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STATE-OF-THE-ART REVIEW

Diagnostic and Therapeutic Aptamers

A Promising Pathway to Improved Cardiovascular Disease Management

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HIGHLIGHTS

- **Aptamers, like monoclonal antibodies, have a unique structure that allows them to bind to their targets with high specificity and selectivity.**
- **Aptamers are small and flexible synthetic molecules with little steric hindrance and scarcely immunogenic.**
- **Aptamer-based drugs encompass a diverse range of technologies, including novel shapes, chemistries, and delivery methods, as well as an assorted use for therapeutic and diagnostic purposes.**
- **Aptamers have the potential to complement and improve the management of CVDs.**

SUMMARY

Despite advances in care, cardiovascular diseases remain the leading cause of death worldwide. As a result, identifying suitable biomarkers for early diagnosis and improving therapeutic and diagnostic strategies is crucial. Because of their significant advantages over other therapeutic approaches, nucleic-based therapies, particularly aptamers, are gaining increased attention. Aptamers are innovative synthetic polymers or oligomers of single-stranded DNA (ssDNA) or RNA molecules that can form 3-dimensional structures and thus interact with their targets with high specificity and affinity. Furthermore, they outperform classical protein-based antibodies in terms of in vitro selection, production, ease of modification and conjugation, high stability, low immunogenicity, and suitability for nanoparticle functionalization for targeted drug delivery. This work aims to review the advances made in the aptamers' field in biomarker detection, diagnosis, imaging, and targeted therapy, which highlight their huge potential in the management of cardiovascular diseases. (J Am Coll Cardiol Basic Trans Science 2024;9:260-277) © 2024 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Cardiovascular diseases (CVDs) are a group of disorders that affect the heart, brain, and blood vessels, resulting in ischemia and tissue death.[1] According to the World Health Organization, the number of CVD-related deaths reached 17.9 million in 2019, accounting for approximately one-third of total deaths. This number is projected to rise to more than 23.6 million deaths per year by 2030, which explains why CVDs remain the leading cause of death worldwide.[2] Moreover, the

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CVD-related global burden is further exacerbated by the number of years lost due to ill health, morbidity, and associated disabilities.[3] From the clinical viewpoint, the etiology of CVDs is complex, encompassing metabolic abnormalities, genetic alterations, environmental and social risk factors, and the recent long-term effects of COVID-19 disease.[4] Furthermore, common clinical CVD diagnostic methods, such as electrocardiography, computed tomography, and cardiac magnetic resonance, have limited sensitivity and specificity, making early diagnosis of CVDs difficult.[4]

Traditional drugs (eg, antiplatelet drugs, anticoagulants, angiotensin-converting enzyme inhibitors, statins, beta-blockers, and nitrates)[4] are currently used to treat CVD symptoms but not the underlying cause of the disease, and thus do not qualify as disease-modifying agents. In addition, they may have a detrimental influence on other organs and affect long-term prognosis, patient life quality, and death rate.[5] At the same time, the clinical application of cardiac surgery is often limited by the complexity of the procedures and the possibility of serious postoperative complications.[4]

Innovative, convenient, and efficient methods for effective risk prediction, early diagnosis, and treatment of CVDs are therefore the need of the hour. We describe here the current state-of-the-art of the aptamer field in biomarker detection, diagnosis, imaging, and targeted therapy in the context of CVDs (**Central Illustration**).

SEARCHING FOR MORE PRECISE AND SAFE TOOLS

Aptamers are RNA- or DNA-based drugs that have attracted great attention in clinical translations as an alternative to classical monoclonal antibody-based agents due to their low manufacturing cost, limited batch-to-batch differences, reversible folding features, small size (~9 kDa vs ~150 kDa of antibodies and ~15 kDa of nanobodies) and low immunogenicity.[6-11]

Aptamers, unlike antibodies, are produced synthetically, as will be described later on, with no need to involve animals or a specific target that can provoke an immune response. In fact, the greatest limitation of antibody therapy is the risk of inducing an immune response in the human body, namely a human anti-murine antibody response, despite the attempts to reduce it through binding of the variable domain to the fragment constant (Fc) regions of human antibodies.[12] These regions are absent in aptamers, and this makes them safer. In addition, the synthetic nature of aptamers makes their reproducibility very accurate, with low variability between batches; their large-scale production more standardized[13]; and their cost less expensive than antibodies, the production of which requires more demanding in vivo conditions. For example, the production cost of the Food and Drug Administration–approved aptamer-based drug Macugen for neovascular age-related macular degeneration is $12,500, while that of trastuzumab, a monoclonal antibody used for the cure of breast cancer, is $70,000.[14-16]

Table 1 collects the studies conducted so far on aptamers for their potential applications in molecular therapy, diagnosis, biosensing, and targeted drug delivery with several basic studies that have successfully translated to clinical trials and some aptamer-based tools that have reached the market.

Despite the promising properties of aptamers discussed previously, their use in diagnostics and therapy remains limited, especially in CVD treatment.[17,18] **Figure 1** shows the total number of publications produced since the discovery of aptamers, which has remained low over time, in contrast to the cancer field. Given that a similar difference is observed not only with aptamers but also in microRNA research, this trend suggests that it may be due to the multiplicity of cancer therapeutic targets compared with CVD-associated ones.

Our aim in this study is to provide an overview of aptamer cardiovascular research (**Central Illustration**), focusing on their unique characteristics and the advances made in their development, selection using bioinformatic tools, and their application in diagnostic and therapeutic fields, including nanodrug delivery, which paves the way to targeted and personalized therapies that address current needs.

APTAMERS: A BRIEF OVERVIEW

The word *aptamers* originates from the Latin *aptus*, meaning fit, and the Greek *meros*, meaning part or region. Depending on the random sequence pools used for the chemical generation, aptamers are classified as either nucleic acid or peptide aptamers.[19] However, unless clearly indicated, the term *aptamers* generally refers to short single-stranded RNA or DNA molecules of 20 to 80 nucleotides in length. These aptamers can fold into unique 3-dimensional conformations, thanks to features such as hairpins, internal loops, bulges, pseudoknots, and G tetramers, and specifically bind to targets with an interaction that resembles the one of an antigen-antibody reaction (**Figure 2**).[20] However, unlike antibodies, aptamers due

