Nanoscale

ROYAL SOCIETY OF CHEMISTRY

REVIEW

View Article Online
View Journal | View Issue

Cite this: *Nanoscale*, 2024, **16**, 4392

Spherical nucleic acids: emerging amplifiers for therapeutic nanoplatforms

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Received 24th November 2023,
Accepted 11th January 2024

DOI: 10.1039/d3nr05971e

rsc.li/nanoscale

Gene therapy is a revolutionary treatment approach in the 21st century, offering significant potential for disease prevention and treatment. However, the efficacy of gene delivery is often compromised by the inherent challenges of gene properties and vector-related defects. It is crucial to explore ways to enhance the curative effect of gene drugs and achieve safer, more widespread, and more efficient utilization, which represents a significant challenge in amplification gene therapy advancements. Spherical nucleic acids (SNAs), with their unique physicochemical properties, are considered an innovative solution for scalable gene therapy. This review aims to comprehensively explore the amplifying contributions of SNAs in gene therapy and emphasize the contribution of SNAs to the amplification effect of gene therapy from the aspects of structure, application, and recent clinical translation – an aspect that has been rarely reported or explored thus far. We begin by elucidating the fundamental characteristics and scaling-up properties of SNAs that distinguish them from traditional linear nucleic acids, followed by an analysis of combined therapy treatment strategies, theranostics, and clinical translation amplified by SNAs. We conclude by discussing the challenges of SNAs and provide a prospect on the amplification characteristics. This review seeks to update the current understanding of the use of SNAs in gene therapy amplification and promote further research into their clinical translation and amplification of gene therapy.

1. Introduction

From the discovery of genes in the early 20th century to the approval of gene therapy products today, the application of genes in the treatment of diseases such as cancer,[1–3] genetic disorders,[4,5] and infections[6,7] marks the dawn of a new era in gene therapy. Gene therapy, with gene drugs as a crucial component, is considered the most promising approach for modulating gene expression levels, drawing significant attention from both the academic and clinical communities.[8,9] Gene drugs provide therapeutic effects through (i) reducing gene expression, (ii) supplementing specific genes and (iii) modifying endogenous gene expression, offering fundamental solutions to diseases. However, in practice, gene drugs face challenges related to suboptimal stability and rapid *in vivo* metabolism during circulation. Reports have shown that the half-life of genes is typically less than 7 minutes due to nucleases in the body.[10] Additionally, the negatively charged nature of genes makes it challenging for them to traverse negatively charged phospholipid bilayers.[11]

In response to this challenge, researchers have explored both viral and non-viral vectors for gene delivery, achieving noteworthy advancements.[12] Viral vectors, including adenoviruses, retroviruses, and lentiviruses, facilitate gene transcription by silencing disease-causing genes and integrating targeted gene sequences into the host genome.[13] Although viral vectors demonstrate high transfection efficiency, their safety remains a subject of debate. In contrast, non-viral vectors, including liposome complexes, cationic polymers, and inorganic nanoparticles, are favored for their low immunogenicity, ease of quality control, and scalability, thereby presenting significant benefits.[14,15] This dichotomy has spurred the development of innovative gene delivery vehicles that optimize gene utilization and enhance quantity, content, and functionality. Numerous gene therapeutic drugs are currently undergoing clinical trials.[9,16] However, most of the reported trials, to our knowledge, remain in the early and preliminary clinical stages likely due to the significantly compromised transfection efficiency for clinical treatment. To move toward better clinical translations, it is crucial to develop approaches that can improve the therapeutic efficacy of gene drugs, ensuring safer, more extensive and efficient utilization, which requires sophisticated design and comprehensive con-

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sideration in the construction of advanced gene delivery vectors.

Over the past decade, the unique structure of spherical nucleic acids (SNAs) has been unveiled, leading to the development of SNA-based platforms with medical implications. Since Mirkin first combined DNA oligonucleotides with gold nanoparticles to define SNA structures,[17] SNAs have been widely recognized as novel nanocarriers consisting of densely packed nucleic acid shells and cores. Owing to their unique three-dimensional structure and highly programmable assembly, SNAs exhibit a range of distinctive physicochemical properties, including reduced degradation risk in the presence of nucleases, efficient transfection of various tissues and cells, and good immunogenicity.[18–21] As an innovative nucleic acid delivery system, SNAs possess immense potential as universal transportation nanoplatforms for nucleic acids, drugs, and proteins, with enhanced clinical translation prospects in biomedical applications; therefore, there have been many reviews with a focus on this hot subject of research. For example, Jiang *et al.* presented thorough elucidation of the selection process concerning the core and densely organized DNA shells within SNAs, and further introduced the applications of SNAs in disease diagnosis and treatment, encompassing *in vitro* biosensing, intracellular assessment, gene regulation, drug delivery, and immune modulation. Stegh *et al.* recently summarized the results of the first-in-human clinical trials involving SNAs in the context of solid tumors with an emphasis on the potential of SNAs as innovative gene regulatory and immunostimulatory structures for overcoming drug resistance and immunosuppression in solid tumors. Very recently, our group delineated significant advances in SNA-based precision therapy and immunotherapy for tumors with a focus on the therapeutic insights derived from gene-level precision fluorescence imaging and the utilization of SNAs for meticulous adjuvant and antigen control to achieve optimal immunomodulation.[19,20,22,23] Despite many reviews on SNAs, there is a lack of reviews, to our knowledge, summarizing the amplification effect of SNAs, particularly in the context of clinical translations, which is considered to be the most important and noteworthy part of gene therapy amplification.

This review aims to explore the amplification role of SNAs and to summarize the reported notable examples with in-depth discussion and concluding remarks. Briefly, we first elaborate the essential characteristics and scaling-up advantages of SNAs relative to the traditionally used linear nucleic acids. Next, we showcase the amplification strategies of SNAs in diverse therapeutic modalities by summarizing combinatory treatment approaches, along with discussion on the theranostics of SNAs and the presentation of the latest reported clinical translations. Finally, we make instructive concluding remarks on the existing challenges and future prospects in this rapidly developing field of SNAs, including the current obstacles encountered as well as the corresponding emerging solutions. Last but not least, this review offers unique insights into how the amplification characteristics of SNAs can facilitate reasonable design outcomes. The significant role and contribution of SNAs to gene therapy undoubtedly require a timely updated review with a main aim to summarize the amplification attributes as valuable guidance for future clinical translations and rational advanced design of SNA nanoplatforms (Fig. 1).

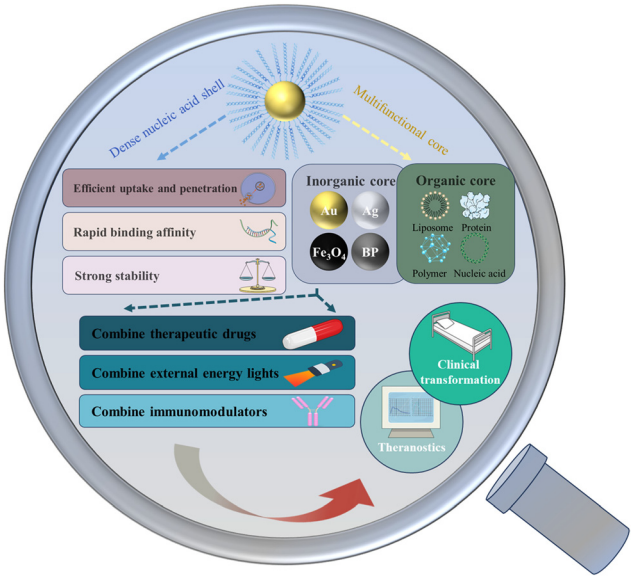

Fig. 1 The amplification process of SNAs in terms of quantity, content, and function in gene delivery. SNAs demonstrate efficient uptake and penetration, rapid binding affinity, and strong stability attributable to the dense nucleic acid shell and the multifunctional core, which are perfectly able to combine a variety of therapeutic options, such as therapeutic drugs, external energy lights, and immunomodulators, increasingly positioning them as valuable tools for theranostics and clinical translations.

2. Amplification of the structure and capacity

The structure of a material determines its properties and leads to further changes in the material functionality. Traditional nucleic acid delivery systems have many drawbacks such as low encapsulation efficiency, poor transfection effects, and strong immune response.[24–26] Moreover, the structure of many nanocarriers only allows nucleic acids to be encapsulated inside,[27,28] which hides the unique advantages of many nucleic acids such as denaturation, renaturation, and hybridization abilities. SNAs overcome the prior structural constraints characteristic of traditional gene therapy. A key feature of SNAs is their densely packed nucleic acid shell, which overcomes the limitations inherent in conventional gene therapy models. This, coupled with a concealed multifunctional core, provides an expansive range of design options, enabling the achievement of enhanced functionality. Consequently, the design and capabilities of SNA structures are closely intertwined. In this section, we will review the unique SNA structure including the dense nucleic acid shell and the multifunctional core, as well as the contribution of structure to the amplification of traditional gene therapy.

2.1. Dense nucleic acid shell

The distinctive structure of SNAs comes from their densely packed nucleic acid shell. This structure not only overcomes the quantity limitations of traditional nucleic acid carriers, but also adds to the charm of SNAs due to their fundamentally unique characteristics compared to linear nucleic acids.[29] In this context, we will provide an overview of the functional amplification achieved through the nucleic acid shell.

2.1.1. Rapid binding affinity. SNAs are renowned for their enhanced binding capacity attributed to the compact nucleic acid shell. They possess the capability of efficiently achieving precise target binding, thereby enhancing the overall rate of target hybridization. This capability is attributed to two main factors: (i) the Watson–Crick base-pairing principle that governs the nucleic acid-specific binding. This principle ensures that nucleic acids can form strong hydrogen bonding with their target sequences for efficient binding;[30,31] (ii) the remarkable one-hundred-fold increase in the binding strength of SNAs compared to that of traditional linear nucleic acids. Overall, the unique structure of SNAs accounts substantially for this significant difference.[32,33] This unique feature makes SNAs exhibit particularly high sensitivity to receptors, enhancing the efficiency of nucleic acid utilization, which provides a new approach for precise and efficient cancer diagnosis or treatment.

For example, Liu successfully developed a low-cost and easily producible integrated SNA by assembling hairpin probes on sea urchin-like DNA nanostructures, which significantly reduces the reaction time compared to traditional chain reaction strategies and enhances the detection sensitivity, therefore facilitating the monitoring of low-abundance tumor-related miRNA in living cells (Fig. 2C).[34] Another innovative sandwich-type electrochemical immunosensor prepared using an SNA-templated silver nanocluster sensing platform and immobilizing the secondary antibody through host–guest recognition demonstrated the application of spherical nucleic acids in the detection of tumor markers, especially the hepatocellular carcinoma marker alpha-fetoprotein.[35] Electrochemical reduction of silver nanoclusters can significantly amplify the immune response signal, which gives the sensor a wide linear range of 0.001 to 100 ng mL^{-1} and a detection limit of 7.74 fg mL^{-1}, which is comparable or even better performance than conventional methods.

