APTAMERS AS USEFUL TOOL FOR THERAPEUTIC AND THERANOSTIC APPLICATIONS

Abstract

Aptamers are short oligonucleotide molecules with the tendency to fold into 3-dimensional structures that can further bind to specific targets with very high affinity and selectivity. The above properties make these aptamers to be termed as antibody analogs. Since the approval of first aptamer-based therapeutic, pegaptanib (Macugen) by US Food and Drug Administration, there has been a long gap exists during which several trials and technological developments in aptamer therapeutics that include their synthetic accessibility and modifications that enhance their therapeutic potentials were developed. In this chapter, we have discussed the significance of aptamers in terms of their structure, functionalities and requirement-based chemical modifications that can overcome the existing limitations are discussed. Representative examples of aptamers with significant therapeutic applications and the challenges that impede the successful application of these aptamers for clinical applications are also discussed.

Keywords: Aptamers, SELEX, Chemical Modification

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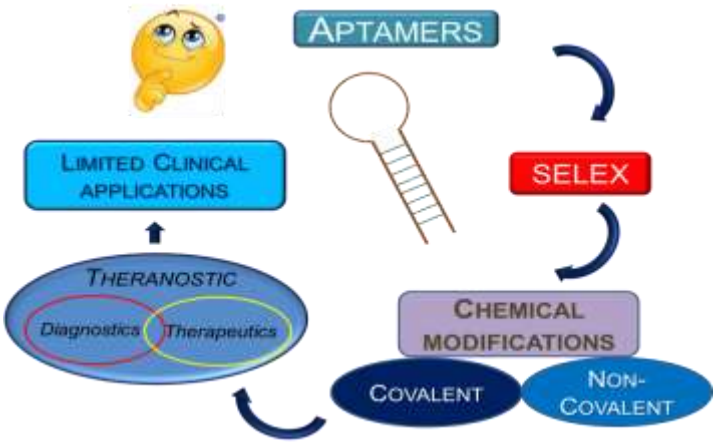
Graphical Abstract



I. INTRODUCTION

Accurate diagnostic methods and successful therapeutics are very important for the development of majority of the clinical applications. Recently, many small molecules with their capabilities to increase/modulate the activities of target sites are being investigated and reported.[1,2]Aptamer is one such molecular probe that is found to play key roles in many of the clinical applications. These are the synthetic units built from single-stranded nucleic acids (DNA or RNA) with the capability to fold uniquely to form secondary and tertiary structures. [3]Particularly, these nucleic acid aptamers were successfully proven for the ability to serve as protein mimics similar to polypeptide release factor in translation machineries. These distinctive features were successfully utilized for their interaction and strong binding with several target molecules like proteins, peptides, metal ions, micro-organisms, viruses and other cellular targets and make them as potential candidates for probe development. Recently, numerous aptamers probes were reported with their remarkable potential as potent anti-tumour agents because of their enhanced circulation stability, biocompatibility, multiple diagnostic functionalities and high drug loading capacities. [4,5]

The negatively charged backbone linkages of the aptamers interact strongly with the positively charged protein surfaces. This has been proven in several cases of aptamer-protein complexes of nucleic acid binding domains of nuclear factor (NF)-κB, bacteriophage MS2 capsid and the heparin binding domain of thrombin, indicating the displayed interaction is majorly induced through electrostatic mode although there are cases where non-electrostatic interactions are also observed. [6,7]

Many of the existing conventional aptamer-based therapies, target towards dysregulating the transcription activator to interfere with cell transfer machineries. They are known to influence the cellular functions by interfering with the binding of nucleic acids to transcription activators. With the advent of many oligonucleotide-based drugs, different ways for treating cancer, autoimmune and inflammatory diseases are available. Pegaptanib sodium is the first FDA approved nucleic acid-based drug that has been successfully demonstrated with the potential for the treatment of age-related macular degeneration. Later, several other aptamer based drugs in various clinical phases like Edifoligide (E2F Decoy), Metastatic Renal Cell Carcinoma (AS1411) and von Willebrand (ARC1779) were reported.[8] Other aptamer-based therapeutic applications include neurodegenerative, bacterial or viral infections, etc. Although aptamers are recognized for their unique roles in diagnostics and therapeutics, their applications are limited as they are easily prone to degradation, poor metabolic clearance and renal filtration, limited control on the nature of interaction, cross-reactivity, irreversible tissue uptake etc. Moreover there are several challenges towards their automated synthetic methods.[9]

Also, these aptamers have shown promising roles as diagnostic and therapeutic agents. [4,8]Although the principle of interaction is very similar to antibodies, aptamers are more preferred as they exhibit several advantages like shorter generation time, lower manufacturing costs, negligible batch-to-batch variation, easy to be modified, high thermal stability and broad spectrum target potentials. In this chapter, an update on the recent developments made in the field of aptamers, application specific chemical modifications that are required for the development of biosensors, therapeutic and theranostic are discussed.

The high specificity displayed by the aptamers to bind to the target depends on its three-dimensional conformation. Furthermore the affinity to the targets is influenced by various interactions including hydrogen bonding, electrostatic, vander Waals, hydrophobic and stacking interactions. Moreover, the ability of these aptamers to easily synthesized and chemically modified allows a variety of customization that can further be utilized to meet specific pharmacokinetics. They reveal high affinity towards small molecules, proteins and live cells. The above advantages of aptamers over antibodies have been utilized for the developing new generation of therapeutics that has recently grabbed the attention of the scientific community. A comparison of aptamers and antibodies are given in table 1.

Table 1: A Comparison of Critical Features between Aptamers and Antibodies

Property	Aptamers	Antibodies
Size	Small	Large bio-macromolecule
Stability	• Withstand broad range of temperatures. • Longer shelf life • Protease resistant • Nuclease degradable	• Stable only at refrigerated conditions. • Shorter shelf life • Degradable by proteases • Nuclease resistant
Specificity	Point mutations are identifiable	Lower specificity – multiple antibodies can bind the same antigen
Affinity	Multivalent aptamers show high affinity	Affinity depends on the number of epitopes
Chemical modifications	Easily modifiable – negligible influence on affinity	Modifications influences the activity
Targeting potential	Broad targeting potential- ions to molecules to cells	Induce strong immune response to generate antibodies
Synthesis feasibility	SELEX – 6 to 8 weeks	6 months
Organelle uptake	Fast	Slow

II. STRUCTURE OF APTAMERS

Aptamers are 15–100 nucleotides long oligonucleotide compounds with the tendency to display complex tertiary or quaternary structures. They usually tend to form complementary base pairs to attain certain structures like loops, stems, hairpins, quadraplexes, etc, which is followed by the formation of tertiary structures with the potential of unique molecular recognitions that are associated with specific targets. Moreover, these aptamers also possess the ability to differentiate conformational isomers and functional groups, identify mutations, recognize epitopes, etc. Since its evolution, several generations of aptamers have evolved following different types of SELEX processes that involve two repetitive stages – selection and amplification. With recent advancements it is possible to develop multivalent aptamers with increase binding affinities towards specific targets. It is also possible to overcome the existing limitations of constructing simple and rigid scaffolds to hold multiple ligating sites. Several bivalent aptamers were rationally designed to control the spatial distance and orientation to strongly bind the target proteins without influencing their native conformations.[10].

