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Review Article

DNA nanostructures-based delivery system for cancer immunotherapy

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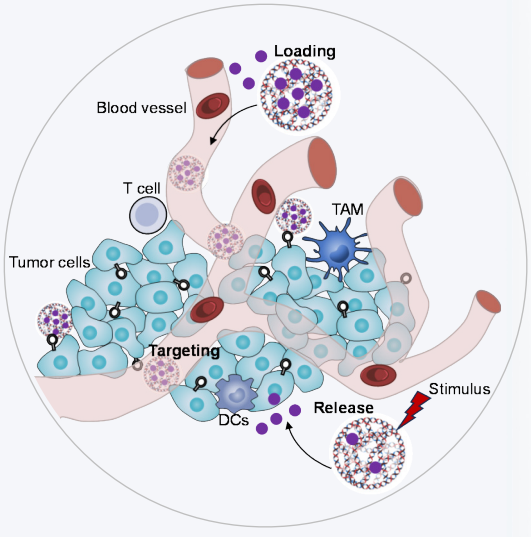
ABSTRACT: Immunotherapy has been emerging as a potent strategy for cancer treatment. However, undesirable therapeutic efficacy remains a challenge, including low drug loading, imprecise targeting, and non-specific releasing. The drug delivery systems of immunotherapy play a key role in improving therapeutic efficacy and reducing side effects. To address these concerns, functional DNA nanostructures-based materials have been explored to achieve high loading capability, precise targeting, and controllable releasing. This review focuses on the crucial issues of delivery system for cancer immunotherapy and the strategies to improve the delivery efficacy. Specifically, recent advances in DNA nanostructures-based materials that promote the therapeutic efficacy of cancer immunotherapy through rational DNA sequence design to regulate the spatial distribution of immunotherapeutics loading are reviewed. The strategies to enhance precise targeting ability basing on nucleic acid aptamers and further enable immune checkpoint inhibitions are presented. The recent progress on the controllable release of immunotherapeutics triggered by specific stimulus is discussed. In the end, we provide insights for the subsequent realization of applications of DNA nanostructures-based materials for cancer immunotherapy in the future.



KEYWORDS: DNA nanotechnology, drug delivery system, loading capability, targeting ability, controllable release, cancer immunotherapy

1 Introduction

Cancer has become a global health issue [1]. Immunotherapy has emerged as a powerful clinical strategy for cancer treatment, which evokes host immunity to fight against tumors cells [2–4]. Nowadays, common immunotherapy strategies, mainly including immunomodulatory drugs [5], cytokines therapeutics [6, 7], immune checkpoint blockade (ICB) [8], chimeric antigen receptor T-cell immunotherapy (CAR-T) [9–11], and cancer vaccines [12], have been explored to constantly evolve and optimize to adapt to more conditions. However, the challenges remain in immunotherapy, especially the existing essential issues of drug delivery for cancer immunotherapy [13]. Current clinical attempts to develop a highly efficient delivery system for immunotherapeutics have not yielded breakthrough outcomes due to significant challenges, including low loading capacity, poor targeting ability, and non-specific drug release.

Insufficient transportation of antigens and adjuvants to the specific sites where immune response is initiated at the right time is a crucial factor contributing to the limited immunotherapeutic efficacy [14, 15], which may also lead to undesirable off-target toxicities, such as cytokine storms [16]. The tumor microenvironment (TME) is a major cause of the failure of both nanomedicines and immunotherapies that not only limits delivery, but also can compromise efficacy, even when therapeutics accumulate in the TME [17]. In addition, the efficient delivery of

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drug molecules with accurate dosing and on-demand release is another major challenge, which mainly depends on the controllable release of drugs from drug carriers. Therefore, to improve therapy efficacy, the highly efficient delivery of immunotherapeutics is the most significant part of treatment process.

Due to the characteristics of deoxyribonucleic acid (DNA), such as sequence programmability, precise and controllable structure, foreseeable functions, biocompatibility and biodegradation [18, 19], the field of DNA nanostructures-based functional materials has been investigated as the delivery system to overcome the above-mentioned hurdles. The sequence programmability of DNA enables construction of structurally well-defined delivery platforms [20]. For example, the angle and arm length of DNA tetrahedral nanostructures can be precisely controlled through the design of the DNA sequence, in addition to excellent structural stability due to their tripod-shaped framework, which is resistant to enzyme degradation [21, 22]. Therefore, rational design and production of nanostructures with controlled size, shape and load capacity enables to achieve effective molecular delivery and treatment [23, 24]. Due to the high affinity and specificity to antigens, nucleic acid aptamers can act as chemical antibodies to recognize and bind to targets [25], contributing to improving the targeting of drug delivery system. DNA nanostructures have garnered significant attention due to their diversity and versatility. Many of DNA nanostructures are stimulus-responsive, such as pH [26–28], metal ions [29, 30], glutathione (GSH) [31–33], and adenosine triphosphate (ATP) [34–36]. Controllable release of therapeutics at specific sites can be accomplished by designing stimulus-responsive DNA nanostructures for different microenvironments, and achieve better therapeutic effects [37]. Thus, numerous DNA nanostructures-based materials have been exploited for the precise and effective delivery of immunotherapy drugs, including food and drug administration (FDA)-approved antibodies [38], nucleic acid immune adjuvants [39, 40], and engineered-cell drugs [41].

This paper reviews the existing issues of immunotherapeutics delivery (Fig. 1). We provide current examples of DNA nanostructures-based biomaterials to solve these issues. Moreover, their applications in immunotherapy are introduced in detail. Lastly, we summarize the prospectives for next-generation strategy of cancer immunotherapy based on DNA nanostructures.

2 DNA nanostructures-based drug delivery system

DNA nanostructures can effectively load tumor therapeutic drugs, achieve targeted delivery of tumor tissues, efficient cell uptake, and stimulus-responsive drug release. The DNA nanostructures-based materials possess a wide range of types, including tetrahedral DNA nanostructures (TDNs), spherical nucleic acids (SNAs), DNA nano-trees, and DNA nanoclusters, which endow them with unique advantages as promising drug delivery system for cancer immunotherapy (Table 1).

2.1 Tunable loading ability

We would like to firstly emphasize that, exploiting the sequence programmability and structural designability, DNA-based nanostructures can be engineered to load drugs with user-defined size, shape, and spatial organization [42, 43]. Owing to their deoxyribonucleotide-comprised backbones, DNA-based nanostructures were readily load nucleic acid drugs through the interactions between bases. For instance, a DNA nanocluster (DNAnc) self-assembled based on complementary base-pairing was demonstrated to load about 8125.5 ± 822.5 copies of cytosine-phosphate-guanine (CpG) oligonucleotides (ODNs) within one single nanostructure. Utilizing lipids functionalized Y-shaped DNA comprising CpG (Y-CpG) as building blocks, the size of DNAnc was adjusted and optimized by altering the concentration of amphiphilic (Fig. 2(a)) [44]. The investigation of cellular internalization suggested that RAW264.7 cells preferred to uptake CpG ODNs in DNAnc rather than ssCpG or Y-CpG, indicating that this DNAnc was an excellent CpG delivery carrier (Fig. 2(b)). In addition, tumor-associated macrophages (TAMs) were the important part in TME. This highly condensed DNAnc exhibited the excellent capability to persistently repolarize TAM into