ABBREVIATIONS AND ACRONYMS

AMI = acute myocardial infarction

CRP = C-reactive protein

CVD = cardiovascular disease

HF = heart failure

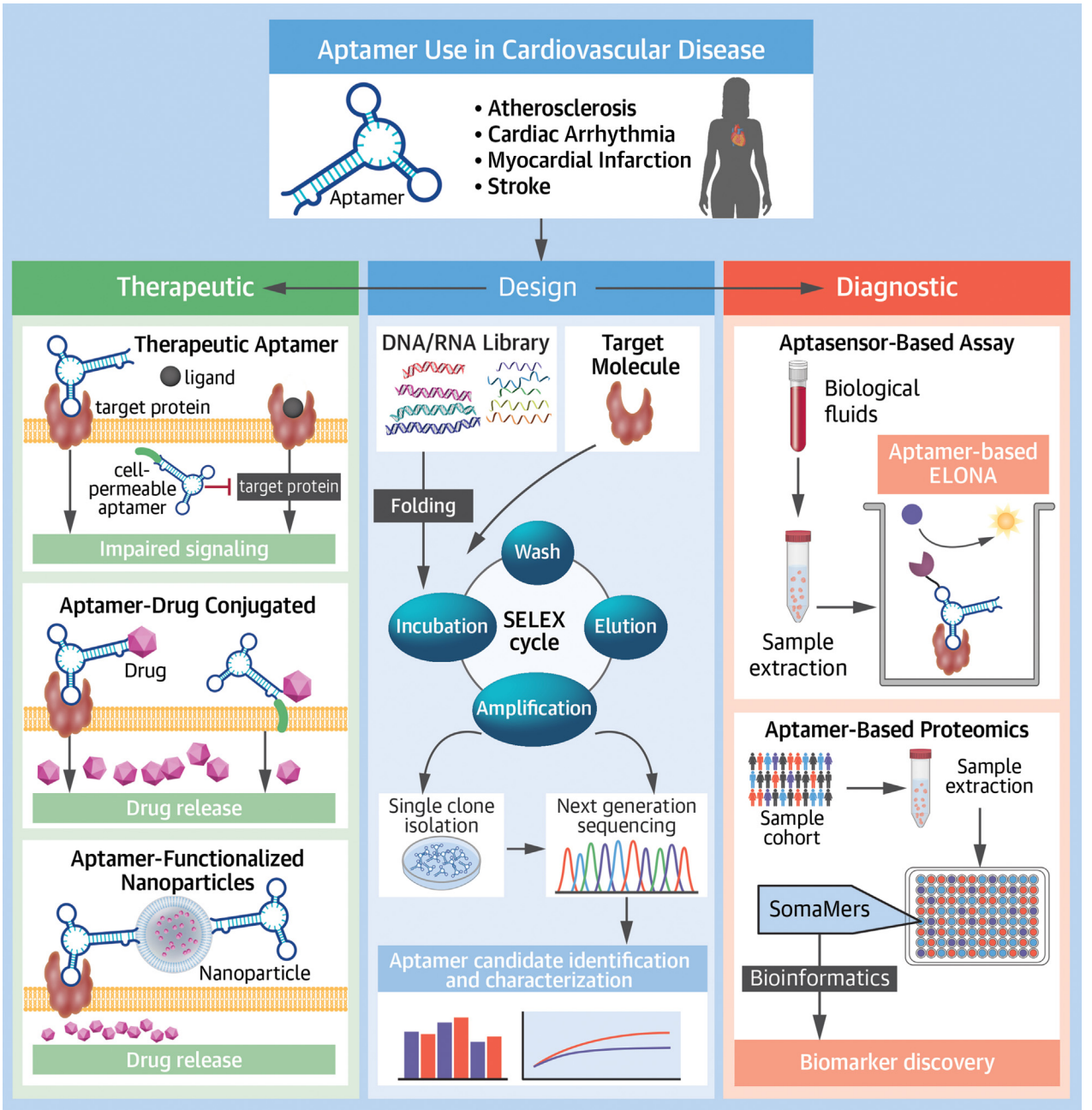
HTS-SELEX = high-throughput sequencing

LOD = limit of detection

SELEX = systematic evolution of ligands by exponential enrichment

SOMAmer = slow off-rate modified aptamer

CENTRAL ILLUSTRATION Use of Aptamers in Cardiovascular Diseases



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Use of aptamers in cardiovascular diseases: from design to their clinical applications as novel therapeutic and diagnostic tools. Created with BioRender.com. ELONA = enzyme-linked oligonucleotide assay; SELEX = systematic evolution of ligands by exponential enrichment; SomaMer = slow off-rate modified aptamer; ssDNA = single-stranded DNA.

TABLE 1 Diagnostic and Therapeutic Aptamers in Clinical Trials

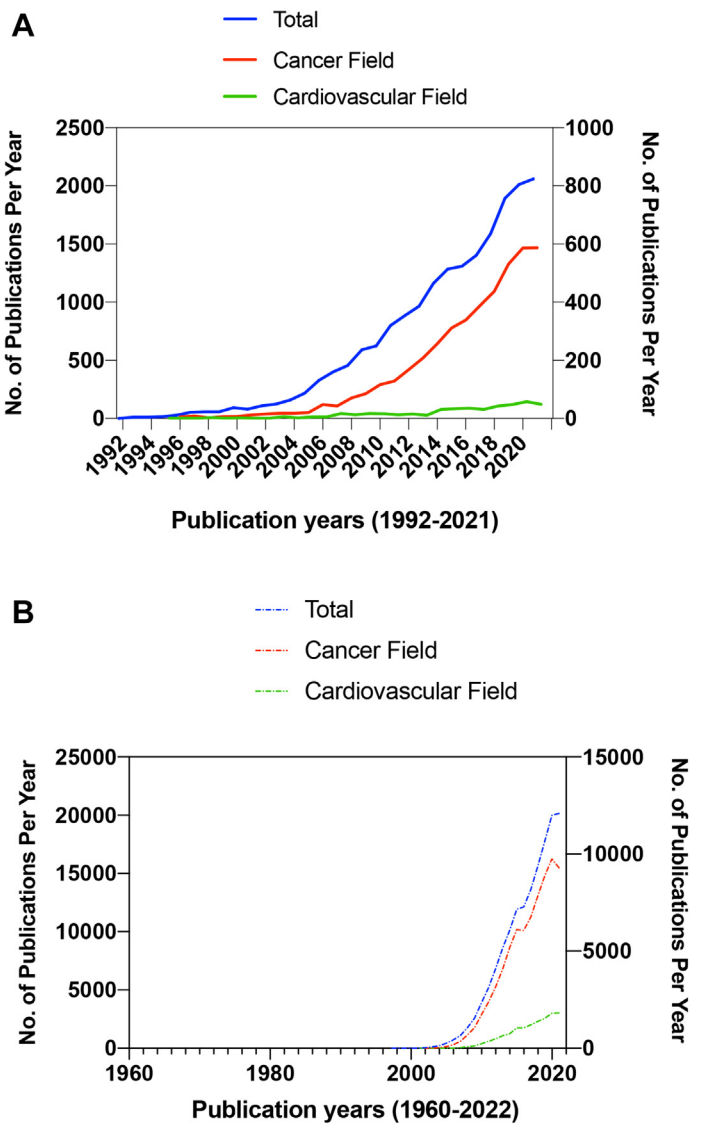
Aptamer	Molecule	Target	Target Organ	Route of Administration	Disease/Application	Status	Sponsor/Company
Macugen (Pegaptanib)	RNA	VEGF	Eye	Intravitreal injection	AMD	Approved[a]	Pfizer
					PDR	Phase 1, completed	Bausch & Lomb Incorporated
					DME	Phase 2, completed	
EYE001	RNA	VEGF	Eye	Intravitreal injection	Macular degeneration	Phase 2, completed	Eyetech Pharmaceuticals
					Choroidal neovascularization	Phase 3, completed	
					Von Hippel-Lindau disease	Phase 1, completed	National Eye Institute
Fovista (E10030)	DNA	PDGF-BB	Eye	Intravitreal injection	AMD	Phase 2, terminated	Ophthotech Corporation
		PDGF			AMD	Phase 2, completed	
AS1411	DNA	Nucleolin	Cancer cells	Intravenous	AML	Phase 2, terminated	Antisoma Research
ARC1779	DNA	vWF	Platelet	Intravenous	von Willebrand disease	Phase 2, withdrawn	Archemix Corp
					Purpura, thrombotic thrombocytopenic von Willebrand disease type-2b	Phase 2, completed	
ARC19499	RNA	TFP1	Platelet	Subcutaneous injection	Hemophilia	Phase 1, terminated	Baxalta now part of Shire
ARC1905 (Zimura)	RNA	C5	Eye	Intravitreal injection	AMD	Phase 2, completed	IVERIC bio, Inc
					Geographic atrophy	Phase 2, completed	
					Macular degeneration	Phase 3, completed	
					Stargardt disease 1	Phase 2, recruiting	
REG1	RNA	Factor IX	Platelet	Intravenous bolus	Coronary artery disease	Phase 3, terminated	Regado Biosciences, Inc
Nox-E36	RNA	MCP-1 (CCL2)	Immune cells	Subcutaneous injection	Chronic inflammatory diseases	Phase 1, completed	TME Pharma AG
					Type 2 diabetes mellitus	Phase 1, completed	
					Autologous stem cell transplantation	Phase 1, completed	
Nox-A12	RNA	SDF-1 (CXCL12)	Blood cells/ lymphocytes	Intravenous	Autologous stem cell transplantation	Phase 1, completed	TME Pharma AG
Nox-H94	RNA	Hepcidin	—	Intravenous	Anemia	Phase 1, completed	TME Pharma AG
					End-stage renal disease	Phase 2, completed	
BT200	RNA	vWF, factor VIII	Platelet	Subcutaneous injection	von Willebrand disease	Phase 2, recruiting	Medical University of Vienna
					Hemophilia A	Phase 2 recruiting	
68Ga-Sgc8	DNA	PTK7 (CCK4)	Cancer cells	Intravenous	Colorectal cancer	Phase 1, unknown	Xijing Hospital
NU172	DNA	Thrombin	—	Intravenous Bolus	Heart disease	Phase 2, unknown	ARCA Biopharma, Inc
ApTOLL	DNA	TLR4	—	Intravenous	Stroke	Phase 1, completed	aptaTargets SL
					COVID-19	Phase 1, recruiting	
AON-D21	L-DNA/RNA	C5a	Hematopoietic stem/progenitor cells	Intravenous	Healthy	Phase 1, recruiting	Aptarion Biotech AG
ADHERE	DNA nanostructures	Tenofovir disoproxil fumarate	—	—	HIV/AIDS	Early phase I, completed	Eastern Virginia Medical School
					Adherence, Medication		
					Drug use		
BC007	DNA	GPCR autoantibodies	—	Intravenous	Heart failure/dilated cardiomyopathy/ autoantibodies	Phase 1, completed	Berlin Cures GmbH
						Phase 2, active not recruiting	
		SARS-Cov-2 spike proteins			Long COVID-19 syndrome	Preclinical, completed	

Updated from Byun et al[13] and ClinicalTrials.gov. [a]Currently, the marketing authorization in the European Union has been withdrawn at the request of the marketing authorization holder.

