Molecular Therapy
Review

Using aptamers for targeted delivery of RNA therapies

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RNA-based treatments that can silence, introduce, or restore gene expression to target human diseases are emerging as a new class of therapeutics. Despite their potential for use in broad applications, their clinical translation has been hampered by a need for delivery to specific cells and tissues. Cell targeting based on the use of aptamers provides an approach for improving their delivery to the desired sites of action. Aptamers are nucleic acid oligonucleotides with structural conformations that provide a robust capacity for the recognition of cell surface molecules and that can be used for directed targeting. Aptamers can be directly conjugated to therapeutic RNA molecules, in the form of aptamer-oligonucleotide chimeras, or incorporated into nanoparticles used as vehicles for the delivery of these therapeutics. Herein, we discuss the use of aptamers for cell-directed RNA therapies, provide an overview of different types of aptamer-targeting RNA therapeutics, and review examples of their therapeutic applications. Challenges associated with manufacturing and scaling up production, and key considerations for their clinical implementation, are also outlined.

INTRODUCTION TO RNA THERAPIES

RNA-based therapies are an emerging class of disease therapeutics. They comprise diverse RNA-based molecules that include anti-sense oligonucleotides (ASOs), small interfering RNAs (siRNAs), short hairpin RNAs (shRNAs), microRNA (miRNA/miR) mimetics, anti-miRs/miR sponges, and clustered regularly interspaced short palindromic repeats (CRISPR) gene-editing constructs.[1,2] Their therapeutic effects can involve gene silencing, gene replacement, or direct genome editing. Their downstream targets include proteins (e.g., with aptamer-based therapies), small molecules (e.g., with riboswitches), or non-coding RNAs (e.g., with anti-miRs). They can also be used to provide or modify protein-encoding mRNA, as with RNA vaccines.[2] The bulk of RNA therapies currently approved or being evaluated in clinical trials comprise nucleic acid-targeting therapeutics.

RNA therapeutics offer several advantages over treatments based on small molecules or monoclonal antibodies (mAb). Most notably, they can be used to target undruggable proteins or modulate protein expression and moreover can be produced in a cost-efficient manner. Other than those based on CRISPR and shRNA, therapeutics based on RNA are likely to have a superior safety profile compared to those based on DNA as they do not integrate into the host genome. For these reasons, RNA-based therapies are an extremely promising class of therapeutics and are being explored for a wide range of diseases.

For therapeutic use, formulations for RNA-based therapies are needed that retain stability in circulation, avoid degradation and rapid elimination, evade immune surveillance and entrapment by Kupffer cells and macrophages, and facilitate uptake by their cellular targets.[3] The use of RNA for therapeutic applications thus requires consideration of RNA stability and degradation in circulation, target organ uptake and biodistribution, hepato-renal clearance, off-target effects, immunogenic response, and intracellular barriers like endosomal escape.[4] Physicochemical properties of RNA such as their hydrophilic nature, molecular weight and polyanionic charge, and their susceptibility to degradation and elimination are important determinants. The zeta potential and negative charge of RNA molecules impact on cell-targeting capabilities and on renal excretion. For example, while their small size may facilitate renal filtration, the negatively charged glomerular filtration barrier may increase circulation time through electrostatic repulsion. Similarly, negatively charged cell membranes create an electrostatic barrier to uptake of "naked" RNA uptake, leading to a short half-life. Manipulating these properties or mitigating their effects is important to strike a balance between effective cellular targeting and minimizing rapid kidney excretion.[5] Moreover, macrophage release of inflammatory cytokines by RNA molecules has been shown to be intricately linked to their size, shape, stoichiometry, and sequence.[6] Thus, the immunogenicity of RNA-based nanoparticles (NPs) could also be fine-tuned by manipulating their composition.

Cell uptake and entry can be enhanced by delivery approaches with positively charged surfaces to promote electrostatic interactions that can facilitate cell entry and by receptor-mediated endocytosis. RNA-based therapies can be incorporated within polymeric NPs (PNPs) or nucleic acid-based NPs, or packaged into delivery vehicles (e.g., synthetic lipid NPs and liposomes or naturally derived extracellular vesicles and exosomes). Once inside the cell, the RNA

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therapeutic must undergo endosomal escape to elicit the desired biological effect(s). Quite surprisingly, only 1%–2% of endocytosed RNA therapeutics escape into the cytoplasm, and these low escape rates remain a major challenge.[7–10] Some types of cells, such as hepatocytes, central nervous system (CNS) neurons, and skeletal muscle cells, are more permissive to endosomal escape. Lipid NPs (LNPs) incorporating ionizable lipids have been successfully used for intracellular mRNA delivery.[11,12] Ionizable LNPs are neutral while in serum, but they become cationic once inside the endosome, which facilitates endosomal escape.[7,13] However, optimization of their formulations is needed since other components such as polyethylene glycol (PEG) within LNP may hinder the endosomal escape of cargo.[14] Other delivery approaches, such as biologically derived vesicles and PNPs/nucleic acid NPs, similarly require careful design.[15,16] Optimizing RNA delivery thus involves fine-tuning the surface charge of delivery vehicles to balance biodistribution and cellular uptake, considering their pKa for efficient endosomal escape, and striving for selective organ targeting.

The targeted delivery of a therapeutic payload to the desired recipient cells or tissues is essential to maximize the clinical efficacy of an RNA therapeutic. To accomplish this, specific targeting ligands can be directly conjugated to the RNA therapeutic or, alternatively, incorporated onto the surface of nanovesicles or NPs used for delivery of the RNA therapeutic.[17–20] Cell-specific targeting can be achieved using a variety of constructs, such as aptamers, mAbs, antibody fragments, or sugar derivatives. The use of nanovesicles functionalized with targeting aptamers has been effective for targeted delivery to hepatocytes or cancer cells expressing specific proteins such as epithelial cell adhesion molecule (EpCAM).[21,22] Herein, we provide a systematic overview of the emerging applications of aptamers for cell-targeted delivery of RNA therapeutics.

APTAMERS AS TARGETING LIGANDS

Aptamers are short (20–100 nucleotides) single-stranded DNA or RNA nucleic acid sequences. They are synthetic ligands that have emerged as powerful tools for targeted delivery of therapeutics, including RNA-based drugs. Aptamers can spontaneously fold into unique secondary and tertiary structures, forming base-paired stem and unpaired loop structures as well as other structural motifs. Their stable 3D conformation enables molecular recognition and the ability to bind to specific target molecules with high affinity and selectivity by enabling them to distinguish between very slight molecular differences in their targets. The aptamer-protein interactions are dominated by non-covalent interactions including hydrogen bonding, electrostatic interactions, π-π stacking, van der Waals forces, and limited hydrophobic interactions.[23] These interactions, along with their unique 3D structural motifs, provide the basis for high binding affinity to specific protein targets. Indeed, dissociation constant (Kd) values for aptamers may range from high picomolar to nanomolar concentrations.[24]

Aptamers can be used as therapeutics that directly target disease-causing molecules, for example, to inhibit coagulation, elicit antiviral effects against several viruses, or reduce the accumulation of neurotoxic proteins such as α-synuclein and amyloid β in neurodegenerative diseases.[25–29] Moreover, their effects can be reversed by the administration of a complementary antidote aptamer.[30] In addition to use for direct therapeutic applications, their high target specificity permits the use of aptamers as specific targeting ligands for delivery of therapeutics in a cell-specific manner. Their effectiveness for this use has been demonstrated in several preclinical studies (Figure 1). Aptamer-based targeted delivery approaches are particularly attractive for therapeutic RNAs. which may be precluded from passive diffusion through cell membranes due to their size or hydrophilic properties. Thus, RNA therapeutics can be directly conjugated to aptamers, incorporated into nanocarriers, or used as building blocks for more complex delivery systems. Approaches used for fabrication of aptamer-targeted RNA therapeutic delivery systems are outlined in Table S1. The ability of aptamers to recognize specific cell types or tissues makes them ideal for directing RNA therapeutics to desired targets while minimizing off-target effects. This targeted approach could improve the efficacy and safety profile of RNA-based therapies across a wide range of applications.

Due to their small size, low immunogenicity, and ease of chemical modification, aptamers have many advantages over other targeting moieties such as antibodies in terms of safety, toxicity, specificity, biodistribution, and organ accumulation.[33,34] Their small size (5–30 kDa) allows for better tissue penetration and access to sterically hindered protein domains. This size difference also impacts biodistribution. Compared with mAbs against the same protein targets, aptamers show improved circulation times and have superior and more rapid tissue penetration rates.[8,35] They demonstrate low immunogenicity, reducing the risk of adverse immune responses. Furthermore, they can be chemically modified to enhance stability and fine-tune their pharmacokinetic properties. Not only are aptamers unique in their ability to recognize and target non-immunogenic/undruggable proteins but they can function as protein agonists or antagonists or serve as a carrier for the targeted delivery of therapeutic cargo.[27,28,36] Aptamers can be denatured and re-folded multiple times and retain their targeting ability, and thus they exhibit superior thermal stability as compared to mAbs.[37] They are also highly amenable to controlled chemical synthesis with modifications that can be used to improve pharmacokinetics, enhance target binding affinity, increase structural and interactome diversity, and enable their conjugation to therapeutic RNAs and delivery vehicles (Figure 2).[38–40] Furthermore, given the synthetic nature of aptamers, they may be relatively inexpensive to produce at scale.

The selection of aptamers is typically performed through an iterative process called systematic evolution of ligands by exponential enrichment (SELEX). This method allows for the identification of aptamers that bind to specific target proteins with nanomolar to picomolar affinities. Selected aptamers can be further optimized to enhance their binding affinity, specificity, and stability in biological environments. SELEX uses large DNA or RNA libraries composed of random nucleotide sequences ranging in size from 20 to 40 nucleic acids flanked by known primer sequences. The nucleic acid library pool

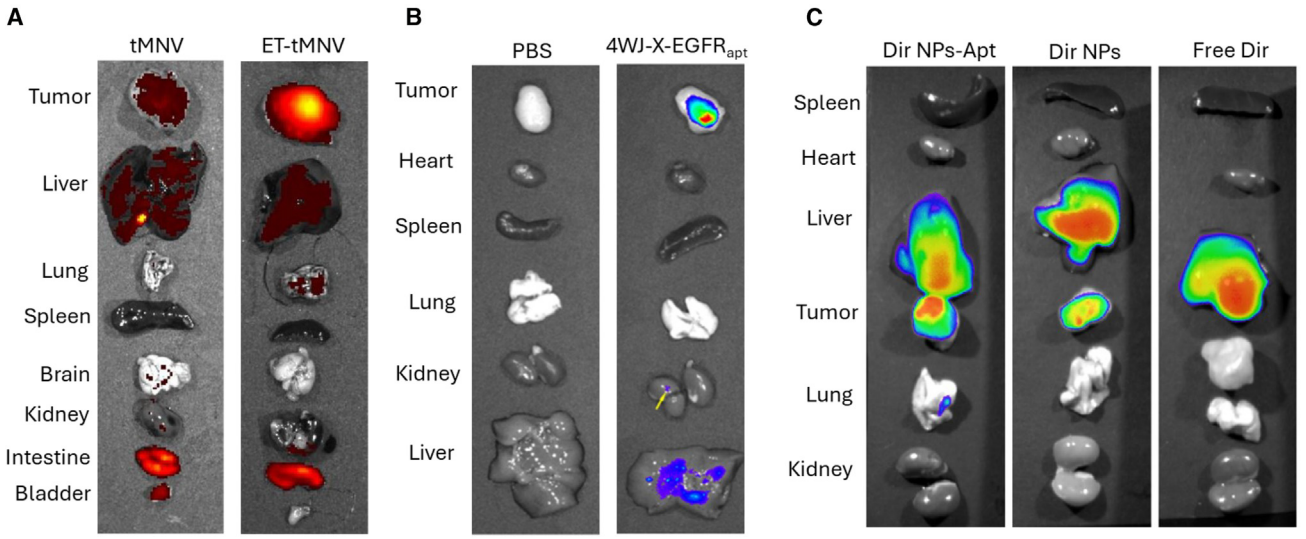

Figure 1. *Ex vivo* imaging of organs to demonstrate tumor-targeting efficacy of aptamer-functionalized therapeutics