The high affinity and ultra-sensitivity of SNAs not only perform well in the field of cancer, but also have a positive impact on other diseases. For example, Li achieved ultra-sensitive detection of low-abundance myocardial infarction-related miRNAs within 30 minutes by using an electrochemiluminescence strategy, which utilized c-SNA enzymes as nanocatalysts.[36] Hu developed a competitive induced fluorescence

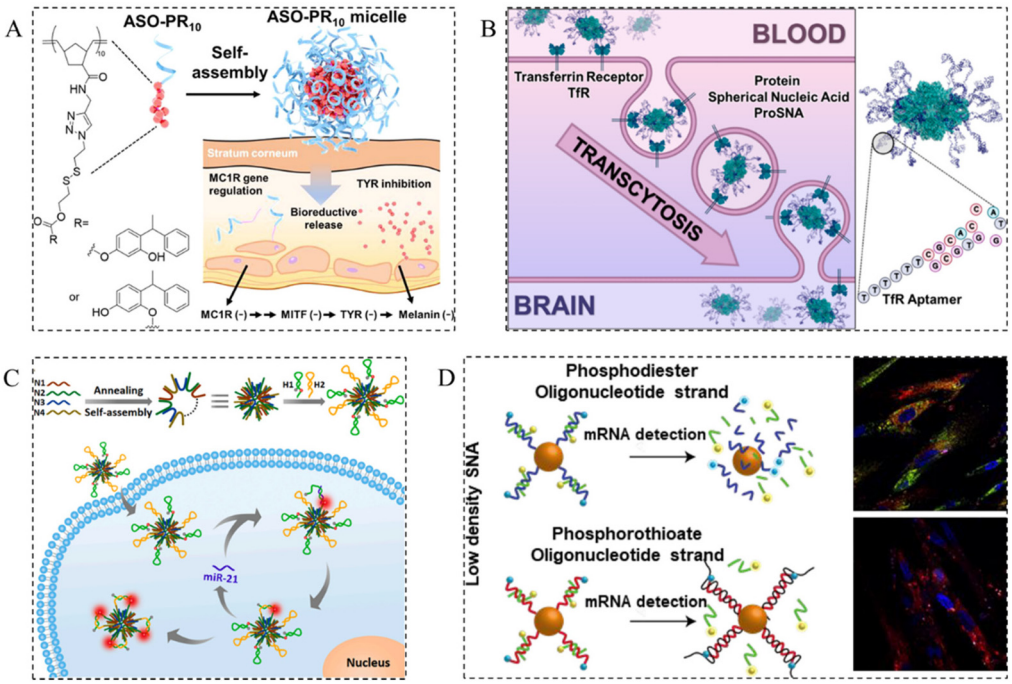

Fig. 2 Typical functional applications of dense nucleic acid shells. (A) SNA containing ASO and tyrosinase inhibitor prodrugs, enhanced skin permeation and reduced melanin content in B16F10 melanoma cells. Reproduced from ref. 46. Copyright 2021 American Chemical Society. (B) Protein SNAs modified with β-galactosidase-conjugated transferrin receptors (TfR), significantly increasing the accumulation in brain tissues and successfully transporting proteins to the brain and central nervous system. Reproduced from ref. 51. Copyright 2022 American Chemical Society. (C) A low-cost and easily producible integrated SNA that significantly reduces the reaction time and enhances the detection sensitivity, therefore facilitating the monitoring of low-abundance tumor-related miRNA in living cells. Reproduced from ref. 34. Copyright 2023 Elsevier B.V. (D) A study conducted a comprehensive analysis on the stability of nucleic acids in spherical nucleic structures, establishing a correlation between the enzyme-mediated DNA degradation and the oligonucleotide density on nanoparticles. Reproduced from ref. 59 Copyright 2022 American Chemical Society.

detection method using sRNA to efficiently identify drinking water standards.[37] This highly sensitive detection capability and simplified process contribute to real-time field and other monitoring tasks.

In a word, rapid binding affinity is an essential breakthrough for an ultra-trace or time-consuming study, and SNAs can increase the productivity and efficiency as a powerful tool for potential clinical translation.

2.1.2. Efficient uptake and penetration. Traditional *in vivo* gene delivery must overcome degradation before cell membrane spanning.[11] Due to the unique three-dimensional structure with a densely packed nucleic acid shell, SNAs could be endocytosed into cells *via* scavenger receptor A.[38] Choi *et al.* engineered miR-146a-functionalized superparamagnetic iron oxide nanoparticles (miR-146a-SPION), a type of spherical nucleic acid (SNA) nanostructure, designed to selectively target scavenger receptor class A (SR-A) on macrophages and endothelial cells (ECs) within atherosclerotic plaques *in vivo*. Despite the high expression level of this receptor on the membranes of macrophages, only approximately 1% of the injected dose of miR-146a-SPIONs accumulated in the aorta. Additionally, Mirkin *et al.* demonstrated that a protein corona formed on the surface of SNAs in the bloodstream further diminishes nonspecific macrophage clearance without significantly affecting the accessibility of the oligonucleotide shell. These studies demonstrate that such high expression in macrophages does not lead to substantial clearance of SNAs by these immune cells,[39,40] which thus promotes the efficient uptake of SNAs by cells without the aid of any transfection agents. This makes SNAs exhibit an enhanced ability to penetrate biological barriers such as the blood–brain barrier and the skin barrier, paving the way for innovative design in localized therapeutic strategies.

Local administration is favored as one of the optimal therapeutic methods due to its convenience and reduced systemic side effects.[41,42] However, one of the major challenges in dermatological therapy has long been low skin permeability due to the sebum membrane and stratum corneum constituting a skin barrier to keep out foreign substances.[43,44] SNA structures exhibit higher penetration capabilities than traditional linear nucleic acids without the need for disruption or transfection agents. For example, Paller's lipid-based SNAs can locally deliver to the epidermis and inhibit the expression of IL-17A to realize successful psoriasis treatment without any transfection agents.[45] Zhang developed a bifunctional oligonucleotide SNA containing antisense oligonucleotides (ASOs) and tyrosinase inhibitor prodrugs, which compared with a single nucleic acid or vehicle enhanced skin permeation and reduced the melanin content in B16F10 melanoma cells (Fig. 2A).[46] Song developed hyaluronic acid-based SNAs for the transdermal delivery of the chemotherapeutic drug doxorubicin (Dox) and metalloproteinase inhibitor 1, which overcame the challenge beyond skin permeability and promoted apoptosis in hypertrophic scar cells.[47]

The blood–brain barrier is a highly regulated barrier that restricts the passive entry of exogenous substances into the central nervous system,[48,49] which has been a bottleneck for brain administration for a long time. SNAs can penetrate the brain and offer a potential new approach to addressing this challenge. For instance, Stegh engineered a gold nanoparticle core with a shell of radially oriented siRNA oligonucleotide nanocomplex capable of crossing the blood–brain barrier for silencing the tumor MGMT protein and achieving glioblastoma treatment.[50] More importantly, higher active targeting can be achieved through the strategy of SNA modification. Mirkin synthesized protein SNAs modified with β-galactosidase-conjugated transferrin receptors (TfRs), significantly increasing the accumulation in brain tissue and successfully transporting proteins to the brain and central nervous system (Fig. 2B).[51] Shi co-encoded the caspase-3 antisense oligonucleotide and a transferrin receptor aptamer into a circle template and developed SNA structures for the treatment of ischemic stroke, which demonstrated a 6.4-fold enhancement in blood–brain barrier penetration capability.[52]

In short, the efficient uptake and penetration of SNAs make them more extensively employed compared to traditional nucleic acid carriers. This unique property of SNAs enhances the prospects for clinical applications.

2.1.3. Strong stability. DNA enzymes play a crucial role *in vivo*, cleaving exogenous DNA to restrain inflammatory responses and maintain homeostasis.[53] Preventing nucleic acids from enzymatic degradation during delivery poses a significant challenge in the field of nucleic acid therapeutics. To address the stability concerns of nucleic acid drugs, researchers have employed various strategies, such as electrostatic adsorption onto carrier systems, core–shell encapsulation, and liposomal nanoparticle protection.[54–56] These methods have become the mainstream approaches for the construction of nucleic acid nanocarriers. However, SNAs significantly reduce the rate and specificity of nucleic acid enzyme cleavage due to their special structure. Several factors contribute to this enhanced stability, such as (i) steric hindrance: the densely packed DNA layer in spherical nucleic structures exhibits steric hindrance to nucleic acid enzymes, restricting their activity and reducing nucleic acid degradation rates; (ii) high salt concentration: the peripheral region of SNA structures creates a localized high salt environment, which inhibits the activity of nucleic acid enzymes, thereby slowing down the degradation of nucleic acids.

The stability of SNAs has been extensively investigated and applied, with recent studies achieving significant breakthroughs. Finn's research team developed a novel class of SNA structures by grafting oligonucleotides onto virus-like particles through copper-catalyzed click chemistry.[57] The virus-SNAs possessed robust cellular uptake capabilities and resistance to nucleic acid enzymatic degradation, with their stability being approximately tenfold that of unmodified virus-like particles. Mirkin quantified the enhanced stability of SNAs in enzyme-catalyzed DNA hydrolysis and provided evidence that the negatively charged surface of nanoparticles and the resulting high local salt concentration are the underlying reasons for stability enhancement.[58] Additionally, Kanaras's research team conducted a comprehensive analysis of nucleic acid stability in

spherical nucleic structures (Fig. 2D).[59] They correlated enzyme-mediated DNA degradation with the oligonucleotide density on nanoparticles, revealing that unmodified SNAs are prone to DNA enzyme degradation at low oligonucleotide density levels. In contrast, phosphorothioate-modified oligonucleotides, such as PS-SNAs, demonstrate enhanced resistance to enzyme degradation.

These findings underscore the critical effect of oligonucleotide density on the stability of SNAs, with SNAs retaining their integrity even in a complex environment, offering an indispensable and important index in future clinical translation.

2.2. Multifunctional core

In the SNA structure, the choice of the core in SNAs is diverse due to their distinct characteristics, different functions, and various purposes. In this context, we will divide cores into organic and inorganic and examine the disparate contributions of these two groups of nanomaterial cores.

