Molecular Therapy
Nucleic Acids
Review

Advantages, applications, and future directions of *in vivo* aptamer SELEX: A review

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***In vivo* systematic evolution of ligands by exponential enrichment (SELEX) emerged as a transformative technique for directly identifying aptamers within living organisms. Unlike traditional *in vitro* methods that often fail to capture the complexity of biological interactions in physiological environments, *in vivo* SELEX leverages whole living organisms as selection targets. This strategy significantly enhances the specificity, functionality, and physiological relevance of the selected aptamers, making them more viable for therapeutic, diagnostic, and imaging applications. The aim of this review is to elucidate the unique advantages of *in vivo* SELEX compared to conventional *in vitro* techniques. It focuses on how *in vivo* SELEX improves aptamer specificity, physiological relevance, and pharmacokinetic properties, thereby enhancing their potential for use in diagnostics and therapeutics. This review highlights the principles, advancements, and challenges of *in vivo* SELEX, while discussing its potential to bridge the gap between aptamer discovery and clinical translation. By enabling the selection of aptamers under real physiological conditions, *in vivo* SELEX addresses critical limitations of *in vitro* methods, such as off-target effects and poor clinical translatability. The review also explores the applications of *in vivo* SELEX in various fields, including neurology, oncology, and cardiovascular diseases, and provides insights into future directions for optimizing this technology.**

INTRODUCTION

Nucleic acid aptamers are single-stranded nucleic acid molecules, typically consisting of 20–100 nucleotides in length, that exhibit specificity and affinity to their targets.[1] Referred to as "chemical antibodies," aptamers share functional similarities with antibodies but offer distinct advantages, including higher specificity, stronger binding affinity, superior stability, and greater ease of chemical modification.[2] These unique characteristics have positioned nucleic acid aptamers as promising tools for a wide range of biomedicine applications, such as therapeutics, targeted drug delivery, and diagnostics.[3]

The process of identifying nucleic acid aptamers, known as systematic evolution of ligands by exponential enrichment (SELEX), involves iterative rounds of selection, enrichment, and amplification from random oligonucleotide libraries to isolate sequences with high affinity for specific targets.[4] Targets of SELEX can vary widely, ranging from small molecules, proteins, antibiotics, and peptides to complex structures such as cells, tissues, viruses, virus-infected cells, and bacteria.[5] Over the years, advancements in SELEX technology, particularly the integration of high-throughput next-generation sequencing, have significantly enhanced the efficiency, accuracy, and scalability of aptamer screening.[6–8]

Despite their broad application potential, the clinical translation of nucleic acid aptamers faces significant challenges. Traditional *in vitro* SELEX methods are limited by their inability to replicate real physiological conditions, difficulties in maintaining complex target structures, and constraints in targeting specific disease states. These limitations often result in aptamers with reduced efficacy or increased off-target effects, hindering their clinical applicability.[9] To address these issues, researchers have increasingly turned to *in vivo* SELEX, a groundbreaking approach that enables aptamer screening within living organisms. By conducting selection under physiological conditions, *in vivo* SELEX not only enhances the relevance and functionality of the identified aptamers but also minimizes off-target effects on healthy tissues. This approach holds particular promise for precision medicine, as it facilitates the identification of aptamers that specifically target diseased tissues or cells.[10]

In this review, we explore the applications of *in-vivo*-selected aptamers in therapeutics and beyond, highlighting the advantages of *in vivo* selection over traditional *in vitro* methods. We emphasize how *in vivo* SELEX can improve the accuracy of therapeutic efficacy predictions in real biological environments in preclinical stages, thereby reducing clinical trial failure rates. Through a comprehensive analysis of recent advancements, we aim to demonstrate the transformative potential of *in vivo* SELEX in developing highly specific and effective aptamers for precision medicine.

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History and development of aptamer

The field of aptamer research began in 1990 when Tuerk and Gold pioneered the SELEX technology, using it to screen RNA aptamers that can specifically bind to bacteriophage T4 DNA polymerase.[11] This groundbreaking work demonstrated the potential of nucleic acids to function as molecular recognition tools, making a significant milestone in molecular biology. Shortly thereafter, Ellington and Szostak independently developed a similar approach, termed *in vitro* selection, to isolate RNA molecules capable of binding to small organic molecules from a pool of random RNA libraries.[12] Their work not only validated the concept of aptamers but also laid the foundation for their widespread application in biomedicine. By 1992, DNA aptamers targeting thrombin and specific dyes were discovered, further expanding the scope of aptamer technology.[13,14] The introduction of cell-SELEX in 1999 represented another major advancement, enabling the selection of aptamers using live cells as the targets and opening new avenues for applications in cancer research and diagnostics.[15]

The clinical potential of aptamers was realized in 2004 with the Food and Drug Administration (FDA) approval of pegaptanib (Macugen), the first aptamer-based therapeutic, for the treatment of age-related macular degeneration (AMD). Pegaptanib functions by selectively inhibiting vascular endothelial growth factor (VEGF), a key driver of abnormal blood vessel growth in AMD.[16] In 2023, avacincaptad pegol (Izervay) became the second FDA-approved aptamer, targeting complement protein C5 for the treatment of geographic atrophy (GA) secondary to AMD.[17] Beyond these approved therapies, several aptamers have advanced through clinical trials. For instance, AS1411, a DNA aptamer targeting nucleolin, has been evaluated in phase I and II trials for various cancers, including acute myeloid leukemia and renal cell carcinoma.[18,19] Similarly, NOX-E36, an aptamer targeting CCL2, has shown promise in preclinical and clinical studies for diabetes and albuminuria, highlighting the versatility of aptamers beyond oncology.[20]

Currently, ClinicalTrials.gov lists 39 different clinical studies involving aptamers, targeting a diverse range of diseases, including ocular diseases, cancer, and cardiovascular diseases. Notably, ocular diseases account for over 50% of these studies, reflecting the success of aptamers in addressing conditions like AMD. Despite their promise, many aptamers remain in the experimental stages, facing challenges such as physiological relevance, stability, and delivery efficiency before achieving widespread clinical adoption.

Comparison between *in vitro* and *in vivo* SELEX

SELEX is a powerful method for identifying aptamers with high specificity and affinity for their targets. It can be broadly categorized into two types based on the selection environment: *in vitro* and *in vivo* SELEX (Figure 1). Each approach has distinct advantages and limitations (Table 1), making them suitable for different applications.

In vitro SELEX

In vitro SELEX is conducted in a controlled laboratory setting, where aptamer libraries are exposed to target molecules such as recombinant proteins, peptides, or whole living cells.[21] The process begins with the synthesis of a diverse oligonucleotide library containing a large number of random sequences ($>10^{15}$) flanked by constant regions for amplification. Initially, the library is incubated with the target molecule, allowing high-affinity aptamers to bind. Unbound sequences are then removed using techniques, such as nitrocellulose filtration, affinity chromatography, or magnetic bead separation. The bound aptamers are amplified via PCR, and the process is repeated iteratively to enrich high-affinity sequences (Figure 1).

In vitro SELEX offers several advantages, including rapid screening, high throughput, and the ability to generate substantial data in a short period of time.[22] The simplicity of the process, which primarily involves chemical synthesis and test-tube experiments, reduces technical variability and enhances reproducibility. This makes *in vitro* SELEX an accessible and efficient tool for aptamer discovery, particularly for well-defined molecular targets.

However, a significant limitation of *in vitro* SELEX is its inability to replicate the complex physiological conditions of living organisms. The absence of factors such as ionic strength, pH, and protein competition and tissue interactions can lead to aptamers that perform poorly *in vivo*.[23] Additionally, some complex targets, such as membrane proteins or disease-specific biomarkers, may be difficult to obtain or maintain *in vitro*, limiting the relevance of selected aptamers.[10,24] Therefore, aptamers identified through *in vitro* SELEX often require extensive validation in biological systems *in vivo* before clinical applications.

In vivo SELEX

In contrast, *in vivo* SELEX is performed within living organisms, providing a physiologically relevant environment for aptamer selection (Figure 1). This method addresses many limitations of *in vitro* SELEX by allowing aptamers to be screened under conditions that closely mimic their intended therapeutic context. The process begins with the construction of a chemically-modified single-stranded RNA or DNA aptamer library, which is injected into an animal model. The aptamers circulate throughout the body, and those that bind to specific tissues or organs are retained, while unbound sequences are cleared via renal filtration. Target tissues are then harvested, and bound aptamers are extracted, amplified via PCR, and further optimized.