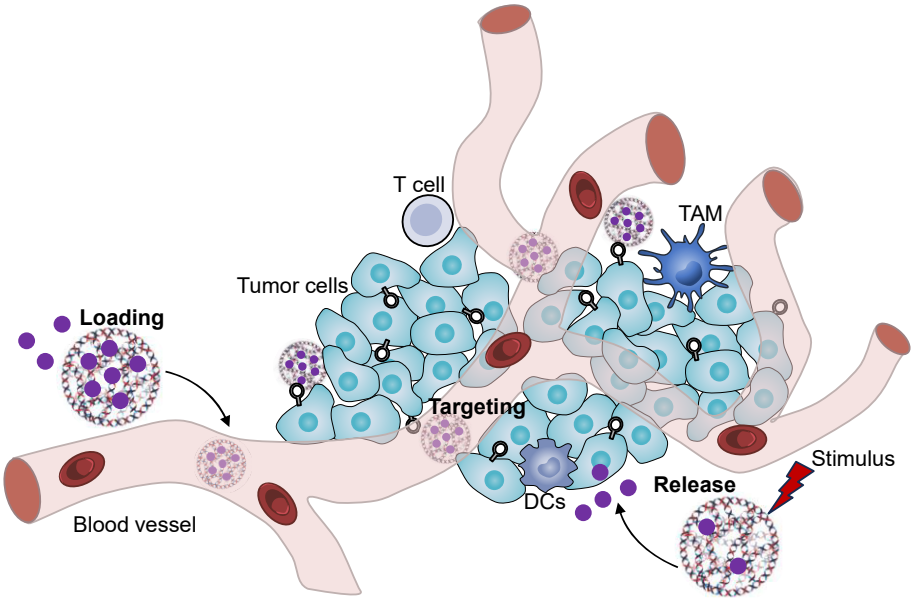

Figure 1 Overview scheme of DNA nanostructures-based delivery system for cancer immunotherapy. The therapeutic efficacy was able to be improved in three ways, including loading, targeting, and releasing.

Table 1 DNA nanostructures-based drug delivery system for cancer immunotherapy

Delivery system	Function	Therapeutic potential	Ref.
TDN	With interferon stimulatory DNA, highly potent toxins delivery, and the cGAS-STING pathway activation	Chemotherapy and immunotherapy	[46]
	Aptamer PL1 and Pcsk9 siRNA delivery	Immune checkpoint blockade	[47]
DNA origami	Adjuvants and antigens delivery	Cancer vaccine	[52]
SNA	Multi-antigen delivery	Cancer vaccine	[55]
DNA nano-tree	Tumor cells recognition, CpG ODNs on-demand release	Macrophages reprogramming, on-demand antitumor immunotherapy	[83]
DNA nanocluster	CpG delivery carrier, TAMs polarization	One-shot radiotherapy enhanced cancer immunotherapy	[44]
DNA nanogel	Two oligo agonists and one antigen peptide delivery	Cancer vaccine	[84]
DNA hydrogel	Tumor infiltrating T lymphocytes isolation, macrophage activation	Cell-based cancer immunotherapy, immune checkpoint blockade	[41]
	Polyvalent PD-1 and CTLA-4 aptamers delivery	Immune checkpoint blockade	[74]
DNA chimera	PD-L1 protein targeting and degradation	Immune checkpoint blockade	[75]

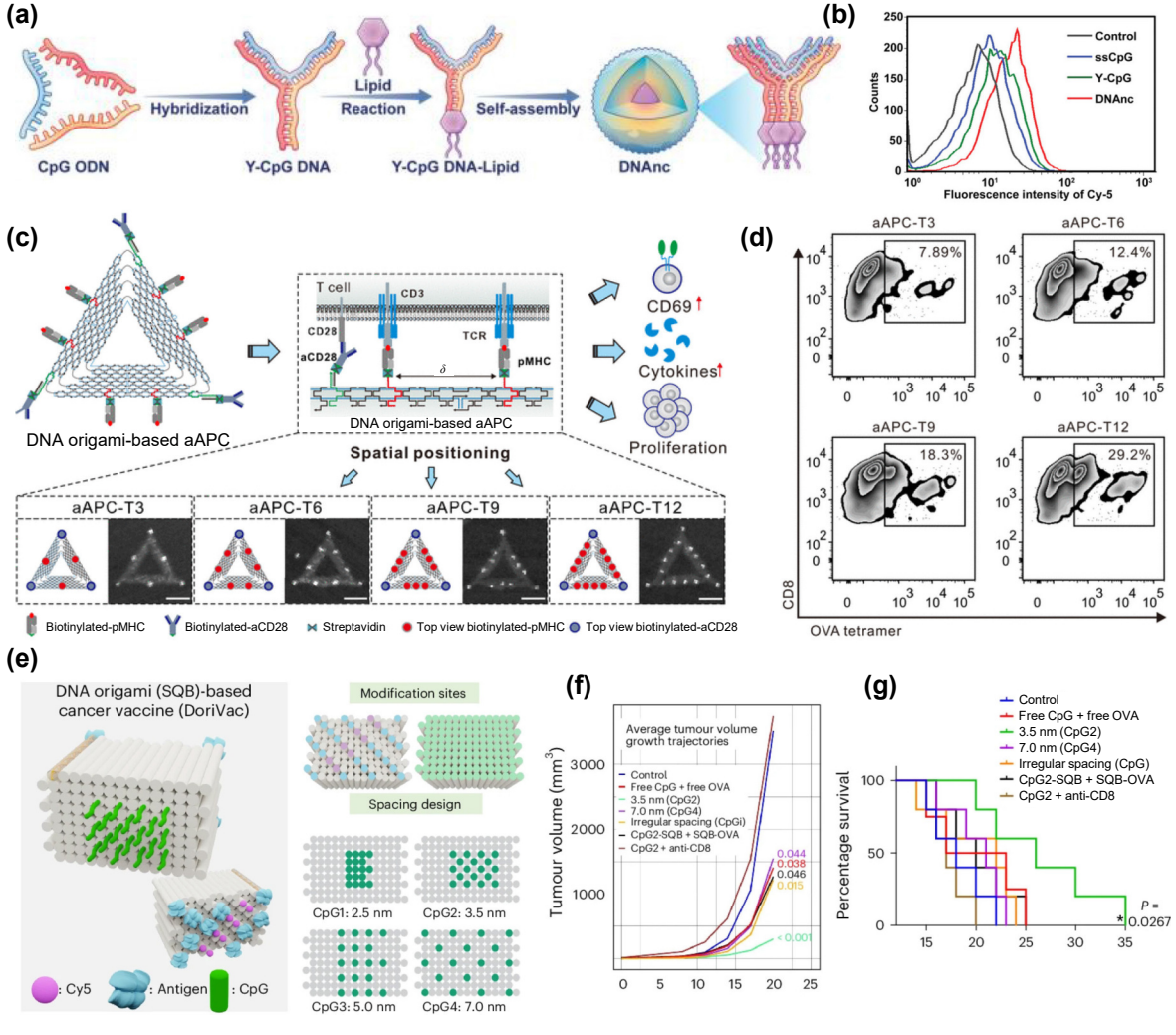

Figure 2 The user-defined DNA nanostructures loading therapeutics for highly efficient cancer immunotherapy. (a) A self-assembled DNA nanocluster with high loading capacity. (b) Cellular uptake of CpG in different formulations. Reproduced with permission from Ref. [44], © Wiley-VCH GmbH 2023. (c) The design of DNA origami-based aAPCs. (d) Flow cytometry analysis of OVA257-264-specific $CD8^+$ T cells stimulated by a series of DNA origami-based aAPCs. Reproduced with permission from Ref. [54], © Sun, Y. Y. et al., some rights reserved; exclusive licensee American Association for the Advancement of Science 2022. (e) The design of DNA origami-based cancer vaccine for fine tuning of CpG spatial distribution. (f) The tumor growth curve of mouse melanoma models treated with DNA origami-based cancer vaccine. (g) The mouse survival curve after treatments. Reproduced with permission from Ref. [57], © Zeng, Y. C. et al., under exclusive licence to Springer Nature Limited 2024.