2.2.1. Inorganic nanomaterial core. Gold, silver, iron, and other elements are commonly used in the design of inorganic nanocarriers due to their high stability and controllable structure. The method of using gold nanoparticles to prepare SNAs is well established and free from major drawbacks. They are both the earliest and most widely employed inorganic nanomaterial. In 1996, Mirkin and colleagues pioneered the construction of SNAs by linking thiolated DNA to 13 nm gold nanoparticles, marking the inception of this field.[17] Since then, methods for preparing SNAs with gold nanoparticles have matured and found extensive applications in targeted drug delivery, diagnostic probes, biosensors, and combination therapies. A typical example is Cui's gold nano-spherical nucleic system, which enabled on-demand release and reversible assembly in response to near-infrared light, thereby effectively modulating catabolic proteases and anabolic components in cartilage over an extended duration of time.[60] This strategy safeguarded chondrocytes from degenerative changes and impedes the progression of osteoarthritis. The exceptional stability and precise control exhibited by this platform establish a solid foundation for localized gene delivery while introducing novel design principles for photothermal gene therapy. Liu carefully designed SNAs based on the programmability and multifunctionality of DNA and gold nanoclusters, which exhibited highly sensitive and specific analysis of flap endonuclease 1 (FEN1). This structure not only observed FEN1 activity in live cells and tumor-bearing mice, but also achieved spatiotemporal control of drug delivery, offering a promising platform for cancer-specific therapy (Fig. 3A).[61]

In addition to gold nanoparticles, silver nanoparticles are also valuable choices as an inorganic nanomaterial for SNA construction. Silver nanoparticles can disrupt bacterial membranes and subcellular structures through various mechanisms, introducing antimicrobial properties into SNA structures.[62,63] The introduction of PS- and PO-Ag-SNAs by Gryaznov represented a novel antibiotic design strategy suitable for Gram-positive and Gram-negative bacteria and demonstrates the unique advantages of SNA structures in enhancing therapeutic efficacy and stability (Fig. 3B).[64]

Various inorganic nanoparticles have been widely employed in the preparation of SNAs. The utilization of diverse elements such as Fe, P, Pt, Al, Pd, Cu, Co, In, Ni, and their mixtures enables the preparation of a wider range of SNA types, conferring them with increasingly specialized functions. Iron oxide nanocrystals (Fe_3O_4 nanoparticles) exhibit magnetism and are extensively used in magnetic structures. In Liu's work, the synthesis of Fe_3O_4@PDA core–shell NPs offered a novel approach for DNA extraction by using magnetic separation and hybridizing complementary nucleic acids, while demonstrating its potential applications in bioanalytical chemistry.[65] What is more, they extended the choice of the core to silicon dioxide and tungsten disulfide materials. Black phosphorus (BP) nanosheets can serve as initiators for photothermal and photodynamic therapies. Wang developed a SNA constructed using BP nanosheets and porphyrin zinc metal–organic framework nanosheets to achieve photothermal photodynamic therapeutic effects on tumors (Fig. 3C).[66] SNAs prepared with these inorganic nanomaterials not only possess high biocompatibility but also demonstrate feasibility in biofunctionalization.

2.2.2. Organic nanomaterial core. After entering cells, oligonucleotide segments in SNAs are typically degraded and removed following their function, while the nanocore is retained. Inorganic nanomaterial cores may present potential long-term toxicity due to poor degradability or toxic substances are easily dissociated.[67,68] With this in mind, numerous organic nanomaterial cores offer biocompatibility and degradability, demonstrating their wider applications than inorganic nanomaterials in the field of biomedicine.

Liposomal nanoparticles exhibit excellent biocompatibility.[69] For example, Mirkin constructed functionalized liposomal SNAs (L-SNAs) from an FDA-approved 1,2-dioleyl-SN-glycerol-3-phospholipid choline lipid monomer.[70] This structure not only preserved the robust internalization capability and stability of metal SNAs, but also promoted broader tissue distribution and longer *in vivo* circulation times through the characteristics of liposomes, offering novel avenues for therapy (Fig. 3D).[71] Furthermore, the structural advantages of L-SNAs were reflected in their delivery efficiency, as evidenced by a significant increase in their distribution across various tissues and circulation within 30 minutes and 24 hours post-injection, highlighting their superior delivery performance compared to linear DNA. Additionally, Mirkin's research successfully reduced the toxicity of siRNA by constructing L-SNAs while retaining their gene-regulatory capacity.[72] Compared to gold-core siRNA-SNAs, these novel siRNA-SNAs, resembling hairpins, exhibited enhanced biocompatibility and greater efficacy in cellular uptake and gene silencing due to the L-SNA structures. This offers a new design and synthesis pathway for therapies based on L-SNA structures, expanding their potential applications in the treatment of various diseases, including cancer.

In biology, proteins are the primary executors of cellular functions, and thus regulating proteins can directly control

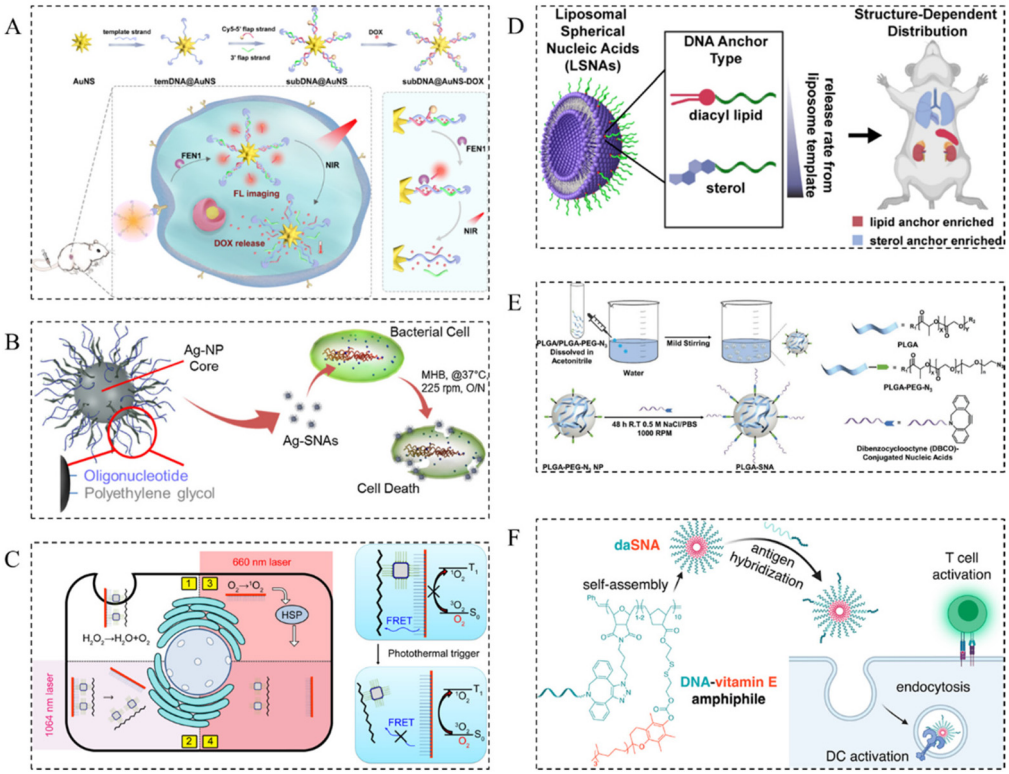

Fig. 3 Some ingenious examples of SNA with different functional cores in practical applications. (A) A carefully designed SNA based on the programmability and multifunctionality of DNA and gold nanoclusters, which achieved spatiotemporal control of drug delivery. Reproduced from ref. 61. Copyright 2021 American Chemical Society. (B) The introduction of PS- and PO-Ag-SNAs represented a novel antibiotic design strategy suitable for Gram-positive and Gram-negative bacteria. Reproduced from ref. 64. Copyright 2023 Elsevier B.V. (C) A SNA constructed from BP nanosheets and porphyrin zinc metal–organic framework nanosheets to achieve photothermal photodynamic therapeutic effects on tumors. Reproduced from ref. 66. Copyright 1999–2023 John Wiley & Sons, Inc. (D) A functionalized L-SNA was constructed from an FDA-approved 1,2 dioleyl-SN-glycerol-3-phospholipid choline lipid monomer, which promoted broader tissue distribution and longer *in vivo* circulation times through the characteristics of liposomes. Reproduced from ref. 71. Copyright 2020 American Chemical Society. (E) PLGA nanoparticles employed to build SNA structures to achieve controllable drug release, which not only enabled independent modulation of drug release kinetics, but also preserved nucleic acid stability on nanoparticle surfaces. Reproduced from ref. 78. Copyright 1999–2023 John Wiley & Sons, Inc. (F) A carrier-free anticancer SNA vaccine developed by utilizing a CpG-rich oligonucleotide and VE as the core, with the VE component serving as the hydrophobic driving force for SNA formation. Reproduced from ref. 82. Copyright 2022 American Chemical Society.

intracellular physiological activities.[73] The introduction of protein cores can expand the functionality of SNAs while SNAs enhance protein stability through their outer shell. Mirkin attached oligonucleotides to the surface of a large homotetrameric enzyme (β-galactosidase), establishing a new pathway for oligonucleotides to be internalized by cells and to perform protein functions.[74] Another work by Mirkin involved designing SNAs with Cas9 as the core, enabling direct execution of Cas9 functions.[75] This design not only demonstrated the direct application of SNAs in gene therapy, but also expanded the potential of SNAs in cellular function manipulation through efficient cellular internalization, endosomal escape, and the nuclear delivery of Cas9 protein SNA. This lays the foundation for the development of protein SNAs as a novel biologically active material, allowing the adjustment of DNA shells and protein cores to amplify functionality.

Using polymer macromolecules as cores to construct SNAs offers a broader space for modification and enhanced loading capacity for controlled drug release.[76,77] Mirkin employed PLGA nanoparticles to build SNA structures to achieve controllable drug release (Fig. 3E).[78] This strategy not only enabled independent modulation of drug release kinetics, but also preserved nucleic acid stability on nanoparticle surfaces, expanding the application scope of SNAs in therapeutic strategies. Zhang constructed a polymer SNA structure with the SNA shell containing antisense oligonucleotides targeting MC1R and prodrug inhibitors of tyrosinase, which successfully reduced melanin production in melanoma cells.[46] Zheng developed a polymer SNA with antisense oligonucleotides and an amphiphilic self-assembling polymer as the core,[79] which efficiently loaded nanoprobes and Dox. After treatment, the quantitative average fluorescence intensity was calculated to be 1.51-fold higher than that of nontumoral sites. Meanwhile, the tumor growth rate in the SNA-treated group was 0.15-fold that of the PBS-treated group.