AMD = age-related macular degeneration; AML = acute myeloid leukemia; DME = diabetic macular edema; GPCR = G protein–coupled receptor; PDGF = platelet-derived growth factor; PDR = proliferative diabetic retinopathy; VEGF = vascular endothelial growth factor; vWF = von Willebrand factor.

to their flexible nature can perfectly fold into or around the surface of target molecules. This means that aptamers can be selected against a wide range of targets, such as peptides, proteins, metabolites, small organic compounds, carbohydrates, biological cofactors, toxins, and whole organisms such as viruses, pathogenic bacteria and yeast, and mammalian cells.[20] Upon binding to targets, aptamers can induce a series of biochemical effects, such as antagonism, agonism, suppression, and destruction.[20]

FIGURE 1 Peer-reviewed Publications on Aptamers and MicroRNA



Schematic plot of annual publications in PubMed indexed to (A) the term "aptamer" during 1992 to 2021 and (B) microRNAs (1960-2021), with aptamer/microRNAs appearing in any field (total values plotted against the left y-axis), cancer and cardiovascular field (values plotted against the right y-axis).

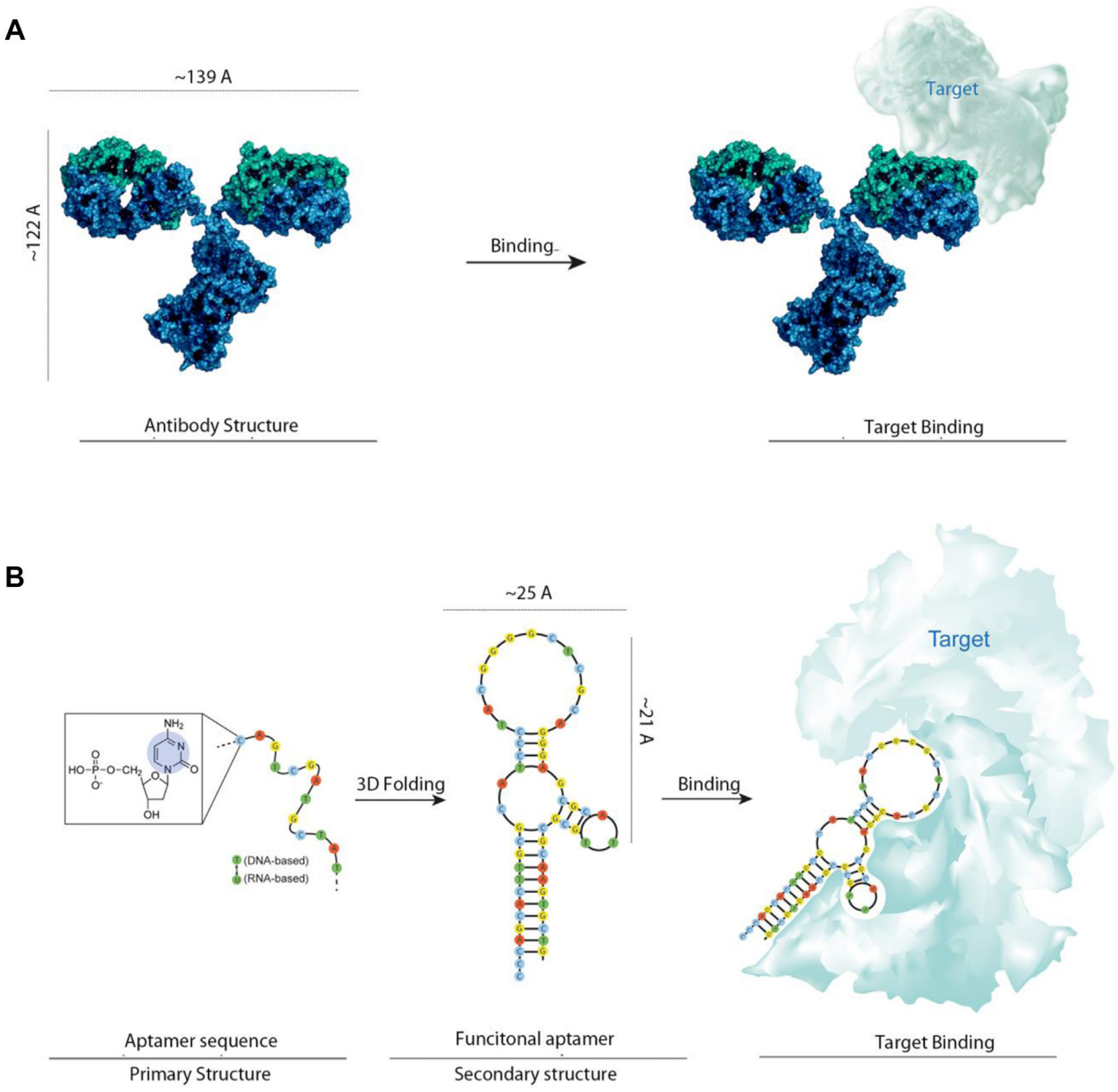
THE SYSTEMATIC EVOLUTION OF LIGANDS BY EXPONENTIAL ENRICHMENT

Aptamer development occurs through the systematic evolution of ligands by exponential enrichment (SELEX) process. SELEX consists of an iterative process in which an oligonucleotide combinatorial library (DNA, RNA, or modified RNA) is challenged to bind against a specific target via 3 steps: 1) selection (sometimes preceded by a counterselection step); 2) partitioning; and 3) amplification.[21] To start, a SELEX protocol oligonucleotide library of high complexity is synthesized, which usually contains up to 10^{14} to 10^{15} different sequences.[22] Each sequence has 2 constant regions at the ends to allow for polymerase chain reaction (PCR) amplification, and a central random region of *n* nucleotides (*n* ranging typically between 20 and 60). In the selection step, the library is incubated with target molecules under the most appropriate conditions of medium and time. Subsequently, several methods are applied to separate the unbound sequences from those that are bound to the target.[23] Those target-bound sequences are amplified by PCR (DNA SELEX) or reverse-transcription PCR (RNA SELEX). The so-obtained PCR products represent a new pool enriched for binding aptamers, which are utilized for the next round of selection.[23] Using SELEX, aptamers have been generated against a wide variety of different targets, from small organic molecules to large proteins, and also complex targets such as cells and tissues.[22-24] To obtain high-quality aptamers against relevant targets, several SELEX methods have been designed and optimized, increasing targeting specificity and reducing the time required for selection (**Figure 3**). Because detailed discussions of the different SELEX methodologies have been extensively reviewed elsewhere,[25,26] **Table 2** collects some of the most effective SELEX variations developed so far.