Recently strategies were designed to develop aptamer-based fluorescent reporters with the potential of switching their adduct structures from DNA-DNA duplexes to DNA-target complexes. In this a duplex is formed between a fluorophore-tagged nucleic acid aptamer and a small quencher-tagged oligonucleotide sequence. In the absence of target, the aptamer binds to the latter oligonucleotide sequence, so as the fluorophore and quenched are in proximity for quenching to be observed. Upon sensing the target, it forms fluorescent aptamer-target complexes. (Figure 1) This strategy can be generalized for any aptamer without prior knowledge of its secondary or tertiary structure and can be utilized for developing aptamer-based reporters for real-time sensing applications.[11]

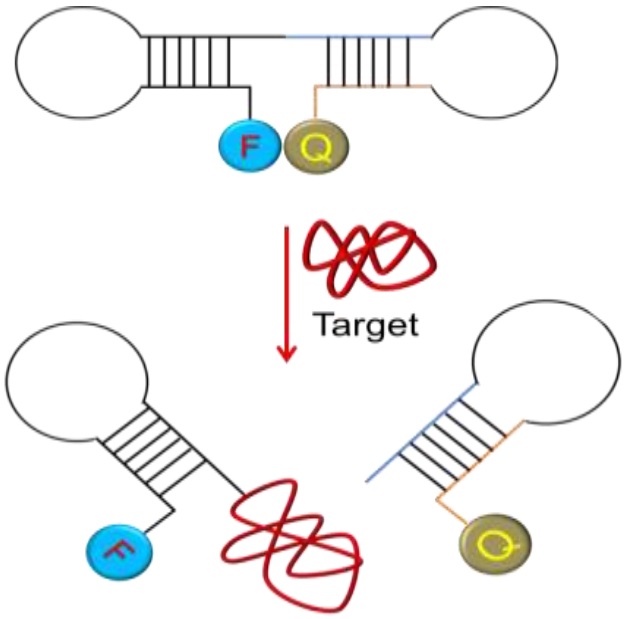


Figure 1: Design of aptamer-based fluorescent reporters with the potential of switching their adduct structures from DNA-DNA duplexes to DNA-target complexes

III. PRINCIPLE – SELEX

SELEX is the standard method that is employed for the generation of aptamers. In this a specific target is incubated with a random pool of 10^{14}~10^{16} single-stranded oligonucleotides. The oligonucleotides in the SELEX library are typically 40 to100 nucleotides long, harbouring a random region at the centre with fixed sequences at both the ends. Following incubation, the unbound oligonucleotides are separated from the target bound ones which are then eluted and taken over for PCR (polymerase chain reaction) amplification. The above steps are repeated several times and the resulting aptamers that display high affinity and specificity are further enriched and sequenced (Figure 2). Recently, the traditional SELEX method is modified further to simplify the above procedures and to obtain aptamers with improved specificity and affinity. Further modifications are being attempted to change the binding conditions, the selection platform, target types, designing of libraries and immobilization matrix. Both DNA and RNA aptamers are reported in the literature. The difficulty with RNA SELEX protocols as compared to their DNA counterparts is that they are more prone towards RNAase degradations, and requires T7 RNA polymerase amplification followed by reverse transcription before PCR.

Numerous strategies are available for the modification and optimization of the conventional SELEX methods. In counter-SELEX, the selection of aptamers is improved through the elimination of nonspecific aptamers. In this structurally analogous molecular targets are used in the pre-clearing step. In capillary electrophoresis method of SELEX the bound targets are separated from unbound aptamers based on their distinct motilities. In electrochemical SELEX approach, the target analytes are immobilized on gold electrodes wherein solid support matrices or fluorescent labels are not required as in other similar methods. Recently cell-SELEX procedures are developed for in vitro selection of aptamers to screen live cells. This approach has been successfully demonstrated for the identification of certain biomarkers in cancer cells and is also utilized for cancer diagnostics.

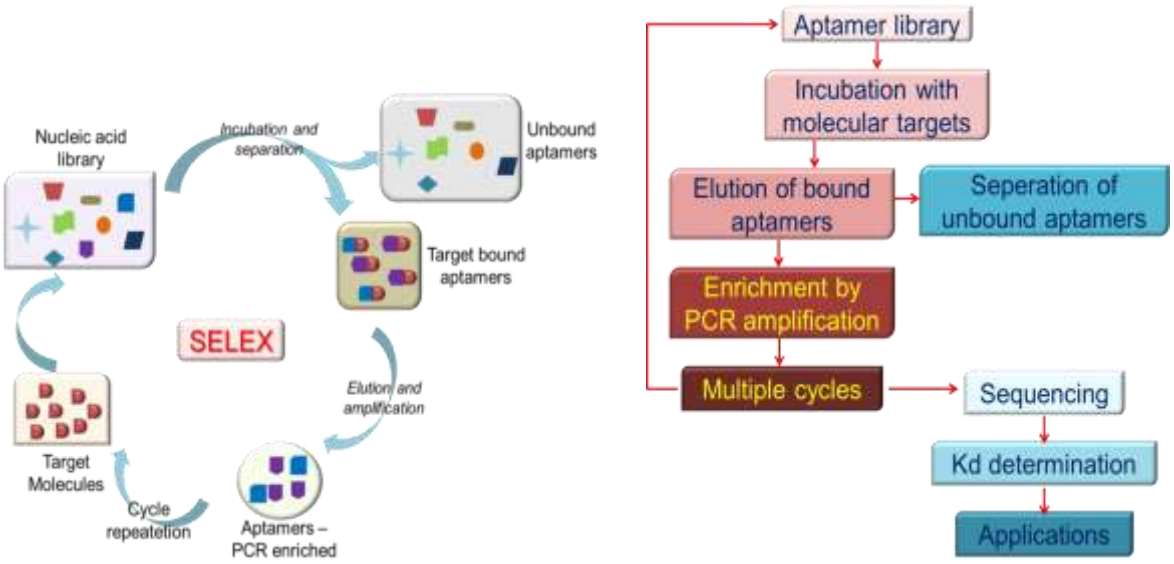


Figure 2: Systematic Evolution of Ligands by Exponential Enrichment (SELEX) (Left). Flow chart representing the various steps involved in SELEX (right). With recent developments in the field of sequencing and computational analyses it is possible to obtain better insights into the structure and dynamics of aptamers during the screening procedures, hence facilitating the identification of highly effective sequences from previous rounds of selection.

IV. LIMITATIONS OF UNMODIFIED APTAMERS

Though several developments and achievements were made in the field of aptamers, till date they are reported with poor clinical therapeutic efficiencies, as compared to that of their antibody counterparts.[12]For example, the anti-vascular endothelial growth factor (VEGF) monoclonal antibody-based drugs - bevacizumab and ranibizumab, display much better therapeutic efficiencies than the federally approved anti-VEGF aptamer-based drugs pegapt anib.[13,14]The observed delay, suppression for clinical translation and distribution of clinically effective aptamers could be due to numerous critical features like intrinsic physicochemical characteristics, poor support from medicinal chemistry, high production costs, hesitancy from the users to change from the available conventional approaches. Several biotechnology and pharmaceutical companies are extensively struggling to take over the conventional antibody-based products in diagnostics and therapeutics. But the recent developments and success revealed through nucleic acid research has invigorated the

researchers to investigate all possible therapeutic aptamers. The inherent physicochemical characteristics of the nucleic acid-based aptamers like metabolic instability, rapid renal filtration, quick distribution to the tissues, non-specific immune activation, polyanionic effects, etc, significantly influence their in vivo therapeutic potency. All the above limitations have paved way for chemical modifications and conjugations to overrule and improve the pharmacokinetic properties of aptamer-based therapeutics.