On the other hand, carrier-free nanomedicines have been extensively studied due to their high biocompatibility, high drug loading, simple drug components, and simplified syn-

thesis procedures.[80,81] Constructing SNAs with the drug itself as the carrier or using nucleic acids as the main component are current directions in carrier-free nanomedicine research. Zhang demonstrated the potential of a carrier-free anticancer vaccine using a CpG-rich oligonucleotide and vitamin E (VE) as the core. Not only does the VE component serve as the hydrophobic driving force for SNA formation, but also the released VE can also work synergically with CpG DNA to amplify immune responses (Fig. 3F).[82] Shi's research demonstrated that the circular template enables the co-encoding of caspase-3 ASO and the TfR aptamer, resulting in the formation of a spherical nucleic acid nanostructure through rolling circle replication. This carrier-free architecture not only simplified the construction, but also mitigated the risk of uncontrolled immune system activation due to the reduced metabolism of natural nucleic acid components.[52]

In summary, the structure of SNAs with either an inorganic nanomaterial core, an organic nanomaterial core, or a carrier-free nanomedicine core reflects a suitable and valuable application requirement, which broadens the prospect of spherical nucleic acids for various therapeutic applications and breakthroughs in gene therapy to clinical translation.

3. Scale-up of therapeutic modalities

SNAs have the potential to function as a multi-modal therapeutic platform apart from their conventional roles such as gene expression suppression, targeted gene supplementation, and intra-genetic modification. In this context, we will summarize the existing neo-modal therapeutic structures of SNAs.

3.1. SNAs with therapeutic drugs

The stability and high configurational flexibility of SNA structures position them as an innovative drug delivery platform, attracting significant attention in the field of combined cancer therapy. SNA structures prevent the strong toxicity and poor targeting of Dox,[83–85] endowing them with unique cellular uptake capabilities and controlled drug delivery. Liang developed pH-responsive Dox–SNA conjugates, facilitating the delivery of Dox to cancer cells and pH-dependent drug release (Fig. 4A).[86] A carrier-free core–shell nanoparticle designed by Liu with a central core and Dox radiating around it, which forms a dual adjuvant SNA,[87] underwent enzymatic degradation in the tumor microenvironment due to matrix metalloproteinase 9 (MMP-9) peptides, resulting in enhanced direct cytotoxicity of Dox against tumor cells.

The poor water solubility and low bioavailability limit the clinical application of paclitaxel (PTX).[88] By binding to the SNA shell, the therapeutic efficacy of PTX is enhanced due to its high stability and cellular uptake. Zhang's amphiphilic DNA–PTX coupling exhibited higher stability against nuclease and faster cellular uptake (100 times) than free DNA, resulting in nearly identical cytotoxicity as a free drug.[89] Moreover, Zhang used benzyl bromide-modified PTX grafted on the DNA skeleton at the PS modification site and loaded into SNA-like micelle nanoparticles with PTX, which realized a high PTX loading rate (≈53%).[90] Subsequently, Zhang used a fluorescent disulfide imine as a linker and combined two PTX molecules with an antisense oligonucleotide integrated with a fluorouracil. This conjugate successfully improved the stability of PTX and effectively inhibited the *p*-glycoprotein expression, leading to the release of FdU and PTX, resulting in a synergistic antitumor effect (Fig. 4B).[91]

Moreover, numerous drugs including coumarin,[78] rapamycin,[92] camptothecin,[93] BKM120 (a lymphocytic leukemia anticancer drug),[94] benzyl 3,4-dihydroxyphenylethylamine (a tyrosine kinase inhibitor),[46] and AS1411 (an aptamer targeting nucleolin)[95] have demonstrated enhanced uptake and stability when delivered *via* SNAs. This targeted drug delivery at the site of the lesion has allowed for the maximization of their therapeutic effects. These investigations highlight the diverse applications of SNAs in combined drug therapy, offering various potential advantages and amplifying the therapeutic efficacy of drugs, thereby accelerating their widespread adoption in the field of medicine.

3.2. SNAs with external energy lights

External energy-based therapies have emerged as promising approaches in contemporary disease treatment. SNAs, leveraging their high specificity and the advantage of shallow skin penetration, offer new prospects for enhancing the therapeutic efficacy and minimizing side effects, paving the way for the future of precision medicine and cancer treatment.

Photothermal therapy is considered one of the most promising cancer treatment methods in recent years, relying on the conversion of light into heat for therapeutic effects.[96,97] The structure of SNAs offers enhanced functionality and heightened targeting capabilities for photothermal therapy. For instance, Wang engineered a "sandwich" SNA structure composed of BP nanosheets and zinc porphyrin metal–organic framework nanosheets, which can finely tune the phase-transition temperature by altering the strand length and the proportion of the nucleic acid and base, achieving photothermal-triggered photodynamic therapy under near-infrared laser irradiation.[66] Zhao developed programmable self-assembling pSNAs that activated local *in situ* photothermal effects upon miRNA-21 expression in the MCF-7 tumor and reduced the side effects of non-specific damage by controllable targeted release capability (Fig. 4C).[98] Li developed AuNS-ASO to achieve the synergistic ablation of tumor cells through gene therapy and photothermal effects under near-infrared laser irradiation. Remarkably, the AuNS-ASO enables precise *in situ* delineation of tumor margins with exceptional spatial resolution (<100 μm) in mice tumors, thereby offering intraoperative guidance for optimal tumor resection.[99]

Photodynamic therapy involves localized irradiation using light of a specific wavelength on applied photosensitizers, generating cytotoxic reactive oxygen species to achieve therapeutic objectives.[100] For example, Zhang prepared PSNAs that generated reactive oxygen species under near-infrared light exposure, leading to the release of siRNA and pASO and

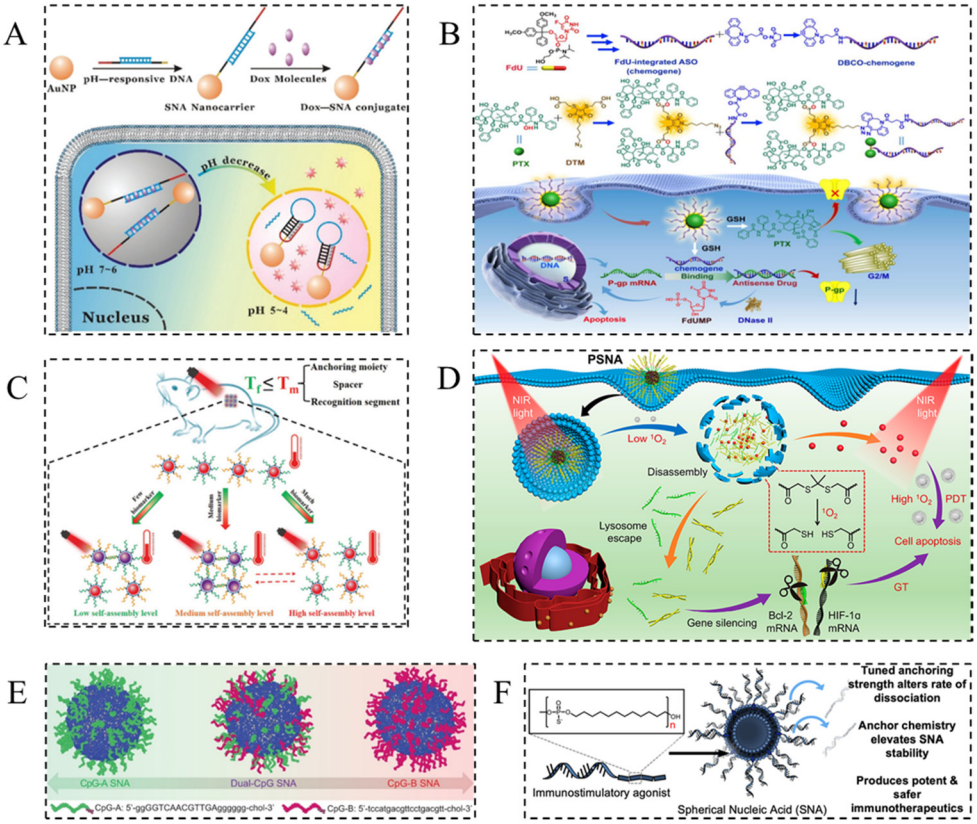

Fig. 4 Some representative cases of the application of spherical nucleic acid multimodal therapy. (A) A pH-responsive Dox–SNA conjugate facilitated the delivery of Dox to cancer cells and pH-dependent drug release. Reproduced from ref. 86. Copyright 2023 Royal Society of Chemistry. (B) A SNA conjugate combined with two PTX molecules and an antisense oligonucleotide integrated with fluorouracil, which successfully improved the stability of PTX and effectively achieved a synergistic anti-tumor effect. Reproduced from ref. 91. Copyright 1999–2023 John Wiley & Sons, Inc. (C) pSNAs activated local *in situ* photothermal effects and reduced side effects of non-specific damage by controllable targeted release capability. Reproduced from ref. 98. Copyright 2023 Royal Society of Chemistry. (D) PSNAs generated reactive oxygen species under near-infrared light exposure, leading to the release of siRNA and pASO and achieving combined photodynamic and gene therapy effects. Reproduced from ref. 101. Copyright 2021 American Chemical Society. (E) A SNA constructed from two CpG immunostimulatory oligonucleotides, termed double CpG-SNA, which enhanced dendritic cell maturation and facilitated a localized immune response against distant tumor cells. Reproduced from ref. 115. Copyright 2020 American Chemical Society. (F) A hydrophobic dodecyl anchored group of liposome SNAs systematically studied, which was found to achieve a strong T cell response after encapsulating its OVA antigen peptide. Reproduced from ref. 119. Copyright 2023 American Chemical Society.

achieving combined photodynamic and gene therapy effects (Fig. 4D).[101] Jiang developed a PSNA nanoplatform for targeted drug release and high-performance cancer treatment, selectively interacting with endogenous ATP to release Dox VE and Ce6 for chemotherapy and photodynamic therapy in synergy.[95]

Radiotherapy is a crucial method for cancer treatment. However, the challenges of normal tissue intolerance and suboptimal treatment effectiveness have driven significant interest in combination therapies.[102,103] Zhou reported a drug delivery strategy that combines radiotherapy with SNA nano-drugs.[104] The SNA structure played a dual role in enhancing radiotherapy and acting as an immunomodulator. The combination of radiotherapy with αPD-L1 exhibited potent anti-tumor effects, completely suppressing tumor growth. Bai developed SNAs that were conjugated with PD-L1 aptamers and indocyanine green (ICG) embedded in mesoporous hafnium oxide nanoparticle cores (Hf@ICG-Apt).[105] Upon irradiation at the tumor site, the nano-system formed a high tumor-to-background ratio (7.97 ± 0.76 fold) and effectively enhanced radiotherapy to combat cancer.