IDENTIFICATION OF APTAMER CANDIDATE: HIGH-THROUGHPUT SEQUENCING AND APTAMER BIOINFORMATICS

Usually, only the last round of SELEX is subjected to cloning and Sanger sequencing for the identification of a few candidate sequences with potential selectivity for their target molecules from an initial library of thousands of random sequences. However, this strategy can be tricky with traditional SELEX, as it may fail to identify sequences with the highest affinity. Indeed, due to the high complexity of their binding domains, many valuable candidates might be lost during previous SELEX steps, preventing their ultimate enrichment.[31,32] Furthermore, sequences with the highest affinity and specificity are not always the most numerous in the final selection phase. In this regard, high-throughput sequencing (HTS-SELEX) has been developed to replace this approach. Indeed, HTS-SELEX allows analyzing millions of sequences of the library across all the selection rounds, enabling to detect very low percentage (below 1%) in fewer selection rounds. Additionally, the high

FIGURE 2 **Aptamers' Structure and Function**

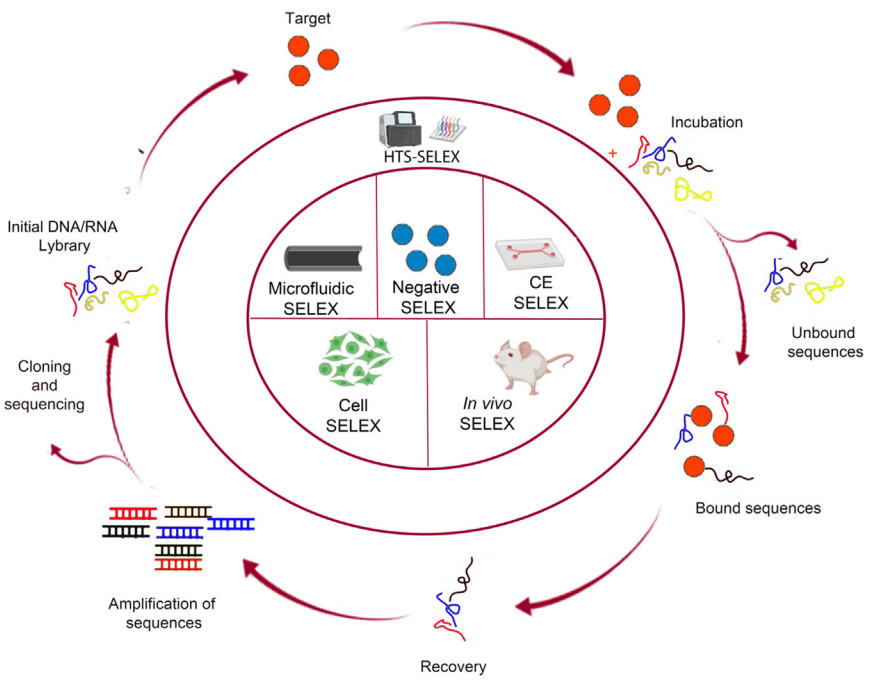


Size and mechanism of action of (A) antibody vs (B) aptamer. Created with BioRender.com. 3D = 3-dimensional.

number of analyzed sequences ensures a statistically robust identification of enriched nucleotide distribution and frequency, rate of molecular enrichment, and primary/secondary sequence motifs.[22] In turn, this robustness also allow a comparison between rounds of SELEX with different conditions of selection in order to determine aptamers with specific skills or to better characterize the binding site of a known aptamer. Ultimately, the mutational landscape can be further explored to find better variants of an aptamer family.[22]

The introduction of HTS-SELEX massively increased the volume of obtained sequences from multiple rounds of selection to hundreds of millions of reads, requiring computational tools able to effectively identify those aptamers with elevated binding

FIGURE 3 Aptamer Selection



Schematic representation of systematic evolution of ligands by exponential enrichment (SELEX) and SELEX variations. Created with BioRender.com. HTS = high-throughput sequencing.

properties.[33] To date, a plethora of dedicated bioinformatic tools have been developed to run several analyses based on: 1) alignment, in order to identify identical/similar sequences and group them in clusters; and 2) folding, by analyzing their secondary structures to get information on relevant structures for binding.[34] To this end, we created the table below to briefly describe the most commonly used in silico tools for sequencing analysis (**Table 3**).

So far, we have described the basic principles for aptamer selection and amplification. However, to achieve an effective and clinically translatable molecule, a number of subsequent chemical manipulations of the structure as well as adjustments of the formulation are needed to: 1) increase the chemical diversity of functional groups; 2) improve in vivo stability against nuclease degradation; and 3) reduce their susceptibility to renal filtration due to their small size.[34] Usually, chemical modifications are introduced in the sugar unit, the nucleobase, and the backbone of the constituting nucleotides and enhance aptamer biostability and binding affinity.[28] Aptamer optimization can be achieved either in the scaffold of selected aptamers through standard solid-phase synthesis (post-SELEX modification) or by using modified nucleoside triphosphates directly in the selection process (pre-SELEX modifications), which are described in detail elsewhere.[50-52]

In the following paragraphs, we go deeper into the biomedical applications of aptamers in the cardiovascular field.

APTAMERS IN CVD BIOMEDICINE: FROM BENCHSIDE TO THEIR CLINICAL APPLICATIONS AND COMMERCIALIZATION

A total of 53 clinical studies are currently registered at ClinicalTrials.gov with the search word "aptamers" (8 if paired with "diagnostic" and 42 with "therapeutic").

APTAMERS AS DIAGNOSTIC TOOLS

Early and quick diagnosis for successful prognosis of pathological CVD states is a crucial point of the modern health care system, and it is strongly

TABLE 2 SELEX Variations and Method Description

SELEX Variation	Method Description	Ref. #
Negative and counter SELEX	During conventional SELEX, whether a DNA or RNA aptamer selection is performed, target molecules are usually attached to an immobilization support, which enables partition. In the selection step, some of the sequences might unspecifically bind to the immobilization matrix, causing false positive results. To avoid such possibility, the library is first incubated with immobilization support alone. In this way, nonspecific binding sequences are removed from each pool, resulting in a 10-fold higher affinity and specificity of the resulting aptamers. An evolution of negative SELEX, called counter-SELEX, extends the classic SELEX approach by incubating structurally identical targets with aptamers to successfully distinguish nonspecific oligonucleotides.	23,27
Capillary electrophoresis SELEX (CE-SELEX)	The aptamer-target complex is separated from unbound oligonucleotides via capillary electrophoresis. according to their different electrophoretic mobility. This approach significantly reduces the rounds of aptamer selection (4 compared with the traditional 16 rounds), without affecting the affinity of the selected aptamer with its target protein.	28
Microfluidic SELEX (mSELEX)	A major drawback to conventional SELEX methods is the high costs due to the large amount of reagent consumption and the tedious, time-consuming process. Microfluidic devices, due to their small dimensions, allowed to reduce sample and reagent consumption, improve speed, and increase resolving power, improving the extent of automation compared with the other conventional SELEX systems.	24
Cell-SELEX	This method employs the whole living cell as a target. One of its main advantages is that target proteins remain in their native environment throughout the selection process, retaining their native folding structure and post-translational modifications, allowing the final selected aptamers to bind to the target's natural folded conformation on cells.[29] Cell-SELEX increases the possibility of the so-obtained aptamer to be used directly for diagnostic and therapeutic applications.	19
In vivo SELEX	This method was developed to overcome the risk that aptamers selected in vitro may not always be functional in vivo. In this case, aptamer libraries are first injected into a mouse model of a specific disease. Subsequently, selected cell/organ-bound aptamers are isolated and amplified, and eventually further screened with a counter selection step in healthy animals. Finally, the so-obtained disease-specific aptamers with high affinity and specificity to target tissues are enriched and identified by sequencing.	30

SELEX = systematic evolution of ligands by exponential enrichment.

correlated to the use of biomarkers. Indeed, the primary prevention of CVDs relies on the ability to identify individuals at high risk long before the appearance of clear pathological events.[53] Therefore, the need for an accurate risk stratification is mandatory. Molecules such as metabolites as well as DNAs, RNAs, proteins, and hormones can serve as biomarkers, and an increase or decrease in their concentration in body fluids can be evaluated as an indication of normal biological processes, severity of diseases, or pharmacological responses to a therapeutic intervention.[54] In this regard, circulating biomarkers such as myoglobin (Myo), B-type natriuretic peptide, cardiac troponin I (cTnI), C-reactive protein (CRP), interleukins, and interferons are the most commonly used in clinical setting for many cardiovascular-specific disorders.[55] However, despite being well established in clinical settings, detection of those biomarkers at very low concentrations might be of great concern. Indeed, most biomarker proteins, especially during the early onset of disease, exist in the blood in trace amounts (0.1 pg mL^{-1} to 1 ng mL^{-1}), which is far below the attainable detection limit of current diagnostic sensors.[56] Thus, development of more sensitive, reliable, and cost-effective diagnostic platforms that can also help in real-time detection and monitoring of CVD patients health is now mandatory. Typically, biosensors for biomarker detection consist of: 1) a biological recognition agent that binds to or recognizes specific analytes; 2) a signal transducer that converts the analyte-receptor interaction into a measurable signal; and 3) a signal readout that displays the transduced signal, which must be easily recognized by users.[56] So far, common approaches used for detection of biomarkers, such as chemiluminescence, fluoro- and radioimmunoassay, colorimetric assay, and conventional enzyme-linked immunosorbent assay (ELISA)-based assays, mainly rely on antibodies as molecular recognition element.[57] Yet, as a diagnostics tool, antibody-based assays present pitfalls, including high production costs, time-consuming processes (requiring several hours or days to reach the final results), and low reliability, due to batch-to-batch variations.[58] However, this hegemony is now being challenged by aptamers that are envisioned as possible superior candidates in the biosensor global market.[59,60] Thus, the number of companies interested in the design of aptamer-based biosensors (also named aptasensors) to monitor the levels of a broad range of targets of clinical interest, such as not only peptides and proteins, but also cells and metal ions, has largely increased in the last few years. An example is represented by Aptamer Group, which is the leading