1. **Sensitivity to Nucleases:** Nuclease mediated degradation is one of the major issues with unmodified aptamers. To overcome this issue, the 3' ends of the aptamers are capped with inverted thymidine that can aid to elevate the nuclease resistance and enhance the binding affinity. Two strategies are employed to incorporate the above modifications in nucleotide sequences into the aptamers - in-SELEX and post-SELEX. In in-SELEX strategy the modified aptamers are directly isolated from the nucleic acid library with modified nucleotides that are compatible with polymerases.[15]Following this method numerous clinically proven aptamers like Spiegelmers®, L-form RNA aptamers that are chiral inverted forms of the natural D-forms, etc are made available. These Spiegelmers® are not recognized by nucleases and display excellent stability under physiological conditions. The only limitation with this Spiegelmers® is that it required enantiomers of target to perform SELEX. [16]

   While is post-SELEX strategies, the modifications are introduced in the aptamers during solid phase chemical synthesis. Using this method it is possible to incorporate multiple modifications to enhance the therapeutic effects. Since the modifications are carried out prior to folding of the aptamer, the structure dependent specificity and affinity may be severely affected. Therefore, precise modifications of the aptamers are required to obtain desired functions. [17]

2. **Elimination by renal filtration:** The unmodified aptamers are generally ~ 5 nm (6 – 30 kDa) in size. When these aptamers are introduced into the blood stream they are prone to rapid excretion through renal filtration resulting the reduced circulation time. In order to overcome this limitation, aptamers modifications are carried out by introducing bulky groups like polyethylene glycol, cholesterol, liposomes, nanomaterials, proteins, etc. Alternatively, these aptamers are multimerized to generate multivalent aptamers, with their sizes well above the cutoff threshold for renal glomerulus (30–50 kDa). For example, polyethylene glycol, a hydrophilic biomaterial and approved for usage in many drug formulations, has proven to enhance solubility, circulation time, in vivo bio-availability and decrease aggregation when administered into the blood stream. Multimerized (tetrameric) aptamer conjugates are reported with increased retention time in circulation and enhanced pharmacokinetic properties. [18]

3. **Toxicity:** Adverse effects on toxicology related events are rarely reported in clinical evaluations. Potential toxicities may arise from unwanted tissue accumulation / chemical modifications / non-specific immune activation / chronic administration. Moreover, the highly negatively charged oligonucleotide-based aptamer can lead to non-specific binding to plasma proteins that can further result in unexpected side effects and poor therapeutic efficiencies. [19]Chemical modifications resulting in unnatural nucleotides, may cause chemical toxicity or even induce immunogenicity. Therefore, adverse care needs to be taken while designing chemically modified aptamers to achieve the desired therapeutic

efficacy. Also modifications that increase the lipophilicity often trigger hepatotoxicity. [20,21]

V. THERAPEUTIC PERSPECTIVES

In order to enhance diagnostic accuracy and successful therapeutics, small molecules with the capability of modulating the target site's activities have been under intense investigation. For the same reason the aptamers were introduced for clinical applications. The aptamers display pharmacological action by activating the targets (agonists), inhibiting the targets (antagonists), or can act as ligands for targeted delivery of therapeutics. Several of the existing aptamers fall into the category of agonists and antagonists. For example the first FDA-approved aptamer Pegaptanib (Macugen®) acts as antagonist for vascular endothelial growth factor (VEGF). On the other hand a variety of diagnostic - fluorescent materials, radioisotopes, nanoparticles and therapeutic molecules - cytotoxic drugs, RNA oligonucleotides, etc can be developed for targeted delivery applications. Further, aptamer-based theranostic conjugates are developed by coupling diagnostic probes and therapeutic molecules for synchronized diagnosis and treatment.

Numerous approaches for the development of aptamers are available to treat various types of cancers particularly those that boost anti-cancer immunity and induce the expression of immunogenic antigens (Figure 3). It is possible to overcome the existing limitations in cancer therapeutics with novel design strategies. There are many aptamers that can target and penetrate tumours and metastasis efficiently. The roles of aptamers in cancer theranostic via targeted delivery are inevitable.

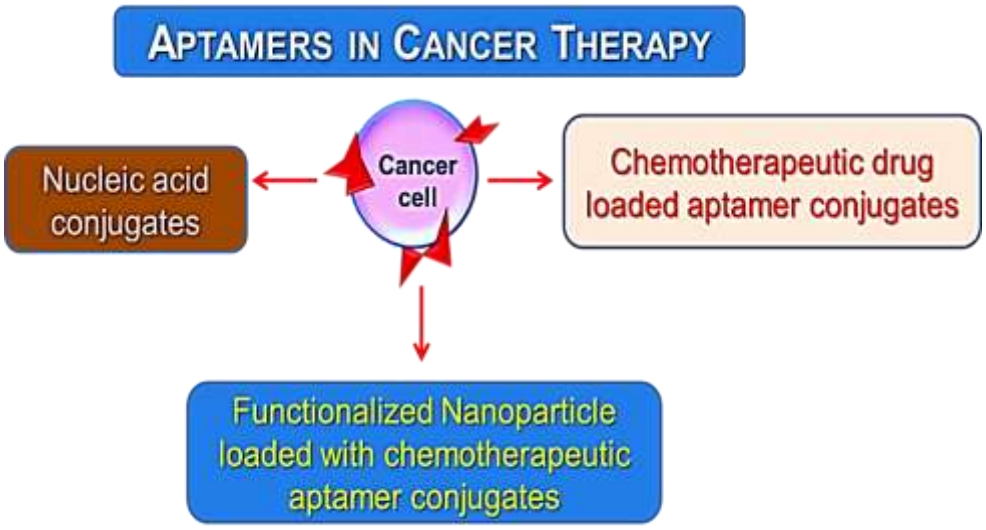
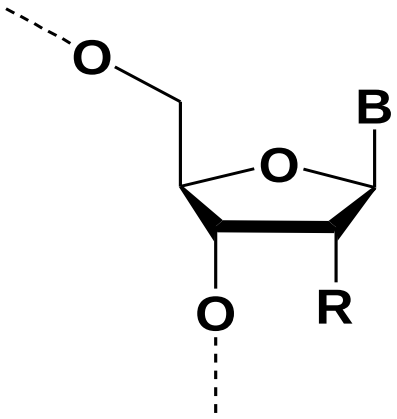


Figure 3: Various types of aptamers available for cancer therapeutics.

There are several other aptamers that can target sites of therapeutic interests - Epidermal growth factor receptor variant III, Receptor tyrosine kinase RETC634Y, Ghrelin, Cytohesin 2, HER3 (ERBB3), Gonadotropin-releasing hormone 1, Platelet-derived growth factor, Mucin 1, etc, can inhibit the growth of tumours. In addition to these there are aptamers for other important targets as listed below.