immunostimulated M1-like phenotype, which was beneficial to enhance the antitumor immunity. The cGAS-STING signalling axis comprised the synthase for the second messenger cyclic GMP-AMP (cGAS) and the cyclic GMP-AMP receptor stimulator of interferon genes (STING), which detects pathogenic DNA to trigger an innate immune reaction involving a strong type I interferon response against microbial infections [45]. TDNs possess the merits of structural stability, cell membrane permeability, and natural biocompatibility as one of the most promising biomedical DNA nanostructures-based materials. Size-tunable DNA tetrahedron (TDN)-based delivery strategy has been developed to activate cGAS-STING signaling pathways [46]. Precisely regulating the size of TDN enables it to load interferon stimulating DNA strands (ISD) with prescribed lengths from 42 to 84 bp, among which the 84 bp-ISD loaded TDNs showed the most significant cGAS-STING pathway activation. This optimized formulation boosted antitumor immune responses presented as stimulating approximately 63% of dendritic cells (DCs) maturation and selected to load 56MESS, a platinum DNA intercalator. Similar to the previous case, 84bp-TDNISD/56MESS treated 4T1 cancer cells internalized slightly more platinum than cells treated by 56MESS alone. These evidences supported that DNA nanostructures with suitable size and physicochemical properties allowed higher delivery efficiency. Despite this, dynamic delivery system was required to overcome the dilemma that nanoparticles in the range of 100 nm favor accumulation of NPs at a tumor site but ultrasmall size assists in tumor penetration [48, 49].

DNA origami is an innovative DNA nanotechnology that use the properties of DNA molecules to create nanostructures with pre-designed specific shapes and functions, invented by Paul Rothemund in 2006 [50]. DNA origami is formed by complementing the bases of a long DNA chain (scaffold chain) with a series of short designed DNA strands (staples strands) at the specific sites, so highly complex nanopatterns or structures can be constructed in a controlled manner. Take advantage of the precise and adjustable properties of DNA origami, researchers used DNA origami to build a cancer vaccine system with precise and controllable size and shape, equipped with tumor antigens and a variety of adjuvants, and to carry out cancer immunotherapy through antigen-specific immune responses. Furthermore, researchers revealed that aptamer-based DNA origami with different geometry has distinct difference in cellular uptake behavior. Rigid tube structure tended to be internalized rapidly while a flexible patch structure preferred to be trapped on the cell surface [51]. Based on this interesting find, a patch-like multivalent formulation was utilized to mediate the interaction of tumor cells and immune cells. With SL1 and XQ-2d aptamers displayed on one side and CLN0020 aptamer on the other side, this formulation facilitated the interaction between MDA-MB-231 (tumor cell) and RAW264.7 (macrophage), consequently decreased the cell viability of tumor cells by 60% *in vitro*. Additionally, researchers demonstrated that a tubular DNA origami carried out significantly more efficient antigen-specific immune responses than the rectangular formulation embedded with same contents, the latter of which may be attributed to degradation of more accessible payload [52].

Inspiringly, addressability endowed DNA origami extraordinary advantages in building a tumor vaccination system with precise valency control [53, 54] and fine spatial distribution for improved immune activation [55–57]. The key of adoptive cell therapy was efficient activation and large-scale expansion of T cells. To address this issue, researchers developed artificial antigen-presenting cells (aAPCs) for stimulating T cell expansion *in vitro*. However, how to accurately construct aAPCs to achieve efficient activation of T cells *in vivo* remained a challenge. Pei et al. used DNA origami nanostructures as two-dimensional scaffolds to regulate the spatial presentation of nanoscale activation ligands to construct efficient aAPC. Specifically, they constructed an aAPC by anchoring costimulatory ligands anti-CD28 antibody (aCD28) at three vertices and displaying T cell receptor (TCR) ligands peptide-major histocompatibility complex (pMHC) at three edges of triangular DNA origami (Fig. 2(c)) [54]. The DNA origami scaffold enabled quantitative analysis of ligand-receptor interactions in T cell activation at the single-particle, single-molecule resolution. With the valency of aCD28 fixed, the pMHC-TCR-binding dwelling time was extended by 2.2 s in a manner that depends on the valency of pMHC, resulting in sequentially promoting T cell activation (Fig. 2(d)). Nanoparticle delivery of CpG molecules with multiple copy numbers was a common approach to develop vaccine adjuvant. However, existing nanoparticles cannot achieve accurate control of CpG space arrangement, and optimized CpG spatial arrangement was of great significance for improving the intensity, duration and polarization of immune response. In response to the above issues, William and his coauthors developed a tumor vaccine development method called DoriVac, which used DNA origami as a platform to precisely regulate the distribution and arrangement of CpG ODNs to enhance cancer vaccine efficacy [57]. Leveraging a square-block (SQB) DNA origami structure, the influence of CpG spacing (2.5 to 7.0 nm) on cancer vaccination was also investigated with nanoscale resolution (Fig. 2(e)). SQB equipped with CpG spaced at 3.5 nm (CpG2) was demonstrated to trigger strongest T helper cell 1 (Th1) polarization. Synergized with ovabumin (OVA), CpG2 exhibited highest performance in the treatment of mouse melanoma and extended the survival period of mice (Figs. 2(f) and 2(g)). In addition, the antigen delivery is another way to enhance immune activation. Mirkin et al. designed and synthesized spherical nucleic acid as a dual-antigen SNA vaccine (DA-SNAs), which carried major histocompatibility complex (MHC)-I restricted and MHC-II restricted antigens at different nanoscale locations [55]. The study found that DA-SNAs were able to significantly increase the activation and proliferation of antigen-specific T cells, compared with single-antigen SNAs. We believed that innovative endeavors in DNA nanotechnology can open a new avenue for personalized and customized cancer immunotherapy by tailoring drug organization to individual characteristics.

2.2 Precise targeting ability

The significance of targeted therapy in the medical field is reflected in the ability to improve drug efficacy, reduce toxicity, improve drug safety and effectiveness, and improve patient compliance [58]. Hence, the development of targeted drugs has become a hot spot in modern drug research. Compared with traditional chemotherapy, targeted therapy has revolutionized cancer treatment by precisely attacking cancer cells with greater therapeutic precision and fewer side effects. Targeted drugs can target specific lesions and accumulate or release active components at the target sites, avoiding damage to normal tissues and cells, thereby reducing side effects. Nucleic acid aptamers are structured oligonucleotide sequences (DNA or RNA) obtained by systematic evolution of ligands by exponential enrichment (SELEX) [59]. Due to the high affinity and