(A) 8 h post intravenous (i.v.) injection of DiI-labeled MNV with siRNA (tMNV) or Alexa 647-labeled EpCAM aptamer-functionalized MNV with siRNA (ET-tMNV) in liver cancer stem cell xenografts in mice (adapted from Ishiguro et al.[22] https://doi.org/10.1002/hep4.1462). (B) 8 h post i.v. injection of RNA NPs functionalized with an EGFR aptamer (4WJ-X-EGFR$_{apt}$) in breast cancer xenograft models (adapted from Guo et al.[31] Published 2020 Feb 20. https://doi.org/10.1038/s41467-020-14780-5) (C) 48 h post i.v. injection of shell-core NPs functionalized without (Dir NPs) or with a PSMA aptamer (Dir NPs-Apt) in prostate cancer xenografts in mice (used with permission from: Guo et al.[32] https://doi.org/10.1021/acsami.1c00852. Copyright American Chemical Society).

is incubated with the desired target, such as a recombinant protein of interest, and any unbound aptamers are discarded. The target-bound aptamers are then amplified by PCR and subjected to iterative rounds of selection until some candidates become highly enriched. A combination of positive and negative selection is used to eliminate non-specific candidates. SELEX can be performed with either naturally occurring nucleic acids (A, T, G, C, and U) or synthetic nucleic acids that are used to enhance aptamer structural motif diversity and increase the potential pool of aptamer interactors.[41] Originally aptamers were composed of a limited pool of naturally existing nucleic acids.[23] Proteins that had low isoelectric points, lacked cationic-rich domains, were heavily glycosylated, or lacked distinct conformational motifs were resistant or weakly interacted with highly anionic aptamers composed of these natural nucleic acids.[42] The incorporation of protein-like, synthetic nitrogenous bases into the aptamer design increased the repertoire of potential non-covalent interactions and improved their structural and functional diversity (examples include slow off-rate modified aptamers or clickamers).[43] A drawback of SELEX is that the selection process is lengthy and may yield only a few promising candidate aptamers. For targeting approaches, aptamer screening performed based on the use of cells expressing the target protein (cell-SELEX) or using a modified approach to directly identify aptamers that can be functionally internalized into cells (cell-internalization SELEX) are more likely to identify aptamers that can be used for targeted therapies. Selection can also be performed using *in vivo* SELEX to more closely mimic physiological settings. These approaches are more likely to yield aptamers with targeting efficacy and are preferable to protein-based approaches to identify aptamers for use in targeted therapies.

As a complementary strategy, computational approaches can be used to optimize aptamer design and for aptamer screening. In one such approach, a 3D folded aptamer is docked with a protein of interest and is subjected to molecular dynamic simulations to model aptamer-protein interactions under physiological conditions. These *in silico* simulations can be used to refine aptamer candidates identified through SELEX.[44] Virtual screening of high-affinity aptamers could be used to determine the nucleic acids and their encoded structural motifs that stabilize aptamer-target interactions, retaining these while mutating or eliminating others to iteratively create novel aptamers with desired binding affinities.[45,46] The advent of neural network-based models such as AlphaFold and Atomic Rotationally Equivariant Scorer, or integration with pretrained transformer-based encoders such as AptaTrans, will likely pave the way for future capabilities for computational design of aptamers with high binding affinity to desired protein-binding domains for targeting and therapeutic applications.[47–49]

APTAMER-OLIGONUCLEOTIDE CHIMERAS FOR RNA THERAPEUTICS

Aptamers can be conjugated to therapeutic oligonucleotides and RNA-based constructs such as siRNA, shRNA, anti-miRs, miR mimics, and ASOs to enable their targeted delivery to desired recipient cells.[29,50–53] Examples of reported therapeutic applications of selected aptamer-oligonucleotide (aptamer-oligo) chimeras are outlined in Table 1. Aptamer-oligo chimeras can be generated by co-transcription with a therapeutic RNA construct, where the two are typically separated by a 3′ polycarbon or nucleotide linker (Figure 3). Alternatively, chimerization can be achieved via non-covalent interactions, such as through

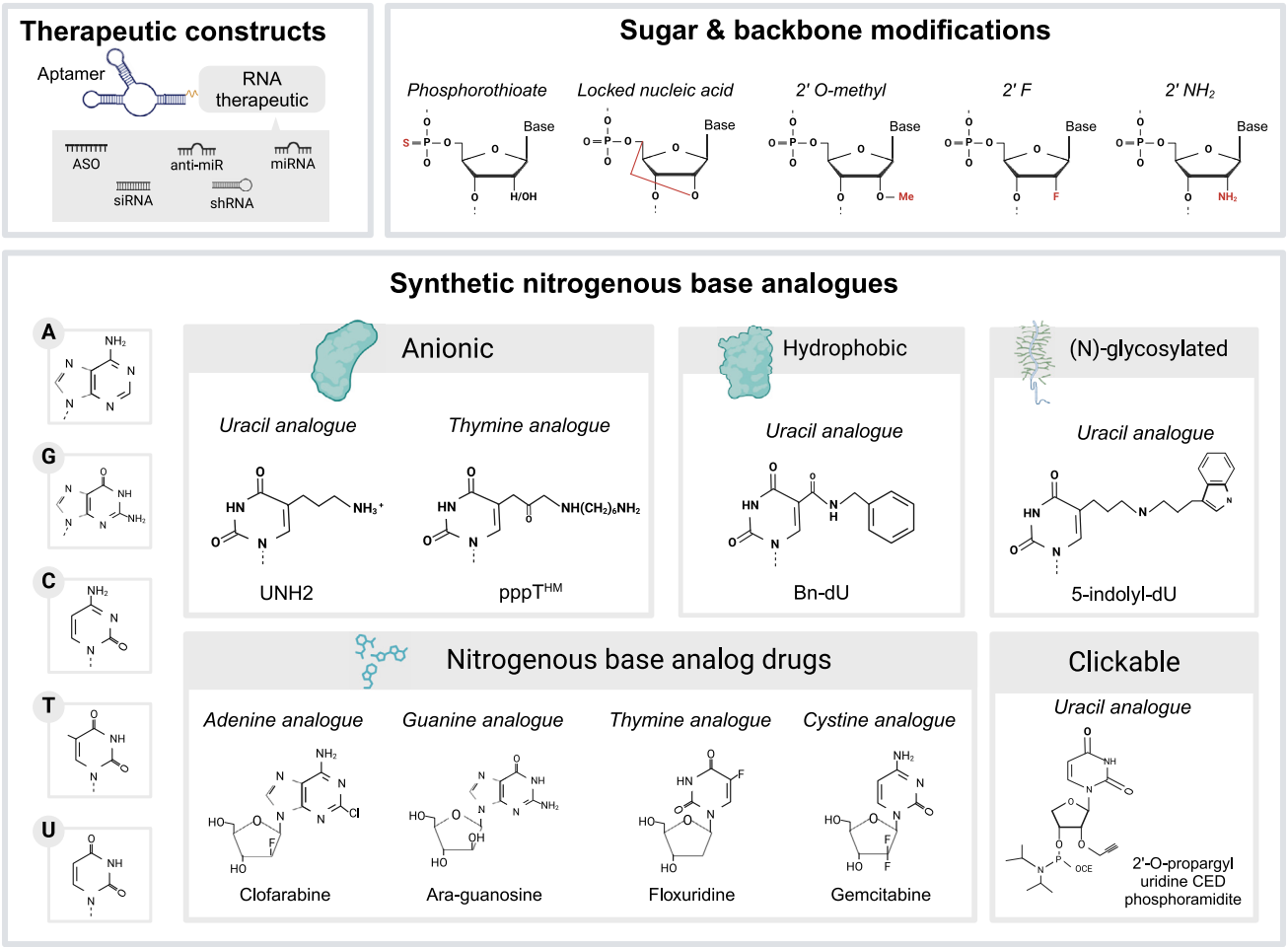

Figure 2. Design considerations

Aptamer-oligo chimeras can be generated by conjugation of aptamers with RNA therapeutic constructs such as anti-sense oligonucleotides (ASOs), siRNA, anti-miRNA (anti-miR), short hairpin RNA (shRNA), or miRNA. Phosphorothioate (DNA/RNA), locked nucleic acid (RNA), 2′O-methyl (RNA), 2′ F (RNA), and 2′ NH_2 (RNA) nucleotide modifications can protect chimeras from serum nuclease-mediated degradation. Incorporation of synthetic nitrogenous base analogs, including drugs, can enable aptamer interactions with anionic, hydrophobic or glycosylated proteins: 5-(3-aminopropyl uridine triphosphate) (UNH_2, 5′ triphosphate of 5-*N*-(6-aminohexyl) carbamoylmethy-2′-deoxyuridine) ($pppT^{HM}$, Bn-dU, benzyl-deoxyuridine (Bn-dU), and 5-indolyl deoxyuridine. Created in BioRender. Driscoll, J. (2025). https://BioRender.com/y93b493.

streptavidin-biotin or "stick-based" methods.[51,77] The latter involves the addition of 3′ polycarbon linkers followed by a complementary 16-base guanine- and cytosine-rich sequence that enables chimerization via Watson-Crick base pairing of the two sequences.[78] Some advantages of non-covalent chimerization over covalent linkages include a lower cost of synthesis and flexibility in the use of both RNA or DNA aptamers and therapeutics.[77,79]

Aptamer-siRNA chimeras

Cell-specific silencing of expression by aptamer-siRNA chimeras (AsiCs) has been demonstrated for several target genes. Such chimeras have been evaluated for extrahepatic delivery of therapeutic siRNA cargo, as intravenously administered nanovesicle-encapsulated formulations preferentially accumulate in the liver.[54] The aptamer and siRNA can be conjugated using covalent or non-covalent linkages. Design approaches have been described that seek to optimize silencing efficacy of the AsiCs generated by co-transcription of the aptamer and one strand of the siRNA.[78] These could be expanded to include non-covalent aptamer-siRNA chimerization.[78] In other AsiC design modifications, addition of a 3′ overhanging di-uridine promoted double-stranded RNA endoribonuclease (DICER) recognition. Introduction of a CΔU mutation to the passenger (sense) strand ensures preferential loading of the guide (anti-sense) strand into the RNA-induced silencing complex. The aptamer can be co-transcribed with the guide strand and subsequently annealed with the passenger stand. Additionally, the hybridized AsiC can be slightly modified to achieve a stem-loop conformation that resembles an endogenous miRNA structure. The identity of the strand conjugated to the aptamer can highly influence the silencing efficiency of the AsiC.[54,78] The thermal stability of the siRNA is also