TABLE 3 Bioinformatic Tools for Aptamer Analyses

Tools	Scope	Description	Ref. #
AptaCLUSTER	Alignment/clustering	It relies on a 2-stage approach to first reduce the size of the aptamer pool and then perform similarity tests with the filtered pool. The aptamer library is iteratively scanned to find potential similar sequences using the locality-sensitive hashing technique; in the next step, these sequences are analyzed to evaluate their actual similarity. Finally, selected matching sequences below a user-defined threshold are grouped, forming the desired clusters.	35
FASTAptamer	Alignment/clustering	An open-source software that consists of several scripts that can count, compare, enrich, and cluster sequencing data coming from selection. FASTAptamer-Count calculates the frequency of each sequence and ranks them by abundance. FASTAptamer-Compare compares populations and generates plots of sequence distribution. FASTAptamer-Cluster performs clustering of similar sequences and reports information about each sequence occurrence in the cluster together with its degree of similarity to the seed sequence. FASTAptamer-Enrich computes fold-enrichment of sequences present in more than one population and, finally, FASTAptamer-Search allows to screen populations to find matches of new or known sequence patterns.	36
Galaxy Workflows	Alignment/clustering	It allows semi-automated analysis of HTS-SELEX data based on: 1) preprocessing, during which multiplexed HTS-FASTA data are scanned to remove adapter and constant sequences. It also isolates variable sequences of defined length and removes duplicates; 2) nonredundant database, in which a database of unique aptamer sequences is created from multiple selection rounds; 3) abundance/persistence analyses, in which aptamers within the nonredundant database are analyzed for abundance and persistence to filter selected sequences from nonselected ones.	37
AptaMotif	Alignment/clustering	It predicts sequence-structure motifs in aptamers in 3 steps: 1) in structural processing, it computes optimal and suboptimal secondary structures, and extracts loops within the aptamer sequence; 2) in seed identification, it performs a user-defined number of iterations in which aptamers are randomly sampled from the pool to extract matching motifs, which are in turn aligned and scored; 3) in the seed extension step, it searches each motif (a seed) against the entire aptamer pool, to assess the degree of abundance of the motif in the library.	37
MPBind	Alignment/clustering	A meta-motif-based statistical framework that relies on the fact that the binding potential of a sequence can be broken down into the combination of the binding potentials of all its *k*-mers. Then, it scores each aptamer binding motif based on cycle-to-cycle enrichment and determines the Meta-Score binding potential of the entire aptamer by combining the scores of all its motifs.	38
APTANI	Alignment/clustering	The analysis relies on the assumption that binding of aptamers to the target molecule is dependent on their structural characteristics rather than on the occurrence of their nucleotide sequences during various SELEX cycles. The input file must have a FASTQ format, and the program workflow involves 3 steps: • Frequency filtering based on a user-defined threshold to remove rare sequences (with a relative frequency $<10^{-7}$); • Prediction of secondary structures RNAsubopt; a tool for calculating suboptimal secondary structures of RNAs; • Prediction of the consensus motifs from the secondary structures identified in the previous step; • Scoring of each aptamer based on the consensus motifs predicted through a match/mismatch scoring scheme.	39;40
RaptRanker	Alignment/clustering	A computational tool based on the concept that structural profiles of important motifs (*k*-mers) are expected to significantly change across SELEX rounds. Its algorithm consists of 5 steps: • Filtering of the sequence data from FASTA or FASTQ input file; • Extraction of unique sequences by removing duplicates, and computation of their profiles (named SSP) based on their nucleotide sequence and secondary structure characteristics; • Determination of subsequences by iteratively cutting out fragments of the unique sequences and computation of sub-SSPs; • Clustering of subsequences based on their sub-SSP similarity; • Scoring of the unique sequences based on the average motif enrichment of each cluster.	41
SFold	Folding	It predicts structural motif probability according to the Boltzmann equilibrium distribution.	42
RNAProfile	Folding	It extracts conserved functional and structural motifs from a set of unaligned RNA sequences, using a similarity measure that considers both the sequence and the possible secondary structures according to base pairing and thermodynamic rules.	43
CentroidFold	Folding	It predicts secondary structure from individual RNA sequences by maximizing the weighted sum of the expected number of true positive base pairings and true negative ones.	44
CentroidHomFold	Folding	By using posterior decoding techniques, it includes in the prediction analysis suboptimal secondary structures and alignments of both the target sequence and its homologous sequences.	44
IPknot	Folding	Integer programming-based computational method that includes in its analysis pseudoknots and RNA secondary structure topologies formed from pairing between unpaired bases of a loop and those outside the loop.	45
CapR	Folding	It calculates the probability that each RNA base position in a given dataset is located within 6 possible secondary structures, according to Turner energy model: stems, hairpin loops, bulge loops, internal loops, multibranch loops, and exterior loops.	46,47
Rtools	Folding	A Web server that integrates several prediction softwares (CentroidFold, CentroidHomfold, IPKnot, CapR, Raccess, Rchange, and RintD) to extract structural information from a single-stranded RNA input sequence.	48
ViennaRNA Web services	Folding	It analyses RNA sequences based on secondary structure information derived from the ViennaRNA package (which includes a C-code library, RNAfold, RNAalifold, Barriers, Locarna, and RNAinverse).	49

HT = high throughput; HTS = high throughput sequencing; SELEX = systematic evolution of ligands by exponential enrichment; SSP = sequence-structure profile.

provider of Optimer, a novel platform based on next-generation aptamer molecules. NeoVentures Biotechnology provides a reinvention of the SELEX selection, and based on their proprietary Neomer platform generates the so-called Neomer. The in vitro diagnostic company Pinpoint Science developed a Low-Cost Handheld COVID-19 Aptamer-based Diagnostic Device, which is an accurate COVID-19 diagnostic test that nonclinicians can execute in humans.

The following are some examples of aptasensors specifically designed to monitor protein markers for CVDs, including cTnI, Myo, and the CRPs.

TROPONINS. The troponin complex, which regulates muscle contraction, is a component of skeletal and cardiac muscle thin filaments. It comprises 3 subunits: troponin C, troponin I, and troponin T. In particular, the cardiac isoforms of troponin I and T (cTnI and cTnT) are considered the "gold standard" for the detection of acute myocardial infarction (AMI) due to their high sensitivity and specificity for cardiac muscle damage.[57] In the clinical setting, myocardial damage causes troponin to be released into the bloodstream, where concentrations begin to rise within 4 to 6 hours from the ischemic event and remain high for more than 2 weeks for cTnT and more than 5 to 7 days for cTnI.[61] cTnT values of 0.05 ng/mL in human serum are considered normal, while values of 0.05 to 0.09 ng/mL and 0.1 ng/mL are borderline and positive for AMI, respectively.[62]

So far, an antibody-based ELISA has been widely used to detect cTnI levels in clinical practice. However, despite ELISA being highly specific and reproducible, the limit of detection (LOD) is at the ng mL^{-1} level, which makes it unsuitable to detect cTnI with concentrations far below this LOD.[63] Therefore, considerable effort has been made to develop more sensitive and faster tools for the detection of early acute rise of cTnI and cTnT. In this regard, several DNA aptamers have synthesized to target cTnI. In 2015, Dorraj et al[64] screened potential cTnI targeting aptamers from a 79 bp ssDNA random library. The final selection process resulted in 4 aptamer sequences (TnIApt23, TnIApt19, TnIApt18, TnIApt11). Among the selected sequences, TnIApt23 exhibited the best affinity in the nanomolar range (2.69 nM) toward the cTnI protein. Moreover, they further developed a rapid colorimetric detection assay for human cTnI using the novel and specific aptamer-AuNanoParticle conjugates (see Aptamer-functionalized nanoparticles) based on a dot blot assay with an LOD of 5 ng/mL for this protein.[64] In another study, 2 high-affinity aptamers (Apt 3 and Apt 6) against cTnI were selected for further development of a dual-aptamer sandwich-based enzyme-linked oligonucleotide assay method. The dual-aptamer sandwich enzyme-linked oligonucleotide assay method was used to detect cTnI in patient serum, with a detection limit of 0.05 ng/mL.[65] More recently, other groups generated additional anti-cTnI aptamers in order to develop novel versions of aptasensors for cTnI to be applied for clinical applications.[66] In this context, Jo et al[67] designed a cTnI aptamer-immobilized screen-printed carbon electrode. They tested this method in a human serum-cTnI added solution with a detection limit of 1.0 pM (24 pg/mL). The same authors also used a gold-based nanoplate with an immobilized anti-cTnI aptamer to allow measurement of cTnI levels. The approach proved to be a reliable method for the diagnosis of AMI patients.[68]