3.3. SNAs with immunomodulators

Immunotherapy has emerged as a pivotal approach in treatment, achieving remarkable success in recent years and attracting significant attention alongside surgery, chemotherapy, and radiation therapy.[106,107] Immunotherapy primarily operates through two key mechanisms: (i) the activation of innate or adaptive immunity and (ii) the modulation of immune responses through antagonizing excessive immune activation. The modular structure and distinctive orientation affinity of SNAs offer a promising avenue for amplifying the positive contributions of immune stimulation and modulation.

Toll-like receptors (TLRs) in endolysosomes play a pivotal role in triggering innate immune responses and promoting

anti-tumor immunity.[108] Reported nucleic acid immune-sensing receptors include TLR3,[109] TLR7/8,[110] and TLR9,[111] which can perceive various nucleic acids from viruses or bacteria, facilitating immune stimulation within the organism. Zhang developed a spherical nucleic acid constructed from CpG polycaprolactones and anti-STAT3 siRNA for synergistic tumor immunotherapy.[112] Mirkin engineered thermo-responsive micelle-based SNAs containing stimulatory TLR-9 CpG sequences, exhibiting superior stability and rapid intracellular uptake, thereby serving as effective TLR-9 immunomodulators.[113] Mirkin subsequently further developed SNAs comprising RNA with TLR-7/8 selectivity. By anchoring cholesterol-terminated oligonucleotides to the lipid core of LSNA, he significantly enhanced cellular uptake and achieved a higher specificity of TLR receptor activation.[114] Moreover, Mirkin developed an SNA constructed from two CpG immunostimulatory oligonucleotides, termed double CpG-SNA, which enhanced dendritic cell maturation (Fig. 4E).[115] This advancement is significant due to the ability of a small quantity of this nanomedicine to induce immune system activation. It mitigates off-target toxicity associated with high doses of conventional nano-chemotherapy drugs and facilitates a localized immune response against distant tumor cells, thereby demonstrating the promising potential of SNAs for immune activation.

In addition, several structural designs have co-functionalized antigen peptides with oligonucleotide adjuvants, resulting in the creation of SNAs that activate APC and tumor-targeted T cells. Zhang developed an SNA-based anti-cancer vaccine, which is comprised of phosphodiesterase oligonucleotides and VE. This formulation was designed to deliver OVA antigen, thereby enhancing TLR9 activation and effectively stimulating adaptive immunity.[82] Liu designed a liposome SNA containing Dox and CpG response peptide coupling, which can enhance the activation of dendritic cells and promote the amplification of $CD8^+$ and $CD4^+$ T cells.[116] Subsequently, Liu constructed a spherical nucleic acid using CpG-ODN and monophosphoryl lipid A double adjuvants, and Dox was radially surrounded as the shell, which can enhance ICD-induced immune responses, achieving synergistic therapeutic effects of chemotherapy and immunotherapy.[87] Mirkin *et al.* enhanced T cell responses by hybridizing antigen peptides onto SNAs.[117] They investigated the immunomodulatory activity of liposome spherical nucleic acids by exchanging liposome components and systematically studied the hydrophobic dodecyl anchored group of liposome SNAs, which was found to achieve a strong T-cell response after encapsulating its OVA antigen peptide (Fig. 4F).[118,119] In addition, they also developed STING-activated spherical nucleic acids, which can effectively activate the cGAS-STING pathway to generate innate immune responses.[120] The development of SNAs that can activate the innate or adaptive immune system and elicit a potent anti-cancer immune response has demonstrated remarkable efficacy.

Innate immunity is one of the weapons in the body's immune defense system, but excessive innate immune responses can lead to autoimmune inflammation.[121,122] In such cases, immune modulation is necessary to reduce excessive autoimmune responses. Psoriasis is a T-cell-mediated skin disease with autoimmune characteristics. Tavoosidana designed a hybrid peptide SNA nanoparticle capable of preventing the excessive gene expression and functional activity of T cells, thereby improving psoriatic skin damage.[123] Paller locally administered L-SNAs targeting the IL-17 receptor gene, successfully reversing the progression of psoriasis.[45] Cui prepared SNAs by regulating the IL-1β mRNA expression in arthritis. They achieved this by downregulating catabolic enzymes in cartilage and upregulating the synthetic part, protecting chondrocytes from degenerative changes and halting the ongoing progression of arthritis.[60]

3.4. SNAs with theranostics

SNAs, with their impressive functionality and unique advantages, have emerged as a cutting-edge technology for integrated cancer treatment and diagnosis. Li's conjugates can simultaneously deliver chemotherapy drugs, contrast agents, or tumor-targeting antibodies, offering a straightforward and innovative pathway toward clinical trials and integrated diagnostics and treatment. Jiang engineered a dual-legged DNA nanowalker, achieving, for the first time, direct intracellular gene excision and repair with fluorescent activation imaging.[124] This innovative technology has been successfully applied in live cells for activity imaging of APE1, associated with BER1. The dual-legged DNA nanowalker system generated through APE1 cleavage achieves high sensitivity detection and imaging of APE1 activity. This study not only furnished novel tools for live cell imaging, but also held promise for the application of dual-legged DNA nanowalkers in the discovery of low-abundance biomarkers and biomedical usage. Bai constructed SNAs by embedding the PDL1 receptor and ICG into mesoporous hafnium oxide, achieving a high tumor-to-background fluorescence ratio upon irradiation at the tumor site.[105] By designing a degradable mesoporous hafnium-based adapter-modified SNA nanosystem for sensitized diagnosis of PD-L1-overexpressing tumors through NIR-II imaging and radiotherapy, the team achieved remarkable anti-tumor effects. This made reliable detection and localization of PDL1 expression possible and facilitated cancer treatment through potent radiotherapy. Zheng engineered an endogenous APE1 enzyme-activated SNA nanosensor for spatiotemporal signal amplification molecular imaging and combined cancer therapy.[79] This intelligent Ep-SNA@Dox nanosensor, containing DNA hairpins with AP sites, ASOs, and Dox, can be specifically cleaved by APE1 in tumor cells, enabling high-sensitivity imaging of mRNA and drug release. *In vitro* and *in vivo* experiments demonstrated that this nanosensor not only possesses high-sensitivity imaging capability for target molecules but also enables precise drug release, offering a new approach to combined therapy.

In conclusion, these studies demonstrate significant breakthroughs in the realm of integrated cancer therapy and diagnosis using SNAs. They not only provide novel directions for

cancer treatment but also lay the foundation for the translation of SNAs into practical tools in clinical practice.

4. Enlargement of clinical translation

Currently, the clinical translation of drugs and carriers requires meticulous consideration of various factors such as precision treatment modalities, real-time monitoring of drug delivery, and the assessment of biocompatibility and safety.[125,126] Research studies into SNAs have revealed their potential to amplify the outcomes of clinical treatment. Day *et al.* provided a comprehensive overview of the distinctive structures and inherent properties of SNAs and a discussion of how these properties enable SNAs' applications.[23] Notably, this paper outlined ongoing initiatives aimed at translating SNAs into clinical practice and offered expert insights into the remaining challenges that need to be addressed in the dynamic pathway toward clinical translations. However, with the rapid development of SNAs in biomedical field, it is necessary to update and summarize the recent clinical translational examples to promote an in-depth understanding of SNAs from a fresh perspective. In light of this, we aim to summarize the latest breakthroughs and contributions of SNAs in clinical therapy.

With in-depth research and successful preliminary preclinical experiments on SNAs, biotechnology companies are driving the clinical translation of SNAs. The unique capabilities of SNAs, particularly their ability to penetrate various tissues and cell types, make them potent tools for treating a wide range of diseases. Furthermore, in current clinical trials, certain SNAs have demonstrated promising performance.

The first phase 0 clinical trial (NCT03020017) involving SNA carrying Bcl2L12 siRNA (drug code: NU-0129) was conducted in recurrent glioblastoma.[127,128] The trial results demonstrated that the intravenous administration of SNAs at the administered dose, which corresponds to the 1/50th of the not observed adverse-event level, exhibited a favorable safety profile and did not exhibit any treatment-related toxicity.

Using ICP-MS and X-ray fluorescence microscopy, it was observed that SNAs accumulated in the tumors of patients and were associated with cytoplasmic accumulation in tumor cells. Quantitative analysis of the gold element concentration indicated SNA uptake in tumor cells, resulting in reduced Bcl2L12 protein expression and the induction of active caspase-3 and p53 proteins. These results demonstrate that using SNAs is a safe and brain-penetrating precision medical approach, enabling the delivery of siRNA oligonucleotide systems to intracranial tumor sites.

Furthermore, a clinical trial investigating the combined use of SNAs with immunotherapy is underway, focusing on immune checkpoint therapy. The study aims to evaluate the effectiveness of an SNA containing a TLR-9 agonist in immune checkpoint therapy. In the phase I human study, an immune-stimulating SNA compound targeting toll-like receptor 9, known as cavrotolimod (formerly AST-008), demonstrated good tolerance and pharmacological characteristics in healthy participants.[129,130] The results indicate that cavrotolimod is an effective innate immune activator, which may exert anti-tumor effects in cancer patients. Based on these findings, cavrotolimod has advanced to phase II clinical trials in combination with PD-1/PD-L1 antibodies for late-stage skin cancer patients.

In studies on psoriasis, a phase I clinical trial involving the topical administration of SNAs carrying siRNA-targeting TNF-α in patients with chronic plaque psoriasis yielded high tolerance and safety. Furthermore, a treatment plan for psoriasis-targeting interleukin-17 receptor alpha is poised to commence phase I clinical trials, marking the clinical therapeutic approach of local SNA administration.[131]

Exicure Inc., a clinical-stage biotechnology company founded by David Giljohann and Chad Mirkin, is expanding the clinical application of SNAs to treat a variety of diseases, including Angelman syndrome, Huntington's disease, Batten disease, alopecia, motor neuron disease, and spinocerebellar ataxia (Table 1).[131] Overall, the translation of SNAs is thriving, and the existing observations will strongly propel the further development of SNA clinical applications, paving the way for future medical advancements.