Table 1. Therapeutic applications of aptamer-oligo chimeras

Chimera type	Aptamer	Target	RNA therapeutic	Therapeutic effect(s)	Reference
Aptamer-siRNA (AsiC)	EpDT3	EpCAM	plk1 siRNA	• selectively targeted EpCAM$^+$ breast cancer cells • promoted tumor regression in subcutaneous EpCAM$^+$ breast cancer tumor-bearing mice	Gilboa-Geffen et al. and Shigdar et al.[54,55]
	AS1411	nucleolin	SMG-1 siRNA	• intratumoral injection of the AsiC greatly reduced tumor burden and increased CD4$^+$ and CD8$^+$ T cell infiltration, while reducing the percentage of infiltrated regulatory T cells in melanoma tumor-bearing mice • systemic combination treatment sensitized immune checkpoint inhibitor refractory murine mammary cancer tumors to CTLA-4 treatment	Meraviglia-Crivelli et al., Bates et al.[56,57]
	PDR3	PDGFRα	STAT3 siRNA	• PDR3 induced cytotoxicity of GBM cells *in vitro*, via suppression of STAT3 and induction of p53-mediated apoptosis • PDR3-STAT3 AsiC treatment reduced STAT3 expression compared to PDR3 aptamer alone	Yoon et al.[58]
	Gint4.T	PDGFRβ	STAT3 siRNA	• AsiC selectively silenced STAT3 expression and reduced migration and viability in PDGFRβ$^+$ tumor cells • intraperitoneal treatment reduced tumor growth and angiogenesis and decreased intratumoral expression of STAT3 and downstream genes	Esposito et al.[59]
	BA	EGFRvIII	cMet siRNA	• BA-cMet AsiC selectively reduced proliferation and induced apoptosis in EGFRvIII$^+$ GBM cells *in vitro*	Zhang et al.[60]
	A-1	HIV-1 GP120 protein	HIV tat/rev site 1 siRNA	• treatment of HIV infected mice sustainably suppressed viral load • AsiC treatment reduced expression of HIV tat/rev in host infected T cells and prevented against virus-induced CD4$^+$ T cell depletion	Neff et al. and Zhau et al.[61,62]
	CD4 clones 9 and 12	CD4	• HIV gag siRNA or vif siRNA • HostCCR5 siRNA	• combination treatment with CD4-gag and CD4-vif AsiCs impaired HIV virus replication in CD4$^+$ T cells • topical application of CD4-gag, CD4-vif and CD4-CCR5 AsiCs prevented vaginal transmission of HIV in mice and reduced p24 antigenemia and viral load • *in situ* treatment of healthy cervicovaginal explant specimens with CD4-CCR5 AsiCs were selectively internalized by CD4$^+$ T cells and macrophages	Wheeler et al., and Davis et al.[63,64]
Aptamer-shRNA	CD30	CD30	RORγt shRNA	• aptamer-shRNA chimera exhibited selective uptake in CD30$^+$ activated T cells • chimera treatment reduced the levels of IL-17 related cytokines in activated human primary CD4$^+$ T cells from healthy donors and those with rheumatoid arthritis	Shi et al., and Mori et al.[65,66]
	A10-3	PSMA	DNAPK siRNA	• aptamer-shRNA chimera treatment sensitized PSMA-expressing prostate cancer cells to radiation-induced cell death • intratumor treatment enhanced the radiosensitivity of PSMA-expressing tumors and stunted tumor growth rate in a subcutaneous prostate cancer xenografts • *ex vivo* treatment reduced the expression of DNAPK in explanted radical prostatectomy tissue specimens	Ni et al.[67]

(*Continued on next page*)

Table 1. *Continued*

Chimera type	Aptamer	Target	RNA therapeutic	Therapeutic effect(s)	Reference
Aptamer-anti-miR	GL21.T	AXL	Anti-miR-222	• chimera treatment reduced miR-222 levels in AXL+ tumor cells and increased expression of miR-222 downstream targets PTPμ and p27 • treatment reduced GBM cell viability and migration, sensitized cells to temozolomide treatment, and reduced tumor volume in GBM subcutaneous tumor-bearing mice	Catuogno et al.[52]
	pRNA-3WJ with CL4	EGFR	Anti-miR-21	• chimera treatment reduced the expression of miR-21 and concomitantly increased the expression of two downstream targets PTEN and PDCD4 in $EGFR^+$ breast cancer cells and in orthotopic breast cancer tumors in mice	Shu et al., and Esposito et al.[68,69]
Aptamer-miR	GL21.T	AXL	Let-7g miRNA	• treatment reduced cell migration and viability of AXL^+ lung cancer cells • intravenous treatment reduced AXL^+ tumor growth and restored the intratumoral let-7g/HMGA2 axis	Esposito et al.[70]
	GL21.T	AXL	miR-34c-3p	• treatment restored miR-34c-3p expression, suppressed migration in NSCLC cells, and reduced proliferation of primary NSCLC AXL^+ cells • treatment sensitized NSCLC cells to erlotinib and reduced colony formation	Russo et al.[53]
	EpDT3	EpCAM	miR-203b-3p	• intraperitoneal treatment with the EpDT3 bivalent chimera reduced metastatic disease and altered the intratumoral miR-203b-3p/CXCL1 axis	Li et al.[71]
	GL21.T and Gint4.T	Axl (GL21.T) PDGFRβ (Gint4.T)	miR-137 mimic and anti-miR10b	• combination treatment of GL21.T-miR137 and Gint4.T-anti10b AsiCs reduced GBM sphere formation and cell migration and downregulated stem cell-associated genes and viability of three patient-derived GBM tumor cell lines	Esposito et al.[72]
	apt69.T	BCMA	miR-137 anti-miR-222	• aptamer chimeras selectively delivered therapeutic RNAs to BCMA-expressing cells • Apt69.T-137 chimera treatment reduced the viability of BCMA+ multiple myeloma B cells and suppressed expression of oncomiR-222 in $BCMA^+$ cells	Catuogno et al.[73]
	c2.min	TfR	pre-miR-126	• TfR-miR126 chimera augmented VEGF-induced angiogenesis and restored the miR-126/VCAM-1 axis in endothelial cells • TfR-miR126 chimera treatment had differential effects on breast cancer cell lines, decreased SK-BR3 cell viability, and reduced MCF7 cell migration	Rohde et al., Wilner et al.[74,75]
Aptamer-ASO	PA9-1	PD-L1	PD-L1 ASO	• pre-treatment of melanoma cells with the chimera potentiated T cell-mediated cytotoxicity • intratumoral injection of the chimera reduced tumor burden and total PD-L1 expression in colorectal syngeneic tumor-bearing mice	Luo et al.[29]
	ST-6-1	SARS-CoV-2 spike protein	SARS-CoV-2 N gene ASO	• the chimera bound to SARS-CoV-2 spike protein and suppressed the N-induced inflammation phenotype in SARS-CoV-2 infected cells • intranasal administration protected Omicron SARS-CoV-2 infected mice, reduced virus loads, and lowered levels of pro-inflammatory cytokines	Yang et al.[76]

AsiC, aptamer-siRNA chimera; ASO, anti-sense oligonucleotide; BCMA, B cell maturation antigen; CCR5 C-C chemokine receptor type 5; CTLA4, cytotoxic t lymphocyte associate protein 4; CXCL1, C-X-C motif chemokine ligand 1; DNAPK, DNA-activated protein kinase catalytic polypeptide; EGFRvIII, epidermal growth factor receptor variant III; EpCAM, epithelial cell adhesion molecule; GBM, glioblastoma; HIV, human immunodeficiency virus; HMGA2, high-mobility group AT-hook 2; miRNA, microRNA; NSCLC, non-small cell lung cancer; PDCD4, programmed cell death protein 4; PDGFRα, platelet-derived growth factor receptor α; PD-L1, programmed death ligand 1; Plk1, polio-like kinase 1; PSMA, prostate-specific membrane antigen; PTEN, phosphatase and tensin homolog; PTPμ, protein tyrosine phosphatase-μ; RORγt, retinoic acid receptor-related orphan receptor gamma t; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; shRNA, short hairpin RNA; siRNA, small interfering RNA; STAT3, signal transducer and activator of transcription 3; SMG-1, suppressor of morphogenesis in genitalia 1; TfR, transferrin receptor; VCAM1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor.

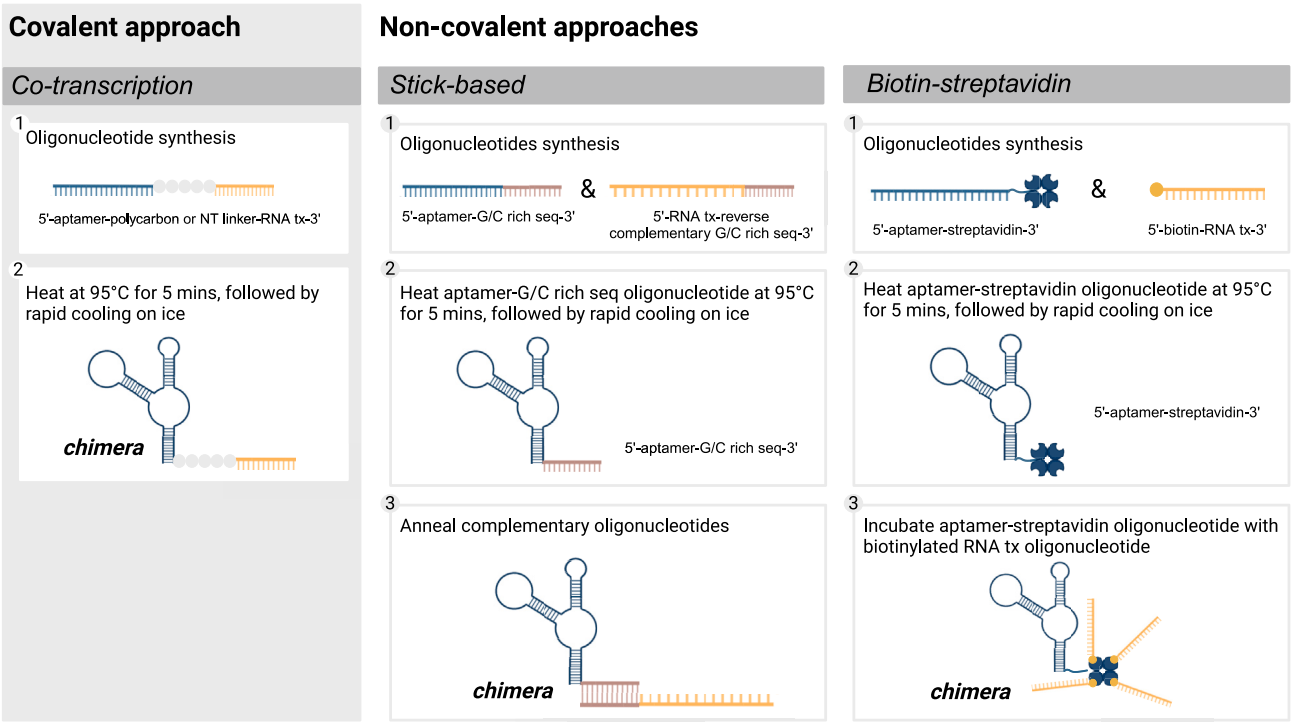

Figure 3. Aptamer-oligo chimerization strategies

Both covalent and non-covalent approaches can be used to link an aptamer to an RNA therapeutic. Linkers may be used for the former. With the stick-based method, individual aptamer and therapeutic strands are designed with a complementary guanine/cytosine (G/C)-rich sequence and are subsequently annealed. Aptamers with a 3′ streptavidin tag can be used to generate a (tetravalent) chimera with biotinylated-RNA therapeutic constructs. For double-stranded RNA (dsRNA) constructs, such as siRNA, miRNA, and anti-miR, either the guide or passenger can be conjugated to the aptamer with the chimera annealed to the other strand. Created in BioRender. Driscoll, J. (2025). https://BioRender.com/a41k699.

important in AsiC design. AsiCs formulated with siRNAs with lower melting temperatures exhibited silencing efficiency comparable to the respective unconjugated siRNA.[80] Thus, the design of AsiCs is highly nuanced, and a thoughtful design approach and testing of several design formulations are required to maximize the silencing efficiency of the AsiC.

AsiCs have shown promise in several preclinical cancer models. An aptamer targeting EpCAM, which is ubiquitously overexpressed in many epithelial solid tumors, was conjugated to an siRNA against Polo-like kinase 1 (Plk1). This AsiC selectively accumulated in distant $EpCAM^+$ breast cancer tumors and promoted tumor regression after subcutaneous administration.[54] AsiCs have also been shown to potentiate the anti-tumor immune response.[56] For example, an AsiC generated with a nucleolin-targeted DNA aptamer, AS1411, was conjugated to an siRNA against suppressor of morphogenesis in genitalia 1 (SMG-1). Intravenous administration of the AS1411-SMG-1 AsiC was safe, did not induce immune activation in healthy mice, and enhanced the antigenicity of immune checkpoint inhibitor refractory tumors, sensitizing these tumors to immune checkpoint blockade.[56]

There is a dearth of studies of therapeutic applications of AsiCs in diseases other than cancer. Achieving biologically meaningful concentrations of therapeutics in the brain remains a major hurdle for the treatment of neurological diseases, as few therapeutic molecules are able to penetrate the blood-brain barrier (BBB) even in the context of a "leaky" barrier in brain cancer and some neurological diseases.[81] Paracellular transport of substances across the BBB is restricted to small, highly lipophilic molecules with molecular weights of 400–500 Da. Thus, aptamers or AsiCs are unlikely to passively traverse the BBB given their typical size of 25–40 nucleotides with molecular weights ranging from ~8,000 to 13,000 Da.[82] However, receptor-mediated transcytosis via binding to the transferrin receptor (TfR) has enabled the accumulation of pharmacologically meaningful concentrations of aptamers/AsiCs in the brain.[83,84] A bivalent TfR-EpCAM-targeting aptamer transcytosed the BBB to bind to $EpCAM^+$ metastatic brain nodules in a model of breast cancer.[84] Thus, the addition of a TfR-targeting aptamer to a therapeutic AsiC could potentially be explored for applications in neuro-oncology or neurodegenerative disease.