The primary advantage of *in vivo* SELEX is its ability to identify aptamers that function effectively in complex biological environments. This approach is particularly valuable for developing targeted therapies and drug delivery systems, as it enables the selection of aptamer that specifically recognize diseased tissues or cells while minimizing off-target effects.[10,25] For example, in drug delivery, many therapeutic agents struggle to effectively reach their targets due to the presence of biological barriers.[26] Notably, *in vivo* SELEX has shown remarkable potential in overcoming biological barriers such as the blood-brain barrier (BBB), blood-retinal barrier (BRB), and bone

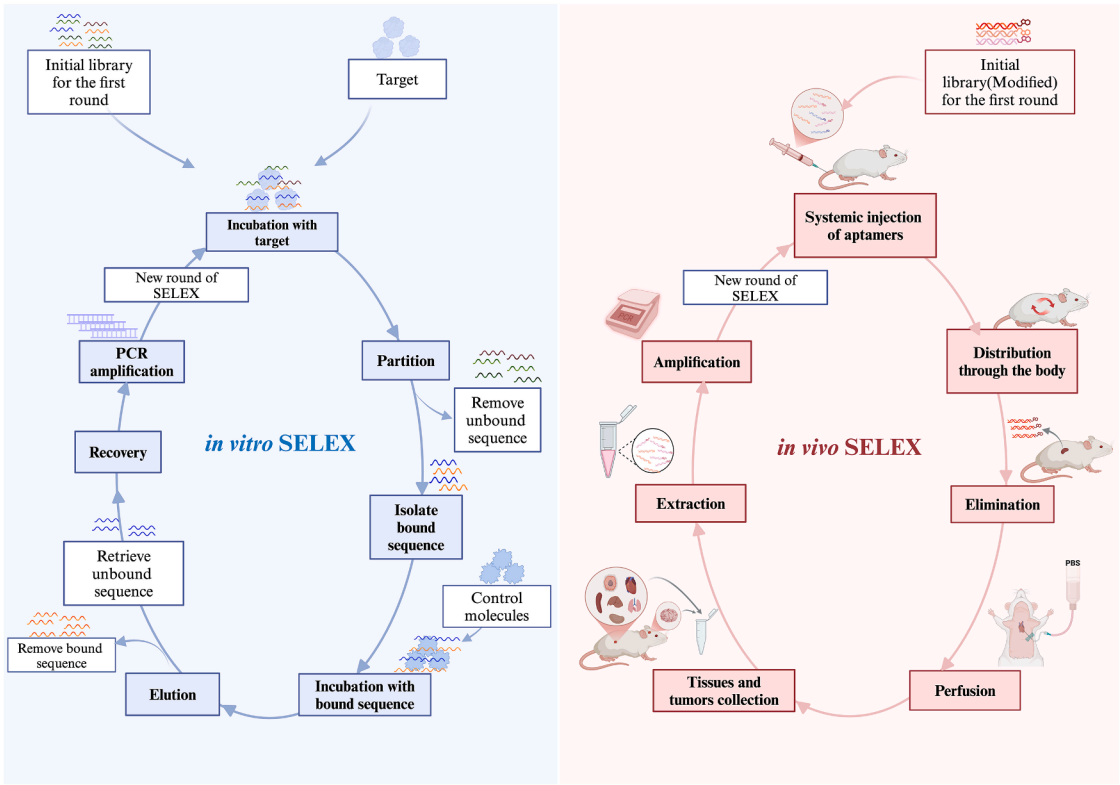

Figure 1. Workflow of *in vitro* and *in vivo* SELEX

For *in vitro* SELEX (left): during the positive selection phase, a random single-stranded DNA/RNA library is incubated with targets, allowing specific sequences to bind. The bound sequences are separated from the unbound ones, and the aptamers attached to the target are recovered and amplified. In the negative selection phase, the amplified sequences from the positive selection are incubated with non-targets. Here, the bound sequences are discarded, whereas the unbound aptamers are collected and amplified via polymerase chain reaction (PCR) to create an enriched pool for the next selection round. These steps are repeated multiple times, followed by sequencing and analysis to isolate aptamers with high affinity and specificity. For *in vivo* SELEX (right): the initial library is injected into a mouse via the tail vein or intraperitoneal route. After 20–90 min of circulation, the mouse is perfused to eliminate non-specifically or weakly bound sequences, and specific tissues and tumors are harvested. Sequences trapped in these tissues and tumors are then isolated and subjected to PCR. Following several rounds of selection, the recovered DNA pool is sequenced for further analysis.

tissues, which are often challenging for traditional therapeutics (Figure 2).[27–29] By screening aptamers under pathological conditions, *in vivo* SELEX facilitates the development of more accurate diagnostic tools and targeted treatments, paving the way for precision medicine.

Despite the ability of *in vivo* SELEX to select aptamers in real physiological environments and enhance the biological relevance of aptamer-target binding, its screening process is typically time-consuming and costly. I*n vivo* SELEX involves additional complexities such as animal handling, tissue extraction, and repeated systemic administration, which further increase both the duration and the experimental costs compared to *in vitro* approaches. Moreover, it faces multiple challenges, including nonspecific binding, low stability by nuclease degradation, and immune clearance, which limit its broad applicability.[10] To address these time and cost bottlenecks, a "combined SELEX" strategy has been introduced in recent years. This strategy integrates both *in vitro* and *in vivo* SELEX to leverage their respective advantages.[30,31] Typically, combined SELEX first employs *in vitro* SELEX to rapidly enrich a preliminary aptamer library with high affinity and specificity, which is then subjected to *in vivo* SELEX for further screening and optimization. This approach significantly shortens the overall screening cycle, reduces experimental costs, and improves the specificity and stability of aptamers in complex physiological environments.

Regarding aptamer stability, chemical modifications such as 2′-fluoropyrimidine nucleotides, 2′-O-methyl modifications, and PEGylation are incorporated to enhance nuclease resistance, prolong circulation time, reduce immune clearance, and optimize pharmacokinetic properties.[32] Specifically, 2′-fluoropyrimidine nucleotides and 2′-O-methyl modifications protect aptamers from nuclease degradation, thereby improving their chemical stability and extending their functional lifespan in biological environments. The 2′-fluoromodification replaces the 2′-hydroxyl (-OH) group with a fluorine atom, preventing nucleophilic attack and enzymatic cleavage by ribonucleases, as the 2′-OH group is a natural cleavage site. Similarly,

Table 1. The advantages and limitations of *in vitro*, *in vivo*, and the combined SELEX

SELEX method	Advantages	Limitations
In vitro SELEX	**precision**: controlled environment (e.g., temperature, pH, buffers). **speed**: fast due to high-throughput screening. **simplified workflow**: no biological variability or ethical constraints.	**artificial conditions**: aptamers may fail *in vivo* due to nuclease degradation, ionic strength, pH shifts, or competition with proteins and biomolecules. **limited biological relevance**: lack of complex physiological conditions of living organisms. **unstable/complex target binding**: poor compatibility with unstable, purified complex targets (e.g., membrane proteins requiring detergents or disease-specific biomarkers).
In vivo SELEX	**biological relevance**: aptamers evolve in native physiological conditions (e.g., nuclease resistance, cellular targeting). **dynamic selection**: maintains targets in their native conformation, reducing off-target binding. **overcomes biological barriers:** overcoming biological barriers (e.g., blood-brain barrier, bone tissues) enables targeted drug delivery to previously inaccessible sites. **therapeutic potential**: identifies aptamers stable in complex environments (e.g., tumor microenvironment).	**complexity**: logistical challenges (delivery into organisms, ethical/regulatory constraints). **variable outcomes**: biological noise (e.g., off-target binding, host immune responses). **cost/time**: resource-intensive and time-consuming due to iterative animal use.
Combined SELEX	**optimized workflow**: leverages *in vitro* speed for initial screening, followed by *in vivo* validation. **robust aptamers**: balances purity and biological relevance. **flexibility**: adaptable to diverse targets (e.g., intracellular molecules).	**resource-heavy**: requires expertise in both methods and extended timelines. **transition challenges**: aptamers selected *in vitro* may lose stability/function *in vivo*. **cost**: higher expenses than stand-alone methods.

the 2′-O-methyl modification replaces the 2′-OH group with a methoxy group (-OCH3), which also blocks the cleavage site, thus increasing the aptamer's resistance to enzymatic degradation. Aptamers with 2′-O-methyl modifications exhibit significantly improved serum stability without compromising their binding affinity, making them well suited for therapeutic applications.[33,34] On the other hand, PEGylation can increase the molecular size of aptamers, reduce renal clearance, and minimize immunogenicity, further extending their circulating half-life. The synergistic application of these chemical modifications with the combined SELEX strategy could effectively overcome key challenges inherent to *in vivo* SELEX, thereby advancing the translational application of aptamers in clinical diagnostics and therapeutics.