specificity of nucleic acid aptamers, they are taken regard as the chemical antibodies to recognize and bind to specific antigens [60]. Therefore, aptamers are introduced to rational design of DNA nanostructures-based delivery systems for targeted cancer immunotherapy. For example, the AS1411 aptamer can specifically bind to the overexpressed nucleolin on the surface of tumor cells, so it can precisely target tumor cells [61]. Yang et al reported a DNA nanoassembly containing multivalent AS1411 aptamers (DNM) generated by clamped hybridization chain reaction (C-HCR) on the interface of Cas9/sgRNA ribonucleoprotein complex (RNP) to complete the delivery of Cas9/sgRNA RNP and achieve accurate gene editing of β-catenin in tumor cells, thus inhibiting the downstream expression of programmed cell death ligand 1 (PD-L1) to achieve cancer immunotherapy (Fig. 3(a)) [62]. The fluorescence images showed that stronger red fluorescence in carboxytetramethylrhodamine (TAMRA)-DNM-treated tumor cells (4T1 cells) than those of TAMRA-DNM-treated normal cells (BEAS-2B cells) and TAMRA-DNM-nApt-treated (without AS1411 aptamers) tumor cells, indicating that nanoassembly containing multivalent AS1411 aptamers promoted the internalization of DNM by 4T1 cells and further enhanced gene editing efficiency (Fig. 3(b)). Rolling circle amplification (RCA) is an efficient isothermal nucleic acid amplification technique, which is able to synthesize a large number of periodically repeated DNA sequences [63]. Leveraging the technology of RCA, the more interesting design was that a DNA hydrogel network was constructed by integrating programmed death receptor 1 (PD-1) aptamer into ultralong single-stranded DNA chains, which can efficiently, specifically and nondestructively isolate T cells from complex systems, such as tumor tissues (Fig. 3(c)) [41]. The captured cell numbers by two different DNA networks (containing or non-containing poly-aptamers) showed significant differences, demonstrating the specificity of the PD-1 containing DNA network to capture T cells (Fig. 3(d)). Additionally, the majority of captured cells in PD-1 containing DNA network were T cells, indicating the high efficiency of this cell capture system (Fig. 3(e)). This PD-1 poly-

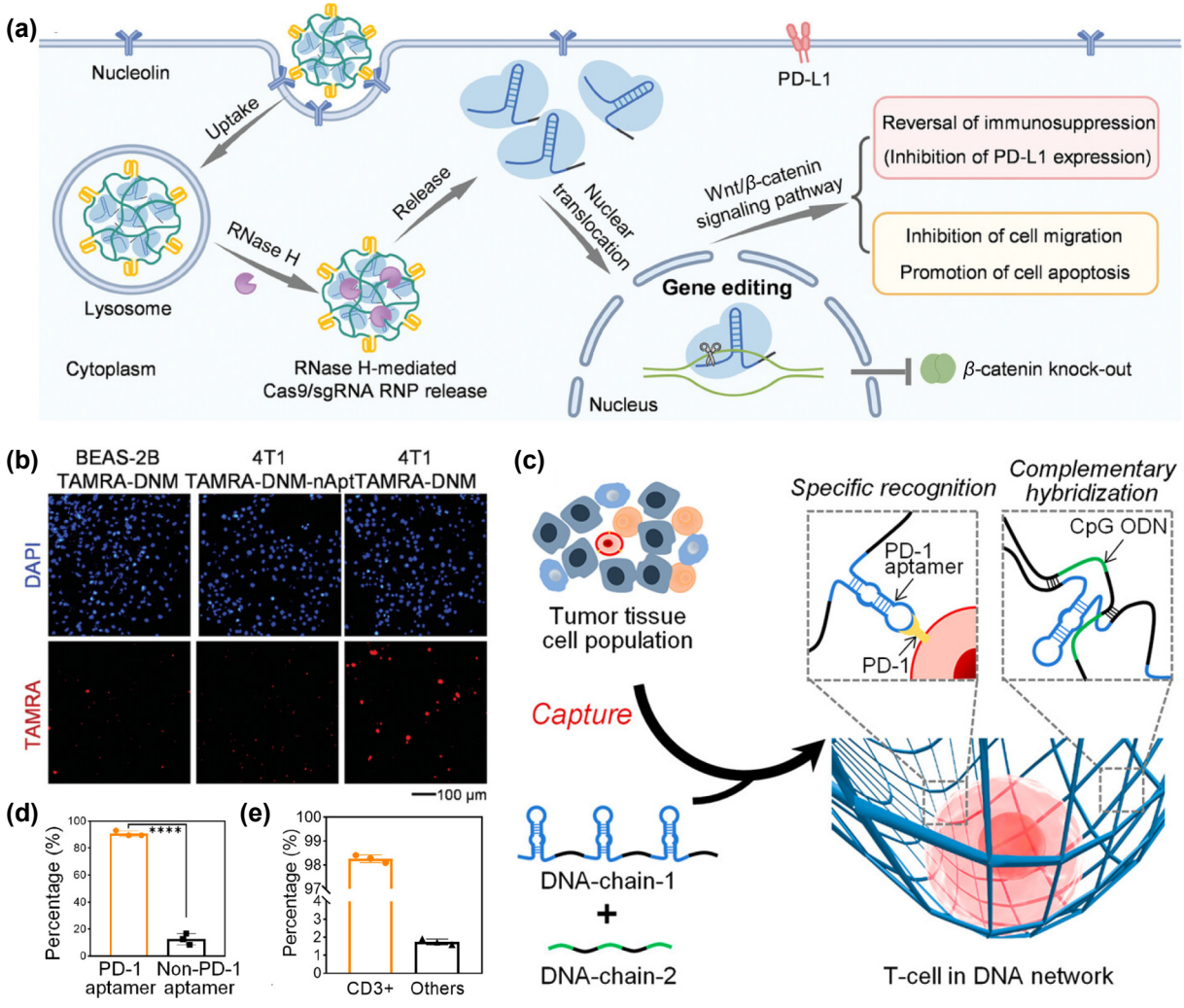

Figure 3 Targeting ability of DNA nanostructures-based materials. (a) A schematic illustration of the nanoassembly containing polyvalent AS1411 aptamers to delivery clustered regularly interspaced short palindromic repeats (CRISPR)-Cas 9 system for targeting gene editing. (b) The fluorescence images of normal cells (BEAS-2B) and cancer cells (4T1) incubated with DNA nano-delivery system mentioned in (a), respectively. Cell nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI), and DNA nano-delivery system was labelled by TAMRA. Reproduced with permission from Ref. [62], © Wiley-VCH GmbH 2023. (c) A schematic representation of a poly-aptamer DNA hydrogel network for the specific and efficient isolation of T cells from tumor tissue to further achieve cancer immunotherapy. (d) The captured cell percentage of PD-1 aptamers-containing DNA network and non-aptamers-containing DNA network. Statistical analysis was performed by the unpaired two-tailed *t* test. ****$P < 0.0001$. (e) The proportion of T cells in the total captured cells. Reproduced with permission from Ref. [41], © American Chemical Society 2021.

aptamers containing DNA hydrogel capturing T cells was further injected into tumor lesions to release T cells and CpG in response to tumor inflammatory environment, and the localized immunotherapy efficacy on the model of mouse melanoma was remarkable. This RCA-based DNA hydrogel provided a feasible platform for the delivery of living cells and other immunotherapeutics. Moreover, because DNA is easily modified, functional groups can be modified to endow materials with diverse natures. For example, to enhance the targeting ability, targeting peptides were modified on the DNA-based delivery system [55, 64].

