 1995 due to the similarities between aptamers and antibodies, and the prevalence of antibody-based therapeutics.\(^{3,39,40}\) It was postulated that, if aptamers could be adapted to be functional in the body, their ability to change the properties or envelop their targets could be beneficial to inhibit diseases.\(^{41}\) The most popular targets for aptamer drugs were therefore diseases with a singular causative protein that could be inhibited by a suitably designed aptamer. Most notably, age-related macular degeneration, which is caused by the angiogenic VEGF, blood clotting disorders, and cancers were targeted by aptamer drug developers.\(^{42}\) Aged related macular degeneration was also selected as a popular target due to the immediacy of intravitreal injections, which allows them to bypass issues such as renal clearance that affect aptamer pharmacokinetics.\(^{43}\) The discovery of aptamers introduced the field of riboswitches and ribozymes, where folded pieces of RNA are capable of catalyzing reactions or altering gene expression.\(^{44}\) Aptamers can interact with a target to change its structure or enable or disable processes.\(^{45}\) This is the mechanism of Macugen, the first approved aptamer drug on the market, which targets VEGF and was approved in 2004. It was commonly used until recent years when antibody-based drugs superseded it in effectiveness.\(^{15,38}\) Additional aptamer-based drugs that are currently in development can be found in Table 3.

Table 3. List of aptamer drugs currently in clinical trials, their developers, their structure, their target, and their progress. Macugen is the only aptamer drug that has been released onto the market.

| Name                  | Developed by            | Form    | Target          | Disease                          | Progress                  |
|-----------------------|-------------------------|---------|-----------------|----------------------------------|---------------------------|
| Macugen (pegaptanib)  | NeXstar Pharmaceuticals | 27-nt RNA | VEGF            | Age-related macular degeneration | On market                 |
| REG-1 (pegnivacogin)  | Regado Biosciences Inc  | 37-nt RNA | Coagulation Factor Ixα | Arterial thrombosis            | Phase III (Suspended)     |
| Zimura (avacincaptad pegol) | IVERIC Bio          | 38-nt RNA | Complement Factor 5 | Age-related macular degeneration/Stargardt disease | Phase IIb                |
| AS1411                | Antisoma Research       | 26-nt DNA | Nucleolin       | Acute myeloid leukemia          | Phase II                  |
| NOX-E36 (emapticap pegol) | NOXXON Pharma          | 40-nt RNA | Chemokine CCL2  | Type 2 Diabetes Mellitus/Albininuria/Liver Cancer | Phase II                  |
| NOX-A12 (olaptesed pegol) | NOXXON Pharma          | 45-nt RNA | CXCL12          | Pancreatic, colorectal, brain cancer/Multiple myeloma | Phase II                  |
| NU172                 | Archemix Corp, Nuvelo   | 26-nt DNA | Thrombin        | Heart disease                   | Phase II                  |
| NOX-H94 (lexaptepid pegol) | Pharma                 | 44-nt RNA | Heparin         | Anemia/renal disease           | Phase II                  |
| RBM-007               | Ribomic                | 37-nt RNA | Fibroblast Growth Factor 2 | Age-related macular degeneration | Phase IIa                 |
Development of Aptamer-Based Drugs

Drug development most commonly begins with target identification. Once a disease or condition has been identified as a possible target for treatment, there are multiple methods that can be used to discover a candidate drug. High-throughput screening is used to test large libraries of potentially therapeutic molecules for efficacy in a disease model. Targeted methods use pharmacological principles to identify a potential drug target for the disease and develop an appropriate drug. Aptamers are best suited for targeted drug discovery since the oligonucleotides are developed against a specific molecule. The SELEX process can be modified to target different types of molecules; for example, cell-SELEX can screen libraries of oligonucleotides against a diseased cell, utilizing a healthy cell as a negative control in order to identify novel drug targets. After candidate aptamers have been identified, they are subject to refinement (Figure 1) in order to alter their properties for in vivo pharmacology. As previously discussed, the properties of aptamers in the body can be substantially modified in order to increase their effectiveness as clinical drugs. The difficulty of transition from in vitro to in vivo has historically been one of the primary causes for aptamer drugs to fail during drug trials. Aptamer drugs are significantly less likely than traditional drugs to have issues with toxicity or immune reaction. In contrast, aptamer drugs are instead more likely than comparable antibodies to have issues with efficacy. Future research is likely to focus on chemical alterations of aptamer drugs during the refinement stage in order to carry over in vitro efficacy in a biological platform. It will also be essential to improve the pharmacokinetic optimization of aptamer drugs so that the administration of these drugs will not be a limiting factor in clinical success. Macugen (pegaptanib), the aptamer drug that has been brought to market, was successful in administration as it is delivered via an injection directly into the eye, bypassing many pharmacokinetic issues that will need to be addressed as a wider range of diseases are targeted. For example, the aptamer used for Macugen or pegaptanib is conjugated to polyethyleneglycol or PEG to increase the intravitreal residence time and inhibiting the activity of the Vascular Endothelial Growth Factor (VEGF) for longer periods.