Aptamer-shRNA chimeras

shRNAs offer some potential advantages over siRNA constructs, and the use of aptamer-shRNA chimeras is a promising approach to cell-targeted RNA interference (RNAi) therapy. shRNA may have superior silencing efficiency compared with siRNA as their 3D folded

structure closely resembles that of endogenous precursor miRNA, which is a better substrate for the RNAi machinery.[85] The incorporation of shRNA into the host genome, following cellular uptake and nuclear translocation, could in theory provide several cycles of gene silencing and can be passed onto daughter cells.[86] Moreover, aptamer-shRNA chimeras can be synthesized as a single strand using PCR-based methods.[65,67,87] In contrast, AsiCs require post-synthesis annealing to the complementary siRNA strand and may be subject to variations in annealing rates.[65]

The therapeutic potency of aptamer-shRNA chimeras has been reported in several disease models.[65] A CD30-targeting aptamer was co-synthesized with an shRNA against RAR-related orphan receptor gamma t (RORγt) by T7-transcription. The CD30-RORγt chimera was internalized by $CD30^+$ activated T helper 17 (Th17) cells and mediated efficient silencing of RORγt expression, concomitant with a reduction in the expression of Th17 associated cytokines.[65] Another example is a chimera formulated with a prostate-specific membrane antigen (PSMA)-targeting aptamer and shRNA against DNA-activated protein kinase catalytic polypeptide (DNAPK), which enhanced radiosensitivity and extended the survival of LnCaP prostate tumor-bearing mice.[67] Although these examples highlight the promising potential of aptamer-shRNA chimera-based RNAi therapies for diverse indications, research to optimize their safety profile is warranted. Toxicity associated with shRNA-based strategies has been attributed to (1) binding of shRNA to off-target mRNAs with partial seed sequence complementarity and (2) the competition of shRNA with the endogenous miRNA pool for loading onto the RNAi machinery.[88]

Aptamer-miR and aptamer-anti-miR chimeras

Once considered "junk" RNA, miRNAs have gained recognition for their ability to regulate the expression of several independent genes. MicroRNAs possess a 2- to 8-nucleotide "seed" region in the 5′ termini that hybridizes to target mRNAs according to Watson and Crick base pairing. The extent of base pairing and the degree of complementarity ultimately determine the fate of the mRNA target. Extensive and complementary binding between the miRNA and the mRNA leads to degradation of the mRNA target, whereas imperfect binding leads to translational repression.[89] MicroRNAs have also been reported to possess non-canonical functionality, including the ability to activate signaling pathways.[89]

Dysregulation in the expression of endogenous miRNAs can contribute to disease pathogenesis and progression. For example, in the cancer setting, there are reduced levels of tumor-suppressor miRNAs and enhanced levels of oncogenic miRNAs. Aptamer-miRNA chimeras represent an attractive approach to therapeutically restore the endogenous expression of miRNA in targeted cells. Aptamer-miRNA chimeras can be generated using various methods, including synthetic co-transcription, the stick-based method described above, or *in vitro* transcription.[53] While several examples have been published, not all reports specify which miRNA mimic strand—the passenger or guide strand—is physically connected to the aptamer. Since the chimera design can influence the RNAi construct silencing efficiency, such information is critical to reproduce the desired biological effects.[54,78] Furthermore, incorporation of the precursor miRNA, as opposed to the mature miRNA, has been shown to ensure the loading of the correct miRNA strand into the DICER complex.[70]

The therapeutic efficacy of aptamer-miRNA chimeras has been studied in models of non-small cell lung cancer and advanced ovarian cancer.[53,70] A bivalent aptamer-miRNA chimera was synthesized with a miR-203b-3p precursor mimic sandwiched between two EpCAM-targeting aptamers. Intraperitoneal administration of the chimera reduced metastatic nodule size and burden, increased intratumoral miR-203b expression, and concomitantly reduced the expression of the downstream target, C-X-C motif chemokine ligand 1 (CXCL1).[71] Aptamer-miRNA chimeras have also been shown to sensitize cells to small-molecule inhibitors, highlighting the potential for synergy between such chimeras and standard-of-care treatments.[53]

Conversely, aptamer-anti-miR chimeras can elicit therapeutic effects by antagonizing endogenous disease-promoting miRNAs in a cell-targeted manner. Aptamer-anti-miR chimerization can be achieved using a stick-based method to generate linear chimeras or three way-junction (3WJ) chimeras.[52,68] The linear chimera can be designed with multiple copies of the desired anti-miR or with different anti-miR constructs (bivalent).[52] While incorporation of multiple copies of an anti-miR construct does not always translate to augmented silencing efficiency, a bivalent design allows for the repression of multiple miRNAs with concomitant increased expression of several downstream targets.[52] Aptamer-anti-miR chimeras have shown promising anti-tumor effects in preclinical models. A linear chimera was generated with an AXL receptor tyrosine kinase (RTK)-targeting aptamer GL21.T and an anti-miR against miR-222 (GL21.T-antimiR222). GL21.T-anti-miR-222 exhibited its therapeutic effects by reducing RTK activity and suppressing the endogenous expression of miR-222 in glioblastoma (GBM) cells. Intravenously administered GL21.T-antimiR222 was stable in serum and significantly reduced tumor burden in a subcutaneous murine model of GBM.[52] However, further studies in orthotopic GBM models will be required to verify the anti-tumor efficacy and therapeutic utility of this chimera.

Aptamer-ASO chimeras

ASOs are short DNA- or RNA-based sequences that hybridize to complementary mRNA to modulate protein expression. Hybridization of the ASO to the cognate mRNA can cause mRNA degradation or translation arrest or regulate alternative splicing, and the latter can stabilize the expression of the mRNA.[90,91] Aptamer-ASO chimeras are developed by co-transcription with the incorporation of various-sized nucleotide-based linkers.[29,76] The linker length can influence the silencing efficiency of some—but not all—aptamer-ASO chimeras. Thus, structural optimization to identify the optimal linker length while preserving the functional activity of the chimera is needed in designing these constructs.[29,76] The reported chimeras have been designed to adopt a conventional linear configuration or become circularized by T4-mediated ligation of the 5′ terminus of

the aptamer to the 3′ terminus of the ASO. Compared with linear chimeras, circular chimeras exhibited superior and more durable silencing efficiency of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) N gene in recipient angiotensin-converting enzyme 2-expressing HEK293T cells. The enhanced functionality of the circular chimera was in part attributed to their improved stability.[76]

Aptamer-ASO chimeras have also shown promising effects in preclinical studies of cancer.[29] Luo et al. developed a linear aptamer-ASO chimera with a programmed death ligand 1 (PD-L1)-targeting aptamer and an ASO against PD-L1. In a murine MC38 colorectal cancer syngeneic model, treatment with this PD-L1 chimera reduced tumor burden to levels similar to those achieved with anti-PD-L1 antibody treatment.[29] Two major challenges associated with conventional PD-L1 therapies are the neutralizing effects of soluble PD-L1 and the endocytic recycling of PD-L1 to maintain its surface expression on cells. The aptamer-ASO chimera effectively addressed these challenges by stably suppressing total PD-L1 expression in recipient cells and consequently reducing the secreted levels of soluble PD-L1. This study highlights the promising therapeutic potential of aptamer-ASO chimeras.

GENERATION OF APTAMER DISPLAYING NANOVESICLES FOR RNA THERAPEUTIC DELIVERY

Therapeutic RNA molecules can be delivered to target sites enclosed within carrier vesicles such as synthetic lipid nanovesicles or naturally derived vesicles such as extracellular vesicles (EVs), biomimetic nanovesicles, plant- or milk-derived nanovesicles, or bacterial outer membrane vesicles (OMVs). Examples of therapeutic applications of aptamer-functionalized nanovesicles are outlined below in Table 2. Aptamer functionalization of nanovesicles can be accomplished through covalent conjugation of modified aptamers to nanovesicles using carbodiimide or maleimide cross-linking, electrostatic interactions with polyethylenimine (PEI)/polyethylene glycol (PEG)-coated nanovesicles, or incorporation of cholesterol-modified aptamers into the nanovesicle membrane (Figure 4).[92,97,104–107]

APTAMER DISPLAY EXOSOMES AND EVs FOR CELL-SPECIFIC TARGETING

Exosomes and EVs released from cells may enclose proteins, nucleic acids, and lipids derived from their cells of origin, some of which retain intrinsic biological properties. These lipid-bilayer-encapsulated nanovesicles of natural origin can also be used for targeted delivery of therapeutic RNA. These include EVs released from mammalian cells of diverse origins or EV-like nanovesicles from edible plants, milk, and bacteria outer membranes. The physiochemical characteristics of these naturally derived nanovesicles are amenable to cell uptake.[92,109–112] Cell-derived nanovesicles (CDNVs)/biomimetic nanovesicles can also be generated by sequential extrusion of cells through a series of filters.[113,114] These nanovesicles can be loaded with therapeutic RNA molecules by engineering the parental cells, thus enabling the selective enrichment of endogenous or exogenous RNA within vesicles during their biogenesis, or by directly loading the RNA into pre-formed vesicles by transfection or electroporation.[8,22,99,106,110]

Milk-derived nanovesicles (MNVs) have also been shown to be potent targeted delivery vehicles for cancer therapeutics.[92] In an orthotopic model of liver cancer, treatment with EpCAM-targeting aptamer-decorated MNVs loaded with siRNA against PD-L1 markedly reduced tumor burden, enhanced tumor infiltration of $CD8^+$ T cells and effector cells, and decreased intratumoral expression of PD-L1.[92]

Aptamer-functionalized EVs have been successfully used for targeted delivery of RNA therapeutics in models of cancer and immune tolerance. To develop cancer cell-targeted RNAi + chemotherapy combination EV-based therapy, survivin siRNA-gemcitabine (gem) and siRNA-paclitaxel (pac) conjugates were created via synthesis of chemically modified siRNA. Gemcitabine cytidine analogs were incorporated into the sense strand of survivin siRNA, and azide-modified paclitaxel was conjugated to a clickable survivin siRNA. The survivin siRNA, siRNA-gem, or siRNA-pac conjugates were loaded into HEK293T-EVs decorated with a cholesterol-modified three-way-junction (3WJ) RNA harboring a CD44-targeting RNA aptamer. Intravenous administration of these CD44-EVs reduced tumor burden in an orthotopic breast cancer mouse model, with the greatest treatment response observed with CD44-EV carrying the siRNA-gem conjugate. Encapsulation of the drugs within the EVs enabled a reduction in the dose needed to elicit a therapeutic response.[100] Similarly, HEK293T-EVs were loaded with RNA scaffolds functionalized with paclitaxel and miR122 and decorated with a 3WJ RNA harboring the ASPGR1-targeting glycoligand, N-galactosamine (GalNAc). Intravenous administration of GalNAc-EVs stunted tumor growth and reduced the intratumoral expression of two miR122 downstream target genes in liver cancer tumor-bearing mice.[101] Likewise, RNAi cargo-loaded EVs functionalized with aptamers targeting PSMA or EGFR also exhibited potent tumor-suppressive effects in preclinical models of prostate and breast cancer, respectively.[97]

Aptamer-functionalized EVs have also shown promising activities in models of immune tolerance. To generate a therapeutic agent to prevent allograft rejection, mesenchymal stem cell (MSC)-derived EVs were first loaded with miR-148a, miR-148b, and miR-152 as an immunosuppressive cargo and surface functionalized with a dendritic cell (DC)-targeting aptamer-mTOR AsiC. Intravenous pre-treatment with this aptamer-functionalized EV therapeutic prevented allograft rejection in mice.[93] This study highlighted some advantages of EV-based delivery vehicles; most notably, the MSC-EV endogenous cargo contains immunosuppressive effectors that act in concert with the exogenous miRNA pool to potentiate immune tolerance in DCs.