The immune checkpoint blockade has attracted much attention on clinical cancer immunotherapy, and has also exhibited encouraging results for cancer immunotherapy [65–67]. Immune checkpoint inhibitors block the interaction of immune checkpoints and their ligands to relieve immune cells from suppression in tumor microenvironment, exerting anti-tumor effects. Therefore, the targeting ability of immune checkpoint inhibitors is one of the keys of therapeutic efficacy. At present, two strategies are widely used to achieve immune checkpoint blockade. One is that the inhibitors compete with original ligands to bind to the specific immune checkpoints, resulting in blocking the original pathway and signal communication, represented by PD-1/PD-L1 inhibitors (Nivolumab) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) inhibitors (ipilimumab) [68]. The other one is to down-regulate or degrade the immune checkpoint-related proteins by means of gene silencing [69], gene editing [70], and targeted protein degradation technology [71, 72]. Based on the first strategy and the excellent targeting ability of DNA aptamers, the researchers designed and constructed a bispecific aptamer for the PD-1/ PD-L1 axis blockade through the connection of PD-1 and PD-L1 aptamer, inducing a potent immunological effect against tumor cells [73]. However, this bispecific aptamer was easily to be digested by nucleases in the physiological environment, and the response to single immune checkpoint inhibition was limited. To address these concerns, Yang et al. synthesized a responsive DNA hydrogel containing polyvalent PD-1 and CTLA-4 aptamers as both a dual-target inhibitor and an inhibitor carrier [74]. In the tumor inflammatory microenvironment, this DNA hydrogel was responsively cleaved into numerous PD-l and CTLA-4 aptamers, which simultaneously blocked the PD-1 and CTLA-4 on the membrane of T cells to enhance the immune functions of T cells (Fig. 4(a)). Based on the SELEX technology, aptamers for different targets can be screened, and integrating aptamers into DNA hydrogels enables to load target inhibitors and resist nucleases degradation. This strategy suggested a great potential in building a database to construct bispecific or multiple-specific immunotherapeutic medicines and promoted the comprehensive clinical application in cancer treatment. Additionally, to deplete the immune checkpoint associated protein PD-L1 on the surface of tumor cells, Liu and his co-authors designed a DNA aptamer-based chimera structure that connected an aptamer capable of targeting the PD-L1 protein on the surface of tumor cells with an aptamer targeting the lysosomal shuttle receptor CI-M6PR to construct a covalent lysosome-targeting chimera (LYTAC) platform (Fig. 4(b)) [75]. While the PD-L1 part of the chimera was targeting cells, the PD-L1 protein on the surface of tumor cells were hijacked into the lysosome by the CI-M6PR part through the lysosomal shuttle receptor-mediated pathway to achieve the degradation of PD-L1 protein, resulting in reducing the expression of PD-L1 protein on tumor cell membrane, and then improving the killing of tumor cells by T cells and enhancing the effect of cancer immunotherapy. In mouse models of CT26 tumor, it was found that the tumor was refractory to PD-L1 antibody blockade therapy, while the PD-L1 degradation triggered by covalent LYTAC significantly reduced the tumor burden (Figs. 4(c) and 4(d)). To confirm the mechanism, the PD-L1 levels in tumor tissues compared to the PD-L1 mAb after treatment were checked. The results showed that PD-L1 mAb failed to downregulate the PD-L1 expression, while about half of PD-L1 was removed by covalent LYTAC (Fig. 4(e)). Furthermore, non-selective suppression of immune checkpoint proteins in all cell types enables the immune system to attack tumor and non-tumor cells indiscriminately, resulting in immune-related adverse events (irAEs) that can affect almost all organ systems and even have fatal toxicity, which is a challenge that cannot be ignored and needs to be addressed urgently. To address this issue, Liu et al reported a DNA "safety catch" made of nanostring (DNS) as an ICB reagent to achieve cell-selective PD-L1 inhibition [76]. Firstly, "safety catch" attached to PD-L1 on the cell surface by DNA aptamers, which was at the "safety on" status. The composition of cancer cell membranes was different from that of normal cell membranes, which can be used as a natural barrier to distinguish them and work as a stimulus signal to manipulate the accessibility of ICB reagent. For cancer cells, one of the specific membrane receptor proteins, cell-mesenchymal epithelial transforming factor (c-Met), which acted as an activation trigger to convert the safety catch into a "safety off" status and restore the accessibility of the ICB reagent to PD-L1. By controlling the accessibility of ICB reagent to PD-L1, selective PD-L1 inhibition of cancer cells was realized, resulting in the enhanced specifically targeting cancer immunotherapy. The above-mentioned works offered novel molecular tools for the construction of targeting immune checkpoint blockade, providing valuable insights in pushing DNA nanostructures-based drugs and drug delivery systems toward comprehensive clinical applications for cancer immunotherapy. With the development of molecular biology technology, the research and application of targeted therapy will continue to be deepened, providing more effective and safer treatment options for cancer patients.

2.3 Controllable release

The controllable release of immunotherapeutics at targeted sites is another obstacle to limit cancer immunotherapy. Due to the complicated environment in the body, drug vehicles may highly likely gradually release the loading therapeutics before reaching tumor tissue, resulting in insufficient payload upon encountering intended targets as well as off-target toxicity [77]. Stimulus-responsive drug release means that the drug delivery system can respond to specific internal and external environmental stimuli to release the loading drugs, such as pH, temperature, light, magnetic field, and biochemical substances, which achieves efficient delivery of drugs to specific patient sites, when needed. DNA nanostructures incorporated with stimulus-responsive modules can undergo disassembly/reassembly in response to a unique signal input in TME or TME-associated cells, such as pH [78], ATP [79, 80], enzymes [26, 81, 82], and tumor-related biomarkers [83, 85]. Therefore, DNA nanostructures-based drug delivery system is a powerful platform allowing programmed drug deployment in specific sites for improved immunotherapy and minimally unexpected side effects. In this part, the design of DNA nanostructures for stimulus-responsive drug release will be highlighted.

The inflammatory tissues, characterized by low pH, oxidative stress, and overexpressed enzymes, also as one of the key