Aptamers in the Drug Screening Process

The selective binding ability of aptamers makes them suitable for assays and screening applications, and in this capacity, they can be exceedingly useful in the development of non-aptamer drugs. For the initial stage of target identification, aptamer microarrays and SOMAscans can be used to measure gene and protein expression and provide comparative information on diseased and non-diseased expression profiles. Aaptamers can also be modified with fluorochromes and quenchers in which the binding of the aptamer to its target protein triggers the uncoupling of the fluorochrome and a quencher allowing to track the aptamer activity in vivo. Most notably, an RNA aptamer (spinach aptamer), was developed to bind the green fluorescence protein (GFP) fluorophore 4-hydroxybenzlidene imidazolinone (HBI), and activate its fluorescence upon binding. The spinach aptamer can be introduced to the cell via vectors or plasmids and expressed for fluorescent visualisation inside the cell. The widely-used enzyme-linked immunosorbent assay or ELISA, which utilises antibodies, can be adapted into an ELONA (enzyme-linked oligonucleotide assay) which allows a greater range of targets and cheaper scaling due to the low synthesis and production costs of aptamers compared to antibodies. The chemical structure of aptamers can also be used to produce aptabeacons, which use the structural change upon target binding to effect a measurable change such as activation of an attached fluorescent molecule. These methods provide a useful toolkit for the identification of potentially novel candidates to be used as drugs. Another use of aptamers for assays is their incorporation in microarrays. Microarrays are commonly used to identify molecules of interest in a mixed solution. Aptamer-based microarrays could bind to a variety of target molecules such as other oligonucleotides, organic and inorganic compounds, and peptides and proteins while antibody-based arrays are limited to capture larger molecules such as proteins. Upon binding, the capture agent releases some kind of signal, such as a fluorescence, which is read by a high-resolution camera and used to quantify how much binding has occurred. Complementary DNA (cDNA) used in DNA microarrays and antibodies are currently the most popular capture agents, but aptamers are equally suitable for this purpose and offer higher shelf stability, and small molecule recognition. Multiple aptamers can be used in concert

Figure 1. The generic drug development pathway.
with each other to test for multiple molecules whereas an antibody array would suffer from severe cross-reactivity. However, aptamer-based microarrays require significant optimization as the microarray format can interfere with the folding and structure adoption of aptamers when bound to their molecules.25 Aptamer-based microarrays could be one of the most robust aptamer biosensor platforms and can be of use in all stages of drug development.

**Aptamers in Drug Purification**

Due to their relative cost-effective synthesis, high-affinity binding to specific targets, and ability to withstand repeated denaturation, aptamers can be utilized in the purification of other drugs. Aptamers have previously been utilized in the purification of the antibody-drug, Avastin, for the purification of the age-based macular degeneration target VEGF, and for the purification of a medley of human proteins from serum using chromatographic methods.51 Their ability to selectively discriminate between enantiomers of a molecule and reach binding constants as low as the femtomolar are also strongly beneficial features when using aptamers for drug purification.52 An aptamer produced for the drug of choice can reach yields that approach 100% recovery of the drug when utilized in affinity chromatography through the methods detailed in this research, and it is likely that aptamers will see greater use in the field of drug purification after this success.63

**Drug Delivery**

Site-specific drug delivery has increasingly become an area of focus in pharmacology as treatment methods are refined. For localized diseases such as cancerous tumors, or for drugs with a high level of off-target effects, it is essential to develop methods to ensure that the drugs are delivered to the correct part of the body in order to maximize efficacy and produce the best health outcome for the patients. The most common use of aptamers in the clinical context is in the delivery of drugs, toxins, liposomes, or siRNAs, using their high specificity to locate the target site and reduce off-target effects.64 Aptamers are well-suited for this purpose as they are simple to manufacture, highly specific for a given target, easily modified, and generally have little to no immunogenicity.