- Plasminogen activator inhibitor 1 – To prevent metastasis
- Amylin – for pancreatic cancer therapeutics
- Immunoglobulin E – To prevent allergies
- l-Selectin - To modulate inflammation
- Interferon-γ - Modulate inflammation and immune response

In general, nucleic acids when used for therapeutic applications, they are very much susceptible to degradation by nucleases. Such oligonucleotides need to be chemically modified with polymerase enzymes that possess the capability of accepting modified nucleotide triphosphates as substrates, to overcome the above limitation. Examples of such modifications include 2′-amino pyrimidines, 2′-fluoro pyrimidines, and 2′-O-methyl ribose purines and pyrimidines (Figure 4). Many chemical reaction-based strategies are available to modify the nucleobases that can stabilize the nucleotides against degradation by nucleases, and imparts enhanced affinities. [12]



$R = H, F, NH_2, OCH_3$

Figure 4: Chemical or enzymatic modification of nucleotides wherein the 2′ position is modified with either a fluoro- (F), amino- (NH_2) or O-methyl (OCH_3) group for enhanced nuclease resistance.

Recent technologies with aptamers have successfully demonstrated the isolation of circulating tumour cells, extracellular vesicles, detection and profiling of biomarkers and therapy. [22-2627]As compared to antibodies these aptamers display some unique advantages that enable their suitability for various biomedical applications. Very recently a microfluidic chip with multivalent aptamer nano-spheres was engineered by Song et al. to capture circulating tumour cells efficiently [25]engineered a microfluidic chip modified with multivalent aptamer nano-spheres as compared to monovalent aptamer-modified chip. By using high density multivalent aptamers and micro-pillar structure, a higher capture efficiency of CTCs was achieved compared with the monovalent counterparts (Figure 5). Moreover, as compared to antibodies, these aptamers display the advantage of label-free release while isolating targets.

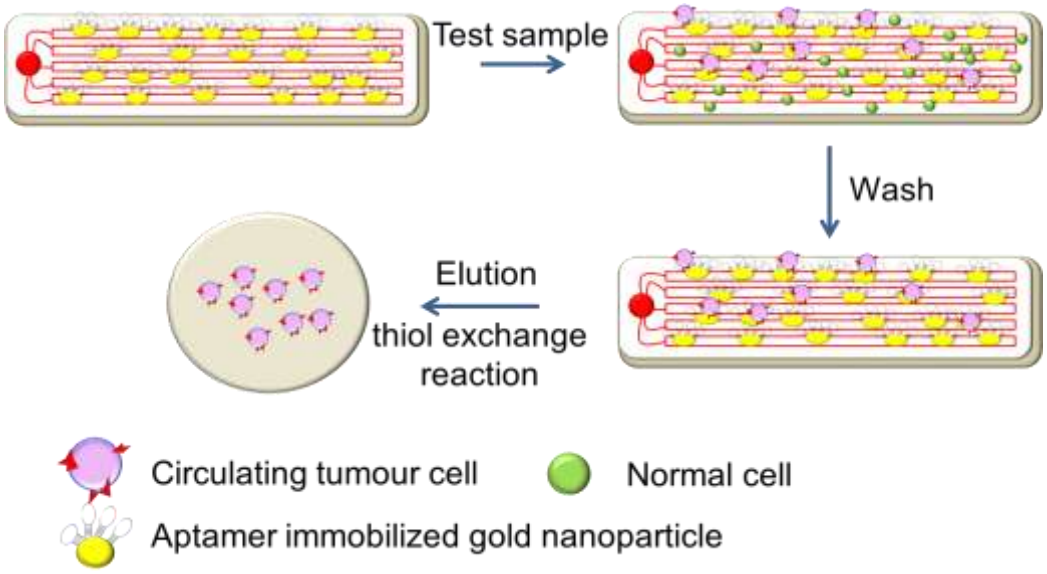


Figure 5: Schematic representing the size selective capture of circulating tumour cells (CTC) with aptamer modified gold nanoparticles embedded on a microfluidic chip. Thus captured CTC can be released through thiol exchange reaction for further analysis.

Similarly another method for rapid capture and non-destructive release of extracellular vesicles was also reported by Zhang et al. using aptamers where the captures targets were eluted with complementary DNA strands. [26]With this method it is possible to isolate pure samples that may further be taken over for subsequent analyses like culturing and therapy. Pun et al. reported an aptamer mediated separation of immune T cells for chimeric antigen receptor (CAR) T-cell therapy. In this the target cells were magnetically labelled by incubating them with aptamer-coated magnetic beads while the unlabelled cells were segregated by applying a magnetic field. The bound cells were eluted with 100-fold excess of complementary agents. This method was considered to display a promising future for sensing small molecules that were otherwise not possible with antibodies. Nakatsuka et al.[28]further modified the above method by replacing the magnetic field with electronic detection system to sense small molecules with the help of aptamer-functionalized field-effect transistor sensors under physiological conditions. Ferguson et al.[55] developed an aptamer-based microfluidic electrochemical biosensor for point of care applications for tracking a variety of circulating drugs in live systems. [29]All the above methods were fine tuned to enhance the efficiencies by using multiple aptamers and can be utilized for early detection and classification of cancers.

VI. CHEMICAL MODIFICATIONS

The drug ability potential and therapeutic effect of aptamer-based drug systems depend on its ability to display a variety of functional abilities like targeted accumulation, drug release, enhanced life span under physiological conditions and high pay load capacity. The major limitations of aptamers in therapeutics include their susceptibility towards nuclease degradation and renal filtration. Chemical modifications are very much necessary to overcome these limitations. Literature reports have revealed negligible effects on the specificity and selectivity of aptamers-small molecule drug conjugates developed through

conventional methods. Hence, for efficient theranostic novel chemical strategies need to be established.

Further these modifications were attempted to enhance the interaction abilities towards a variety of target molecules and hence to increase their diagnostic and therapeutic potentials. Particularly with nucleic acid-based aptamer systems, these modifications augment their resistance towards nuclease degradation and lower renal filtration in addition to increased binding affinity. [6]Click reaction, oxidation induced coupling reactions, avidin-biotin and thio-gold reactions are some of the methods that are generally employed for such chemical modifications. [30,31]On the other hand non-covalent coupling of drugs with aptamers are limited with the availability of poorly efficient synthetic procedures, low yields, low payloads, low spatiotemporal controllability, etc.

The physiological applications of unmodified-aptamers are limited by their short life time. Therefore, to enhance their pharmacodynamics and pharmacokinetics properties, the biochemical modifications including polymerization, nucleic acid sequence truncation, functional group optimization, etc.are being carried out.[12]Moreover, these modifications are known to improve the binding efficiency and stability of the aptamers further. Till date numerous conjugation methods and modification designs are reported to serve the purpose with negligible residual components other than the target molecules. [32]All the modifications are expected to dominate other non-specific interactions from the surrounding molecules.