The therapeutic applications of aptamer-targeted EVs warrant further evaluation in other disease indications. Intravenous prophylactic treatment of myelin-targeting aptamer-functionalized MSC-EVs promoted potent immunosuppression and reduced CNS demyelination lesions in an experimental murine model of multiple sclerosis.[95] Aptamer-functionalized EVs derived from MSCs, as well as other cell types, have been loaded with exogenous RNA cargo and effectively induced immune tolerance, mitigated inflammatory bowel disease severity, and reduced tumor growth.[93,97,103] Other biological

Table 2. Aptamer-functionalized biological and synthetic nanovesicles

Nanovesicle	Aptamer	Target	RNA therapeutic	Therapeutic effect(s)	Reference
MNVs	3WJ RNA with EpDT3	EpCAM	β-catenin siRNA	• enhanced tumor accumulation in EpCAM$^+$ liver cancer stem cell orthotopic tumors. Reduced tumor burden and intratumoral expression of β-catenin	Ishiguro et al., and Shigdar et al.[22,55]
MNVs	3WJ RNA with EpDT3	EpCAM	PD-L1 siRNA (siRNA-tMNVs) and cas9/gRNA against PD-L1 (RNP-tMNVs)	• siRNA-tMNVs potentiated T cell and NK cell-mediated anti-tumor cytotoxicity against CCA cells in 2D and 3D *in vitro* models, the effects were less pronounced for RNP-tMNVs • siRNA-tMNVs reduced tumor burden, decreased intratumoral PD-L1 expression, and enhanced CD8+ and NK cell tumor infiltration in orthotopic CCA tumor-bearing mice • siRNA-tMNVs exhibited synergistic effects with gemcitabine treatment *in vivo*	Gondaliya et al., and Shigdar et al.[55,92]
MSCs EVs	SIGN-mTOR AsiC	dendritic cell SIGN	miR-148a, miR-148b, miR-152	• MSC-EVs loaded with anti-inflammatory miRs were coated with PEG/PEI to enable functionalization with SIGN-mTOR AsiC • treatment with MSC-EVs conferred immune tolerance in DC and inhibited DC-mediated T cell activation in allogenic mixed lymphocyte co-culture • systemic treatment with MSC-EVs prevented gross and immune rejection of allogeneic skin grafts and suppressed the infiltration of T cells into the allografts	Li et al., and Hui et al.[93,94]
MSC-EVs	LJM-3064-COOH	murine myelin	intrinsic cargo	• prophylactic treatment with MSC-EVs delayed disease onset and reduced the disease severity in an MS mouse model • prophylactic treatment reduced CNS inflammatory cell infiltration, inhibited demyelination, and decreased circulating Th17 and Th1 helper T cells	Hosseini Shamili et al., and Nastasijevic et al.[95,96]
HEK293T cell EVs	cholesterol-tagged pRNA-3WJ with A9g or CL4 aptamers	A9g: PSMA; CL4: EGFR	survivin siRNA	• systemic treatment with PSMA-EVs stunted tumor growth in subcutaneous prostate tumor-bearing mice • systemic treatment with EGFR-EVs halted tumor growth in orthotopic breast cancer tumor-bearing mice	Pi et al., Esposito et al., and Rockey et al.[69,97,98]
HEK293T cell EVs	AS1411-cholesterol	nucleolin	anti-miR-21	• HEK293T cells were engineered to release EVs enriched with anti-miR-21 in response to blue light exposure. The cholesterol-tagged AS1411 aptamer was incorporated into the EV membrane • treatment reduced the level of oncomiR-21 and increased expression of downstream target, PTEN in leukemia cells	Huang et al.[99]
HEK293T cell EVs	3WJ RNA with CD44 aptamer	CD44	survivin siRNA-drug (gemcitabine or paclitaxel) conjugates	• systemic treatment with CD44-EVs harboring the siRNA-drug conjugates reduced tumor growth in orthotopic breast cancer tumor-bearing mice	Bhullar et al.[100]
HEK293T cell EVs	3WJ RNA with GalNAc	ASGPR1	RNA scaffold conjugated to paclitaxel and miR122	• intravenous treatment with GalNAc-EVs stunted tumor growth in HepG2 liver cancer tumor-bearing mice in a dose-dependent manner and reduced intratumoral expression of downstream miR122 target genes, MDR1 and ADAM10	Ellipilli et al.[101]

(*Continued on next page*)

Table 2. *Continued*

Nanovesicle	Aptamer	Target	RNA therapeutic	Therapeutic effect(s)	Reference
HEK293T cell EVs	3WJ with EGFR aptamer	EGFR	survivin siRNA	• intravenous treatment led to tumor regression in NSCLC tumor-bearing mice and reduced intratumoral expression of survivin	Li et al.[102]
HEK293T cell EVs	dual-targeting aptamer	ZF motifs and target mRNA	IL-10 mRNA	• HEK293T cells were engineered to sort selected target mRNA into EVs via CD9-ZF motif fusion protein and a dual-targeting aptamer that binds to EV ZF motifs and target mRNA, IL-10 • systemic treatment reduced disease activity in experimental murine IBD and rescued IBD-induced colon shortening and inflammation	Zhang et al.[103]
Ginger NVs	folic acid displaying pRNA-3WJ-cholesterol	FR	survivin siRNA	• pRNA-3WJ aptamers were conjugated with folate and cholesterol. The cholesterol tag was used for aptamer functionalization of the ginger EVs • 3WJ-NV-survivin reduced tumor growth and intratumoral expression of survivin in KB cell tumor-bearing mice	Li et al.[104]
Grapefruit NVs	LA1	multidrug resistant (MDR) human colon cancer cells	P-gp siRNA and doxorubicin (dox)	• Dox-loaded grapefruit NVs were coated with PEI and subsequently functionalized with LA1 aptamer and loaded with P-gp siRNA • treatment impaired colony formation of MDR colon cancer cells. Systemic treatment with LA1-NV-Pgp/Dox suppressed tumor growth	Yan et al.[105]
E. coli OMV	AS1411-cholesterol	nucleolin	Pre-mir-126	• AS1411 aptamers were grafted onto the OMV via the incorporation of cholesterol into the OMV membrane • AS1411-OMV-miR-126 accumulated in the tumor tissue in breast cancer tumor-bearing mice, reduced tumor burden and restored the intratumoral expression of miR-126, with reduction in downstream C-X-C chemokine receptor type 4	Cui et al.[106]
human red blood cells (RBC) CDNV	AS1411-cholesterol	nucleolin	P-gp siRNA and dox	• RBC biomimetic vesicles were loaded with cholesterol-tagged P-gp siRNA and dox and functionalized with AS1411-cholesterol aptamer • AS1411-CDNV-Pgp/Dox treatment reduced the endogenous expression of P-gp in MDR breast cancer cells and re-sensitized cells to doxorubicin. Treatment improved the penetration of siRNA and dox into multicellular MDR breast cancer spheroids	Wang et al.[107]
ionizable cationic LNPs	Ch6-thiol	rat and human osteoblasts	Plekho1 siRNA	• Ch6-LNP-Plekho1 promoted bone formation and improved bone mass in ovariectomized rats to levels comparable to the sham group • a single high-dose treatment with Ch6-LNP-Plekho1 induced sustained silencing of endogenous Plekho1 in healthy rats for up to 12 days	Liang et al.[108]

3WJ, three-way junction; ADAM10, A disintegrin and metalloprotease 10; AR, androgen receptor; BIM, B cell lymphoma 2-interacting mediator; CCA, cholangiocarcinoma; DC-SIGN, dendritic cell-specific intercellular adhesion molecule 3 grabbing non-integrin; Dox, doxorubicin; GalNAc, N-acetylgalactosamine; EDC/NHS, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide)/N-hydroxysuccinimide; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; EVs, extracellular vesicles; FR, folate receptor; IBD, inflammatory bowel disease; IL-10, interleukin-10; LNPs, lipid nanoparticles; MDR, multidrug resistant; MMP-9, matrix metalloprotease-9; MS, multiple sclerosis; MNV, milk-derived nanovesicles; MSC, mesenchymal stem cell; MSNPs, mesoporous silica NPs; mTOR, mammalian target of rapamycin; NK, natural killer; NSCLC, non-small cell lung cancer; NVs, nanovesicles; OMVs, outer membrane vesicles; PDCD4, programmed cell death protein 4; PD-L1, programmed death ligand 1; PEG, polyethylene glycol; PEI, polyethylenimine; Plekho1, pleckstrin homology domain-containing family O member 1; PSMA, prostate-specific membrane antigen; P-gp, P-glycoprotein; RBC, red blood cell; VEGF, vascular endothelial growth factor.

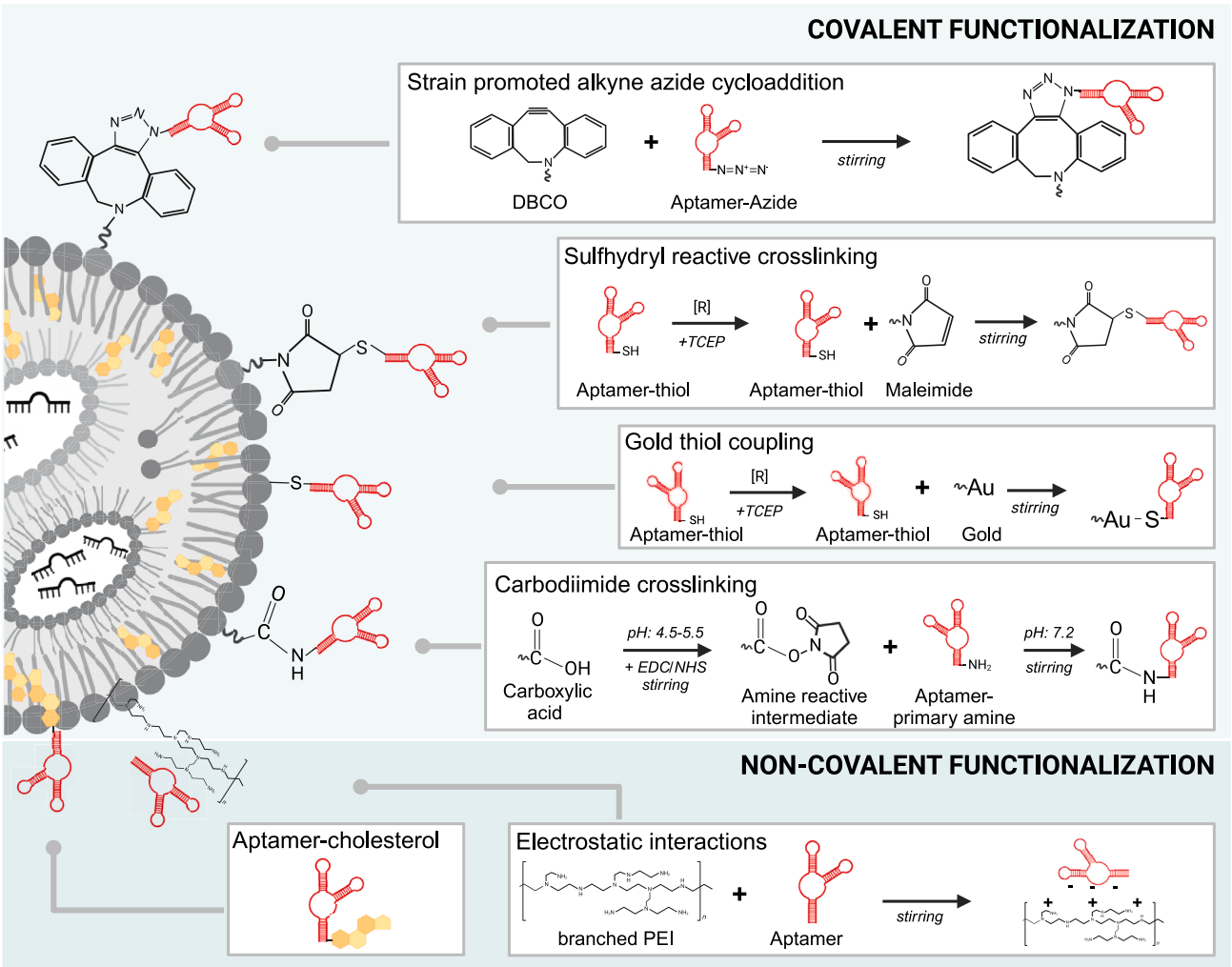

Figure 4. Chemical strategies for aptamer functionalization of nanovesicles and NPs