The most notable example of a drug delivered by aptamer is doxorubicin, an anti-cancer drug that is only delivered to cancerous cells due to the aptamer’s specific binding to PMSA-positive cells.15 The versatility of the aptamer structure means that they can be conjugated to a given drug in a variety of different ways in order to reduce the impact that aptamers could have on the efficacy and stericity of the drug. Aptamers are most frequently used for the delivery of anticancer drugs since cancerous cells typically display a unique set of antigens that allows them to be distinguished from healthy cells by aptamers.85 Small-interfering RNAs (siRNAs), which are a part of the RNA interference pathway, can be delivered to cells using aptamers as a targeting method, and since siRNAs and RNA oligonucleotides are both composed of RNA, the two are easily conjugated together.16,16 The use of aptamers for drug delivery is likely to increase as the need for targeted drug delivery increases in the future.

**Conclusion**

Aptamers are currently being utilized in different capacities at all stages of drug development. However, they have yet to be adopted universally into this process and will require additional research in order to reach maximum effectiveness. Aptamers have shown some effectiveness as drugs and have strengths in their low toxicity and easy manufacture. It is clear that the promise of aptamers in the drug development field has yet to be utilized, which paves the way for future discoveries that may have significant impacts on the field.

**Author Contributions**

All authors contributed equally to this work. (acquisition and interpretation of data and drafting). Dr. Kumar as the correspondence author designed and revised the manuscript. They have read and agreed to the published version of the manuscript.

**Conflict of Interest**

The authors report no conflicts of interest.

**References**

1. Hughes JP, Rees S, Kalindjian SB, Philpott KL. Principles of early drug discovery. Br J Pharmacol. 2011;162(6):1239-49. doi:10.1111/j.1476-5381.2010.01127.x
2. Jedd I, Saiz L. Three-dimensional modeling of single stranded DNA hairpins for aptamer-based biosensors. Sci Rep. 2017;7:1178. doi:10.1038/s41598-017-01348-5
3. Brody EN, Gold L. Aptamers as therapeutic and diagnostic agents. J Biotechnol. 2000;74(1):5-13. doi:10.1016/S1389-0352(99)00004-5
4. Ellington AD, Szostak JW. In vitro selection of RNA molecules that bind specific ligands. Nature. 1990;346(6287):818-22. doi:10.1038/346818a0
5. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: Rna ligands to bacteriophage t4 DNA polymerase. Science. 1990;249(4968):505-10. doi:10.1126/science.2200121
6. Liu Y-J, Lu S, Tong S, Chen X, Chen CP, Li D-J. Adaptive control-based barrier lyapunov functions for a class of stochastic nonlinear systems with full state constraints. Automatica. 2018;87:83-93. doi:10.1016/j.automatica.2017.07.028
7. Pfeiffer F, Mayer G. Selection and biosensor application of aptamers for small molecules. Front Chem. 2016;4:25. doi:10.3389/fchem.2016.00025
8. Ku T-H, Zhang T, Luo H, Yen TM, Chen P-W, Han Y, et al. Nucleic acid aptamers: An emerging tool for biotechnology and biomedical sensing. Sensors. 2015;15(7):16281-313. doi:10.3390/s150716281
9. McKeague M, DeRosa MC. Challenges and opportunities for small molecule aptamer development. J Nucleic Acids. 2012;2012:478913. doi:10.1155/2012/478913
10. Zhuo Z, Yu Y, Wang M, Li J, Zhang Z, Liu J, et al. Recent advances in selex technology and aptamer applications in biomedicine. Int J Mol Sci. 2017;18(10):2142. doi:10.3390/ijms18102142

11. Jayasena SD. Aptamers: An emerging class of molecules that rival antibodies in diagnostics. Clin Chem. 1999;45(9):1628-50. doi:10.1093/clinchem/45.9.1628

12. Stoltenburg R, Reinemann C, Strehlitz B. Selex—a (r) evolutionary method to generate high-affinity nucleic acid ligands. Biomol Eng. 2007;24(4):381-403. doi:10.1016/j.bioeng.2007.06.001

13. Komarova N, Kuznetsov A. Inside the black box: What makes selex better? Molecules. 2019;24(19):3598. doi:10.3390/molecules24193598

14. Kong HY, Byun J. Nucleic acid aptamers: New methods for selection, stabilization, and application in biomedical science. Biomol Ther. 2013;21(6):423. doi:10.4062/Fbiomothler.2013.085