Applications of *in vivo* SELEX in different diseases

As a cutting-edge technique, *in vivo* SELEX ensures that the identified aptamers are highly relevant to real biological environments. Nowadays, *in vivo* SELEX has been widely applied in various fields, including neurological disorders and cancers (Figure 3), offering innovative solutions for medical research and precision medicine.

Neurological disorders

The BBB is a selectively permeable physiological barrier that separates the bloodstream from the CNS. Composed of endothelial cells, a basement membrane, and astrocytes, the BBB plays a critical role in maintaining brain homeostasis by blocking harmful substances while allowing essential nutrients to pass through.[35,36] However, this protective function also poses a significant challenge for drug delivery, as over 95% of therapeutic macromolecules are unable to cross the BBB.[36,37] This limitation has hindered the development of effective treatments for neurological disorders.

The permeability of the BBB can change under various pathological conditions. For example, in Alzheimer disease, BBB dysfunction leads to increased vascular permeability, impairing the clearance of neurotoxic substances and allowing harmful molecules and pathogens to enter the brain. This triggers neuroinflammatory responses that exacerbate neurodegeneration.[38] In contrast, in brain tumors such as glioblastoma, the vascular structure of the BBB is often altered, forming a blood-tumor barrier (BTB).[39] Although the increased permeability of the BTB may facilitate drug delivery to tumor cells, it also raises concerns about non-specific uptake and potential toxicity to healthy brain tissue.[40]

Aptamers, with their small size, hydrophilicity, and ability to fold into specific three-dimensional structures, offer a promising solution for overcoming the BBB. Unlike traditional drugs, which are often hydrophobic and require invasive delivery methods,[41] aptamers can cross the BBB without compromising its integrity, thereby maintaining its protective role while facilitating drug delivery to the CNS.[42] This is crucial in treating neurological conditions

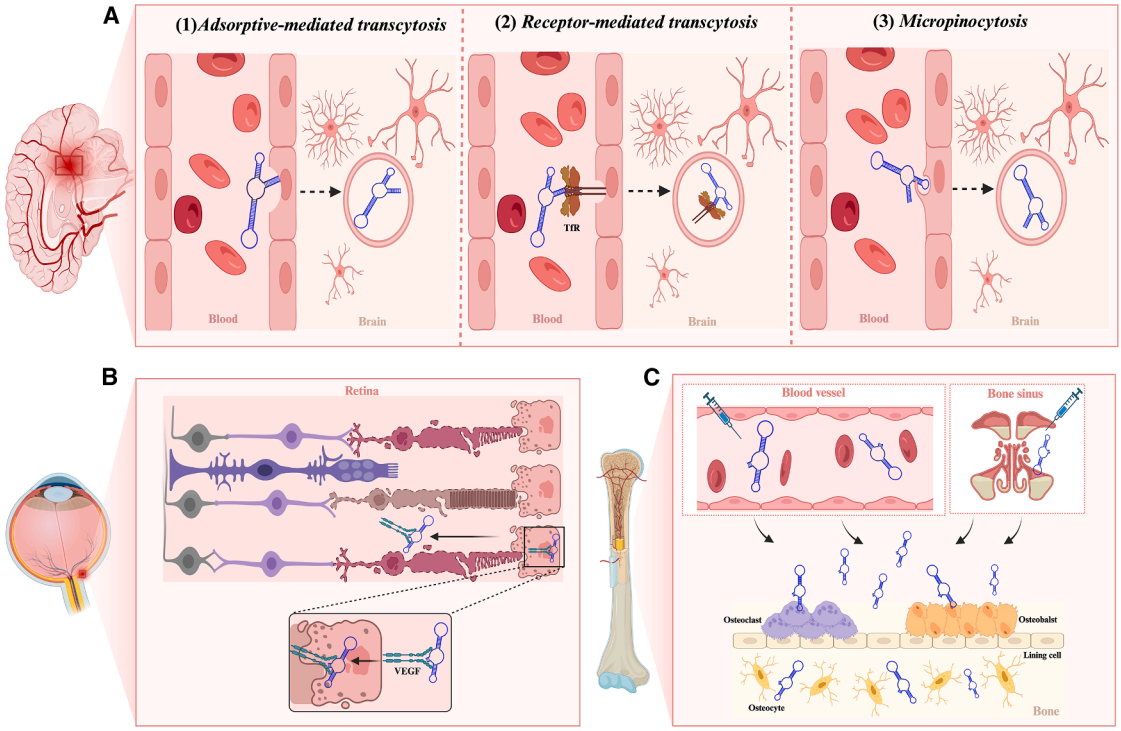

Figure 2. The adaptability of aptamers in overcoming biological barriers for tissue-specific delivery

(A) Aptamers are designed to target blood-brain barrier (BBB) by binding to specific receptors, such as transferrin receptor (TfR), to facilitate receptor-mediated transcytosis. This mechanism enables the delivery of therapeutic cargo across the BBB to treat brain diseases. Proposed mechanisms for aptamer traversal across the BBB include adsorptive-mediated transcytosis, receptor-mediated endocytosis, and micropinocytosis. (B) For the blood-retinal barrier (BRB), aptamers target markers like VEGF receptors, allowing the delivery of anti-angiogenic drugs or gene therapies directly to the retina. This strategy can be used to address conditions such as diabetic retinopathy and age-related macular degeneration. (C) In bone tissue targeting, aptamers bind to bone-specific proteins, such as osteocalcin, to deliver therapeutics precisely to bone lesions, effectively addressing diseases like osteoporosis and bone metastases.

where preserving BBB integrity is essential for preventing further complications. *In vivo* SELEX offers a more effective solution by enabling the selection of aptamers based on various pathological states affecting the BBB and allowing for the direct evaluation of their functionality under specific physiological conditions. This capability enhances targeted therapy by ensuring that aptamers can effectively bind to specific receptors on endothelial cells of the BBB, facilitating efficient transport of therapeutic agents into the brain through specific mechanisms such as receptor-mediated endocytosis.[43]

Early research has demonstrated that *in vivo* SELEX can serve as an effective tool for discovering high-specificity aptamers that target the brain. Cheng et al.[28] developed an aptamer named A15 using *in vivo* SELEX. They designed 40-nucleotide RNA library with 2′-fluoropyrimidine modifications to enhance stability against RNase A degradation. After intravenous injection into mice, brain tissues were harvested to recover bound aptamers. Although initial rounds of selection showed no significant enrichment, a negative selection step was introduced in which the recovered sequences were incubated with mouse serum to remove serum-bound sequences. This step aimed to reduce nonspecific binding and enhance the selection process for brain-targeting aptamers. After 22 rounds of selection, the A15 aptamer was identified, demonstrating BBB penetration and distribution in brain regions such as the cortex, hippocampus, cerebellum, and striatum. However, the molecular target of A15 remains unidentified. Future studies should focus on elucidating the specific receptors or molecular targets on the surface of brain endothelial cells to which A15 binds, such as membrane proteins or transporters. Defining these targets is essential for further optimizing the design and functionality of A15, thereby enhancing its targeting specificity and therapeutic efficacy, and ultimately unlocking its full clinical potential.

Choi et al.[31] integrated BBB microphysiological systems (MPS) with *in vivo* SELEX to identify single-stranded DNA aptamers capable of crossing the BBB while preserving its barrier function. They first performed five rounds of *in vitro* MPS-SELEX using brain microvascular endothelial cells (BMECs) and identified the aptamer hBS01. Subsequent two *in vivo* SELEX rounds yielded hmBS03, which showed higher brain specificity and uptake in astrocytes compared to neurons. This study highlights the advantages of combining *in vitro* and *in vivo* SELEX to identify aptamers with distinct BBB-penetrating mechanisms.