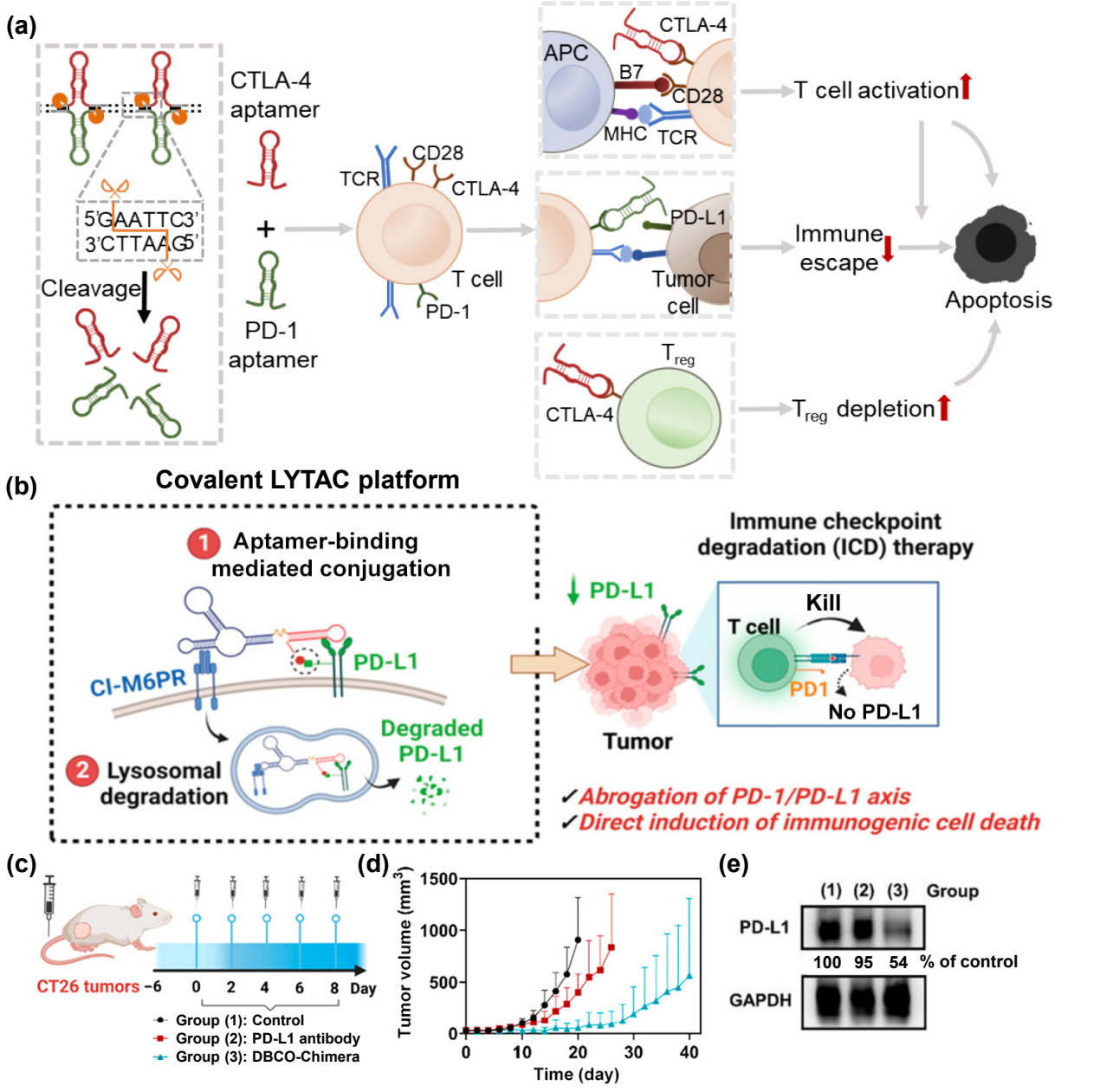

Figure 4 Targeting DNA nanostructures-based materials for immune checkpoint blockade. (a) Schematic illustration of the responsive DNA hydrogel with the function of dual-target immune checkpoint inhibition. Reproduced with permission from Ref. [74], © Wiley-VCH GmbH 2024. (b) A covalent LYTAC platform based on DNA chimera for immune checkpoint degradation therapy. (c) The timeline of treatment procedure for B16F10 tumor-bearing C57BL/6mice. (d) The tumor volume curves of CT26 tumor-bearing BALB/c mice after different treatments. (e) The PD-L1 level in tumor tissue after treatments analyzed by western blot. Reproduced with permission from Ref. [75], © American Chemical Society 2023.

characteristics of the TME, has been extensively targeted as a trigger for site-selectively drug release with controlled manner [86–88]. An innovative carrier (DNA nano-cocoons, DNCs) loaded with HhaI caged into triglycerol monostearate (TGMS) and anti-PD-1 antibody (aPD1) was assembled by synthesized ultralong single-stranded DNA containing repeated CpG sequences and cutting sites of restriction enzyme HhaI (Fig. 5(a)) [38]. TGMS was cleaved by proteolytic enzymes in the inflammation environment, thereby releasing encapsulated HhaI, which sequentially released CpG and aPD1 through fragmentation of DNCs, while DNCs were not able to degraded in PBS or non-inflammation environment (Figs. 5(b) and 5(c)). Compared to free CpG and aPD1, controllably released CpG and aPD1 elicited enhanced antitumor immune response in incomplete tumor resection model and tumor metastasis model of B16F10 mouse melanoma. This inflammation-triggered DNCs offered a flexible strategy to controllably delivery immunotherapeutic drugs. Interestingly, DNA nanostructures underwent conformational changes in respond to subcellular stimulus, also contributing to avoid undesired immunogenicity. Ding et al. reported a reconfigurable tubular DNA origami as a cancer vaccine to transport tumor antigen peptide (e.g. OVA) and Toll-like receptor (TLR) agonists within its inner cavity to draining lymph nodes (dLNs) [52]. The researchers selected OVA covalently coupling with single-stranded DNA, and selected nucleic acid immune adjuvant double-stranded RNA and CpG for immune pathway receptors TLR3 and TLR9 located in the endosomes of