In general the aptamer conjugations are classified into non-covalent and covalent conjugation. The effects of covalently conjugated aptamers systems are determined by the linkers while the drug load depends on the intercalation potential of the aptamer sequences. Therefore the therapeutic potential of any aptamer-drug system depends on both the above parameters. Few representative types of chemical modifications along with their therapeutic functions are depicted in figure 6.[33]

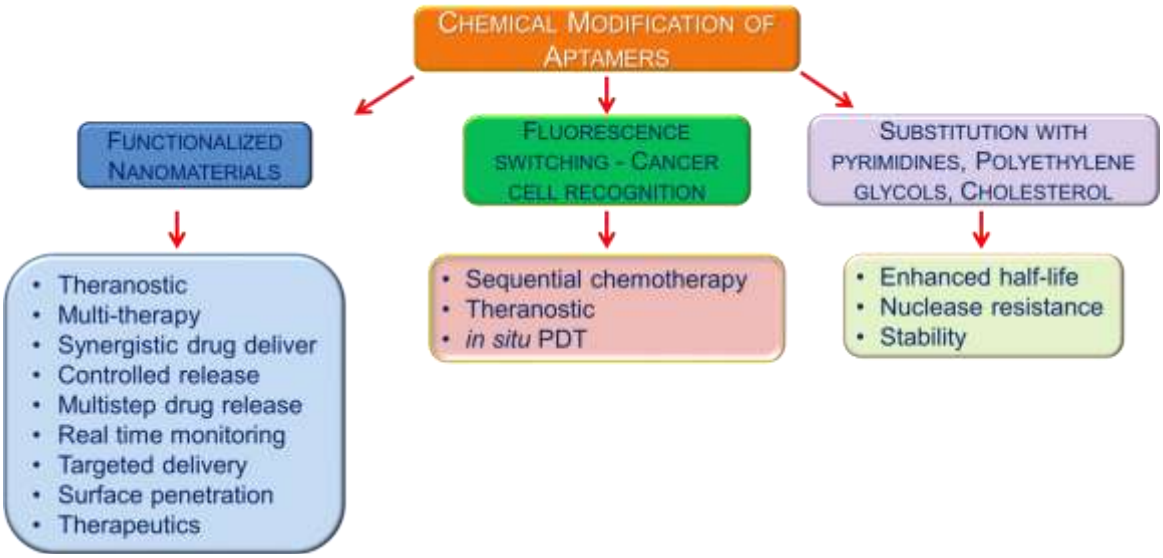


Figure 6: Representative types of chemical modifications in aptamers to enhance therapeutic efficiencies.

Covalent conjugation: Huang et al. demonstrate the first covalent conjugation of a chemotherapeutic doxorubicin (DOX) drug (figure 3) with protein tyrosine kinase (PTK7) targeting aptamer via a hydrazone linker to specifically target the cellular membrane of lymphoblastic leukaemia cells.[34] The above conjugate revealed better cytotoxicity than its unconjugated counterpart. Moreover, with the above conjugate system it is possible to monitor on real time, the drug uptake by cancer cells by utilizing their intrinsic luminescent properties.[35] But the use of this conjugate system was limited by the poor payload on each aptamer. (Figure 7)

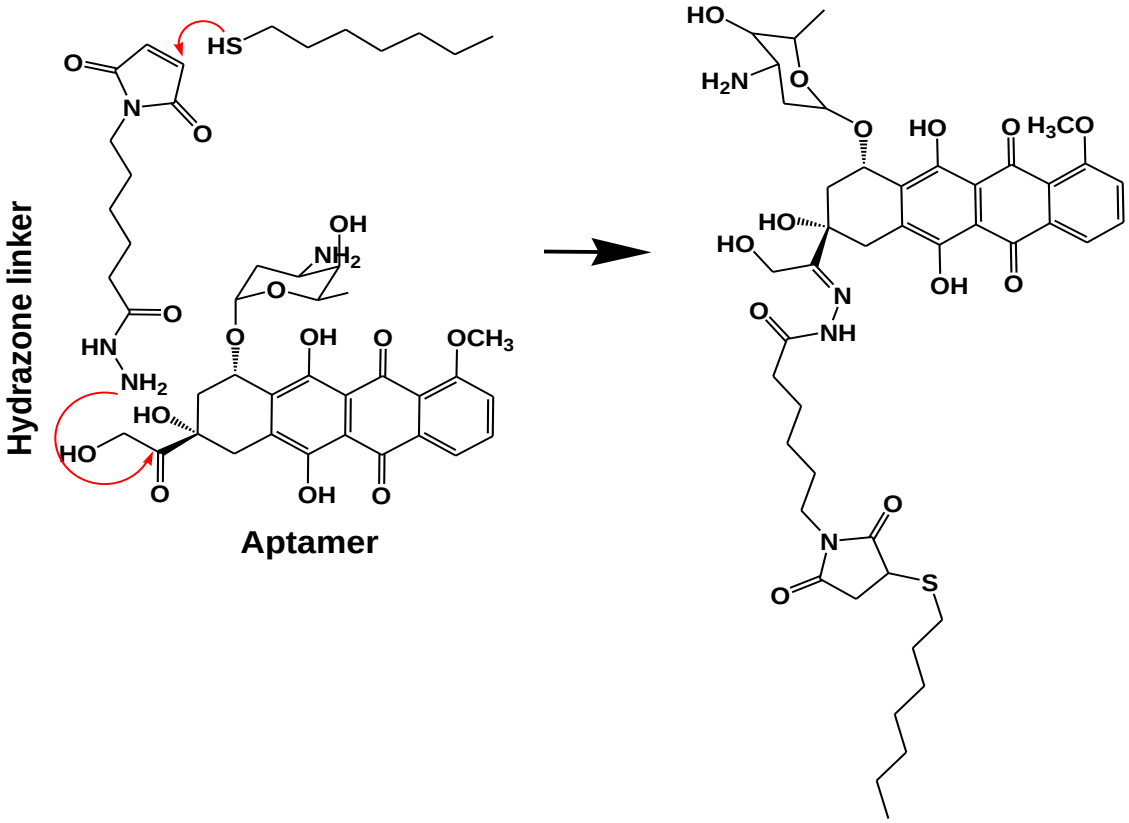


Figure 7: Synthesis of Aptamer-Dox drug conjugate with the potential to target cancer cells with high specificity and affinity

Following an automated and modular solid phase synthetic methodology, Wang et al. developed a next generation aptamer drug conjugates (ApDCs) for drug delivery applications.[9]The modular design of the above conjugate is shown in figure 8. Here one end of the aptamer is linked to tandem drug-molecular trains wherein the spatiotemporal controllability was maintained to release the drug at therapeutic targets via a photo-cleavable linker between the drugs and vehicles. This highly efficient method enabled the possibility of loading multiple drugs onto an aptamer with exceptional high drug payloads.