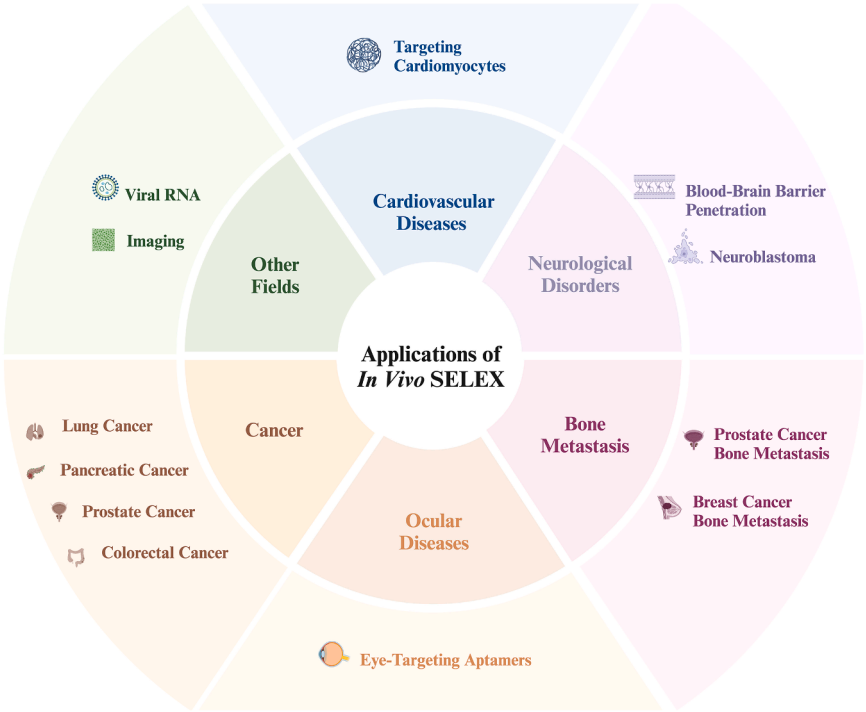

Figure 3. Applications of *in vivo* SELEX across various diseases

Aptamers selected via the *in vivo* SELEX methodology have demonstrated significant potential in addressing a wide range of diseases, including neurological disorders, cardiovascular diseases, cancers, bone metastasis, ocular diseases, and more.

The presence of BBB presents significant challenges for the treatment of leptomeningeal carcinomatosis (LM), leading to a limited number of clinically available treatment options.[44] Huang et al.[45] applied *in vivo* SELEX in a lung cancer LM orthotopic mouse model and identified the aptamer AptB1, which binds to EAAT2, nucleolin, and YB-1 proteins. Conjugating AptB1 with cisplatin (AptBCis1) significantly increased cisplatin concentration in the cerebrospinal fluid and demonstrated superior tumor suppression compared to cisplatin alone, showcasing the therapeutic potential of aptamer-drug conjugates.

Neuroblastoma (NB) originates from neural crest progenitor cells of the sympathetic nervous system and primarily affects young children. It is the most common extracranial solid tumor in this age group, accounting for approximately 15% of childhood cancer deaths worldwide.[46] The high expression of the glycolipid antigen GD2 on neuroblastoma cells makes it a promising target for therapeutic interventions.[47] However, since the glycolipid GD2 is expressed not only in tumor cells but also presents in normal tissues, targeting GD2 can lead to severe side effects due to a lack of specificity.[48,49] To address this challenge, Zhang et al.[30] discovered a GD2-specific aptamer DB99 using a combination of *in vivo* and *in vitro* SELEX. By substituting adenine (dA) residues with 5′-thiophosphoramide, they enhanced the stability of the DNA pool for *in vivo* selection in GD2-positive tumor-bearing mice. Subsequent *in vitro* selection ensured high specificity for GD2-positive neuroblastoma cells. DB99 demonstrated strong binding affinity and specificity, offering a promising therapeutic candidate for neuroblastoma with reduced off-target effects. Recently, Doherty et al.[50] utilized an orthotopic patient-derived xenograft (PDX) model to select DNA aptamers specifically targeting glioblastoma (GBM) cells *in vivo*. To avoid triggering innate immune responses, the DNA aptamers were modified by replacing 5′-CpG dinucleotides with 5-methyl-dC, reducing recognition as pathogen-associated molecular patterns (PAMPs). After eight rounds of *in vivo* selection, aptamer A and E demonstrated specificity for the target GBM cell line without binding to other human tumor cells. These selected aptamers showed rapid accumulation in GBM tumors relative to other tissues after intraperitoneal injection, highlighting their potential as vehicles for delivering therapeutic agents directly to glioblastoma cells. This targeted accumulation underscores their promise for enhancing the efficacy of glioblastoma targeted therapies.

Cardiovascular diseases

Cardiovascular diseases (CVDs) are the leading cause of death globally, accounting for nearly one-third of all fatalities. They impose a significant health and economic burden on societies worldwide.[51,52] Despite their prevalence, the development of new cardiovascular drugs has been slow, with less than 10% of FDA-approved new drugs between 2012 and 2019 targeting CVDs.[53] In recent years, aptamers have emerged as a promising tool for CVD treatment due to their ability to specifically bind to cardiomyocytes and facilitate targeted drug delivery.[54,55] Compared to aptamers selected through traditional *in vitro* methods, those identified via *in vivo* SELEX exhibit enhanced internalization capabilities and greater physiological relevance, making them more effective for therapeutic applications.[56]

Philippou et al.[57] pioneered the use of *in vivo* SELEX to develop cardiomyocyte-specific aptamers for the treatment of cardiomyopathy. By incorporating a cell enrichment step, they enhanced the localization of RNA aptamers specifically within cardiomyocytes (CMs), rather than other cardiac cell types. This approach involved intravenous administration of a 2′-fluoropyrimidine-modified RNA library into a Duchenne muscular dystrophy (DMD) animal model, followed by the recovery from heart tissue. After seven rounds of selection, they successfully identified aptamers capable of differentiating between various cardiac cell types. The study demonstrated that adding a cell enrichment step before recovering aptamers from tissue significantly improved the specificity of the selection process. These

cardiomyocyte-specific aptamers hold great potential as targeted ligands for therapeutic strategies, enabling precise drug delivery to diseased tissues while minimizing off-target effects. This is particularly advantageous for chronic CVDs, where long-term treatment requires reduced dosage and dose-dependent toxicity to improve patient tolerance and safety. However, this study did not evaluate the binding capacity of the enriched aptamers to cardiomyocyte surfaces or their endosomal escape capabilities, leaving their therapeutic applications uncertain.

Cancers

In cancer research, *in vivo* SELEX has proven invaluable for identifying aptamers that function effectively within the complex tumor microenvironment. This approach allows for the screening of aptamers under physiological conditions, providing a more accurate reflection of their binding properties and interactions with tumor cells. It also facilitates the discovery of novel therapeutic targets that are difficult to identify using traditional *in vitro* methods.

Colorectal cancer is one of the most common cancers worldwide,[58] with RNA helicases p68 (DDX5) and DHX9 identified as key therapeutic targets due to their role in promoting tumor progression.[59–61] Mi et al. utilized *in vivo* SELEX to identify RNA aptamers targeting helicases in an intrahepatic colorectal cancer metastasis model.[62] They injected a 2′-fluoropyrimidine-modified RNA library into the model, allowed the molecules to interact with the tumor, and then harvested liver tissues for aptamer recovery. After 14 rounds of selection, they identified RNA 14-16 that bind to DDX5 and inhibits its APTase activity, demonstrating its potential as a therapeutic agent by binding assays. For DHX9, the research team established an intrahepatic xenograft model using cell lines derived from colorectal liver metastasis patients. After 12 rounds of *in vivo* screening, they identified the aptamer S-1, which showed high affinity and specificity for DHX9. These findings highlighted the ability of *in vivo* SELEX to identify aptamers that function effectively under physiological conditions, addressing the limitations of *in vitro* methods that rely on purified proteins.[63]

Prostate cancer, the most common cancer among men, is characterized by high heterogeneity and a complex tumor microenvironment that poses significant challenges for precision therapy.[64] Civit et al.[65] explored *in vivo* SELEX to develop aptamers for hormone-refractory prostate cancer (HRPC) treatment. They created an orthotopic xenograft mouse model by implanting HRPC cells into the prostate of immunocompromised mice, closely mimicking the human disease environment. To address the rapid clearance of nucleic acids, they used a PEG-modified DNA library and a naive library for screening. After 10 rounds of selection, aptamers from the PEGylated library showed significantly higher binding affinity to HRPC cells compared to normal prostate cells. Notably, aptamer D3P exhibited balanced enrichment and enhanced therapeutic efficacy when combined with therapeutic agents, reducing tumor growth and improving survival in xenograft models. This study underscores the importance of PEGylation in improving the pharmacokinetic properties of aptamers for *in vivo* applications.

Non-small cell lung cancer (NSCLC) accounts for 85%–90% of lung cancer cases worldwide.[66] Wang et al.[67] performed *in vivo* SELEX using a PEGylated RNA library in an NCI-H460 bearing xenograft NSCLC mouse model. After 11 rounds of selection, they successfully identified the aptamer RA16, which demonstrated strong specificity for NCI-H460 NSCLC cells. RA16 showed significant accumulation at the tumor site over time, confirming its effectiveness in targeting NSCLC tumors.