A variety of chemistries can be used to covalently bind nanovesicles or NPs with aptamers. Non-covalent approaches include the use of cholesterol tagging to enable incorporation into lipid membranes or the use of linear or branched polyethylenimine (PEI), which form electrostatic interactions with aptamers. EDC/NHS, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/*N*-hydroxysuccinimide; TCEP-HCl, Tris(2-carboxyethyl)phosphine hydrochloride; [R], reduction reaction. Created in BioRender. Driscoll, J. (2025). https://BioRender.com/u47d31.

nanocarriers, including edible plant-derived nanovesicles, bacterial OMVs, and biomimetic vesicles, have also served as effective cell-targeted delivery vehicles for RNA therapeutics.[104–107]

APTAMER-FUNCTIONALIZED LIPID NPs FOR RNA DELIVERY

Lipid NPs (LNPs) and liposomes are attractive nanocarriers for the delivery of RNA therapeutics. Like EVs, LNPs offer protection of a therapeutic RNA cargo and can enable targeted delivery to the desired cell(s) of interest. Due to electrostatic interactions, cationic LNPs exhibit superb loading efficiency for RNA-based therapeutic cargoes. They can be synthesized through co-assembly of aqueous phases (containing therapeutic RNA cargo dissolved in acidic buffer) with ethanol phase (containing the lipid mixture), resulting in LNPs with narrow polydispersity and encapsulation of RNA cargo within their lumen. Synthesis can be performed by rapid solvent mixing or by using microfluidic-based mixing, with the latter being preferred for size uniformity and scalability.[115] Several LNP formulations have received US Food and Drug Administration (FDA) approval, and many others are in clinical trials.[116] Their use highlights the ability to successfully address several challenges in delivering RNA cargo, such as enzymatic degradation, inability to penetrate membrane barriers, and off-target effects.[117]

Cationic LNPs are formulated with cationic or ionizable lipids, phospholipids, cholesterol, and PEGylated lipids, and they can be loaded with therapeutic RNA cargoes. Each component has a specific effect in facilitating loading and delivery of an RNA cargo. Cholesterol

provides structural stability and rigidity to the lipid bilayer and enhances LNP integrity. Helper phospholipids can enhance cellular uptake and improve stability, thereby preventing leakage of RNA cargoes. PEGylated lipids can impact LNP size and zeta potential while improving stability and minimizing particle aggregation at physiological pH. They also impact the circulation, reducing LNP clearance by the mononuclear phagocyte system (MPS) and can serve as a scaffold for functionalization with targeting ligands.[118] LNPs that evade detection by the MPS will be destined for the liver or excreted by the kidneys. Cationic lipids can be either permanently positively charged lipids or ionizable lipids with the latter being favored due to their improved serum half-life, superior safety profiles, and their ability to promote endosomal escape of the therapeutic cargo.[118] While ionizable lipids are mostly neutral at physiological pH, they become protonated and gain a positive charge within the low-pH environment inside endosomes, leading to membrane destabilization and endosomal escape of LNPs. Maintaining a neutral pH while in circulation can reduce LNP attachment to negatively charged biological molecules, enhancing biocompatibility and reducing immune clearance and thereby extending circulation time.

Within the circulation, LNPs can become opsonized by plasma proteins—mostly apolipoprotein E (ApoE), which attracts the binding of circulating lipids—and causes hepatic accumulation of LNPs due to the ubiquitous expression of the low-density lipoprotein receptor on hepatocytes.[119] To potentially achieve extrahepatic delivery, LNPs could be formulated with lipids that enhance the formation of a distinct protein corona with tissue-tropic distribution, and LNPs can be functionalized with aptamers to ensure cell-specific delivery of their therapeutic payloads.[119,120] Aptamer functionalization of LNPs can be achieved using a one pot assembly method, in which the structural lipids, helper lipids, and aptamers are combined and assembled into aptamer-decorated LNPs. Drawbacks of this approach are that the aptamer may be improperly oriented toward the LNP lumen, and the conditions required for LNP synthesis may affect the aptamer-LNP conjugation chemistry.[120] Alternatively, with post-insertion methods, the LNPs are first assembled and then subsequently functionalized with the aptamer. This method ensures the correct outward-facing orientation of the aptamer and imparts flexibility into the design, as the targeting ligands can be interchangeable.[120] To functionalize aptamers onto the surface of the LNPs, the aptamers are synthesized with either a cholesterol tag or a functional group. Cholesterol-modified aptamers naturally incorporate into the LNP membrane, but this does not ensure an external facing orientation. Alternatively, aptamers synthesized with a thiol, azide, or primary amine group can be covalently conjugated to LNPs formulated with various PEGylated lipid derivatives possessing functional groups such as maleimide, dibenzocyclooctyne, or 1-ethyl-3-[3 dimethylaminopropyl] carbodiimide hydrochloride (EDC)/N-hydroxysuccinimide (NHS), respectively.[120–124]

Aptamer-functionalized RNA-loaded LNPs and liposomal formulations have shown promising success in several disease models.[108] Kim et al. evaluated cationic liposomes loaded with doxorubicin (Dox) and siRNA to indoleamine 2,3-dioxygenase-1 (IDO1) that were conjugated with anti-CD44 and anti-PD-L1 DNA aptamers seeking to enhance cellular uptake of siRNA through aptamer-mediated endocytosis and to inhibit PD-1/PD-L1 interactions between breast cancer cells and T cells. These targeted liposomes showed increased binding and cellular uptake in MDA-MB-231 and 4T1 breast cancer cells, suppressed IDO1 expression, and led to higher cytotoxicity in cancer cells compared with non-targeted liposomes. Tumor-site accumulation and a reduced size of tumor xenografts in Balb/c mice were noted after systemic administration.[125] Similarly, mRNA-loaded LNPs conjugated with DNA aptamers to PD-L1 were used to deliver phosphatase and tensin homolog (PTEN) mRNA to castration-resistant prostate cancer (CRPC) cells. The aptamer-conjugated LNPs could efficiently and selectively deliver their cargo to PD-L1-expressing CRPC cells and increase PTEN expression compared to non-targeted LNPs, resulting in phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathway inhibition, increased cytotoxicity, and reduction in tumor size.[11]

The use of aptamer-functionalized LNPs has been evaluated for conditions other than cancer. For example, LNPs functionalized with an osteoblast-specific aptamer (CH6) were loaded with either siRNA to NLRP3 or siRNA to pleckstrin homology domain-containing family O member 1 (Plekho1). These CH6-LNPs had high accumulation in the femurs of healthy rats and suppressed target gene (NLRP3 or Plekho1) expression. Compared with non-functionalized LNPs, weekly treatment with Ch6-LNP-siRNAs was more effective in stimulating bone reformation and increased bone mass without observable toxicity despite repeated dosing in a rat model of postmenopausal osteoporosis.[108,126]

APTAMER-FUNCTIONALIZED POLYMERIC NANOPARTICLES FOR RNA DELIVERY

Therapeutic RNA molecules can also be encapsulated into PNPs and selectively delivered to target sites. Examples of therapeutic applications of aptamer-functionalized PNPs are provided in Table 3. Aptamer functionalization of these NPs can be accomplished through carbodiimide or maleimide crosslinking of modified aptamers bearing primary amine or thiol functional groups, respectively.[127,129–132]

Organic polymeric nanoparticles

Given their versatility, PNPs are an attractive alternative delivery vehicle for RNA therapeutics. While both cationic and non-cationic organic polymers exist, the former are more frequently used for the encapsulation of RNA payloads because cationic NPs can naturally self-assemble around anionic RNA molecules.[140] Organic cationic PNPs can be synthesized using PEI, polyamidoamine, or poly DL-lactic-co-glycolic acid, among others. These polymers exhibit superb loading efficiency of RNA molecules and promote endosomal escape of encapsulated RNA via the proton-sponge effect. However, due to their highly cationic nature, these polymers exhibit poor safety profiles *in vivo*. The use of lower-molecular-weight organic polymers, inclusion of biodegradable linkers, and hybridization with more biocompatible materials may reduce the toxicity with a minimal impact on RNA loading and delivery.[141] Biodegradable organic cationic polymers such as chitosan or hyaluronic acid also exhibit

Table 3. Aptamer-functionalized PNPs or nucleic acid-based NPs

PNP	Composition	Aptamer	Target	RNA therapeutic	Therapeutic effect(s)	Citations
Organic PNPs	PLGA	A10-primary amine	PSMA	AR shRNA	A10-PNP-AR eradicated androgen-responsive tumors in subcutaneous prostate cancer tumor-bearing mice and reduced serum PSMA levels and intratumoral expression of AR in orthotopic prostate cancer tumors	Yang et al., and Lupold et al.[127,128]
	PLGA	sTN145-primary amine	TNBCs	PD-L1 siRNA	sTN145-NP-PDL1 reduced endogenous expression of PD-L1 and SNAIL transcription factor in recipient TNBCs	Camorani et al.[129]
	DOTAP/PLGA lipid-polymer hybrid	A6-thiol	Her2 receptor	P-gp siRNA	A6-NP-Pgp reduced endogenous expression of P-gp in recipient MDR breast cancer cells and sensitized them to Dox treatment	Chandra et al.[130]
Inorganic PNPs	Au nanocages formed using MMP-9 cleavable peptides	AS1411-primary amine	nucleolin	VEGF siRNA-thiol and dox	AS1411-Au-VEGF/Dox by inhalation combined with photothermal therapy exhibited the greatest reduction in orthotopic lung adenocarcinoma tumor burden	Yang et al.[131]
	Au iron oxide (Fe_3O_4)	VEGF-notch3 AsiC	VEGF	Notch3 siRNA (AsiC)	VEGF-AuFeO3-NOTCH sensitized MDR ovarian cancer cells to cisplatin treatment	Chen et al., and Ruckman et al.[132,133]
RNA NPs	3WJ RNA	CD133	CD133	Anti-miR21	selectively accumulated in triple negative breast cancer orthotopic tumors. Reduced tumor burden and upregulated intratumor expression of downstream targets, PTEN and PDCD4	Yin et al., and Shigdar et al.[134,135]
	pRNA	CD4	CD4	BIM siRNA	protected $CD4^+$ B cells from IL-3 deprivation-induced cell death	Khaled et al. and Kraus et al.[136,137]
	3WJ RNA	A9g	PSMA	anti-miR21 or anti-miR17	selectively accumulated in tumor tissue of LNCaP tumor-bearing mice, reduced tumor burden, and increased expression of downstream targets, PTEN, and PDCD4	Binzel et al.[138]
	3WJ RNA	EGFR	EGFR	$KRAS^{G12C}$ siRNA	pre-treatment sensitized NSCLC cells to radio- and chemotherapy via suppression of hyperactive KRAS. Intravenous administration reduced tumor growth in NSCLC xenografts	Yang et al.[139]
	3WJ RNA	EGFR	EGFR	anti-miR21	selectively accumulated in tumor tissue in orthotopic triple negative breast cancer tumor-bearing mice, reduced the tumor growth, and increased intratumoral expression of PTEN and PDCD4	Shu et al.[68]

3WJ, three-way junction; AR, androgen receptor; AsiC, aptamer-siRNA chimera; Au, gold; BIM, B cell lymphoma 2-interacting mediator; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; Dox, doxorubicin; EGFR, epidermal growth factor receptor; Her-2, Herceptin-2; IL, interleukin; MDR, multidrug resistant; MMP-9, matrix metalloprotease-9, NSCLC, non-small cell lung cancer; P-gp, P-glycoprotein; PEI, polyethylenimine; PLGA, poly(lactic-co-glycolic) acid; PNPs, polymeric nanoparticles; PD-L1, programmed death ligand 1; PDCD4, programmed cell death protein 4; PSMA, prostate-specific membrane antigen; PTEN, phosphatase and tensin homolog; TNBCs, triple negative breast cancer cells; VEGF, vascular endothelial growth factor.

excellent RNA encapsulation and delivery efficiency with better safety profiles. Moreover, NPs can also be developed with stimuli-responsive polymers that alter their physiochemical properties in response to endogenous or exogenous stimuli. While PNPs can be synthesized by top-down or bottom-up approaches, the latter approach requires the use of harsh solvents and surfactants and is seldom used for preparation of RNA PNP-delivery vehicles.[141] A detailed overview of PNP-synthesis methods is provided by Jiang et al.[141]

Aptamer-functionalized PNPs have shown promising effects in several cancer models.[127,129] Biweekly systemic treatment with PSMA aptamer-functionalized PNPs encapsulated with shRNA against the

androgen receptor (A10-PNP-shRNA) reduced the tumor burden in CRPC and androgen-responsive prostate cancer tumor-bearing mice.[127] These therapeutic approaches provide a safer but efficacious alternative to adeno-associated virus gene therapy. Aptamer-functionalized PNP therapies have also shown promise in preclinical models of GBM.[142–144] Remarkably, systemically administered PEG-polycaprolactone PNPs functionalized with the U87 GBM-targeting aptamer GMT8 could penetrate the BBB and exhibited sustained brain accumulation at 24 h post treatment. GMT8 PNPs loaded with coumarin-6 greatly extended the median overall survival of orthotopic GBM tumor-bearing mice.[144] Although the aptamer targeting NP GBM-related studies reported to date mostly involve the delivery of drugs and small molecules, they provide evidence that these nanocarriers could be used for neurotherapeutic applications.