15. Zhou J, Rossi J. Aptamers as targeted therapeutics: Current potential and challenges. Nat Rev Drug Discov. 2017;16(3):181-202. doi:10.1038/nrd.2016.199

16. Gao S, Zheng X, Hu B, Sun M, Wu J, Jiao B, et al. Enzyme-linked, aptamer-based, competitive biosensor for quantitative detection of plasmodium falciparum glutamate dehydrogenase in serum samples. Biosens Bioelectron. 2019;133:50-55. doi:10.1016/j.bios.2018.09.085

17. Bar-Or D, Rael LT, Madayag RM, Banton KL, Tanner AI, Acuna DL, et al. Stress hyperglycemia in critically ill patients: Insight into possible molecular pathways. Front Med. 2019;6:54. doi:10.3389/fmed.2019.00054

18. Kaur H, Bruno JG, Kumar A, Sharma TK. Aptamers in the therapeutics and diagnostics pipelines. Theranostics. 2018;8(15):4016-32. doi:10.7150/thno.25958

19. Bai Y, Feng F, Zhao L, Wang G, Wang H, Tian M, et al. Aptamer/thrombin/aptamer-aunps sandwich enhanced surface plasmon resonance sensor for the detection of subnanomolar thrombin. Biosens Bioelectron. 2013;47:265-70. doi:10.1016/j.bios.2013.02.004

20. Maier KE, Levy M. From selection hits to clinical leads: Progress in aptamer discovery. Mol Ther Methods Cl Dev. 2016;3:16014. doi:10.1038/mtm.2016.14

21. Minunni M, Tombelli S, Gullotto A, Luzzi E, Mascini M. Development of biosensors with aptamers as bio-recognition element: The case of HIV-1 tat protein. Biosens Bioelectron. 2004;20(6):1149-56. doi:10.1016/j.bios.2004.03.037

22. Ni S, Yao H, Wang L, Lu J, Jiange F, Lu A, et al. Chemical modifications of nucleic acid aptamers for therapeutic purposes. Int J Mol Sci. 2017;18(8):1683. doi:10.3390/ijms18081683

23. Wu X, Shaikh AB, Yu Y, Li Y, Ni S, Lu A, et al. Potential diagnostic and therapeutic applications of oligonucleotide aptamers in breast cancer. Int J Mol Sci. 2017;18(9):1851. doi:10.3390/ijms18091851

24. Sharma TK, Bruno JG, Dhiman A. Abcs of DNA aptamer and related assay development. Biotechnol Adv. 2017;35(2):275-301. doi:10.1016/j.biotechadv.2017.01.003

25. Kratschmer C, Levy M. Effect of chemical modifications on aptamer stability in serum. Nucleic Acid Ther. 2017;27(6):335-44. doi:10.1089/nat.2017.0680

26. Yan AC, Levy M. Aptamer-mediated delivery and cell-targeting aptamers: Room for improvement. Nucleic Acid Ther. 2018;28(3):194-9. doi:10.1089/nat.2018.0732

27. Appella DH. Non-natural nucleic acids for synthetic biology. Curr Opin Chem Biol. 2009;13(5-6):687-96. doi:10.1016/j.cob.2009.09.030

28. Sedighian H, Halabian R, Amani J, Heiat M, Amin M, Fooladi AA. Staggered target selex, a novel approach to isolate non-cross-reactive aptamer for detection of sea by apta-qpcr. J Biotechnol. 2018;286:45-55. doi:10.1016/j.jbiotec.2018.09.006

29. Thivyanathan V, Gorenstein DG. Aptamers and the next generation of diagnostic reagents. Proteomics Clin Appl. 2012;6(11-12):563-73. doi:10.1002/prca.201200042

30. Singh NK, Thungon PD, Estrela P, Goswami P. Development of an aptamer-based field effect transistor biosensor for quantitative detection of plasmodium falciparum glutamate dehydrogenase in serum samples. Biosens Bioelectron. 2019;123:30-5. doi:10.1016/j.bios.2018.09.085

31. Ruscito A, DeRosa MC. Small-molecule binding aptamers: Selection strategies, characterization, and applications. Front Chem. 2016;4:14. doi:10.3389/fchem.2016.00014

32. Ruscito A, McConnell EM, Koudrina A, Velu R, Mattice C, Hunt V, et al. In vitro selection and characterization of DNA aptamers to a small molecule target. Curr Protoc Chem Biol. 2017;9(4):233-68. doi:10.1002/cpb.c.28