Table 1 Summary of the clinical disease research on SNAs and their pipeline

Disease mode	Drug/target	Phase
Melanoma	AST-008 (TLR9)	Clinical phase II
Head and neck squamous cell carcinoma	AST-008 (TLR9)	Clinical phase II
Merkel cell carcinoma	AST-008 (TLR9)	Clinical phase II
Solid tumors	AST-008 (TLR9)	Clinical phase I
Neoplastic hematologic disorder	AST-008 (TLR9)	Preclinical
Neuropathic pain	SCN9A Candidate (Nav1.7)	Unknown
Psoriasis	XCUR-17 (IL-17RA)	Clinical phase I
Alopecia	XCUR-17 (IL-17RA)	Preclinical
Angelman syndrome	Oligonucleotide	Preclinical
Huntington's disease	Oligonucleotide	Preclinical
Batten disease	Oligonucleotide (CLN3)	Preclinical
Friedreich's ataxia	XCUR FXN (ATXN)	Preclinical
Psoriasis	AST-005 (TNF)	Clinical phase I
Diabetic foot	AST-006	Drug discovery
Dermatosis	DMX-102 (SPINK5)	Unknown

5. Conclusion and future perspectives

In conclusion, SNAs represent a distinct nucleic acid structure with substantial promise in therapeutic amplification. This paper has explored SNAs' structure, functionality, therapeutic applications, and their progress towards clinical translation. Their notable properties, including high penetrability, strong affinity, stability, and versatile functionalization through structural amplification, render them exceptionally suitable for nucleic acid-based drug delivery, diagnostics, and treatment. Furthermore, the integration of SNAs with conventional therapeutic drugs, external energy sources, and immune modulation in combinatorial therapies significantly overcome the limitations of traditional nucleic acid therapeutics, thereby advancing SNA-based therapy to fulfill clinical demands.

However, there are still challenges to overcome. Firstly, it is crucial to address the impact of the protein corona during circulation and prevent lysosome clearance after cellular uptake. The protein corona refers to the dynamic protein layer that forms around nanoparticles or nucleic acids upon exposure to biological fluids. This corona can significantly alter the interaction of nucleic acids with target cells and tissues, thereby impacting their therapeutic efficacy. It has been reported that the defects of the protein corona can be utilized to actively adsorb functional proteins on the surface of SNAs, leading to less protein loss and stronger anti-degradation ability. Mirkin designed and synthesized SNAs with a predefined protein coronae consisting of functional proteins (monoclonal antibodies targeting human epithelial growth factor receptor 2) immobilized on the oligonucleotide shell. These structures exhibited enhanced stability in buffer and human serum, as well as selectivity for HER2-positive breast cancer cells in mixed cell cultures with HER2-negative breast cancer cells. This strategy of using the protein corona to regulate the *in vivo* fate of SNAs will improve the design methods of such carriers and enhance the pharmacokinetic characteristics of SNAs.[132] This strategy of using the protein corona to regulate the *in vivo* fate of SNAs will improve the design methods of such carriers and enhance the pharmacokinetic characteristics of SNAs. Lysosomes are cellular organelles that break down and recycle various molecules and particles, including foreign substances like SNAs. Regarding the issue of lysosome degradation, utilizing certain substances to disrupt the lysosomal membrane, thereby causing the release of SNAs into the cytoplasm to exert their effects, is a common approach. It has been reported that photo-induced production of $^1O_2$ can enable lysosome escape.[133] Alternatively, the introduction of lanthanide particles binding to the phospholipid head of the endosome membrane has been demonstrated to disrupt the membrane structure and facilitate rapid escape from the lysosome.[134] However, the bodily reactions caused by excessive lysosomal disruption still need to be carefully considered.

Secondly, it is necessary to develop optimized synthetic processes that can yield large quantities of SNAs at a reasonable cost for successful commercialization. The first on the list is the synthesis of SNAs without compromising their quality or functionality. This necessitates a fine balance between the use of efficient, cost-effective materials and methods, and the preservation of the structural integrity and bioactivity of SNAs. Advanced techniques in nanotechnology and molecular engineering are key to developing such optimized processes. Notably, recent advancements such as the freeze-anchoring method have been reported as a simple and time-saving alternative to traditional SNA synthesis modes. By reducing the temperature, DNA can be conjugated to AuNPs during freezing without the need for additional reagents, enabling the completion of the conjugation process within a few minutes. Moreover, this method yields a DNA density that is 20–30% higher compared to that achieved using the typical salt-aging approach.[135,136] By achieving this objective, SNAs can transition from a promising scientific concept to a widely available commercial product, unlocking new possibilities in science and medicine.

Finally, the *in vivo* safety of SNAs still requires long-term attention. The core of SNAs is generally composed of metals such as gold, iron, and silver, which can accumulate in the body and lead to poisoning. Additionally, the off-target effects and immunogenicity of nucleic acids themselves are issues that need to be considered when using SNAs. Carrier-free self-delivery strategies have been shown to reduce the defects and maximize the function of the drug due to simplified construction and mitigate the risk of uncontrolled immune system activation. This strategy may become the most ideal way for clinical treatment and an important development direction for SNAs in the future.[52,82]

In conclusion, the amplification contribution of SNAs for potential clinical translations should not be overlooked. Through an examination of the distinctive amplification structure inherent in SNAs, the distinctive features outlined below undoubtedly strengthen the significant clinical translational potential of SNAs: (i) spherical structure: the spherical structure is chosen due to their minimal potential energy and stability against external forces; furthermore, spheroids, acting as isotropic particles, increase the probability of effective particle collisions, (ii) drug loading form: external binding of high-density nucleic acids for drug loading offers unique opportunities for nucleic acid pairing and amplified SNA binding; moreover, a higher nucleic acid density enhances nanoparticles' capacity to load various cargoes with greater loading contents, and (iii) flexible and tailor-made compositions: the shell and core components of SNAs can be tailor-made to meet a broad range of different requirements. This adaptability allows for different solutions to be implemented based on distinct disease models.

All in all, the amplification of SNAs is an ongoing process, with the continuous advancement of technology and a deeper understanding of SNAs, more innovations and breakthroughs are expected in the future. This holds the potential to make the treatment of various human diseases a reality in the future.

Author contributions

We report author contributions using the credit model as follows. Z. T.: conceptualization, data curation, formal analysis, methodology, software, writing – original draft, and writing – review and editing. H. Z.: funding acquisition, methodology, software, supervision, and writing – review and editing. S. W.: investigation and visualization. J. Z.: investigation and visualization. Y. C.: validation and visualization. L. L.: validation and visualization. Y. Q.: validation and visualization. H. W.: funding acquisition, project administration, resources, supervision, and writing – review and editing. C. Y.: funding acquisition, project administration, resources, supervision, and writing – review and editing.

Conflicts of interest

The authors declare that there are no known competing financial interests or personal relationships that could influence the work reported in this paper.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (82373826), the Key R&D Program of Hunan Province (2023SK2043, 2021SK2036), the Hunan Science and Technology Innovation Leading Talent Project (2022RC3080), the Natural Science Foundation of Hunan Province (2021JJ30603, 2022JJ40381), the Research Foundation of Education Bureau of Hunan Province (No. 21A0284), the Scientific Research Project of Hunan Provincial Health Commission (No. 202113021875), and the Innovation and Entrepreneurship Training Program for College Students in Hunan Province (No. S202210555281).

References

1 G. Dranoff, *J. Clin. Oncol.*, 1998, **16**, 2548–2556.
2 I. F. Parney and L. J. Chang, *J. Biomed. Sci.*, 2003, **10**, 37–43.
3 C. Liu, Q. Shi, X. Huang, S. Koo, N. Kong and W. Tao, *Nat. Rev. Cancer*, 2023, **23**, 526–543.
4 T. L. Roth and A. Marson, *Annu. Rev. Pathol.: Mech. Dis.*, 2021, **16**, 145–166.
5 R. Palanki, W. H. Peranteau and M. J. Mitchell, *Adv. Drug Delivery Rev.*, 2021, **169**, 51–62.
6 C. Govers, Z. Sebestyen, M. Coccoris, R. A. Willemsen and R. Debets, *Trends Mol. Med.*, 2010, **16**, 77–87.
7 A. Rodriguez-Gascon, P. A. Del, A. Isla and M. A. Solinis, *Adv. Drug Delivery Rev.*, 2015, **92**, 71–83.
8 A. Rolland, *Adv. Drug Delivery Rev.*, 2005, **57**, 669–673.
9 C. C. Ma, Z. L. Wang, T. Xu, Z. Y. He and Y. Q. Wei, *Biotechnol. Adv.*, 2020, **40**, 107502.
10 J. Soutschek, A. Akinc, B. Bramlage, K. Charisse, R. Constien, M. Donoghue, S. Elbashir, A. Geick, P. Hadwiger, J. Harborth, M. John, V. Kesavan, G. Lavine, R. K. Pandey, T. Racie, K. G. Rajeev, I. Rohl, I. Toudjarska, G. Wang, S. Wuschko, D. Bumcrot, V. Koteliansky, S. Limmer, M. Manoharan and H. P. Vornlocher, *Nature*, 2004, **432**, 173–178.
11 M. Elsabahy, A. Nazarali and M. Foldvari, *Curr. Drug Delivery*, 2011, **8**, 235–244.
12 H. Kamiya, H. Tsuchiya, J. Yamazaki and H. Harashima, *Adv. Drug Delivery Rev.*, 2001, **52**, 153–164.
13 G. Palu, C. Parolin, Y. Takeuchi and M. Pizzato, *Rev. Med. Virol.*, 2000, **10**, 185–202.
14 Z. Zhou, X. Liu, D. Zhu, Y. Wang, Z. Zhang, X. Zhou, N. Qiu, X. Chen and Y. Shen, *Adv. Drug Delivery Rev.*, 2017, **115**, 115–154.
15 R. Mohammadinejad, A. Dehshahri, M. V. Sagar, M. Zahmatkeshan, S. Tavakol, P. Makvandi, D. Khorsandi, A. Pardakhty, M. Ashrafizadeh, A. E. Ghasemipour and A. Zarrabi, *J. Controlled Release*, 2020, **325**, 249–275.
16 J. A. Kulkarni, D. Witzigmann, S. B. Thomson, S. Chen, B. R. Leavitt, P. R. Cullis and R. van der Meel, *Nat. Nanotechnol.*, 2021, **16**, 630–643.
17 C. A. Mirkin, R. L. Letsinger, R. C. Mucic and J. J. Storhoff, *Nature*, 1996, **382**, 607–609.
18 J. I. Cutler, E. Auyeung and C. A. Mirkin, *J. Am. Chem. Soc.*, 2012, **134**, 1376–1391.
19 Y. Song, W. Song, X. Lan, W. Cai and D. Jiang, *Aggregate*, 2022, **3**, e120.
20 S. Liu, C. Y. Yu and H. Wei, *Mater. Today Bio*, 2023, **22**, 100750.
21 C. A. Mirkin and S. H. Petrosko, *ACS Nano*, 2023, **17**, 16291–16307.
22 A. S. Mahajan and A. H. Stegh, *Cancers*, 2022, **14**, 1615.
23 C. H. Kapadia, J. R. Melamed and E. S. Day, *BioDrugs*, 2018, **32**, 297–309.
24 S. Matosevic, *J. Immunol. Res.*, 2018, **2018**, 4054815.
25 E. Check, *Nature*, 2005, **433**, 561.
26 N. Attia, M. Mashal, G. Puras and J. L. Pedraz, *Pharmaceutics*, 2021, **13**, 843.
27 K. Y. Choi, S. Correa, J. Min, J. Li, S. Roy, K. H. Laccetti, E. Dreaden, S. Kong, R. Heo, Y. H. Roh, E. C. Lawson, P. A. Palmer and P. T. Hammond, *Adv. Funct. Mater.*, 2019, **29**, 1900018.
28 M. F. Coutinho, J. I. Santos, S. Mendonça, L. Matos, M. J. Prata, A. S. Jurado, M. C. Pedroso De Lima and S. Alves, *Int. J. Mol. Sci.*, 2020, **21**, 5732.
29 H. D. Hill, J. E. Millstone, M. J. Banholzer and C. A. Mirkin, *ACS Nano*, 2009, **3**, 418–424.
30 A. Krissanaprasit, C. M. Key, S. Pontula and T. H. LaBean, *Chem. Rev.*, 2021, **121**, 13797–13868.
31 S. Hoshika, I. Singh, C. Switzer, R. J. Molt, N. A. Leal, M. J. Kim, M. S. Kim, H. J. Kim, M. M. Georgiadis and S. A. Benner, *J. Am. Chem. Soc.*, 2018, **140**, 11655–11660.
32 J. I. Cutler, E. Auyeung and C. A. Mirkin, *J. Am. Chem. Soc.*, 2012, **134**, 1376–1391.