Inorganic polymeric nanoparticles

PNPs can also be synthesized with inorganic materials such as gold (Au), silver, iron oxide, and silica. These PNPs possess unique physiochemical properties endowing them with multifunctionality. Namely, they are highly effective nucleic acid-delivery vehicles, and they can be utilized as theragnostic agents for imaging and, when loaded with photosensitizer agents, photodynamic therapy.[145] While toxicity related to oxidative stress may be associated with inorganic PNPs, their biocompatibility can be increased by coating the PNPs with antioxidants or PEG.[146] Au PNPs have been extensively studied, as they exhibit uniform size profiles, biocompatibility, and enable facile loading of therapeutic payloads and conjugation to targeting ligands.[131] Therapeutic RNA molecules and aptamers can be incorporated into and onto AuNPs using covalent and non-covalent strategies. Most studies report the use of the former method, which takes advantage of the strong chemical attraction between Au and sulfur. Aptamers and therapeutic RNA constructs modified with a thiol tag are effectively incorporated onto or into Au PNPs and are protected against nuclease-mediated degradation. The conjugated RNA molecules are suspected to be released from the Au PNPs via the abundant cytoplasmic reducing agent, glutathione.[131]

Mesoporous silica NPs (MSNPs) are biodegradable PNPs that exhibit a high surface area, a porous structure, and have good safety profiles in preclinical models.[147] MSNPs possess high cargo-loading efficiency and can be functionalized with targeting constructs as well as photosensitizers, making them excellent multifunctional nucleic acid-delivery vehicles. In a study by Wang et al., MSNPs were passively loaded with miR200c and siRNA against Plk1, coated with a lipid film and a photosensitizer agent (indocyanine green), and functionalized with a tumor-targeting peptide (iRGD) using strain-promoted cycloaddition (iRGD-MSNP-RNA). Upon internalization of the iRGD-MSNP-RNA by MDA-MB-231 cells, the RNA cargo was mostly entrapped in endosomes. Transient exposure to near-infrared light promoted endosomal escape of the MSNP-encapsulated RNA cargo via endosomal rupture induced by light-irradiation-generated reactive oxygen species (ROS). In an orthotopic breast cancer model, the most pronounced anti-tumor effects were observed in mice treated with MSNPs and irradiated at four hours post treatment.[147] This study highlighted the promising potential of MSNPs to serve as both RNA therapeutic-delivery vehicles and photodynamic therapy agents. Other studies have shown that MSNPs can be covalently functionalized with aptamers using carbodiimide EDC/NHS chemistry.[148,149]

RNA NANOPARTICLES INCORPORATING TARGETING APTAMERS

RNA can be used as a scaffold to create NPs with different architectures, sizes, and functional groups.[150] The packaging RNA (pRNA) from the DNA packaging motor from the phi29 bacteriophage has been used to create RNA NPs for selective delivery of therapeutic RNA to cells.[151] One pRNA unit consists of a complementary stem region that truncates into two ends defined by hairpin loops, termed right and left loops.[152] Hand-in-hand intermolecular interactions between the loops of pRNA monomers results in the assembly of pRNA dimers, trimers, tetramers, and hexamers.[153] The 3WJ motif in pRNA can also be used to generate RNA NPs of different geometries, notably triangles, squares, pentagons, nanocages, and dendrimers.[154–156] These RNA NPs exhibit exceptional thermal stability as well as metabolic stability against nucleases when chemically modified to include 2′-fluoro nucleotides.[134] Functional groups, such as aptamers and therapeutic RNA constructs, can be added at the termini of the 3WJ RNA to enable cell-targeted delivery of RNA payloads.[136,157] RNA NPs can be used as a scaffold to enable targeted delivery of therapeutic drugs or for controlled release of therapeutic RNA payloads.[158,159] For example, RNA nanocages assembled with a combination of nuclease-sensitive and -resistant 3WJs enabled the release of the entrapped RNA payload upon exposure to serum.[154]

These RNA NPs are safe and well tolerated. Their size and shape can influence immunogenicity and safety profiles, *in vivo* circulation half-life and biodistribution, and uptake by the liver, spleen, kidneys, or tumor tissues, thus providing an opportunity to design them to enhance desirable properties.[134,155,160,161] Unlike other types of NPs, the stoichiometry of targeting ligands and therapeutic constructs incorporated into RNA NPs is highly controllable.[160] They are immunologically inert with systemic administration unless functionalized with immunostimulatory single-stranded RNA (ssRNA).[6,160] Comprehensive toxicological analyses revealed an absence of toxicity with repeated dosing of high doses of pRNA RNA NPs.[162]

Therapeutic effects have been demonstrated in several preclinical studies, with examples listed in Table 3.[160,162–164] RNA NPs assembled using the pRNA 3WJ motif as a scaffold with a CD133-targeting RNA aptamer and an anti-miR-21 sequence selectively accumulated within orthotopic triple-negative breast cancer tumor xenografts in mice following systemic administration. Repeated dosing reduced tumor burden, increased the intratumoral expression of PTEN and programmed cell death protein 4 (PDCD4), and promoted apoptosis. Notably, non-targeting RNA tetrahedrons were rapidly cleared from circulation.[150]

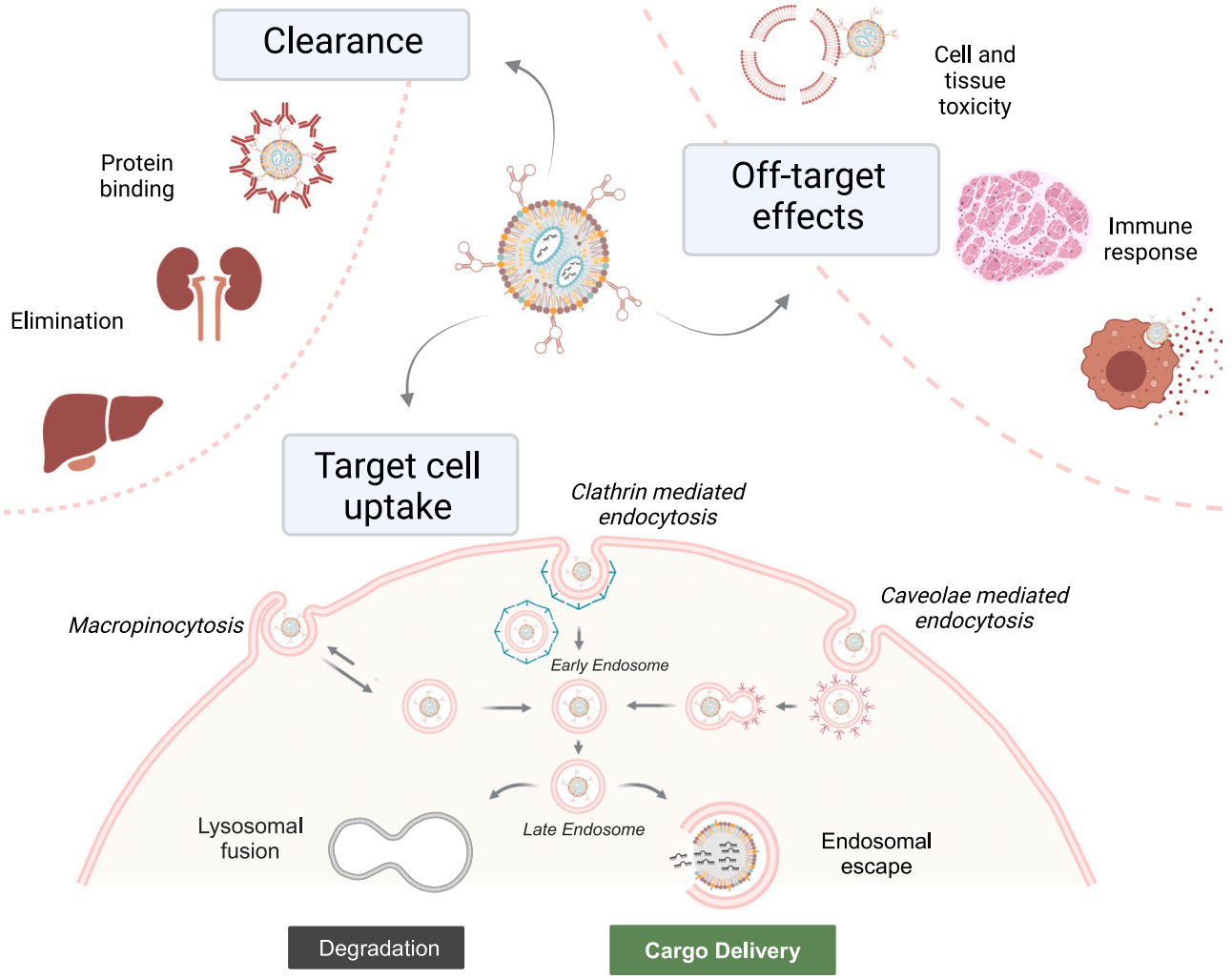

Figure 5. Biological considerations for use of aptamer-targeted RNA therapies

Following systemic administration, clearance can occur by the mononuclear phagocytic system, opsonization by serum proteins, or elimination by the kidneys or liver. Cell uptake can occur via different mechanisms, following which endosomal escape results in delivery of therapeutic cargo to the cytoplasm. Off-target effects on non-target or immune cells or tissues could result in toxicity or immune responses. Created in BioRender. Driscoll, J. (2025). https://BioRender.com/h34i136.

BIOLOGICAL CONSIDERATIONS

Cellular internalization

Aptamer-functionalized materials can be internalized by recipient cells via clathrin-mediated endocytosis, caveolae-mediated endocytosis, or macropinocytosis (Figure 5).[165] The dominant mechanism of endocytosis may vary with aptamers, and multiple mechanisms can simultaneously be involved in aptamer internalization. Moreover, the mechanisms of endocytosis may vary between malignant and non-malignant cells.[165–167]

Routes of cellular internalization have been extensively characterized for the nucleolin-targeting aptamer, AS1411. In Du145 prostate cancer cells, AS1411 is predominantly internalized by macropinocytosis, and its internalization is minimally affected by clathrin- or caveolae-dependent endocytosis inhibitors. Conversely, HS27 non-malignant skin fibroblast cells mainly internalized AS1411 by clathrin- or caveolae-dependent endocytosis. Remarkably, AS1411 was also observed to be predominantly internalized by macropinocytosis by breast cancer cells. Uniquely, internalization promoted hyperstimulation of macropinocytosis in these malignant breast and prostate cancer cells, thus augmenting further internalization of AS1411.[166] However, in a different prostate cancer cell line, PC3 cells, AS1411 was predominantly internalized by clathrin-dependent endocytosis and macropinocytosis.[167] These studies highlight the complexity and cell-type dependency of the internalization mechanisms of aptamer-functionalized materials.