Barbas et al.[68] developed aptamers targeting human pancreatic cancer tissue using *in vivo* SELEX. They injected BXPC-3 human pancreatic cancer cells into nude mice to create xenografts and administered two nuclease-resistant RNA libraries (containing either 2′ fluoropyrimidine or 2’ O-methyl modified pyrimidines). After 10 rounds of screening, tumor-binding aptamers were enriched, with several sequences showing high expression. However, to comprehensively validate the functionality and efficacy of the identified aptamers, further research must prioritize target identification and functional validation.

Bone metastasis

In vivo SELEX has demonstrated exceptional efficacy in developing bone-targeted drugs for cancer bone metastasis. The bone represents one of the most challenging biological barriers in the human body due to its highly mineralized and complex structure, which hinders effective drug penetration and distribution.[69] Traditional *in vitro* SELEX methods often fail to replicate the physiological environment of bone tissue, resulting in aptamers that perform suboptimally in practical applications.[70,71] In contrast, *in vivo* SELEX enables the selection of aptamers that specifically bind to bone tissue under real physiological conditions, significantly enhancing drug concentration in the bone and improving therapeutic outcomes.[27,72]

Bone metastasis is a severe complication of advanced prostate cancer, significantly impacting patients’ quality of life. Researchers have utilized *in vivo* SELEX in a PC3-derived mouse model of prostate cancer bone metastasis to identify aptamers specifically targeting bone tissue.[27] The process involved injecting a thiolated aptamer library into nude mice bearing PC3 tumors, followed by the recovery and amplification of aptamers bound to bone marrow. After 10 rounds of selection, an aptamer PB was identified, demonstrating the highest targeting frequency for bone tissue. PB exhibited strong affinity for endothelial cells associated with cancer cells in the bone. When conjugated with gold nanoparticles, PB demonstrated significant targeting efficiency *in vivo*, highlighting its potential for therapeutic applications.

In another study, Liu et al.[72] screened a DNA thioaptamer candidate, T1, targeting the tumor microenvironment (TME) in a breast cancer bone metastasis model. The results showed that T1 aptamers

exhibited significantly higher tumor-tropic accumulation compared with random sequences.

These studies underscore the ability of *in vivo* SELEX to develop efficient and specific targeted delivery systems capable of overcoming strong biological barriers, offering new treatment strategies for cancers and with bone metastases.

Ocular diseases

In vivo SELEX has been applied to discover targeting ocular tissues, leveraging the structural and functional similarities between BBB and BRB.[73] Korhonen et al. pioneered the use of *in vivo* SELEX to screen aptamers from multiple ocular tissues.[29] They introduced a 2′-fluoropyrimidine-modified RNA library into rats by tail vein injection and, after 14 rounds of selection, identified Apt2 and Apt5, which demonstrated significant homing to eye tissues. These aptamers effectively addressed the challenge of insufficient permeability of macromolecules in ocular tissues following intravenous administration, enabling large, negatively charged macromolecules to target complex and difficult-to-reach ocular tissue structures. The development of this aptamer pool paves the way for creating aptamer-drug combinations for treating ocular diseases.

Other applications

Beyond therapeutic applications, *in vivo* SELEX has played a pivotal role in studying viral RNA structures and advancing imaging techniques.

Murawski et al. utilized *in vivo* SELEX to select RNA sequences capable of replacing approximately 20% of a subviral RNA genome. By introducing viral RNA into a living organism, they observed real-time evolutionary changes under natural selection pressures, providing insights often overlooked in *in vitro* studies. This work highlights the significance of *in vivo* approaches in virology and reveals how structural plasticity and rapid evolutionary processes enable viruses to thrive under selective pressures.[74]

Guo et al. investigated the fitness of various RNA conformations through *in vivo* SELEX. By substituting specific regions of satellite RNA with randomized sequences, they identified sequences that preserved critical structural features necessary for fitness in plant hosts. This research enhances our understanding of how viruses adapt to their hosts and environments through genetic and structural innovations.[75]

Melchers et al. performed *in vivo* SELEX to select RNA aptamers, emphasizing the dynamic nature of viral RNA structures and their evolutionary adaptations. They identified a GCUA tetranucleotide loop in the poliovirus oriL, which plays a critical role in viral replication. This discovery provides new insights into viral replication mechanisms and potential therapeutic targets.[76]

Nikki et al. conducted i*n vivo* SELEX to identify RNA aptamers that specifically bind to regions of HIV-1 leader RNA. By introducing an RNA library into a living model, they facilitated interactions with viral RNA and selected high-affinity binders. The most effective aptamer inhibited over 75% of HIV-1 production in cell lines, demonstrating its potential as a therapeutic agent. This study highlights the utility of *in vivo* SELEX in developing aptamers for both imaging and therapeutic applications.[77]

CONCLUDING REMARKS AND PROSPECTS

In vivo SELEX represents a transformative advancement in aptamer technology, offering significant advantages over traditional *in vitro* methods. By conducting selections within living organisms or tissues, researchers can identify aptamers with enhanced specificity, functionality, and physiological relevance. This approach addresses critical limitations of *in vitro* SELEX, such as the inability to replicate complex biological environments and the risk of off-target effects. The ability of directly targeting diseased tissues or organs under real physiological conditions opens new avenues for developing highly specific diagnostic tools and targeted therapies, ultimately improving patient outcomes.

The applications of *in vivo* SELEX span a wide range of fields, including neurology, oncology, cardiovascular diseases, and infectious diseases. By enabling the selection of aptamers that function effectively in complex biological systems, this technology holds the potential to revolutionize precision medicine. For instance, *in vivo* SELEX has already demonstrated success in overcoming biological barriers such as BBB and BRB, as well as in targeting challenging environments like bone metastases and tumor microenvironments. These achievements underscore the potential to address critical unmet clinical needs, including the effective delivery of therapeutics across the BBB for neurological disorders and intracranial tumors, precise targeting of metastatic tumors and their microenvironmental alterations, and significantly enhanced *in vivo* functionality compared to aptamers selected via conventional *in vitro* methods. These advances not only overcome existing limitations in biological barrier penetration and target specificity in targeted therapies but also provide a robust foundation for developing safer and more efficacious treatment strategies, thereby facilitating the clinical translation and application of precision medicine for complex diseases.

Future research should focus on the following key aspects to further advance *in vivo* SELEX technology. First, advanced technologies such as machine learning (ML), computational modeling, and artificial intelligence (AI) could be combined with *in vivo* SELEX to accelerate aptamer discovery and optimization. ML algorithms can design compact, structurally diverse libraries by learning from existing aptamer databases, thereby reducing selection cycles.[78] Computational approaches, including secondary and tertiary structure prediction, molecular docking, and molecular dynamics simulations, provide mechanistic insights into aptamer-target binding.[79] Additionally, AI-driven models can guide rational chemical modifications to

improve aptamer stability and specificity *in vivo*. Iterative feedback loops combining experimental data with ML further refine aptamer selection, transforming the process into a data-driven pipeline that significantly improves targeting accuracy and therapeutic potential in complex biological environments.[80]

Second, continuous refinement of enrichment procedures, particularly the optimization of selection strategies targeting complex biological molecules, is essential. Recent study has demonstrated that modified *in vivo* SELEX protocols can successfully isolate aptamers with selective affinity for cardiomyocytes, offering novel therapeutic avenues for neuromuscular disorders.[57] By optimizing cellular enrichment steps to enhance aptamer recognition of specific cell types within tissues, targeting precision is significantly improved, providing an effective approach to augment the efficiency of *in vivo* SELEX. Building on this, the integration of hybrid selection strategies, such as combining *in vivo* SELEX with *in vitro* selection techniques, leverages the strengths of each method, overcoming the limitations inherent to single approaches and markedly enhancing the functional relevance and clinical applicability of selected aptamers.

Third, the design of aptamers capable of multi-target or multi-receptor recognition is emerging as a transformative direction. Such multidimensional recognition systems enhance the ability of aptamers to identify and therapeutically address complex pathologies, including cancer and immune-related diseases, by simultaneously blocking multiple pathogenic pathways or synergistically modulating cellular functions. This effectively broadens the clinical utility of *in vivo* SELEX-derived aptamers. Notably, the deep integration of animal models with high-throughput screening platforms is poised to overcome current technical bottlenecks: on one hand, by precisely recapitulating human pathological microenvironments to improve selection efficiency; on the other, through automation enabling large-scale candidate validation. This convergence of technologies is expected to drive breakthrough advancements in nucleic acid aptamer development for precision medicine and targeted therapies, furnishing smarter molecular tools for disease treatment.

The integration of *in vivo* SELEX into the broader landscape of biomedical research holds promise for advancing personalized medicine. By enabling the development of highly specific and effective therapeutic agents, this technology has the potential to transform the treatment of complex diseases and improve global health outcomes. As research continues to refine and expand the applications of *in vivo* SELEX, it will undoubtedly play a pivotal role in shaping the future of targeted and precision medicine.