MYOGLOBIN. Myo is a single-stranded hemeprotein of both cardiac and skeletal muscle that belongs to the globin family and is characterized by the presence of a porphyrin ring with a central ferrous iron molecule.[69] As a result of muscle damage, Myo is released into the bloodstream, and because its serum levels significantly increase right after an acute AMI, it is considered a valuable early marker. Indeed, the normal level of Myo in the blood is 6 to 85 ng/mL, and after 2 hours from the onset of AMI, it starts to rise up to 70 to 200 ng/mL, with a peak concentration after 6 to 9 hours and a return to baseline after 1 day.[70]

One of the first successful aptamer isolations against Myo aptamer was reported in 2014 by Wang et al,[71] who first generated an anti-Myo aptamer with a dissociation constant in the low nanomolar range (KD of 4.93 nM). By using it as a probe for an electrochemical biosensor, the authors showed that the aptamer was functional for Myo detection in a target-induced aptamer displacement assay.[72] The same team subsequently created an antibody-Myo-aptamer sandwich test for AMI in which the aptamer and antibody do not compete for the same binding site in Myo.[73] The authors then developed a polystyrene microplate coated with commercial anti-Myo monoclonal antibodies as capture targets and an invertase-aptamer conjugate as a secondary probe for signal amplification. Therefore, in order to develop a glucose-meter device, the same group designed an antibody-Myo-aptamer sandwich assay to monitor AMI.[73] Further, by using surface plasmon resonance, it was shown that binding of the anti-Myo antibody did not interfere with binding of the aptamer to the same Myo target. The authors then developed a polystyrene microplate coated with commercial anti-

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 9, NO. 2, 2024
FEBRUARY 2024:260–277

Myo monoclonal antibodies as capture targets and an invertase-aptamer conjugate as a secondary probe for signal amplification. Another example of a developed aptamer for Myo is represented by a 72 nt DNA-based molecule produced by OTC Biotech. In this regard, this aptamer was applied to develop a differential pulse voltammetry-based electrochemical sensor with a 2.1 pg/mL LOD from 10-fold diluted human serum.[74]

C-REACTIVE PROTEINS. CRP is a 125 kDa homopentameric protein produced in liver hepatocytes and in other cell types in response to any inflammation, including cardiac damage.[57] Despite the fact that CRP is quite unspecific, it is considered a well-established cardiac outcome predictor in CVDs, including AMI and acute coronary syndrome. The normal levels of CRP in the blood serum of healthy individuals are <10 μg/mL; however, during AMI, the CRP levels rise above this value, reaching about 200 μg mL^{-1} in severe inflammation condition.[75] Based on the widespread use of this biomarker in clinical diagnostics, the number of aptamers targeting CRP is relatively high and has been extensively reviewed elsewhere.[76] Here, we just provide some examples of successfully designed CRP aptamers. Yang et al,[77] by using the graphene oxide-SELEX method, generated 2 RNA aptamers, CRP-80-17 (characterized by a KD of 3.9 nM) and also a truncated version, named CRP-40-17, with better selectivity against the target despite a slightly higher KD (16.2 nM).

An example of aptamers for biosensor development is represented by a CRP DNA aptamer of 20 nt produced by OTC Biotech and further applied for the signal recognition particle-based detection of CRP using an antibody-aptamer sandwich with an LOD of 5 fg/mL.[78] Another DNA-based aptamer named 6th-62-40 was identified using a microfluidic SELEX device.[77] The aptamer exhibited high selectivity to CRP, which was determined against immunoglobulin G, human serum albumin, hemoglobin, transferrin, and Myo. Its binding to CRP is characterized by a KD of 16.2 nM.

APTAMER-BASED HIGH-THROUGHPUT PROTEOMIC SCREENING FOR MEASURING THE CARDIOVASCULAR RISK: FROM CLINICAL TRIALS TO MARKETING

It is well recognized that having access to a greater variety of protein targets that are changed in the mammalian proteome during the early start or course of a certain ailment might lead to the discovery of novel important biomarkers with potential predictive value for disease characterisation.[79] Indeed, multiplex approaches have been widely used in exploratory studies for biomarker discovery, and to date, the best example of a successful use of diagnostic/prognostic aptamers has been shown with Slow off-rate modified aptamers (SOMAmers).

SomaLogic, a pioneering company in the aptamer industry, developed an affinity- and nucleic acid-based proteomic platform called SOMAscan for the direct detection of numerous proteins (approximately thousands of different targets) with a single binding reagent since 2012.[80,81] The assay takes advantage of the unique binding capabilities of a large library of modified DNA aptamers known as SOMAmer, which are generated for a tight and specific binding (range of detection from fM to μM) to more than 7,000 (10,000 in 2023) protein targets in body fluids. SOMAmers consist in a short ssDNA sequence, incorporate modified deoxyuracils with amino acid-like side chains at the C5 position, and are selected for slow off-rates in the SELEX procedure. As a result, SOMAmers have a low off-rate and high binding specificity, making them comparable to monoclonal antibodies as capture molecules for protein detection. Another advantage of using SOMAmers in SOMAscan assays is that the nucleic acid nature of SOMAmers allows for direct quantification by standard DNA detection methodologies, such as DNA microarrays. Furthermore, because of their high binding specificity, SOMAmer ligands do not cross-react with each other and can be combined in the highly multiplexed protein detection SOMAscan assay.

In 2016, Ganz et al[82] used the SOMAscan platform to generate an effective prediction score in a large-scale study designed to improve the risk prediction of cardiovascular outcomes in plasma samples. The authors measured the levels of 1,130 circulating proteins in plasma samples from 2 prospective cohorts of patients with stable coronary heart disease: 938 samples from patients in the derivation cohort and 971 samples from patients in the validation cohort. The authors identified 9 proteins with prognostic value (angiopoietin-2, matrix metalloproteinase-12, CCL18, C7, SERPINA3, troponin I, TNNI3, growth differentiation factor 8/11, and SERPINA3), whose levels are associated with a risk score reflecting the probability of an adverse cardiovascular event to occur over a period of 4 years. Although the increase in the predictive power of the group of 9 proteins was modest, this report highlights the reliability and accuracy of measurements using SOMAscan as a proteomic platform to monitor the progress of a disease in even complex plasma samples.

A similar SOMAscan-based approach was performed in a prospective study of acute cardiovascular events by Mosley et al,[83] who took advantage of the SOMAscan proteomic assay to develop a "virtual proteomic" approach in 2018. To this end, the group used a dataset derived from participants in the Framingham Heart Study Offspring cohort. Upon the profiling of a total of 1,129 plasma proteins, the study showed 268 proteins to be regulated by single nucleotide polymorphisms. A "virtual biomarker" strategy was consequently generated to genetically link the predicted protein levels to the clinical diagnoses previously obtained from large electronic health datasets. This whole process identified and validated CLC1B and platelet-derived growth factor receptor-β as putative biomarkers of atherosclerosis. In a recent report, Williams et al[82] further applied the SOMAscan platform as a quantitative health assessment of multiple health indicators. By profiling 5,000 plasma proteins in 16,894 participants, the authors validated the protein-phenotype model for 11 different health states, including diabetes risk and primary cardiovascular event risk.[82]

In another study, Egerstedt et al[82] took advantage of SOMAscan-based proteomics to profile a systematic characterization of circulating proteins at different stages of heart failure (HF). To this end, the authors analyzed blood samples from different cohorts of HF patients, across populations at risk of HF, with manifest HF, heart transplant recipients, and respective control subjects. By measuring 1,305 proteins, the authors demonstrated a blueprint for plasma biomarker discovery for HF and implicated several proteins associated with HF development, including natriuretic peptides and immunological mediators.[84]

To sum up, we have shown in this section how important biomarkers are in medicine, especially in the realm of personalized medicine, which is also one of the major challenges in the clinical cardiovascular field. Indeed, up to now, establishing the a standard therapeutic regimen entails carrying out a lengthy trial-and-error process of applying and tailoring a drug on the basis of a patient's responses and the outcome.[85] In this context, the use of large-scale proteomic platforms, such as somaScan, may have the potential to predict prognosis and drug-dose selection by measuring therapeutic and adverse responses in patients, as also demonstrated by 10 ongoing clinical trials (NCT04496830, NCT04655729, NCT05809583, NCT02050100, NCT02760277, NCT03646708, NCT05050240, NCT04781062, NCT05755997, NCT05094024) based on the use of this technology. Additionally, somaScan as a high-throughput technology that exposes multiple disease-causing pathways, may lead to the discovery of novel drug targets, highlighting new therapeutic indications, and identifying clinically relevant biomarkers.[86]

APTAMER-BASED TARGETED THERAPEUTICS

Due to their ability to compete with small molecules and protein ligands and to inhibit their targets, aptamers are considered to be promising therapeutics that can be applied either as stand-alone therapeutics or as adjuvants for another therapeutic.[11]

This section contains brief introductions to the specific cardiovascular disorders to identify the settings in which the aptamers were investigated.