Cytosolic delivery

Endosomal escape remains the major hurdle for the cytoplasmic delivery of therapeutic RNA molecules.[9] Following endocytosis,

the aptamer-functionalized materials (i.e., aptamer-oligo chimeras and aptamer-functionalized nanovesicles/NPs) are trafficked to the early endosome, where they have one of two fates.[165] The material can be recycled back to the plasma membrane or trafficked to the late endosome, which, upon fusion with lysosomes, is degraded. The release (endosomal escape) of the material into the cytoplasm occurs following transient disruption or rupture of the endosomal membrane, which can have negative consequences. While much research has sought to improve the endosomal escape of NP-encapsulated RNA therapeutics, few strategies have been devised to enhance the escape of aptamer-oligo chimeras.[9] LNPs formulated with ionizable lipids can disrupt the endosomal membrane. Within the endosomes' acidic environment, the ionizable lipids become protonated, enabling them to interact with negatively charged phospholipids on the endosomal membrane. The LNPs can then fuse with the endosome and can cause a lamellar to inverted hexagonal phase transition in the endosomal membrane. Both events can cause temporary disruption of the endosomal membrane, followed by release of the intravesicular therapeutic RNA cargo into the cytoplasm. Conversely, PNPs have been shown to promote endosomal membrane rupture. Organic PNPs formulated with organic cationic polymers such as PEI become protonated once inside the endosome, which significantly increases the osmotic pressure and can result in the rupture of the endosomal membrane via a process called the proton sponge effect.[140] Inorganic PNPs coated with photosensitizers can improve the cytoplasmic delivery of therapeutic RNAs by irradiation-induced ROS generation, which also causes endosomal membrane rupture.[168]

Immune system responses

Aptamer-oligo chimeras are regarded to have little to no immunogenicity.[52,78] Treatment of mice with AsiCs or aptamer-anti-miR chimeras did not induce any noticeable immunotoxicity in mice, despite repeated dosing.[52,78] Furthermore, aptamer-oligo chimeras modified with 2′F or 2′O bases do not appear to trigger an intracellular innate immune response. Conversely, immunogenicity concerns with LNPs and PNPs might be resolved by the engraftment of different molecules onto their surface.

The MPS represents a front-line defense for clearing systemic exogenous materials; activation of the MPS results in the release of pro-inflammatory cytokines that can induce inflammation and tissue damage.[145] Therefore, systemically administered therapeutic delivery vectors must evade detection by the cells of the MPS. Cationic materials become rapidly opsonized in the blood and are subsequently cleared from circulation. Strategies to evade detection by the MPS and thus improve their biocompatibility include formulation of LNPs/PNPs with zwitterionic lipids, grafting CD47 onto the surface of the NPs, and coating the NPs with portions of blood cell membranes.[119] Incorporation of PEGylated lipids neutralizes the surface charge of the NPs, thus minimizing their interaction with plasma opsonins.[169] However, it is important to keep in mind that PEGylated materials can be immunogenic in patients who have received prior PEGylated therapies.[170]

Toxicity

Cellular and systemic toxicity has been associated with some—but not all—aptamer-based RNA therapeutics. Most of the reported toxicity has been observed with LNPs and PNPs and is attributed to the method of synthesis used, the formulation of the NPs, or the presence of modifications such as PEG. While cationic LNPs and PNPs have been known to induce toxicity through opsonization of plasma proteins, destabilization of cell membranes, and induction of undesirable immune responses, their biocompatibility can be improved through PEG cloaking and inclusion of biodegradable polymers.[141,171] Inorganic PNPs, such as metal PNPs, have unique physiochemical properties that enable their use for a wide range of therapeutic and diagnostic applications. However, toxicity concerns over their bioretention time, and undesirable immune activation, necessitate that these NPs be coated to improve their colloidal stability, biocompatibility, and bioavailability.[172,173] Only a few of the studies reviewed herein reported on the treatment-induced toxicity of aptamer-targeted RNA therapeutics in preclinical animal models. Remarkably, those that did include toxicity profiling reported no gross toxicity or cytotoxicity despite repeated dosing.[97,108,168] Inclusion of comprehensive toxicity profiling in future *in vivo* studies is critical for the advancement of these therapies to the clinic.

Biodistribution

Off-target tissue distribution is often observed to some degree for aptamer-oligo chimeras and aptamer-functionalized LNPs and PNPs. Systemically administered aptamer-oligo chimeras, due to their small size, tend to accumulate in and are subsequently cleared by the kidneys. However, systemically administered LNPs and PNPs that evade detection by the MPS will accumulate in the liver and/or kidneys. Despite PEG-mediated cloaking, opsonization with plasma proteins on the surface of the NPs can lead to an ApoE-dominant protein corona and hepatic accumulation of circulating NPs.[119] Despite this initial off-target accumulation, ideally over time, the desired tissue to liver and kidney ratio of accumulation of aptamer-functionalized NPs tends to increase. Tissue-tropic biodistribution can be enabled by the synthesis of LNPs using different lipids that attract the formation of a unique enabling protein corona.[119]

PATHWAYS TOWARD CLINICAL APPLICATION

Aptamer-based therapies have promise for the targeted treatment of a host of diseases and conditions. A PEGylated anti-vascular endothelial growth factor (VEGF) aptamer therapeutic, pegaptanib, was approved by the FDA in 2004 as an antagonist to VEGF. While its use was overshadowed by the subsequent availability of more-effective anti-VEGF drugs, several aptamer therapeutics are undergoing evaluation in early-phase trials. In contrast, the use of aptamers as targeting ligands for RNA therapeutics remains underexplored. There are several considerations for advancing clinical implementation of aptamer-directed RNA-based therapies.

For clinical translation of aptamer-oligo chimera therapies, cytoplasmic delivery and pharmacodynamics are barriers that need to be addressed.[36,174] Their susceptibility to nuclease-mediated degradation

and high renal clearance rates lead to low circulation half-lives. Moreover, undesirable immunogenicity, off-target binding, inefficient cellular internalization, and poor endosomal escape rates pose additional limitations.[2] Several strategies have been proposed to address these challenges and provide optimism for the continued development and generation of newer, effective RNA-based therapies. These include incorporation of inter- and/or intra-nucleoside modifications that promote resistance to nuclease activity, or, as with the latter, can mitigate the immunogenicity arising from the stimulation of toll-like receptors.[1,175] Other efforts to extend the circulation half-life seek to limit renal clearance by incorporating phosphorothioate-modified nucleosides or conjugating the chimera to PEG to increase the molecular weight or to lipids to enable the binding of the RNA therapeutic to plasma proteins.[176,177] However, given the potential immunogenicity of PEGylated materials in a small population of patients, it is desirable to minimize such modifications.

Despite the success of the COVID-19 mRNA-LNP vaccines, the successful clinical implementation of LNP-based therapeutics also faces several challenges. Passive targeting can be used to achieve LNP tropism to desired tissue sites. Although aptamer functionalization could potentially promote their cell-specific uptake, LNPs tend to accumulate in the liver following intravenous administration due to the abundance of adsorbed ApoE and plasma lipids on the surface of the LNPs. Thus, effective strategies to enable extrahepatic delivery of systemically administered LNP therapeutics are needed. By altering the composition of their lipid formulation, other serum proteins could preferentially be adsorbed and alter the biodistribution of the LNPs. Changing the route of administration may also be considered. LNPs introduced by intramuscular and intradermal administration tend to accumulate in the lymphoid tissues, whereas nebulized formulations of LNPs accumulate in the lungs.[178] Lastly, undesirable effects of repeated dosing of LNPs such as stimulation of undesirable immune responses and/or accelerated blood clearance of LNPs warrant consideration as they may reduce the therapeutic efficacy.[178]

Aptamer-functionalized PNPs face some of the same challenges as LNPs. While aptamer functionalization can somewhat reduce hepatic accumulation of PNPs, this could be further mitigated by taking advantage of passive targeting strategies as outlined above. Furthermore, while cationic PNPs are the most widely used for the delivery of RNA therapeutics, they have inherent toxicity. The biocompatibility of cationic PNPs can be improved by the incorporation of biodegradable linkers. Moreover, a challenge inherent to all the aptamer therapeutics discussed herein is the delivery of a biologically meaningful concentration of therapeutics in the cytoplasm. Studies have suggested that inclusion of zwitterionic groups that become cationic in the presence of an acidic environment can aid in endosomal escape.[177]

Scalability and manufacturing

Aptamers are easily modifiable and have minimal batch-to-batch variation. Aptamer-oligo chimeras are synthetic nucleic acids, and their production is highly scalable, albeit costly. However, biomanufacturing of other aptamer-directed therapeutics based on EVs, LNPs, or PNPs is more challenging. Production of naturally derived vesicles may require dedicated cell banks and their scale-up can be challenging with batch-to-batch variation and high costs.[179] For LNPs and organic PNPs, the challenges lie in scaling up the production of the NPs while retaining their original properties.[180] Small-scale LNP and organic PNP synthesis methods may involve bulk mixing or microfluidic mixing, with the latter being preferable as it can generate consistently sized NPs that are reproducible. While microfluidic mixing allows for rapid and reproducible production of NPs, they have low throughput. Their adaption for large-scale NP production would require modifications such as the use of chips in parallel, adjustments to flow rates, aqueous to organic ratios, and the architecture of the mixing chips, such as the use of T-junction chips or confined impinging jet mixers. In transitioning from small- to large-scale production of LNPs, ensuring consistency of size, polydispersity, and loading efficiency is essential.[181] With other aptamer-based approaches such as inorganic PNPs, scaling up production is challenged by a need to prevent NP aggregation, minimize batch-to-batch variation, and to develop environmentally friendly synthesis methods and avoid high costs.[182,183] The FDA has emphasized a transition to the use of continuous synthesis-based methods to minimize batch-to-batch variation of organic and inorganic PNPs. However, issues such as reactor fouling can slow down production efficiency and introduce impurities, and therefore there is a need to improve some of the current continuous production methods.[141,182]

Conclusions and future directions

Aptamers have several attractive properties that justify their further development for use in targeted RNA therapeutics. Most notably, aptamers (1) exhibit high binding affinity and specificity for a protein target, (2) can serve as both a targeting ligand and a therapeutic, (3) are modifiable and have scalable production platforms, and (4) can be conjugated to therapeutic oligonucleotides or RNA encapsulated NPs to enable targeted delivery of these therapeutics. Herein we highlight several possible approaches to enhance the desired biological effects of aptamer-targeted RNA therapeutics. One example is the creation of bivalent chimera therapeutics formulated with either multiple copies of the aptamer or with different therapeutic RNA constructs. This strategy can be advantageous by simultaneously targeting different members of a pathway that can ultimately enhance the desired biological effect.[52] Another approach is the use of circularized aptamer-oligo chimeras. These could augment the desired biological effect in comparison to the linear chimera, in part due to nuclease resistance of the circularized chimera.[76] Circularization is a promising alternative to the incorporation of nuclease-resistant modified bases as the latter could, in high numbers, stimulate undesired immune responses. Additionally, functionalization of NPs using a combination of aptamers and other targeting ligands could improve their delivery and uptake at desired tissue sites. For example, functionalization of NPs using both a cancer cell-targeting aptamer and a TfR-targeting ligand could enable their passage across the BBB for delivery to primary brain tumors or metastatic nodules. Continued research and development to improve the pharmacokinetics, safety profile, biodistribution, and

cytoplasmic delivery of these therapeutics is warranted. The clinical applications of aptamer-targeted RNA therapeutics are expected to expand as these issues are addressed and the results of ongoing preclinical studies and early-phase clinical trials become available.

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AUTHOR CONTRIBUTIONS

T.P., conceptualization, supervision, funding acquisition, writing – original draft, writing –review & editing; J.D., writing – original draft, writing – review & editing; P.G., D.A.Z., I.K.Y., R.J., and H.D., writing – reviewing & editing. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

SUPPLEMENTAL INFORMATION

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