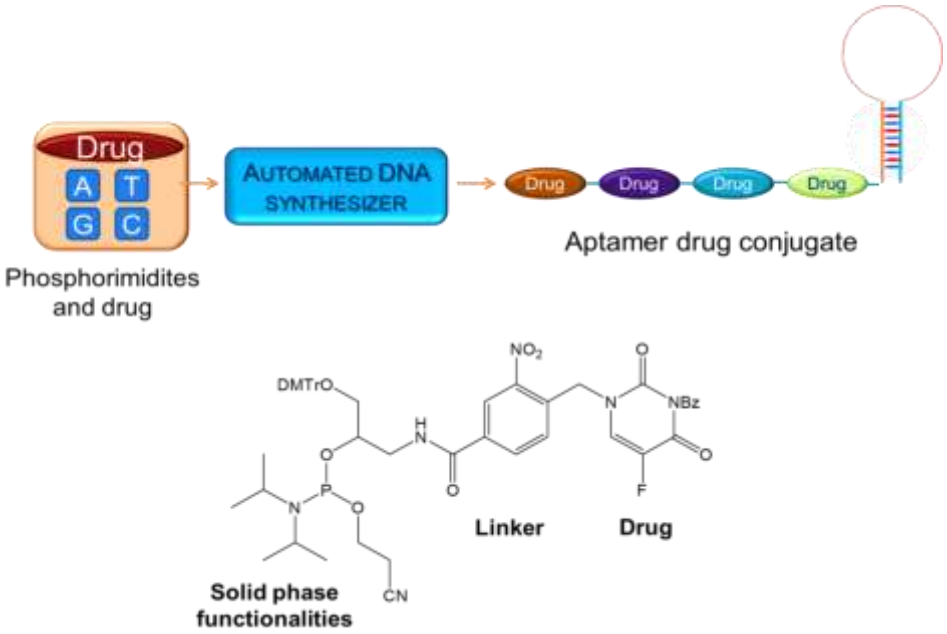
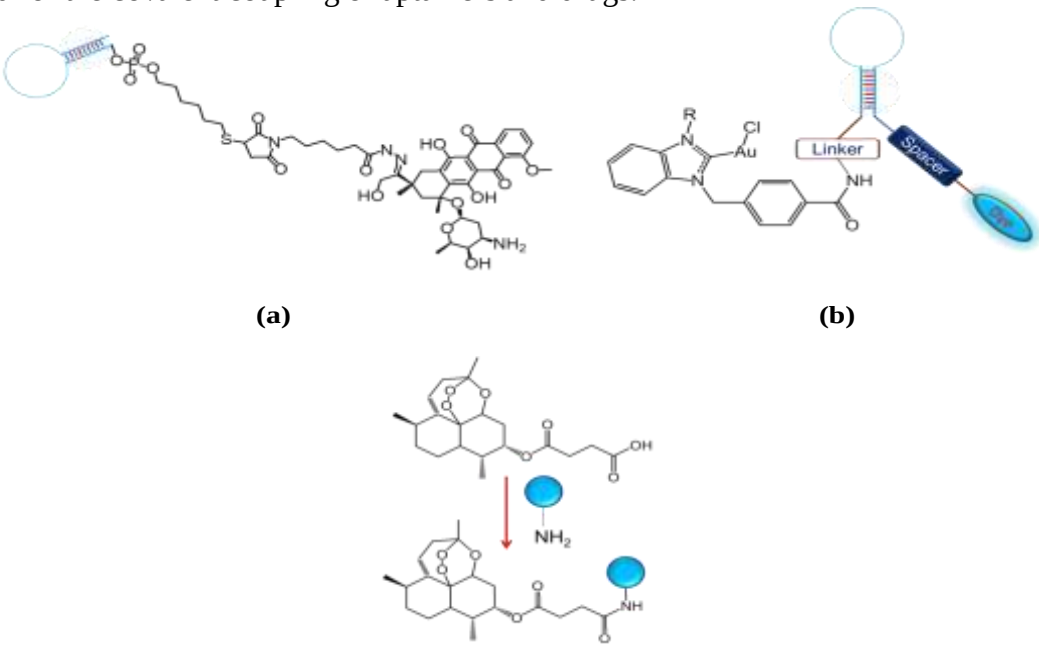


Figure 8: Schematic representation phosphorimidite derived ApDCs with nucleobases A, T, G, C and drug moieties (Top); Modular design of phosphorimidite drug (Bottom).

Further increase in the payload of the above aptamer system was revealed with another well-known anticancer drug 5-fluorouracil which was later modified by including a photo-cleavable linker to connect the aptamer and 5-fluorouracil.[9] Zhu et al., extended the above work by constructing ApDCs with multiple drugs that can potentially inhibit cancer progression at different levels. Examples of other covalently conjugated ApDCs are depicted in figure 9. Reactive functional groups like dibenzocyclooctyne, thiols, amines, etc. were utilized for the covalent coupling of aptamers and drugs. [33]



(a) **(b)**

(c)

Figure 9: Examples of covalently coupled aptamer-drug conjugates with proven anticancer therapeutics. (a) DOX, (b) (NHC)–Au(I) complex and (c) Artesunate

Further advancements in the field led to the development of highly stable cyclic bivalent aptamer-drug conjugates (cbApDC), wherein the release of drug is mediated by enzymatic cleavage. These kinds of aptamers displayed higher selectivity and enhanced target localization. The possibility for managing the medication ratio precisely with these cbApDCs further paved way for combinatorial and combination therapies.

Non-covalent conjugation: Unlike covalent conjugation, here the interactions are mediated through physical phenomena like intercalative binding, van der Waals, hydrogen bonding, etc. The applications of these non-covalent conjugates are limited by their poor drug pay load for in vivo applications. [36]Moreover, the reversible nature of these conjugates diminishes their stability significantly. Few examples of non-covalent modifications of aptamers are shown in figure 10. The well-known anticancer drug DOX was non-covalently intercalated between CG/GC rich oligonucleotide sequences.[37] There are several other examples of non-covalently coupled aptamer drugs that were used in (i) the treatment of retinoblastoma with enhanced therapeutic efficacy, (ii) for the inhibition of overexpressed B-cell activating factor receptor protein (BAFF-R) in B-cell malignancies, and other gene mediated therapies. The therapeutic efficacies of the conjugates were enhanced with multivalent aptamers via non-covalent coupling using biotin-streptavidin linker.

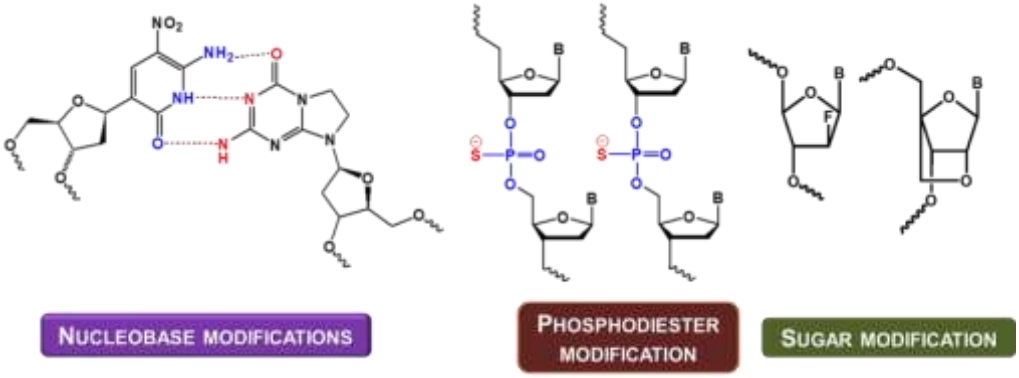


Figure 10: Examples of non-covalently modified aptamer-drug conjugates.

Further to increase the limitations of poor pay loads with oligonucleotide-based aptamer-drug conjugates, DNA-nano-trains were developed by Tan et al. for targeted drug delivery (figure 11).[36] In order to enhance the specificity with the above drug conjugates multivalent aptamers were used along with nano-trains.