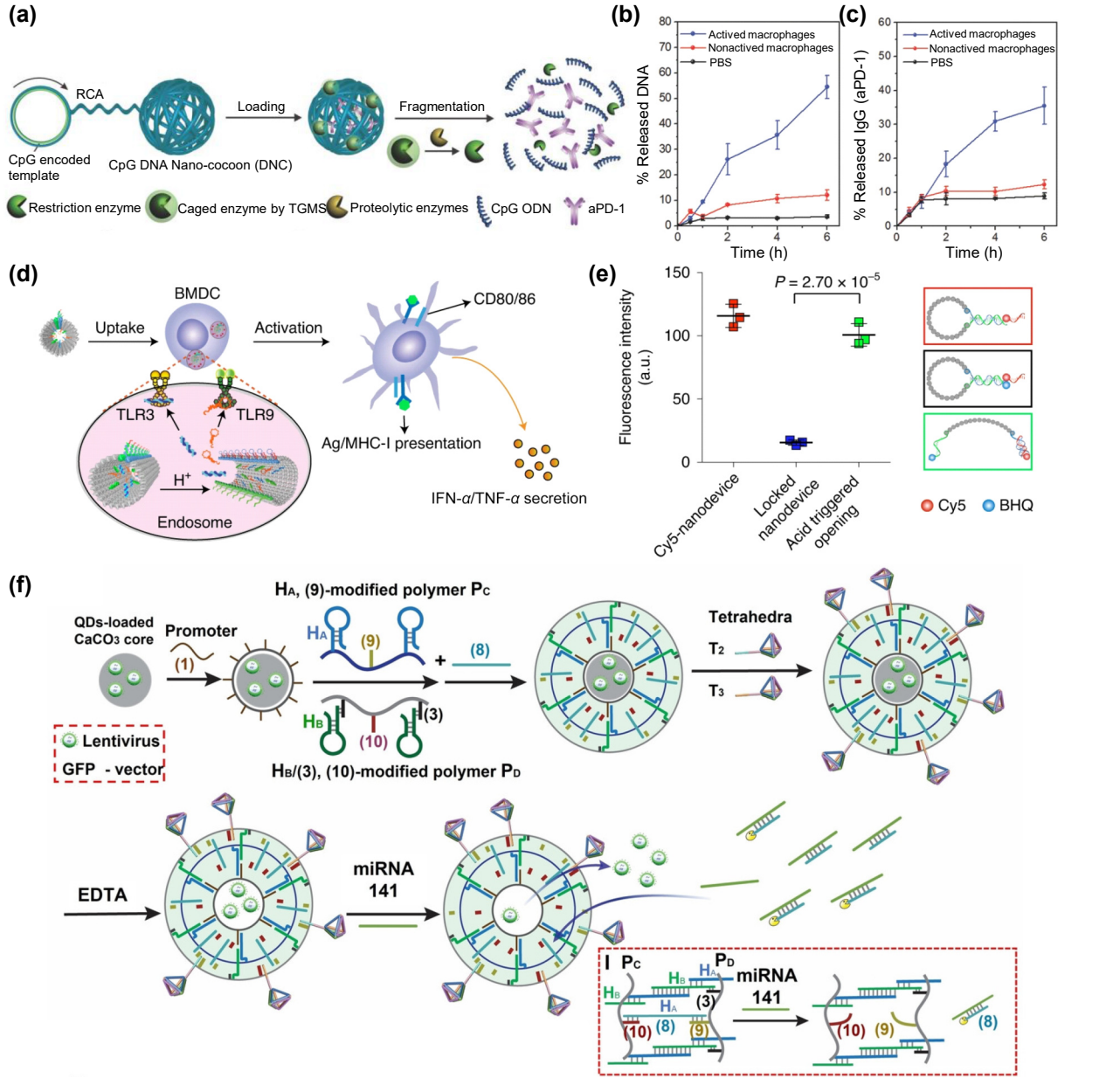

Figure 5 The release of immunotherapeutics loaded in DNA nanostructures-based delivery system responded to endogenous stimulus. (a) DNA "nano-cocoons" loaded with CpG and aPD1 disassembled in response to inflammatory tumor microenvironment. Releasing profile of (b) CpG and (c) aPD1 in different milieu. Reproduced with permission from Ref. [38], © WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 2016. (d) A DNA origami-based cancer vaccine undergoing conformation transformation induced by H^+ in the endosomal of DC to release Toll-like receptor agonists. (e) The fluorescence signals of CpG in lymph nodes after treated with free CpG and DNA nanodevices. Reproduced with permission from Ref. [52], © Springer Nature 2020. (f) The illustration of a customizable hydrogel microcapsules responding to miRNA-141. Reproduced with permission from Ref. [89], © Fischer, A. et al., Angewandte Chemie International Edition published by Wiley-VCH GmbH 2023.

immune cells (Fig. 5(d)). These immunotherapeutics were site-directedly and quantitatively loaded inside the DNA origami-based nanomachines through hybridization of nucleic acid molecules. Acid-responsive DNA molecular locks were designed to seal the DNA structures, forming a completely closed DNA nanomachine to protect the internal immune functional components. To verify the effectiveness of the DNA molecular lock, researchers used a fluorophore-quencher pairs to detect the change of fluorescent signal. When this DNA molecular lock was exposed to acidic conditions (pH 5.5), the increased fluorescent signal demonstrated the responsive opening of the nanodevice (Fig. 5(e)). When entered into dendritic cells in lymph nodes, and triggered by the acidic endosomal environment, allosteric DNA locks reassembled and unmasked the active payloads, stimulating the activation of dendritic cells and antigen presentation, inducing antigen-specific immune response, and killing tumor cells effectively. Tumor-related biomarkers are produced during tumor progression, which can be used as triggers for designing intelligent DNA nanostructures to achieve precise control over drug release. Willner et al. fabricated a stimuli-responsive hydrogel microcapsules encapsulating lentivirus to selectively stimulate an immune response in TME [89] (Fig. 5(f)). The surface of microcapsules was functionalized by HCR to release the immunogenetic content through recognizing H^+, alpha-fetoprotein (AFP) or miRNA-141. This sophisticated vector

provides a versatile and customizable drug delivery system for targeted immunotherapy applications. Chang et al. developed a smart tumor cell-derived anchored non-linear hybridization chain reaction (Panel-HCR) strategy for on-demand regulation of TAMs [83]. This Panel-HCR consisted two modules, including a recognition-then-assembly module and a release-then-regulation module. Upon recognizing tumor cells, DNA nano-trees were assembled on the tumor cell surface and released by-product chains loaded with CpG ODN depending on tumor cell concentration. The on-demand release of CpG-ODN can effectively regulate the M2-TAM to M1 phenotype by stimulating TLR9 to activate the nuclear factor kappa-B (NF-κB) pathway and increase inflammatory cytokines. This proposed strategy held a great promise for on-demand anti-tumor immunotherapy.

Alternatively, immune drug release can be designed as the follow-up treatment of chemotherapy, radiotherapy or photodynamic therapy (PDT) to enhance the synergistic effects of combination therapy. The heart of this strategy lies on ensuring that the therapeutics are released in the proper dose at the desirable time and sites after other treatments. An example for this was an alginate (ALG)-based hydrogel *in situ* formed in the presence of calcium ions in solid tumors [90]. The side chains of ALG were coupled with ATP-specific aptamers (Aapt) hybridized with CpG ODN. Once systemic chemotherapy with oxaliplatin (OxPt) or X-ray-triggered local radiotherapy induced immunogenic cell death (ICD) of tumor cells, releasing enormous ATP from dying cells that displaced CpG ODNs from Aapt due to the higher binding energy between ATP and Aapt, so as to the released CpG boosted the antitumor immunotherapy (Fig. 6(a)). Given that the imaging data *in vivo*, CpG ODNs assembled in hydrogel were able to retain in