ATHEROSCLEROSIS. Atherosclerosis is a complex, multifactorial disease responsible for events such as ischemic heart disease, stroke, and sudden death. All these conditions are responsible for the total mortality rate of over 50% in the Western world.[87] To date, antithrombotic and thrombolytic therapeutic agents have been developed to treat thrombotic conditions, including the recombinant tissue plasminogen activator. However, due to the lack of efficient antidotes able to reverse their action, occurrence of hemorrhage represents a major risk that strongly limits their use. In a recent report, Nimjee et al[88] addressed targeting of the von Willebrand factor (vWF), a glycoprotein that is reduced in von Willebrand disease and involved in thrombus formation by inducing platelet adhesion to the vessel walls. They designed and tested in vitro 90 truncates derived from the 2′-fluoropyrimidine-modified RNA aptamer, named 9.14T10.[88] The resulting aptamer DTRI-031 was shown to be a dose-dependent inhibitor of platelet aggregation, able to induce vascular recanalization of platelet-rich thrombotic occlusions. On the other hand, the base pair complementarity of nucleic acids gives a unique practical advantage to aptamers by naturally providing each aptamer with its own antidote.[89] Therefore, to reduce the risk of complications, the authors also showed the rapid reversibility of the DTRI-031 aptamer action by an antidote oligonucleotide, making the vWFaptamer-antidote pair a powerful and innovative anti-thrombotic therapeutic. A potential use of vWFaptamer as an adjunct in the treatment of myocardial ischemia was also proposed.[90] A randomized, double-blind, single-center, placebo-controlled phase 1 study in healthy volunteers is currently ongoing to assess safety, tolerability, pharmacokinetics, and pharmacodynamics of a single intravenous injection of DTRI-031 (NCT05005520).

VASCULAR DISORDERS. A frequent pathological process in vascular disorders, including arteriosclerosis, restenosis, and vein-bypass graft disease, involves the induction of vascular smooth muscle cell (VSMC) activation. The therapies currently adopted to prevent VSMC migration and proliferation involve use of drug-eluting stents for the local administration of inhibitory drugs such as paclitaxel. However, treatments with drug inhibitors of proliferation, while efficiently acting on VSMC activation, may result in adverse, undesired effects inhibiting endothelial cell proliferation, leading to thrombosis as a complication of drug-eluting stents.[91] To overcome limits imposed by drugs' antiproliferative effects, Thiel et al[37] addressed the development of therapeutic aptamers that selectively inhibit VSMC cell proliferation but have no effect on endothelial cells, allowing rapid re-endothelialization. To this end, taking advantage of previously selected rat VSMC-targeting aptamers with rapid uptake in VSMC cells, they showed that, by binding to platelet-derived growth factor receptor β, Apt 14 inhibits VSMC migration with no effects on endothelial cells.[92] The authors also evaluated the in vivo effects of treatments on VSMC proliferation and migration. They used a mixture of Pluronic F-127 gel for the delivery of Apt 14 to the vessel wall, showing that the aptamer was effective to prevent cell migration and the formation of neointima upon vascular injury. Furthermore, they demonstrated that direct delivery of Apt 14 to the medial layer of the porcine carotid artery via catheter improved retention in the vessel wall. As a result, the authors emphasized Apt 14's potential to act as VSMC-specific drugs for vascular diseases.[93]

AUTOIMMUNE CARDIOMYOPATHIES. Autoimmune abnormalities appear among the major predisposing factors for the development of specific heart diseases, such as idiopathic dilated cardiomyopathy, Chagas' cardiomyopathy, and peripartum cardiomyopathy.[94] All these pathological conditions are characterized by the production of autoantibodies against G protein-coupled receptors that agonistically activate their receptors.[95]

Immunoadsorption (IA), also known as immunoapheresis, is a clinical procedure used to remove circulating antibodies and antibody complexes from blood of patients with different immune disorders or rejection reactions, including removal of autoantibodies against G protein-coupled receptors in idiopathic dilated cardiomyopathy, with studies reporting convincing patient benefit.[96] Despite promising results, the high cost and complexity of IA treatment will hamper its widespread application, especially in countries with restricted health care budgets and problems realizing the logistics of IA.[96] Therefore, to overcome IA problems, Berlin Cures developed a novel ssDNA aptamer, also named BC 007, which in the ongoing 2-arm randomized open-label phase 2a clinical trial showed positive results in terms of neutralization of autoantibodies in the long term after a single dose. In addition, there was a significant improvement in cardiac function (measured as ejection fraction).[95] BC 007 might provide a novel aptameric drug that has the potential to revolutionize the treatment of a number of incurable diseases caused by pathogenic functional autoantibodies.

APTAMERS FOR SMART DRUG DELIVERY

In the early 20th century, Dr Paul Ehrlich proposed the "magic bullet" concept for cancer therapy, in which an ideal therapeutic agent would only kill the specific tumor cells to which it was targeted. Since that moment, the selective delivery of drugs in a cell- or tissue-specific manner to reduce the occurrence of unwanted off-target effects has become the long-standing goal of modern medicine.[97] Indeed, one of the main bottlenecks of traditional medicine for CVD treatment is the widely used systemic administration of drugs, which in turn can negatively affect the bioavailability or concentration in the diseased regions. Moreover, systemic administration implies that drugs circulate in the bloodstream, affecting the function of healthy tissues, which can result in off-target adverse effects.[1] In this context, the advent of aptamer technology has inspired many groundbreaking studies that have successfully adapted the idea of "targeted therapy" into the development of novel "on-demand" drug delivery systems, in which aptamers have also been developed as the vehicles for targeted delivery tools conjugated to oligonucleotides, drugs, and nanoparticles.[98]

APTAMER-DRUG CONJUGATES. The onset and progression of CVDs rely on the deregulated activity of intracellular targets, such as components of numerous signal transduction pathways, cell metabolism regulators, and different enzymes.[99] Thus, the increasing request for novel drugs intended to influence intracellular components led the scientific community to put a lot of effort into developing new, in-demand drugs able to exert effects within cells.

For instance, regardless of the cause, HF patients in the late stage of the pathology have a common feature: defective Ca^{2+} signaling in cardiomyocytes, which results in impaired cardiac muscle contraction and relaxation as well as in development of cardiac