33. Nimjee SM, White RR, Becker RC, Sullivan BA. Aptamers as therapeutics. Annu Rev Pharmacol Toxicol. 2017;57:61-79. doi:10.1146/annurev-pharmaco-010716-104558

34. Trapaidze A, Hérault J-P, Herbert J-M, Bancaud A, Gué A-M. Investigation of the selectivity of thrombin-binding aptamers for thrombin titration in murine plasma. Biosens Bioelectron. 2016;78:58-66. doi:10.1016/j.bios.2015.11.017

35. Woodruff R, Ivanov I, Verhamme I, Sun M-F, Gailani D, Sullenger B. Generation and characterization of aptamers targeting factor xia. Thromb Res. 2017;156:134-41. doi:10.1016/j.thromres.2017.06.015

36. Zavalyova E, Samoylenkova N, Revischin A, Tarashev A, Gordeychuk I, Golovin A, et al. The evaluation of pharmacodynamics and pharmacokinetics of antithrombin DNA aptamer RA-36. Front Pharmacol. 2017;8:922. doi:10.3389/fphar.2017.00922

37. Gunaratne V, Kumar S, Frederiksen JW, Stayrook S, Sullenger BA, Gué A-M, et al. Combination of aptamer and drug for reversible anticoagulation in cardiopulmonary bypass. Nat Biotechnol. 2012;30(7):606. doi:10.1038/nbt.4153

38. Hori S-i, Herrera A, Rossi JJ, Zhou J. Current advances in aptamers for cancer diagnosis and therapy. Cancers. 2018;10(1):9. doi:10.3390/cancers10010009

39. Huo Y, Li L, Lv X-J, Lai T, Zhang J, Zhang Z-Q. A sensitive aptasensor for colorimetric detection of adenosine triphosphate based on the protective effect of apt-aptamer complexes on unmodified gold
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nanoparticles. Biosens Bioelectron. 2016;78:315-20. doi:10.1016/j.bios.2015.11.043

40. Wang C, Zhang M, Yang G, Zhang D, Ding H, Wang H, et al. Single-stranded DNA aptamers that bind differentiated but not parental cells: Subtractive systematic evolution of ligands by exponential enrichment. J Biotechnol. 2003;102(1):15-22. doi:10.1016/S0168-1656(02)00360-7

41. Azhdarzadeh M, Atyabi F, Saei AA, Varanakkhasti BS, Omidi Y, Fateh M, et al. Theranostic muc-1 aptamer targeted gold coated superparamagnetic iron oxide nanoparticles for magnetic resonance imaging and photothermal therapy of colon cancer. Colloids Surf B: Biointerfaces. 2016;143:224-32. doi:10.1016/j.colsurfb.2016.02.058

42. Vandghanooni S, Eskandani M, Barar J, Omidi Y. As1411 aptamer-decorated cisplatin-loaded poly (lactic-co-glycolic acid) nanoparticles for targeted therapy of mir-21-inhibited cancer cells. Nanomed J. 2018;13(21):2729-58. doi:10.2217/nmm-2018-0205

43. Ebrahimi M, Johari-Ahar M, Hamzeiy H, Barar J, Mashinchian O, Omidi Y. Electrochemical impedance spectroscopy of methamphetamine by a specific aptamer. Bioimpacts. 2012;2(2):91. doi:10.5681/bi.2012.013

44. Weigand JE, Wittmann A, Suess B. RNA-based networks: Using RNA aptamers and ribozymes as synthetic genetic devices. Methods Mol Biol. 2012;813:157-68. doi:10.1007/978-1-61779-412-4_9

45. Yokobayashi Y. Aptamer-based and aptazyme-based riboswitches in mammalian cells. Curr Opin Chem Biol. 2019;52:72-8. doi:10.1016/j.cbpa.2019.05.018

46. Desbordes SC, Placantonakis DG, Ciro A, Socci ND, Lee G, Djaballah H, et al. High-throughput screening assay for the identification of compounds regulating self-renewal and differentiation in human embryonic stem cells. Cell Stem Cell. 2008;2(6):602-12. doi:10.1016/j.stem.2008.05.010

47. Zhu S, Rooney S, Michlewski G. RNA-targeted therapies and high-throughput screening methods. Int J Mol Sci. 2020;21(8):2996. doi:10.3390/ijms21082996

48. Layzer JM, Sullenger BA. Simultaneous generation of aptamers to multiple gamma-carboxyglutamic acid proteins from a focused aptamer library using deselx and convergent selection. Oligonucleotides. 2007;17(1):1-11. doi:10.1089/oli.2006.0059