33 A. E. Prigodich, O. Lee, W. L. Daniel, D. S. Seferos, G. C. Schatz and C. A. Mirkin, *J. Am. Chem. Soc.*, 2010, **132**, 10638–10641.
34 L. Duan, Y. Hong, W. Yang, L. Zhang and J. Liu, *Chem. Eng. J.*, 2023, **473**, 145418.
35 H. Chen, Y. Li, Y. Song, F. Liu, D. Deng, X. Zhu, H. He, X. Yan and L. Luo, *Biosens. Bioelectron.*, 2023, **223**, 115029.
36 L. Shi, C. Liu, H. Wang, J. Zheng, Q. Wang, L. Shi and T. Li, *Anal. Chem.*, 2022, **94**, 14394–14401.
37 L. Yuan, D. Ji, Q. Fu and M. Hu, *Nanomaterials*, 2022, **12**, 2196.
38 C. H. Choi, L. Hao, S. P. Narayan, E. Auyeung and C. A. Mirkin, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 7625–7630.
39 Q. Bai, Y. Xiao, H. Hong, X. Cao, L. Zhang, R. Han, L. K. C. Lee, E. Y. Xue, X. Y. Tian and C. H. J. Choi, *Proc. Natl. Acad. Sci. U. S. A.*, 2022, **119**, e2093524177.
40 W. Zhang, B. Meckes and C. A. Mirkin, *ACS Cent. Sci.*, 2019, **5**, 1983–1990.
41 V. Krishnan and S. Mitragotri, *Adv. Drug Delivery Rev.*, 2020, **153**, 87–108.
42 R. J. Kulchar, R. Singh, S. Ding, E. Alexander, K. W. Leong and H. Daniell, *Biomaterials*, 2023, **302**, 122312.
43 R. Jamaledin, C. Yiu, E. N. Zare, L. N. Niu, R. Vecchione, G. Chen, Z. Gu, F. R. Tay and P. Makvandi, *Adv. Mater.*, 2020, **32**, e2002129.
44 Q. Qi, Y. Wei, X. Zhang, J. Guan and S. Mao, *J. Controlled Release*, 2023, **361**, 191–211.
45 H. Liu, R. S. Kang, K. Bagnowski, J. M. Yu, S. Radecki, W. L. Daniel, B. R. Anderson, S. Nallagatla, A. Schook, R. Agarwal, D. A. Giljohann and A. S. Paller, *J. Invest. Dermatol.*, 2020, **140**, 435–444.
46 Y. Fang, X. Lu, D. Wang, J. Cai, Y. Wang, P. Chen, M. Ren, H. Lu, J. Union, L. Zhang, Y. Sun, F. Jia, X. Kang, X. Tan and K. Zhang, *J. Am. Chem. Soc.*, 2021, **143**, 1296–1300.
47 K. Jiang, D. Zhao, R. Ye, X. Liu, C. Gao, Y. Guo, C. Zhang, J. Zeng, S. Wang and J. Song, *Nanoscale*, 2022, **14**, 1834–1846.
48 Y. Zhou, Z. Peng, E. S. Seven and R. M. Leblanc, *J. Controlled Release*, 2018, **270**, 290–303.
49 Q. Tan, S. Zhao, T. Xu, Q. Wang, M. Zhang, L. Yan, X. Chen and M. Lan, *Coord. Chem. Rev.*, 2023, **494**, 215344.
50 T. L. Sita, F. M. Kouri, L. A. Hurley, T. J. Merkel, A. Chalastanis, J. L. May, S. T. Ghelfi, L. E. Cole, T. C. Cayton, S. N. Barnaby, A. J. Sprangers, N. Savalia, C. D. James, A. Lee, C. A. Mirkin and A. H. Stegh, *Proc. Natl. Acad. Sci. U. S. A.*, 2017, **114**, 4129–4134.
51 C. D. Kusmierz, C. E. Callmann, S. Kudruk, M. E. Distler and C. A. Mirkin, *Bioconjugate Chem.*, 2022, **33**, 1803–1810.
52 W. Yu, C. Xuan, B. Liu, L. Zhou, N. Yin, E. Gong, Z. Zhang, Y. Li, K. Zhang and J. Shi, *Nano Res.*, 2023, **16**, 735–745.
53 J. Yan, M. Ran, X. Shen and H. Zhang, *Adv. Mater.*, 2023, **35**, e2300374.
54 C. Liu, F. Liu, L. Feng, M. Li, J. Zhang and N. Zhang, *Biomaterials*, 2013, **34**, 2547–2564.
55 A. R. Chandrasekaran, *Nat. Rev. Chem.*, 2021, **5**, 225–239.
56 Y. Zhang, C. Sun, C. Wang, K. E. Jankovic and Y. Dong, *Chem. Rev.*, 2021, **121**, 12181–12277.
57 R. Hincapie, S. Bhattacharya, P. Keshavarz-Joud, A. P. Chapman, S. N. Crooke and M. G. Finn, *Biomacromolecules*, 2023, **24**, 2766–2776.
58 D. S. Seferos, A. E. Prigodich, D. A. Giljohann, P. C. Patel and C. A. Mirkin, *Nano Lett.*, 2009, **9**, 308–311.
59 M. E. Kyriazi, A. H. El-Sagheer, I. L. Medintz, T. Brown and A. G. Kanaras, *Bioconjugate Chem.*, 2022, **33**, 219–225.
60 Z. Chen, F. Zhang, H. Zhang, L. Cheng, K. Chen, J. Shen, J. Qi, L. Deng, C. He, H. A. Santos and W. Cui, *Adv. Sci.*, 2021, **8**, 2004793.
61 S. Li, Q. Jiang, Y. Liu, W. Wang, W. Yu, F. Wang and X. Liu, *Anal. Chem.*, 2021, **93**, 11275–11283.
62 K. Zheng, M. I. Setyawati, D. T. Leong and J. Xie, *Coord. Chem. Rev.*, 2018, **357**, 1–17.
63 Z. Qin, Y. Zheng, Y. Wang, T. Du, C. Li, X. Wang and H. Jiang, *Coord. Chem. Rev.*, 2021, **449**, 214218.
64 C. H. Rische, A. Goel, A. F. Radovic-Moreno and S. M. Gryaznov, *Mater. Today Commun.*, 2016, **9**, 30–40.
65 M. Zandieh and J. Liu, *Bioconjugate Chem.*, 2021, **32**, 801–809.
66 T. Du, Z. Shi, Z. Qin, Y. Hu, Y. Zhu, H. Jiang and X. Wang, *Adv. Funct. Mater.*, 2022, **32**, 2207410.
67 J. Li, X. Chang, X. Chen, Z. Gu, F. Zhao, Z. Chai and Y. Zhao, *Biotechnol. Adv.*, 2014, **32**, 727–743.
68 R. Mohammadpour, M. A. Dobrovolskaia, D. L. Cheney, K. F. Greish and H. Ghandehari, *Adv. Drug Delivery Rev.*, 2019, **144**, 112–132.
69 R. Cheng, L. Liu, Y. Xiang, Y. Lu, L. Deng, H. Zhang, H. A. Santos and W. Cui, *Biomaterials*, 2020, **232**, 119706.
70 R. J. Banga, N. Chernyak, S. P. Narayan, S. T. Nguyen and C. A. Mirkin, *J. Am. Chem. Soc.*, 2014, **136**, 9866–9869.
71 J. R. Ferrer, A. J. Sinegra, D. Ivancic, X. Y. Yeap, L. Qiu, J. J. Wang, Z. J. Zhang, J. A. Wertheim and C. A. Mirkin, *ACS Nano*, 2020, **14**, 1682–1693.
72 M. K. Vasher, M. Evangelopoulos and C. A. Mirkin, *ACS Appl. Bio Mater.*, 2023, **6**, 3912–3918.
73 P. Song, F. Yang, H. Jin and X. Wang, *Signal Transduction Targeted Ther.*, 2021, **6**, 68.
74 J. D. Brodin, A. J. Sprangers, J. R. McMillan and C. A. Mirkin, *J. Am. Chem. Soc.*, 2015, **137**, 14838–14841.
75 C. Huang, Z. Han, M. Evangelopoulos and C. A. Mirkin, *J. Am. Chem. Soc.*, 2022, **144**, 18756–18760.
76 X. Li, T. Yamazaki, M. Ebara, N. Shirahata and N. Hanagata, *Mater. Today*, 2023, **67**, 127–150.
77 H. Zhu, X. J. Loh, E. Ye and Z. Li, *ACS Mater. Lett.*, 2022, **4**, 21–48.
78 S. Zhu, H. Xing, P. Gordiichuk, J. Park and C. A. Mirkin, *Adv. Mater.*, 2018, **30**, e1707113.
79 Y. Zeng, R. Peng, Y. Hu, P. Luo, R. Yang, J. Li and J. Zheng, *Anal. Chem.*, 2023, **95**, 14710–14719.
80 H. Mei, S. Cai, D. Huang, H. Gao, J. Cao and B. He, *Bioact. Mater.*, 2022, **8**, 220–240.