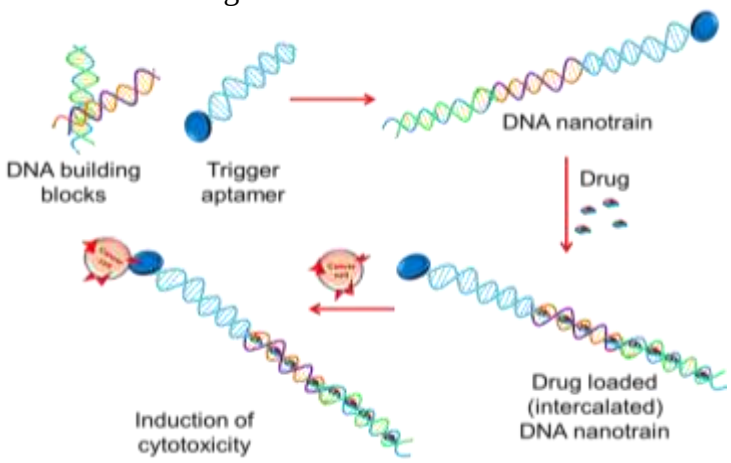


Figure 11. Schematic representing the modular design of DNA-nanotrains for targeted drug delivery with enhanced specificity.

The main purpose of chemical modifications is to protect nucleic acid-based aptamers from degradation by nucleases and the resistance for renal filtration. Attempts were made not only to fine tune the already existing aptamers but also for those that are in clinical trials. Moreover, efforts to improve the half-life under physiological conditions also fall into the scope of aptamer chemical modifications. The table 2 shows the various drugs along with their targets and attempted modifications. [38]

Table 2: List of Aptamer-Based Drugs, Their Targets and Chemical Modifications.[38]

Drug	Chemical modification	Target
ACT-GRO-777	26 nucleotide sequence rich in guanine base	Nucleolin
Anti-CD33	DNA aptamer	CD33: transmembrane protein
Anti-CD30 aptamer	ssDNA aptamer	CD30-transmembrane protein
BAFF-R-specific aptamer	RNA aptamers	B-cell activating factor receptor
NOX-A12	45 nucleotide RNA, L-ribonucleic acid and Spiegelmer	Stromal cell-derived factor-1
TD05 aptamer	ssDNA	Immunoglobulin heavy mu chain
Sgc8 aptamer	ssDNA	Membrane receptor, protein tyrosine kinase 7
BAX499	32 nucleotides	Tissue factor (TF)
NOX-H94, (Spiegelmer® lexaptepidpegol)	44 nucleotide RNA and L-ribonucleic acid	Hepcidin peptide
DGB-1, DBG-2, DBG-4, and DBG-5	ssDNA aptamers	Direct oral anticoagulants
Macugen (pegaptanib sodium)	• 27-nt RNA • 2′-fluoropyrimidines • 2′-O-methylpurines	VEGF
REG1	• 37-nt RNA aptamer • 2′-ribopurine or 2′-fluoropyrimidine	coagulation factor IXa
NU172	26-nt DNA	Thrombin

VII. CONCLUSIONS

For the two decades tremendous developments and applications were reported with in vitro-selected aptamers. These aptamers have proven to serve as high affinity synthetic reagents and are considered as antibody analogs. But as compared to antibodies, these aptamers are still in their early stages of development when considered to therapeutic and / or diagnostic applications. The areas wherein improvements are expected include controlled downstream functions under physiological conditions, stable scaffolds that can resist nuclease digestion, ease for chemical modifications, etc. Moreover, efforts to optimize aptamers that can function in antibody inaccessible areas like small-molecule targets and cellular toxins. The aptamer design strategies target applications like drug delivery, diagnostics, imaging, biomarker development, etc. Investigations are carried out to resolve the existing flaws and limitations. For the past twenty years there has been no new aptamer-based therapeutic and many of them have failed to prove in clinical evaluations. Though numerous chemical modifications strategies are proposed to enhance their half-life, reduce their toxicity, stability,

etc, the gap for practical applications still exists. Recently, the use of aptamers for targeted therapy that required high binding specificity as comparable with antibodies is being investigated. These properties of aptamers fill the therapeutic gap between small-molecule drugs and bio-macromolecules like antibodies. It is possible to achieve good pharmacokinetic clinical performance through rational design and development for therapeutic applications. This chapter summarizes the various advancements in the field of aptamers that were successfully screened for different therapeutic targets. Additional experiments and clinical trials are required for further progression and commercialization of these aptamer-based drugs.

REFERENCES

[1] Beck, H.; Harter, M.; Hass, B.; Schmeck, C.; Baerfacker, L. Small molecules and their impact in drug discovery: A perspective on the occasion of the 125th anniversary of the Bayer Chemical Research Laboratory. Drug Discov Today 2022,27 (6), 1560.
[2] Li, Q.; Kang, C. Mechanisms of Action for Small Molecules Revealed by Structural Biology in Drug Discovery. Int J Mol Sci 2020,21 (15).
[3] Chen, W.; Zhao, X.; Yang, N.; Li, X. Single mRNA Imaging with Fluorogenic RNA Aptamers and Small-molecule Fluorophores. Angew Chem Int Ed Engl 2023,62 (7), e202209813.
[4] Hu, X.; Zhang, D.; Zeng, Z.; Huang, L.; Lin, X.; Hong, S. Aptamer-Based Probes for Cancer Diagnostics and Treatment. Life (Basel) 2022,12 (11).
[5] Yuhan, J.; Zhu, L.; Zhu, L.; Huang, K.; He, X.; Xu, W. Cell-specific aptamers as potential drugs in therapeutic applications: A review of current progress. J Control Release 2022,346, 405.
[6] Odeh, F.; Nsairat, H.; Alshaer, W.; Ismail, M. A.; Esawi, E.; Qaqish, B.; Bawab, A. A.; Ismail, S. I. Aptamers Chemistry: Chemical Modifications and Conjugation Strategies. Molecules 2019,25 (1).
[7] Shraim, A. S.; Abdel Majeed, B. A.; Al-Binni, M. A.; Hunaiti, A. Therapeutic Potential of Aptamer-Protein Interactions. ACS Pharmacol Transl Sci 2022,5 (12), 1211.
[8] Khan S., H. A., Fahimi H., Aliakbari F., Haj Bloukh S., Edis Z., Mahdi Nejadi Babadaei M., Izadi Z., Shiri Varnamkhasti B., Jahanshahi F., Lin Y., Hao X., Hasan Khan R., Rasti B., Vaghar-Lahijani G., Hua L., Derakhshankhah H., Sharifi M., Falahati M. A review on the therapeutic applications of aptamers and aptamer-conjugated nanoparticles in cancer, inflammatory and viral diseases. Arabian Journal of Chemistry 2022,15 (2).
[9] Wang, R.; Zhu, G.; Mei, L.; Xie, Y.; Ma, H.; Ye, M.; Qing, F. L.; Tan, W. Automated modular synthesis of aptamer-drug conjugates for targeted drug delivery. J Am Chem Soc 2014,136 (7), 2731.
[10] Hu, X.; Tang, L.; Zheng, M.; Liu, J.; Zhang, Z.; Li, Z.; Yang, Q.; Xiang, S.; Fang, L.; Ren, Q.et al. Structure-Guided Designing Pre-Organization in Bivalent Aptamers. J Am Chem Soc 2022,144 (10), 4507.
[11] Nutiu, R.; Li, Y. Structure-switching signaling aptamers. J Am Chem Soc 2003,125 (16), 4771.
[12] Keefe, A. D.; Pai, S.; Ellington, A. Aptamers as therapeutics. Nat Rev Drug Discov 2010,9 (7), 537.
[13] Ferrara, N.; Adamis, A. P. Ten years of anti-vascular endothelial growth factor therapy. Nat Rev Drug Discov 2016,15 (6), 385.
[14] Ng, E. W.; Shima, D. T.; Calias, P.; Cunningham, E. T., Jr.; Guyer, D. R.; Adamis, A. P. Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease. Nat Rev Drug Discov 2006,5 (2), 123.
[15] Jager, S.; Rasched, G.; Kornreich-Leshem, H.; Engeser, M.; Thum, O.; Famulok, M. A versatile toolbox for variable DNA functionalization at high density. J Am Chem Soc 2005,127 (43), 15071.
[16] Vater, A.; Klussmann, S. Toward third-generation aptamers: Spiegelmers and their therapeutic prospects. Curr Opin Drug Discov Devel 2003,6 (2), 253.
[17] Lee, Y.; Urban, J. H.; Xu, L.; Sullenger, B. A.; Lee, J. 2'Fluoro Modification Differentially Modulates the Ability of RNAs to Activate Pattern Recognition Receptors. Nucleic Acid Ther 2016,26 (3), 173.
[18] Borbas, K. E.; Ferreira, C. S.; Perkins, A.; Bruce, J. I.; Missailidis, S. Design and synthesis of mono- and multimeric targeted radiopharmaceuticals based on novel cyclen ligands coupled to anti-MUC1 aptamers for the diagnostic imaging and targeted radiotherapy of cancer. Bioconjug Chem 2007,18 (4), 1205.
[19] Henry, S. P.; Giclas, P. C.; Leeds, J.; Pangburn, M.; Auletta, C.; Levin, A. A.; Kornbrust, D. J. Activation of the alternative pathway of complement by a phosphorothioate oligonucleotide: potential mechanism of action. J Pharmacol Exp Ther 1997,281 (2), 810.