49. Xiang Z, Wan R, Zou B, Qi X, Huang Q, Kumar S, et al. Highly sensitive and specific real-time PCR by employing serial invasive reaction as a sequence identifier for quantifying egfr mutation abundance employing serial invasive reaction as a sequence identifier for quantifying egfr mutation abundance. Anal Chem. 2018;410(26):6751-9. doi:10.1021/acs.analchem.8b03946

50. Xu X, Makaraviciute A, Kumar S, Wen C, Sjödin M, Abdurakhmanov E, et al. Structural changes of mercaptohexanol self-assembled monolayers on gold and their influence on impedimetric aptamer sensors. Anal Chem. 2019;91(22):14697-704. doi:10.1021/acs.analchem.9b03946

51. Lollo B, Steele F, Gold L. Beyond antibodies: New affinity reagents to unlock the proteome. Proteomics. 2014;14(6):638-44. doi:10.1002/pmic.201300187

52. Candia J, Cheung F, Kotliarov Y, Fantoni G, Sellers B, Griesman T, et al. Assessment of variability in the somanasc assay. Sci Rep. 2017;7(1):14248. doi:10.1038/s41598-017-14755-5

53. Fu H, Guthrie JW, Le XC. Study of binding stoichiometries of the human immunodeficiency virus type 1 reverse transcriptase by capillary electrophoresis and laser-induced fluorescence polarization using aptamers as probes. Electrophoresis. 2006;27(2):433-41. doi:10.1002/elps.200500460

54. James W. Aptamers in the virologists’ toolkit. J Gen Virol. 2007;88(2):351-64. doi:10.1099/vir.0.82442-0

55. Paige JS, Wu KY, Jaffrey SR. RNA mimics of green fluorescent protein. Science. 2011;333(6042):642-6. doi:10.1126/science.1207339

56. Kudlak B, Wieczerzak M. Aptamer based tools for environmental and therapeutic monitoring: A review of developments, applications, future perspectives. Crit Rev Environ Sci Technol. 2020;50(8):816-67. doi:10.1080/10643389.2019.1634457

57. Hanif A, Farooq R, Rehman MU, Khan R, Majid S, Ganaie MA. Aptamer based nanobiosensors: Promising healthcare devices. Saudi Pharm J. 2019;27(3):312-9. doi:10.1016/j.jsps.2018.11.013

58. Li Y, Lee HJ, Corn RM. Fabrication and characterization of rna aptamer microarrays for the study of protein–aptamer interactions with spr imaging. Nucleic Acids Res. 2006;34(22):6416-24. doi:10.1093/nar/gkl738

59. Witt M, Walter J-G, Stahl F. Aptamer microarrays—current status and future prospects. Microarrays. 2015;4(2):115-32. doi:10.3390/microarrays4020115

60. Taguchi K, Yamagishi S-i, Yokoro M, Ito S, Kodama G, Kaida Y, et al. Rage-aptamer attenuates deoxycorticosterone acetate/salt-induced renal injury in mice. Sci Rep. 2018;8(1):2686. doi:10.1038/s41598-018-21176-5

61. Tabasi A, Noorbakhsh A, Sharifi E. Reduced graphene oxide-chitosan-aptamer interface as new platform for ultrasensitive detection of human epidural growth factor receptor 2. Biosens Bioelectron. 2017;95:117-23. doi:10.1016/j.bios.2017.04.020

62. Schax E, Lönne M, Scheper T, Belkin S, Walter J-G. Aptamer-based depletion of small molecular contaminants: A case study using ochratoxin a. J Biotechnol. 2003;102(1):15-22. doi:10.1016/j.bios.2007.02.018

63. Alsgar OA, Kumar S, Hodgkiss JM. Lateral flow aptasensor for small molecule targets exploiting adsorption and desorption interactions on gold nanoparticles. Anal Chem. 2017;89(14):7416-24. doi:10.1021/acs.analchem.7b00906
65. Jo N, Kim B, Lee S-M, Oh J, Park IH, Lim KJ, et al. Aptamer-functionalized capacitance sensors for real-time monitoring of bacterial growth and antibiotic susceptibility. Biosens Bioelectron. 2018;102:164-70. doi:10.1016/j.bios.2017.11.010
66. Rossi J, Zhou J, Weinberg M, Morris K. Cell-specific internalizing ma aptamers against human ccr5 and uses therefore. United States Patent US20180080026A1. 2017.