81 L. Liu and X. Zhang, *Prog. Mater. Sci.*, 2022, **125**, 100919.
82 P. Chen, D. Wang, Y. Wang, L. Zhang, Q. Wang, L. Liu, J. Li, X. Sun, M. Ren, R. Wang, Y. Fang, J. J. Zhao and K. Zhang, *Nano Lett.*, 2022, **22**, 4058–4066.
83 F. B. Manalo, A. Marks and H. J. Davis, *JAMA, J. Am. Med. Assoc.*, 1975, **233**, 56–57.
84 L. Lothstein, M. Israel and T. W. Sweatman, *Drug Resistance Updates*, 2001, **4**, 169–177.
85 M. Mohammadi, L. Arabi and M. Alibolandi, *J. Controlled Release*, 2020, **328**, 171–191.
86 H. Li, X. Zhou, D. Yao and H. Liang, *Chem. Commun.*, 2018, **54**, 3520–3523.
87 B. Ma, Y. Ma, B. Deng, P. Xiao, P. Huang, D. Wang and L. Liu, *J. Nanobiotechnol.*, 2023, **21**, 171.
88 S. Ezrahi, A. Aserin and N. Garti, *Adv. Colloid Interface Sci.*, 2019, **263**, 95–130.
89 X. Tan, X. Lu, F. Jia, X. Liu, Y. Sun, J. K. Logan and K. Zhang, *J. Am. Chem. Soc.*, 2016, **138**, 10834–10837.
90 Y. Guo, J. Zhang, F. Ding, G. Pan, J. Li, J. Feng, X. Zhu and C. Zhang, *Adv. Mater.*, 2019, **31**, e1807533.
91 L. Zhu, Y. Guo, Q. Qian, D. Yan, Y. Li, X. Zhu and C. Zhang, *Angew. Chem., Int. Ed.*, 2020, **59**, 17944–17950.
92 Y. Guo, J. Qin, Q. Zhao, J. Yang, X. Wei, Y. Huang, M. Xie, C. Zhang and Y. Li, *Adv. Sci.*, 2022, **9**, e2105875.
93 S. P. Narayan, C. H. Choi, L. Hao, C. M. Calabrese, E. Auyeung, C. Zhang, O. J. Goor and C. A. Mirkin, *Small*, 2015, **11**, 4173–4182.
94 D. Bousmail, L. Amrein, J. J. Fakhoury, H. H. Fakih, J. Hsu, L. Panasci and H. F. Sleiman, *Chem. Sci.*, 2017, **8**, 6218–6229.
95 N. Li, M. H. Xiang, J. W. Liu, H. Tang and J. H. Jiang, *Anal. Chem.*, 2018, **90**, 12951–12958.
96 J. Chen, C. Ning, Z. Zhou, P. Yu, Y. Zhu, G. Tan and C. Mao, *Prog. Mater. Sci.*, 2019, **99**, 1–26.
97 L. Zhao, X. Zhang, X. Wang, X. Guan, W. Zhang and J. Ma, *J. Nanobiotechnol.*, 2021, **19**, 335.
98 L. Huang, J. Zhang, L. Pang, S. Hu, L. Zhang and S. Zhao, *Chem. Commun.*, 2021, **57**, 11617–11620.
99 R. Yan, J. Chen, J. Wang, J. Rao, X. Du, Y. Liu, L. Zhang, L. Qiu, B. Liu, Y. D. Zhao, P. Jiang, C. Chen and Y. Q. Li, *Small*, 2018, **14**, e1802745.
100 M. Kolarikova, B. Hosikova, H. Dilenko, K. Barton-Tomankova, L. Valkova, R. Bajgar, L. Malina and H. Kolarova, *Med. Res. Rev.*, 2023, **43**, 717–774.
101 L. Chen, G. Li, X. Wang, J. Li and Y. Zhang, *ACS Nano*, 2021, **15**, 11929–11939.
102 A. N. DuRoss, M. J. Neufeld, S. Rana, C. J. Thomas and C. Sun, *Adv. Drug Delivery Rev.*, 2019, **144**, 35–56.
103 Y. Pan, W. Tang, W. Fan, J. Zhang and X. Chen, *Chem. Soc. Rev.*, 2022, **51**, 9759–9830.
104 J. Liu, L. Guo, Z. Mi, Z. Liu, P. Rong and W. Zhou, *J. Controlled Release*, 2022, **348**, 1050–1065.
105 M. Wei, X. Shen, X. Fan, J. Li and J. Bai, *Front. Bioeng. Biotechnol.*, 2023, **11**, 1224339.
106 J. Nam, S. Son, K. S. Park, W. Zou, L. D. Shea and J. J. Moon, *Nat. Rev. Mater.*, 2019, **4**, 398–414.
107 B. Zhou, J. Liu, M. Lin, J. Zhu and W. R. Chen, *Coord. Chem. Rev.*, 2021, **442**, 214009.
108 M. C. Patra, M. Shah and S. Choi, *Semin. Cancer Biol.*, 2020, **64**, 61–82.
109 E. Vercammen, J. Staal and R. Beyaert, *Clin. Microbiol. Rev.*, 2008, **21**, 13–25.
110 H. Sun, Y. Li, P. Zhang, H. Xing, S. Zhao, Y. Song, D. Wan and J. Yu, *Biomark. Res.*, 2022, **10**, 89.
111 A. M. Krieg, *Nat. Rev. Drug Discovery*, 2006, **5**, 471–484.
112 Q. Zhang, Y. Guo, L. Zhu, X. Liu, J. Yang, Y. Li, X. Zhu and C. Zhang, *Biomater. Sci.*, 2021, **9**, 4755–4764.
113 R. J. Banga, B. Meckes, S. P. Narayan, A. J. Sprangers, S. T. Nguyen and C. A. Mirkin, *J. Am. Chem. Soc.*, 2017, **139**, 4278–4281.
114 C. Guan, N. Chernyak, D. Dominguez, L. Cole, B. Zhang and C. A. Mirkin, *Small*, 2018, **14**, e1803284.
115 Z. N. Huang, L. E. Cole, C. E. Callmann, S. Wang and C. A. Mirkin, *ACS Nano*, 2020, **14**, 1084–1092.
116 B. Deng, B. Ma, Y. Ma, P. Cao, X. Leng, P. Huang, Y. Zhao, T. Ji, X. Lu and L. Liu, *J. Nanobiotechnol.*, 2022, **20**, 140.
117 C. E. Callmann, C. D. Kusmierz, J. W. Dittmar, L. Broger and C. A. Mirkin, *ACS Cent. Sci.*, 2021, **7**, 892–899.
118 M. H. Teplensky, M. Evangelopoulos, J. W. Dittmar, C. M. Forsyth, A. J. Sinegra, S. Wang and C. A. Mirkin, *Nat. Biomed. Eng.*, 2023, **7**, 911–927.
119 J. W. Dittmar, M. H. Teplensky, M. Evangelopoulos, L. Qin, B. Zhang and C. A. Mirkin, *ACS Nano*, 2023, **17**, 17996–18007.
120 A. Mahajan, L. Hurley, S. Tommasini-Ghelfi, C. Dussold, A. Stegh and C. Mirkin, *Neuro-Oncology*, 2021, **23**, vi104–vi105.
121 W. T. Ma, C. Chang, M. E. Gershwin and Z. X. Lian, *J. Autoimmun.*, 2017, **83**, 95–112.
122 G. Azizi, R. Yazdani, W. Rae, H. Abolhassani, M. Rojas, A. Aghamohammadi and J. M. Anaya, *Autoimmun. Rev.*, 2018, **17**, 1028–1039.
123 H. Nemati, M. H. Ghahramani, R. Faridi-Majidi, B. Izadi, G. Bahrami, S. H. Madani and G. Tavoosidana, *J. Controlled Release*, 2017, **268**, 259–268.
124 M. M. Lv, J. W. Liu, R. Q. Yu and J. H. Jiang, *Chem. Sci.*, 2020, **11**, 10361–10366.
125 S. Sindhwani and W. Chan, *J. Intern. Med.*, 2021, **290**, 486–498.
126 P. Zhang, Y. Xiao, X. Sun, X. Lin, S. Koo, A. V. Yaremenko, D. Qin, N. Kong, O. C. Farokhzad and W. Tao, *Med*, 2023, **4**, 147–167.
127 P. Kumthekar, A. Rademaker, C. Ko, K. Dixit, M. A. Schwartz, A. M. Sonabend, L. Sharp, R. V. Lukas, R. Stupp, C. Horbinski, K. McCortney and A. H. Stegh, *J. Clin. Oncol.*, 2019, **37**, 3012.
128 P. Kumthekar, C. H. Ko, T. Paunesku, K. Dixit, A. M. Sonabend, O. Bloch, M. Tate, M. Schwartz, L. Zuckerman, R. Lezon, R. V. Lukas, B. Jovanovic, K. McCortney, H. Colman, S. Chen, B. Lai, O. Antipova, J. Deng, L. Li, S. Tommasini-Ghelfi, L. A. Hurley, D. Unruh, N. V. Sharma, M. Kandpal, F. M. Kouri,

R. V. Davuluri, D. J. Brat, M. Muzzio, M. Glass, V. Vijayakumar, J. Heidel, F. J. Giles, A. K. Adams, C. D. James, G. E. Woloschak, C. Horbinski and A. H. Stegh, *Sci. Transl. Med.*, 2021, **13**, eabb3945.

129 S. O. Day, C. Perez, T. Wise-Draper, G. Hanna, S. Bhatia, C. Kelly, T. Medina, D. Laux, A. Daud, S. Chandra, M. Shaheen, L. Gao, M. Burgess, L. Hernandez-Aya, C. Yeung, K. Smythe, E. DeGoma, W. Daniel, D. Feltner, L. Sindelar, R. Michel, A. Bexon, M. Bexon and M. Milhem, *J. Immunother. Cancer*, 2020, **8**, A257–A258.

130 W. L. Daniel, U. Lorch, S. Mix and A. S. Bexon, *Front. Immunol.*, 2022, **13**, 1073777.

131 Exicure, **https://www.exicuretx.com/**.

132 W. Zhang, B. Meckes and C. A. Mirkin, *ACS Cent. Sci.*, 2019, **5**, 1983–1990.

133 L. Shi, W. Wu, Y. Duan, L. Xu, Y. Xu, L. Hou, X. Meng, X. Zhu and B. Liu, *Angew. Chem., Int. Ed.*, 2020, **59**, 19168–19174.

134 C. Yu, K. Li, L. Xu, B. Li, C. Li, S. Guo, Z. Li, Y. Zhang, A. Hussain, H. Tan, M. Zhang, Y. Zhao, Y. Huang and X. Liang, *Nano Res.*, 2022, **15**, 9160–9168.

135 B. Liu and J. Liu, *J. Am. Chem. Soc.*, 2017, **139**, 9471–9474.

136 X. Wang, Z. Yang, Y. Li, K. Huang and N. Cheng, *J. Colloid Interface Sci.*, 2024, **655**, 830–840.