[20] Santos, A.; Soares, J. X.; Cravo, S.; Tiritan, M. E.; Reis, S.; Afonso, C.; Fernandes, C.; Pinto, M. M. M. Lipophilicity assessement in drug discovery: Experimental and theoretical methods applied to xanthone derivatives. J Chromatogr B Analyt Technol Biomed Life Sci 2018,1072, 182.
[21] Waring, M. J. Lipophilicity in drug discovery. Expert Opin Drug Discov 2010,5 (3), 235.
[22] Jiang, Y.; Shi, M.; Liu, Y.; Wan, S.; Cui, C.; Zhang, L.; Tan, W. Aptamer/AuNP Biosensor for Colorimetric Profiling of Exosomal Proteins. Angew Chem Int Ed Engl 2017,56 (39), 11916.
[23] Kacherovsky, N.; Cardle, II; Cheng, E. L.; Yu, J. L.; Baldwin, M. L.; Salipante, S. J.; Jensen, M. C.; Pun, S. H. Traceless aptamer-mediated isolation of CD8(+) T cells for chimeric antigen receptor T-cell therapy. Nat Biomed Eng 2019,3 (10), 783.
[24] Neff, C. P.; Zhou, J.; Remling, L.; Kuruvilla, J.; Zhang, J.; Li, H.; Smith, D. D.; Swiderski, P.; Rossi, J. J.; Akkina, R. An aptamer-siRNA chimera suppresses HIV-1 viral loads and protects from helper CD4(+) T cell decline in humanized mice. Sci Transl Med 2011,3 (66), 66ra6.
[25] Song, Y.; Shi, Y.; Huang, M.; Wang, W.; Wang, Y.; Cheng, J.; Lei, Z.; Zhu, Z.; Yang, C. Bioinspired Engineering of a Multivalent Aptamer-Functionalized Nanointerface to Enhance the Capture and Release of Circulating Tumor Cells. Angew Chem Int Ed Engl 2019,58 (8), 2236.
[26] Zhang, K.; Yue, Y.; Wu, S.; Liu, W.; Shi, J.; Zhang, Z. Rapid Capture and Nondestructive Release of Extracellular Vesicles Using Aptamer-Based Magnetic Isolation. ACS Sens 2019,4 (5), 1245.
[27] Liu, C.; Zhao, J.; Tian, F.; Cai, L.; Zhang, W.; Feng, Q.; Chang, J.; Wan, F.; Yang, Y.; Dai, B.et al. Low-cost thermophoretic profiling of extracellular-vesicle surface proteins for the early detection and classification of cancers. Nat Biomed Eng 2019,3 (3), 183.
[28] Nakatsuka, N.; Yang, K. A.; Abendroth, J. M.; Cheung, K. M.; Xu, X.; Yang, H.; Zhao, C.; Zhu, B.; Rim, Y. S.; Yang, Y.et al. Aptamer-field-effect transistors overcome Debye length limitations for small-molecule sensing. Science 2018,362 (6412), 319.
[29] Ferguson, B. S.; Hoggarth, D. A.; Maliniak, D.; Ploense, K.; White, R. J.; Woodward, N.; Hsieh, K.; Bonham, A. J.; Eisenstein, M.; Kippin, T. E.et al. Real-time, aptamer-based tracking of circulating therapeutic agents in living animals. Sci Transl Med 2013,5 (213), 213ra165.
[30] Ahmad, M. G.; Balamurali, M. M.; Chanda, K. Click-derived multifunctional metal complexes for diverse applications. Chem Soc Rev 2023, DOI:10.1039/d3cs00343d 10.1039/d3cs00343d.
[31] Stephanopoulos, N.; Tong, G. J.; Hsiao, S. C.; Francis, M. B. Dual-surface modified virus capsids for targeted delivery of photodynamic agents to cancer cells. ACS Nano 2010,4 (10), 6014.
[32] Espah Borujeni, A.; Mishler, D. M.; Wang, J.; Huso, W.; Salis, H. M. Automated physics-based design of synthetic riboswitches from diverse RNA aptamers. Nucleic Acids Res 2016,44 (1), 1.
[33] Roberts, T. C.; Langer, R.; Wood, M. J. A. Advances in oligonucleotide drug delivery. Nat Rev Drug Discov 2020,19 (10), 673.
[34] Huang, Y. F.; Shangguan, D.; Liu, H.; Phillips, J. A.; Zhang, X.; Chen, Y.; Tan, W. Molecular assembly of an aptamer-drug conjugate for targeted drug delivery to tumor cells. Chembiochem 2009,10 (5), 862.
[35] Chanda, K.; Mm, B. Light emitting probes - approaches for interdisciplinary applications. Chem Soc Rev 2021,50 (6), 3706.
[36] Zhu, G.; Zheng, J.; Song, E.; Donovan, M.; Zhang, K.; Liu, C.; Tan, W. Self-assembled, aptamer-tethered DNA nanotrains for targeted transport of molecular drugs in cancer theranostics. Proc Natl Acad Sci U S A 2013,110 (20), 7998.
[37] Prathima T.S., C. B., Ahmad M.G., Chanda K., Balamurali M.M. Recent developments on other platinum metal complexes as target-specific anticancer therapeutics. Coordination Chemistry Reviews 2023,490.
[38] Ni, S.; Zhuo, Z.; Pan, Y.; Yu, Y.; Li, F.; Liu, J.; Wang, L.; Wu, X.; Li, D.; Wan, Y.et al. Recent Progress in Aptamer Discoveries and Modifications for Therapeutic Applications. ACS Appl Mater Interfaces 2021,13 (8), 9500.