arrhythmias and adverse remodelling.[100] Indeed, a hallmark of failing cardiomyocytes is a change in excitatory contraction coupling, including reduced amplitude of Ca^{2+} transients and delayed onset and decay kinetics of Ca^{2+} transients. All these changes in Ca^{2+} handling can be attributed, among others, to the impaired function of L-type calcium channel (LTCC), RyR2, SERCA, phospholamban (PLN), and the Na^{+}-Ca^{2+} exchanger.[100] In this context, nanobodies (nAbs), small recombinant antigen-binding fragments derived from the atypical monomeric immunoglobulins present in camelid mammals and cartilaginous fish, have been proposed as diagnostic/therapeutic tools to visualize and/or modulate endogenous targets within living cells.[101-103] Compared with traditional antibodies, nAbs, also called intrabodies, have the advantages of low MW (~15 kDa vs the conventional antibodies immunoglobulin Gs, ~150 kDa), high affinity, high stability, low immunogenicity, and strong penetrability.[101-103] Although promising, only 1 nanobody-based drug has been approved so far (caplacizumab [Sanofi] for targeting the vWF), whereas to the best of our knowledge, only 2 works describing the preclinical use of nAbs for defective calcium signaling components (RyR2 and PLN specific nanobodies) have been shown.[100,104] In fact, the main problem with regard to the use of this technology is the requirement of a safe, efficient, and cell-specific gene delivery method for their effective clinical translation. As a matter of fact, nAbs, like any other antibody, rely on conserved disulfide bonds, which in the reducing milieu of the cytosol strongly impair the formation of disulfide bonds. In turn, this can affect the overall folding and, consequently, the successful applicability of the molecule itself.[102] Moreover, transport of nAbs inside cells using precise genomic insertion of intrabody-encoding sequences via CRISPR (clustered regularly interspaced short palindromic repeats) gene editing or alternatively adeno-associated viruses is still questionable in terms of safety for effective therapeutic applications.[102] In this scenario, aptamers, as drug conjugates (aptamer-drug conjugates), may again represent a promising alternative, providing effective delivery of nAbs within the intended targeted cells. Despite the fact that no such examples are currently publicly available, other examples of aptamer-based guides for the intracellular delivery of therapeutic drugs have been proposed. In the work of Romanelli et al,[105] a novel aptamer-peptide chimera was developed to guide a therapeutic mimetic peptide targeting a cytosolic subunit of the LTCC. In this study, the nuclease-resistant single-stranded RNA aptamer Gint4 was used as a cell-permeant carrier to allow the internalized peptide to bind to its cytosolic target and restore the LTCC membrane density in stressed cardiomyocytes.[105,106] On the other hand, Sakai et al[107] created a specific RNA aptamer called Apt30 that targets the small intracellular protein PLN, whose main purpose is to govern cardiomyocyte calcium management by inhibiting SERCA. While Apt30 is the therapeutic moiety in this aptamer-drug conjugate, the cell-penetrating TAT peptide is the drug carrier and allows the aptamer delivery into adult rat cardiomyocytes, where it enhances both cardiac Ca^{2+} transients and contractility.[107]

APTAMER-FUNCTIONALIZED NANOPARTICLES. Because of the extraordinary properties of nanoparticles, which can be shaped to fulfill specific therapeutic applications, nanomedicine has become a highly researched approach to cardiovascular diseases.[108]

When combined with their high payload and release capacities, their size, shape, structure, surface charge, and modularity allow them to overcome the existing strategies in terms of solubility, diffusivity, half-life, drug release, and biodistribution patterns. As a result, when an appropriate drug delivery system is built, nanoparticle-based therapies give superior and longer-lasting therapeutic efficacy with limited side effects.[109] Moreover, their inherent sensor capabilities and/or payload capacity can be exploited for ex vivo diagnostic techniques and in vivo nanoparticle-based contrast agents.

As described in the very recently published review conducted by Smith and Edelman[110] nanoparticles have been studied in a variety of applications, ranging from the treatment of ischemia-reperfusion conditions through intravenous and intracardiac administrations to support for the function of bulk biomaterials to improve their efficiency, efficacy, and durability, such as heart valves, cardiac patches, and vascular grafts and stents. Moreover, immune system modulation appears to be a particularly promising strategy for CVDs due to its involvement in the pathological processes, and this can be accomplished by targeting specific cell subsets to improve efficacy and reduce the dose of the administered drug while avoiding systemic effects on the body's immune defenses with a risk of infection and sepsis.

As previously stated in the introduction regarding aptamer research, nanomedicines have been studied more in the cancer field than in CVDs, which can be attributed to several factors, including investment in research and enthusiasm and momentum related to early clinical trials that successfully drove regulatory approvals of Doxil and Abraxane's cancer nanotherapies in the mid-1990s to 2000s.[110] However, it should be borne in mind that the knowledge gained

over the years in the field of oncological nanotechnology is not limited to that field; rather, it may inspire therapeutic approaches also for CVDs due to similarities in their inflammatory disease pathogenesis.[110]

Furthermore, the first nanodrug used in CVD has recently been approved by the Food and Drug Administration and European Medicines Agency, indicating that cardiovascular nanomedicines are of current interest and can reach the market. We are referring to Leqvio (inclisiran), which is used to treat atherosclerotic CVDs. In particular, by inhibiting the translation of the protein PCSK9 in the liver, Leqvio lowers circulating low-density lipoprotein cholesterol levels and thus lowers the consequences of cardiovascular risk.

Anti-tumor nanomedicines often exploit the enhanced permeability and retention (EPR) effect, which occurs as a consequence of abnormal or highly permeable vasculature at sites of injury or inflammation,[111] particularly present in tumors, where an increased vascularization associated with marked and chaotic leaky vessels results from the rapid proliferation of cancer cells. However, this condition is less pronounced in the heart, even after myocardial infarction, in which a relatively poor EPR effect for nanoparticle-based drug delivery has been observed.[111] Despite the widely accepted notion that the release of cytokines during ischemia-reperfusion after myocardial infarction increases local vascular permeability and inflammation, only 0.2% of the injected fluorescent-labeled PEG-modified nanoparticles were able to reach the infarcted zone, as demonstrated in the study by Lundy et al.[112]

Alternative strategies that allow nanoparticles to accumulate at the desired target are thus required. An intriguing strategy made possible by nanomedicine is the use of unconventional routes of administration such as inhalation, as investigated in the study conducted by Modica et al,[113] in which biocompatible and biodegradable calcium phosphate-based nanoparticles were used to deliver a microRNA to the heart, and it was found that they preferentially targeted cardiomyocytes without significant accumulation in other myocardial cells or organs. NanoPhoria biotech startup is currently investing in this technology for the delivery of biologics directly into cardiomyocytes considering the advantages of biomimetic nanoparticles over viral vectors.[114]

Moreover, active targeting can improve nanoparticle-based drug delivery beyond passive uptake in CVDs, and this is the field in which aptamers could make their valuable contribution. In fact, nanoparticles can be functionalized with a variety of ligands, including aptamers, to improve their tissue- or cell-specific uptake. A good example is represented by the work of Patrick Hsieh's group, in which aptamers were specifically functionalized on the surface of circulating monocytes for the delivery of nanocarriers to the infarct site.[115] Although improvements of the proposed formulation are necessary to make it clinically relevant, the selected aptamer allowed around 5% of the injected nanoparticles to reach the infarct site, which is far more than EPR-based uptake alone could accomplish. Unfortunately, at present, there are no further publications available on the use of aptamers for nanoparticle drug delivery so it would be worthwhile to investigate this aspect in greater depth.

CONCLUSIONS: WHY TO USE APTAMERS

Aptamers, like monoclonal antibodies, have a unique structure that allows them to bind to their targets with high specificity; however, unlike monoclonal antibodies, aptamers are small and flexible molecules with little steric hindrance, are scarcely immunogenic, and are less expensive, making them potentially suitable for a broader range of applications.

This review focused specifically on the use of aptamers as alternatives to traditional therapeutic approaches in a field that desperately needs improvement, that is, the management of CVDs, in terms of both diagnostics and therapy.

They have yielded interesting results in diagnostics in that they have enabled the monitoring of levels of specific protein markers for CVDs as well as the simultaneous detection of thousands of proteins for proteomic screening via innovative platforms that revolutionize CVD risk prediction.

In therapeutics, they have been used to inhibit platelet aggregation in atherosclerosis and VSMC cell proliferation in vascular disorders, thus avoiding adverse effects due to their highly specific targeting, and to neutralize pathogenic functional autoantibodies in autoimmune cardiomyopathies. Unfortunately, their potential in nanoparticle drug delivery has been little explored, despite the leading role nanomedicine plays today in CVD treatment and diagnosis. Thus, associating nanomedicine with aptamer technology would pave the way to countless solutions aimed at making chosen strategies smart and patient-personalized where the therapeutic outcomes are programmed as opposed to merely tolerated. Moreover, it should be borne in mind that

multinational pharmaceutical companies are making major investments in aptamers, including agreements recently stipulated with aptamer companies,[116] which could significantly speed up the future development of innovative treatment methods in various fields, possibly including CVDs.

For the reasons discussed previously, aptamer research has not yet come to an end. On the contrary, the potential benefits that could be attained from their use in the cardiovascular field certainly justify further lines of research aimed, on the one hand, at identifying new targets to be reached with aptamers for both diagnostic and therapeutic purposes and, on the other hand, at improving the existing drug delivery systems to perform efficient targeted delivery in the CVD field, in which their potential could be fully realized as a promising innovation for human health.

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