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AUTHOR CONTRIBUTIONS

M.L., writing, editing, and visualization; C.H.C., Z.C., and Y.L., investigation and visualization; Y.Y., writing, editing, supervision, project administration, and funding acquisition.

DECLARATION OF INTERESTS

The authors declare no competing interest.

REFERENCES

1. Rothlisberger, P., Gasse, C., and Hollenstein, M. (2017). Nucleic Acid Aptamers: Emerging Applications in Medical Imaging, Nanotechnology, Neurosciences, and Drug Delivery. Int. J. Mol. Sci. *18*, 2430. https://doi.org/10.3390/ijms18112430.
2. Shi, Y., Zhen, X., Zhang, Y., Li, Y., Koo, S., Saiding, Q., Kong, N., Liu, G., Chen, W., and Tao, W. (2024). Chemically Modified Platforms for Better RNA Therapeutics. Chem. Rev. *124*, 929–1033. https://doi.org/10.1021/acs.chemrev.3c00611.
3. Yu, Y., Liang, C., Lv, Q., Li, D., Xu, X., Liu, B., Lu, A., and Zhang, G. (2016). Molecular Selection, Modification and Development of Therapeutic Oligonucleotide Aptamers. Int. J. Mol. Sci. *17*, 358. https://doi.org/10.3390/ijms17030358.
4. Brown, A., Brill, J., Amini, R., Nurmi, C., and Li, Y. (2024). Development of Better Aptamers: Structured Library Approaches, Selection Methods, and Chemical Modifications. Angew Chem. Int. Ed. Engl. *63*, e202318665. https://doi.org/10.1002/anie.202318665.
5. Sefah, K., Shangguan, D., Xiong, X., O'Donoghue, M.B., and Tan, W. (2010). Development of DNA aptamers using Cell-SELEX. Nat. Protoc. *5*, 1169–1185. https://doi.org/10.1038/nprot.2010.66.
6. Xiao, X., Li, H., Zhao, L., Zhang, Y., and Liu, Z. (2021). Oligonucleotide aptamers: Recent advances in their screening, molecular conformation and therapeutic applications. Biomed. Pharmacother. *143*, 112232. https://doi.org/10.1016/j.biopha.2021.112232.
7. Blind, M., and Blank, M. (2015). Aptamer Selection Technology and Recent Advances. Mol. Ther. Nucleic Acids *4*, e223. https://doi.org/10.1038/mtna.2014.74.
8. Bashir, A., Yang, Q., Wang, J., Hoyer, S., Chou, W., McLean, C., Davis, G., Gong, Q., Armstrong, Z., Jang, J., et al. (2021). Machine learning guided aptamer refinement and discovery. Nat. Commun. *12*, 2366. https://doi.org/10.1038/s41467-021-22555-9.
9. Zhou, J., and Rossi, J. (2017). Aptamers as targeted therapeutics: current potential and challenges. Nat. Rev. Drug Discov. *16*, 181–202. https://doi.org/10.1038/nrd.2016.199.
10. Sola, M., Menon, A.P., Moreno, B., Meraviglia-Crivelli, D., Soldevilla, M.M., Cartón-García, F., and Pastor, F. (2020). Aptamers Against Live Targets: Is In Vivo SELEX Finally Coming to the Edge? Mol. Ther. Nucleic Acids *21*, 192–204. https://doi.org/10.1016/j.omtn.2020.05.025.
11. Tuerk, C., and Gold, L. (1990). Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science *249*, 505–510. https://doi.org/10.1126/science.2200121.
12. Ellington, A.D., and Szostak, J.W. (1990). In vitro selection of RNA molecules that bind specific ligands. Nature *346*, 818–822. https://doi.org/10.1038/346818a0.
13. Bock, L.C., Griffin, L.C., Latham, J.A., Vermaas, E.H., and Toole, J.J. (1992). Selection of single-stranded DNA molecules that bind and inhibit human thrombin. Nature *355*, 564–566. https://doi.org/10.1038/355564a0.
14. Ellington, A.D., and Szostak, J.W. (1992). Selection in vitro of single-stranded DNA molecules that fold into specific ligand-binding structures. Nature *355*, 850–852. https://doi.org/10.1038/355850a0.
15. Homann, M., and Göringer, H.U. (1999). Combinatorial selection of high affinity RNA ligands to live African trypanosomes. Nucleic Acids Res. *27*, 2006–2014. https://doi.org/10.1093/nar/27.9.2006.
16. Ng, E.W.M., Shima, D.T., Calias, P., Cunningham, E.T., Jr., Guyer, D.R., and Adamis, A.P. (2006). Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease. Nat. Rev. Drug Discov. *5*, 123–132. https://doi.org/10.1038/nrd1955.

17. Kang, C. (2023). Avacincaptad Pegol: First Approval. Drugs (Basel) *83*, 1447–1453. https://doi.org/10.1007/s40265-023-01948-8.

18. Rosenberg, J.E., Bambury, R.M., Van Allen, E.M., Drabkin, H.A., Lara, P.N., Jr., Harzstark, A.L., Wagle, N., Figlin, R.A., Smith, G.W., Garraway, L.A., et al. (2014). A phase II trial of AS1411 (a novel nucleolin-targeted DNA aptamer) in metastatic renal cell carcinoma. Invest. N. Drugs *32*, 178–187. https://doi.org/10.1007/s10637-013-0045-6.

19. Mongelard, F., and Bouvet, P. (2010). AS-1411, a guanosine-rich oligonucleotide aptamer targeting nucleolin for the potential treatment of cancer, including acute myeloid leukemia. Curr. Opin. Mol. Therapeut. *12*, 107–114.

20. Kaur, H., Bruno, J.G., Kumar, A., and Sharma, T.K. (2018). Aptamers in the Therapeutics and Diagnostics Pipelines. Theranostics *8*, 4016–4032. https://doi.org/10.7150/thno.25958.

21. Darmostuk, M., Rimpelova, S., Gbelcova, H., and Ruml, T. (2015). Current approaches in SELEX: An update to aptamer selection technology. Biotechnol. Adv. *33*, 1141–1161. https://doi.org/10.1016/j.biotechadv.2015.02.008.

22. DeRosa, M.C., Lin, A., Mallikaratchy, P., McConnell, E.M., McKeague, M., Patel, R., and Shigdar, S. (2023). In vitro selection of aptamers and their applications. Nat. Rev. Methods Primers *3*, 55. https://doi.org/10.1038/s43586-023-00247-6.

23. Famulok, M., Hartig, J.S., and Mayer, G. (2007). Functional aptamers and aptazymes in biotechnology, diagnostics, and therapy. Chem. Rev. *107*, 3715–3743. https://doi.org/10.1021/cr0306743.

24. Jin, B., Guo, Z., Chen, Z., Chen, H., Li, S., Deng, Y., Jin, L., Liu, Y., Zhang, Y., and He, N. (2023). Aptamers in cancer therapy: problems and new breakthroughs. J. Mater. Chem. B *11*, 1609–1627. https://doi.org/10.1039/d2tb02579e.

25. Zhuo, Z., Yu, Y., Wang, M., Li, J., Zhang, Z., Liu, J., Wu, X., Lu, A., Zhang, G., and Zhang, B. (2017). Recent Advances in SELEX Technology and Aptamer Applications in Biomedicine. Int. J. Mol. Sci. *18*, 2142. https://doi.org/10.3390/ijms18102142.

26. Yang, R., Wei, T., Goldberg, H., Wang, W., Cullion, K., and Kohane, D.S. (2017). Getting Drugs Across Biological Barriers. Adv. Mater. *29*. https://doi.org/10.1002/adma.201606596.

27. Chen, L., He, W., Jiang, H., Wu, L., Xiong, W., Li, B., Zhou, Z., and Qian, Y. (2019). In vivo SELEX of bone targeting aptamer in prostate cancer bone metastasis model. Int. J. Nanomed. *14*, 149–159. https://doi.org/10.2147/IJN.S188003.

28. Cheng, C., Chen, Y.H., Lennox, K.A., Behlke, M.A., and Davidson, B.L. (2013). In vivo SELEX for Identification of Brain-penetrating Aptamers. Mol. Ther. Nucleic Acids *2*, e67. https://doi.org/10.1038/mtna.2012.59.

29. Korhonen, S., Stenberg, K., Seemab, U., Bartos, P., Mäkiniemi, K., Kjems, J., Dupont, D.M., and Subrizi, A. (2024). Targeting ocular tissues with intravenously administered aptamers selected by in vivo SELEX. Mol. Ther. Nucleic Acids *35*, 102352. https://doi.org/10.1016/j.omtn.2024.102352.

30. Zhang, L., Wang, M., Zhu, Z., Chen, S., Wu, H., Yang, Y., Che, F., Li, Q., and Li, H. (2021). A GD2-aptamer-mediated, self-assembling nanomedicine for targeted multiple treatments in neuroblastoma theranostics. Mol. Ther. Nucleic Acids *26*, 732–748. https://doi.org/10.1016/j.omtn.2021.08.021.

31. Choi, J.W., Seo, M., Kim, K., Kim, A.R., Lee, H., Kim, H.S., Park, C.G., Cho, S.W., Kang, J.H., Joo, J., and Park, T.E. (2023). Aptamer Nanoconstructs Crossing Human Blood-Brain Barrier Discovered via Microphysiological System-Based SELEX Technology. ACS Nano *17*, 8153–8166. https://doi.org/10.1021/acsnano.2c11675.

32. Qi, S., Duan, N., Khan, I.M., Dong, X., Zhang, Y., Wu, S., and Wang, Z. (2022). Strategies to manipulate the performance of aptamers in SELEX, post-SELEX and microenvironment. Biotechnol. Adv. *55*, 107902. https://doi.org/10.1016/j.biotechadv.2021.107902.

33. Kratschmer, C., and Levy, M. (2017). Effect of Chemical Modifications on Aptamer Stability in Serum. Nucleic Acid Therapeut. *27*, 335–344. https://doi.org/10.1089/nat.2017.0680.

34. Ni, S., Zhuo, Z., Pan, Y., Yu, Y., Li, F., Liu, J., Wang, L., Wu, X., Li, D., Wan, Y., et al. (2021). Recent Progress in Aptamer Discoveries and Modifications for Therapeutic Applications. ACS Appl. Mater. Interfaces *13*, 9500–9519. https://doi.org/10.1021/acsami.0c05750.

35. Pandit, R., Chen, L., and Götz, J. (2020). The blood-brain barrier: Physiology and strategies for drug delivery. Adv. Drug Deliv. Rev. *165–166*, 1–14. https://doi.org/10.1016/j.addr.2019.11.009.

36. Terstappen, G.C., Meyer, A.H., Bell, R.D., and Zhang, W. (2021). Strategies for delivering therapeutics across the blood-brain barrier. Nat. Rev. Drug Discov. *20*, 362–383. https://doi.org/10.1038/s41573-021-00139-y.

37. Yu, S., Xu, X., Feng, J., Liu, M., and Hu, K. (2019). Chitosan and chitosan coating nanoparticles for the treatment of brain disease. Int. J. Pharm. *560*, 282–293. https://doi.org/10.1016/j.ijpharm.2019.02.012.

38. Huang, Z., Wong, L.W., Su, Y., Huang, X., Wang, N., Chen, H., and Yi, C. (2020). Blood-brain barrier integrity in the pathogenesis of Alzheimer's disease. Front. Neuroendocrinol. *59*, 100857. https://doi.org/10.1016/j.yfrne.2020.100857.

39. Arvanitis, C.D., Ferraro, G.B., and Jain, R.K. (2020). The blood-brain barrier and blood-tumour barrier in brain tumours and metastases. Nat. Rev. Cancer *20*, 26–41. https://doi.org/10.1038/s41568-019-0205-x.

40. Bauer, N., Beckmann, D., Reinhardt, D., Frost, N., Bobe, S., Erapaneedi, R., Risse, B., and Kiefer, F. (2024). Therapy-induced modulation of tumor vasculature and oxygenation in a murine glioblastoma model quantified by deep learning-based feature extraction. Sci. Rep. *14*, 2034. https://doi.org/10.1038/s41598-024-52268-0.

41. Bukari, B., Samarasinghe, R.M., Noibanchong, J., and Shigdar, S.L. (2020). Non-Invasive Delivery of Therapeutics into the Brain: The Potential of Aptamers for Targeted Delivery. Biomedicines *8*, 120. https://doi.org/10.3390/biomedicines8050120.

42. Kong, A.H.Y., Wu, A.J., Ho, O.K.Y., Leung, M.M.K., Huang, A.S., Yu, Y., Zhang, G., Lyu, A., Li, M., and Cheung, K.H. (2023). Exploring the Potential of Aptamers in Targeting Neuroinflammation and Neurodegenerative Disorders: Opportunities and Challenges. Int. J. Mol. Sci. *24*, 11780. https://doi.org/10.3390/ijms241411780.

43. Doherty, C., Wilbanks, B., Khatua, S., and Maher, L.J., 3rd (2024). Aptamers in neuro-oncology: An emerging therapeutic modality. Neuro Oncol. *26*, 38–54. https://doi.org/10.1093/neuonc/noad156.

44. Ozcan, G., Singh, M., and Vredenburgh, J.J. (2023). Leptomeningeal Metastasis from Non-Small Cell Lung Cancer and Current Landscape of Treatments. Clin. Cancer Res. *29*, 11–29. https://doi.org/10.1158/1078-0432.CCR-22-1585.

45. Huang, B.T., Lai, W.Y., Yeh, C.L., Tseng, Y.T., Peck, K., Yang, P.C., and Lin, E.P.Y. (2024). AptBCis1, An Aptamer-Cisplatin Conjugate, Is Effective in Lung Cancer Leptomeningeal Carcinomatosis. ACS Nano *18*, 27905–27916. https://doi.org/10.1021/acsnano.4c04680.

46. Matthay, K.K., Maris, J.M., Schleiermacher, G., Nakagawara, A., Mackall, C.L., Diller, L., and Weiss, W.A. (2016). Neuroblastoma. Nat. Rev. Dis. Primers *2*, 16078. https://doi.org/10.1038/nrdp.2016.78.

47. Cheever, M.A., Allison, J.P., Ferris, A.S., Finn, O.J., Hastings, B.M., Hecht, T.T., Mellman, I., Prindiville, S.A., Viner, J.L., Weiner, L.M., and Matrisian, L.M. (2009). The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. Clin. Cancer Res. *15*, 5323–5337. https://doi.org/10.1158/1078-0432.CCR-09-0737.

48. Sabbih, G.O., and Danquah, M.K. (2021). Neuroblastoma GD2 Expression and Computational Analysis of Aptamer-Based Bioaffinity Targeting. Int. J. Mol. Sci. *22*, 9101. https://doi.org/10.3390/ijms22169101.

49. Zhang, L., Wang, M., Zhu, Z., Ding, C., Chen, S., Wu, H., Yang, Y., Che, F., Li, Q., and Li, H. (2021). A Novel pH-Sensitive Multifunctional DNA Nanomedicine: An Enhanced and Harmless GD2 Aptamer-Mediated Strategy for Guiding Neuroblastoma Antitumor Therapy. Int. J. Nanomed. *16*, 3217–3240. https://doi.org/10.2147/IJN.S302450.

50. Doherty, C.D., Wilbanks, B.A., Jain, S., S Pearson, K., Bakken, K.K., Burgenske, D. M., Lett, N.W., Sarkaria, J.N., and Maher, L.J., 3rd (2025). In vivo selection of anti-glioblastoma DNA aptamers in an orthotopic patient-derived xenograft model. NAR Cancer *7*, zcaf005. https://doi.org/10.1093/narcan/zcaf005.

51. Vaduganathan, M., Mensah, G.A., Turco, J.V., Fuster, V., and Roth, G.A. (2022). The Global Burden of Cardiovascular Diseases and Risk: A Compass for Future Health. J. Am. Coll. Cardiol. *80*, 2361–2371. https://doi.org/10.1016/j.jacc.2022.11.005.

52. Global Cardiovascular Risk, C., Magnussen, C., Ojeda, F.M., Leong, D.P., Alegre-Diaz, J., Amouyel, P., Aviles-Santa, L., De Bacquer, D., Ballantyne, C.M., Bernabe-Ortiz, A., et al. (2023). Global Effect of Modifiable Risk Factors on Cardiovascular

Disease and Mortality. N. Engl. J. Med. *389*, 1273–1285. https://doi.org/10.1056/NEJMoa2206916.

53. Zhao, C., Li, S., Zhang, J., Huang, Y., Zhang, L., Zhao, F., Du, X., Hou, J., Zhang, T., Shi, C., et al. (2020). Current state and future perspective of cardiovascular medicines derived from natural products. Pharmacol. Ther. *216*, 107698. https://doi.org/10.1016/j.pharmthera.2020.107698.

54. Zhu, G., and Chen, X. (2018). Aptamer-based targeted therapy. Adv. Drug Deliv. Rev. *134*, 65–78. https://doi.org/10.1016/j.addr.2018.08.005.

55. Komarova, N., Panova, O., Titov, A., and Kuznetsov, A. (2022). Aptamers Targeting Cardiac Biomarkers as an Analytical Tool for the Diagnostics of Cardiovascular Diseases: A Review. Biomedicines *10*, 1085. https://doi.org/10.3390/biomedicines10051085.

56. Narayan, C., Lin, L.H., Barros, M.N., Gilbert, T.C., Brown, C.R., Reddin, D., London, B., Chen, Y., Wilson, M.E., Streeter, J., et al. (2024). Identification of In Vivo Internalizing Cardiac-Specific RNA Aptamers. Preprint at bioRxiv. https://doi.org/10.1101/2024.08.13.607054.

57. Philippou, S., Mastroyiannopoulos, N.P., Tomazou, M., Oulas, A., Ackers-Johnson, M., Foo, R.S., Spyrou, G.M., and Phylactou, L.A. (2023). Selective Delivery to Cardiac Muscle Cells Using Cell-Specific Aptamers. Pharmaceuticals *16*, 1264. https://doi.org/10.3390/ph16091264.

58. Rawla, P., Sunkara, T., and Barsouk, A. (2019). Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Przegląd Gastroenterol. *14*, 89–103. https://doi.org/10.5114/pg.2018.81072.

59. Liu, S., He, L., Wu, J., Wu, X., Xie, L., Dai, W., Chen, L., Xie, F., and Liu, Z. (2021). DHX9 contributes to the malignant phenotypes of colorectal cancer via activating NF-kappaB signaling pathway. Cell. Mol. Life Sci. *78*, 8261–8281. https://doi.org/10.1007/s00018-021-04013-3.

60. Xie, J., Wen, M., Zhang, J., Wang, Z., Wang, M., Qiu, Y., Zhao, W., Zhu, F., Yao, M., Rong, Z., et al. (2022). The Roles of RNA Helicases in DNA Damage Repair and Tumorigenesis Reveal Precision Therapeutic Strategies. Cancer Res. *82*, 872–884. https://doi.org/10.1158/0008-5472.CAN-21-2187.

61. Li, F., Ling, X., Chakraborty, S., Fountzilas, C., Wang, J., Jamroze, A., Liu, X., Kalinski, P., and Tang, D.G. (2023). Role of the DEAD-box RNA helicase DDX5 (p68) in cancer DNA repair, immune suppression, cancer metabolic control, virus infection promotion, and human microbiome (microbiota) negative influence. J. Exp. Clin. Cancer Res. *42*, 213. https://doi.org/10.1186/s13046-023-02787-x.

62. Mi, J., Liu, Y., Rabbani, Z.N., Yang, Z., Urban, J.H., Sullenger, B.A., and Clary, B.M. (2010). In vivo selection of tumor-targeting RNA motifs. Nat. Chem. Biol. *6*, 22–24. https://doi.org/10.1038/nchembio.277.

63. Mi, J., Ray, P., Liu, J., Kuan, C.T., Xu, J., Hsu, D., Sullenger, B.A., White, R.R., and Clary, B.M. (2016). In Vivo Selection Against Human Colorectal Cancer Xenografts Identifies an Aptamer That Targets RNA Helicase Protein DHX9. Mol. Ther. Nucleic Acids *5*, e315. https://doi.org/10.1038/mtna.2016.27.

64. Siegel, R.L., Miller, K.D., Wagle, N.S., and Jemal, A. (2023). Cancer statistics, 2023. CA Cancer J. Clin. *73*, 17–48. https://doi.org/10.3322/caac.21763.

65. Civit, L., Theodorou, I., Frey, F., Weber, H., Lingnau, A., Gröber, C., Blank, M., Dambrune, C., Stunden, J., Beyer, M., et al. (2019). Targeting hormone refractory prostate cancer by in vivo selected DNA libraries in an orthotopic xenograft mouse model. Sci. Rep. *9*, 4976. https://doi.org/10.1038/s41598-019-41460-2.

66. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. *68*, 394–424. https://doi.org/10.3322/caac.21492.

67. Wang, H., Zhang, Y., Yang, H., Qin, M., Ding, X., Liu, R., and Jiang, Y. (2018). In Vivo SELEX of an Inhibitory NSCLC-Specific RNA Aptamer from PEGylated RNA Library. Mol. Ther. Nucleic Acids *10*, 187–198. https://doi.org/10.1016/j.omtn.2017.12.003.

68. Barbas, A., Viles, K., Rialon, K., Mi, J., Clary, B., Sullenger, B., and White, R. (2011). In-vivo SELEX Against Pancreatic Cancer Xenografts. J. Surg. Res. *165*, 307. https://doi.org/10.1016/j.jss.2010.11.362.

69. Wong, S.K., Mohamad, N.V., Giaze, T.R., Chin, K.Y., Mohamed, N., and Ima-Nirwana, S. (2019). Prostate Cancer and Bone Metastases: The Underlying Mechanisms. Int. J. Mol. Sci. *20*, 2587. https://doi.org/10.3390/ijms20102587.

70. Xu, H., Wang, W., Liu, X., Huang, W., Zhu, C., Xu, Y., Yang, H., Bai, J., and Geng, D. (2023). Targeting strategies for bone diseases: signaling pathways and clinical studies. Signal Transduct. Targeted Ther. *8*, 202. https://doi.org/10.1038/s41392-023-01467-8.

71. Gisbert-Garzaran, M., Gomez-Cerezo, M.N., and Vallet-Regi, M. (2023). Targeting Agents in Biomaterial-Mediated Bone Regeneration. Int. J. Mol. Sci. *24*, 2007. https://doi.org/10.3390/ijms24032007.

72. Liu, H., Mai, J., Shen, J., Wolfram, J., Li, Z., Zhang, G., Xu, R., Li, Y., Mu, C., Zu, Y., et al. (2018). A Novel DNA Aptamer for Dual Targeting of Polymorphonuclear Myeloid-derived Suppressor Cells and Tumor Cells. Theranostics *8*, 31–44. https://doi.org/10.7150/thno.21342.

73. Nguyen, C.T.O., Hui, F., Charng, J., Velaedan, S., van Koeverden, A.K., Lim, J.K.H., He, Z., Wong, V.H.Y., Vingrys, A.J., Bui, B.V., and Ivarsson, M. (2017). Retinal biomarkers provide "insight" into cortical pharmacology and disease. Pharmacol. Ther. *175*, 151–177. https://doi.org/10.1016/j.pharmthera.2017.02.009.

74. Murawski, A.M., Nieves, J.L., Chattopadhyay, M., Young, M.Y., Szarko, C., Tajalli, H.F., Azad, T., Jean-Jacques, N.B., Simon, A.E., and Kushner, D.B. (2015). Rapid evolution of in vivo-selected sequences and structures replacing 20% of a subviral RNA. Virology *483*, 149–162. https://doi.org/10.1016/j.virol.2015.04.002.

75. Guo, R., Lin, W., Zhang, J., Simon, A.E., and Kushner, D.B. (2009). Structural plasticity and rapid evolution in a viral RNA revealed by in vivo genetic selection. J. Virol. *83*, 927–939. https://doi.org/10.1128/JVI.02060-08.

76. Melchers, W.J.G., Zoll, J., Tessari, M., Bakhmutov, D.V., Gmyl, A.P., Agol, V.I., and Heus, H.A. (2006). A GCUA tetranucleotide loop found in the poliovirus oriL by in vivo SELEX (un)expectedly forms a YNMG-like structure: Extending the YNMG family with GYYA. RNA *12*, 1671–1682. https://doi.org/10.1261/rna.113106.

77. van Bel, N., Das, A.T., and Berkhout, B. (2014). In vivo SELEX of single-stranded domains in the HIV-1 leader RNA. J. Virol. *88*, 1870–1880. https://doi.org/10.1128/JVI.02942-13.

78. Salimi, A., Jang, J.H., and Lee, J.Y. (2024). Leveraging attention-enhanced variational autoencoders: Novel approach for investigating latent space of aptamer sequences. Int. J. Biol. Macromol. *255*, 127884. https://doi.org/10.1016/j.ijbiomac.2023.127884.

79. Iwano, N., Adachi, T., Aoki, K., Nakamura, Y., and Hamada, M. (2022). Generative aptamer discovery using RaptGen. Nat. Comput. Sci. *2*, 378–386. https://doi.org/10.1038/s43588-022-00249-6.

80. Chen, Z., Hu, L., Zhang, B.T., Lu, A., Wang, Y., Yu, Y., and Zhang, G. (2021). Artificial Intelligence in Aptamer-Target Binding Prediction. Int. J. Mol. Sci. *22*, 3605. https://doi.org/10.3390/ijms22073605.