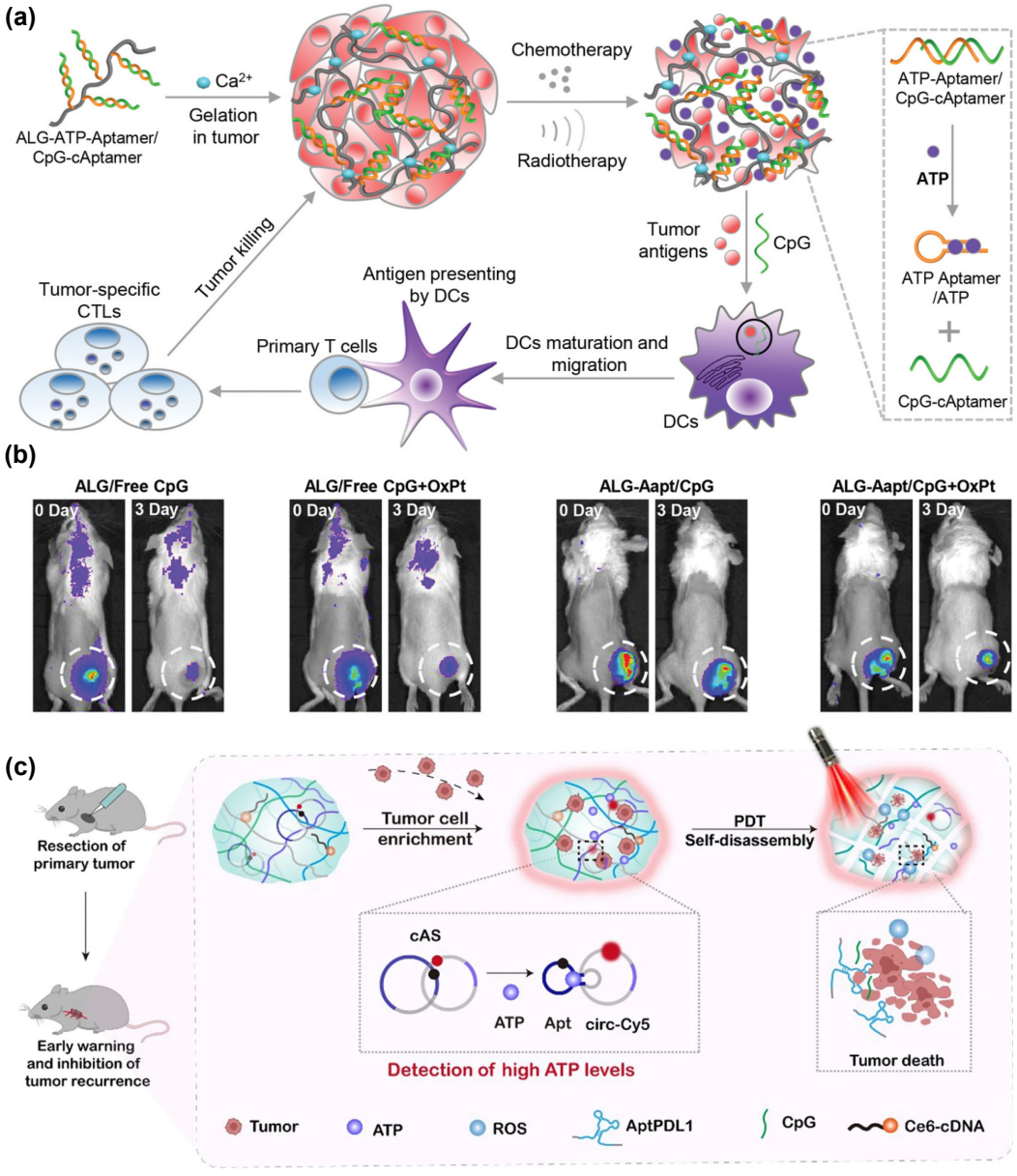

Figure 6 Stimulus-responsive release of immunotherapeutics after other treatment methods. (a) An intelligent hydrogel releasing CpG as the follow-up treatment of chemotherapy or radiotherapy. (b) The accumulation of CpG at the tumor site after different treatments analyzed by imaging data *in vivo*. Reproduced with permission from Ref. [90], © Wiley-VCH GmbH 2021. (c) A multifunctional hydrogel self-disassembled in the trigger of ROS produced by PDT after local ATP concentration increasing for the detection and inhibition of postoperative tumor recurrence. Reproduced with permission from Ref. [91], © Wang, D. Y. et al. 2023.

the tumors and released in response to the OxPt or X-ray treatment, while the free ones were rapidly released (Fig. 6(b)). Powered by the ability to release immune adjuvants in response to repeatedly applied chemotherapeutics or radiation treatments, this intelligent hydrogel held great clinical significance. DNA hydrogel integrated with cascade stimulus-responsive reaction was also devised to achieve the detection and inhibition of postoperative tumor recurrence [91] (Fig. 6(c)). The DNA hydrogel constructed by RCA reaction was functionalized with PD-L1 aptamers, ATP sensor (cAS), photosensitizer Ce6 and CpG ODNs. Relapsed tumor cells enriched by PD-L1 aptamers elevated local ATP levels in DNA hydrogel. When cAS sensed such change and transmitted warning signal, PDT was performed. Generated reactive oxygen species (ROS) by PDT triggered the self-disassembly of hydrogel, releasing PD-L1 aptamer and CpG that exerted ICB and enhanced immune response. This spatiotemporally controlled system showed an ultimate inhibition rate of approximately 88.1% against recurrent tumors in a mice melanoma model, which provides a promising way for timely and effective treatment of postoperative tumor recurrence.

In addition to the above-mentioned strategies, emerging targets, such as mechanical phenotypes, are increasingly being used in the design of drug delivery [92, 93]. Furthermore, the combination of different stimuli triggering strategy is necessary for spatiotemporally controlled drug release to adapt to the dynamic and heterogeneous TME.

3 Summary and future perspectives

Although cancer immunotherapy has exhibited a profound impact on clinical applications, the therapeutic efficacy is still limited by chronic issues. Among them, the efficiency of drug delivery is an urgent problem to be solved. In this review, these studies demonstrate the potential of DNA nanostructures-based delivery systems for cancer immunotherapy, particularly in improving drug delivery efficiency, including enhancing drug loading efficacy, targeting ability, and controllable release.

We are also aware of other immunotherapy approaches based on DNA nanostructures. Using DNA nanostructures to manipulate the distribution of proteins on the surface of immune cells [94, 95] or to regulate the interactions between immune cells and tumor cells [96–98] can effectively regulate the activation of immune cells, thereby promoting immune responses. This approach leverages the precise control over the structure and surface chemistry of DNA nanostructures, which can be designed to specifically target and interact with immune cells in a controlled manner. Based on the addressability of DNA origami and the specific hybridization of DNA, Tan and Qiu et al. reported a series of DNA nanoconnections (DNJS) with controllable sizes to precisely regulate the membrane spacing at the interface of antigen-presenting cells and T cells, which is potential to the research of T cells immune responses [99]. Additionally, DNA-based nanosystems were reported to realize efficient and enhanced mRNA transfection in cells [31, 100]. These works boosted the applications of mRNA nanodelivery systems in promoting the functions of immune cells for enhanced cancer immunotherapy. In the context of novel glycan-based immune checkpoints, the application of DNA nanostructures to form chimeras with checkpoint blockade-like properties has emerged as a promising approach for developing new immunotherapeutic drugs and drug delivery vehicles [101]. With the advances of DNA nanotechnology, these systems are expected to provide more therapeutic options for cancer treatment. We will continue to pay attention to these developing technologies.

Overall, DNA nanostructured delivery systems have great potential to enhance cancer immunotherapy, and dozens of studies have demonstrated their ability to effectively load therapeutics, improve tumor tissue targeting, enhance cargo internalization, and control stimulus-responsive drug release. In addition, recent combination approaches have shown even better results, producing encouraging results for a variety of cancer types that are often difficult to manage. These innovative strategies require in-depth testing for further clinical applications. Despite these advantages for drug delivery, DNA nanostructures still face several challenges in clinical translation, including stability, yield, the presence of foreign DNA, and ethical considerations. At present, only a small amount of work has systematically evaluated the biosafety of DNA materials. For example, Yang et al. evaluated the pharmacokinetics, immunogenicity and immunotoxicity of TDN and DNA polymeric nanostructures (DNP), proving that TDN and DNP were safe for biological systems [102], so further researches about the biosafety assessment of other DNA nanostructures-based materials are needed in this area. Chemical modifications can be used to improve the structural stability of DNA nanostructures, such as PEGylation, and other biopolymers coating, or incorporation of stabilizing motifs, which is also a strategy to reduce immunogenicity of DNA nanostructures, improve their circulation time and tumor targeting capacity. A large amount of biomass DNA exists in nature, if it can be utilized, it will greatly reduce the production cost of DNA material, which is conducive to downstream applications. Moreover, scalable production techniques, including automated synthesis and assembly methods, could address cost and reproducibility concerns, paving the way for clinical applications.

Data availability

Not applicable.

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Declaration of competing interest

All the contributing authors report no conflict of interests in this work.

Author contribution statement

R. Z.: Figure curation, writing manuscript. T. L. X.: Figure curation, writing manuscript. D. Y. Y.: Project administration, funding acquisition, writing manuscript.

Informed consent

Not applicable.

Ethics statement

Not applicable.

Use of AI statement

None.

References

[1] Siegel, R. L.; Miller, K. D.; Fuchs, H. E.; Jemal, A. Cancer statistics, 2022. *CA Cancer J. Clin.* **2022**, *72*, 7–33.

[2] Saxena, M.; van der Burg, S. H.; Melief, C. J. M.; Bhardwaj, N. Therapeutic cancer vaccines. *Nat. Rev. Cancer* **2021**, *21*, 360–378.

[3] Ott, P. A.; Hu, Z. T.; Keskin, D. B.; Shukla, S. A.; Sun, J.; Bozym, D. J.; Zhang, W. D.; Luoma, A.; Giobbie-Hurder, A.; Peter, L. et al. An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature* **2017**, *547*, 217–221.

[4] Mellman, I.; Coukos, G.; Dranoff, G. Cancer immunotherapy comes of age. *Nature* **2011**, *480*, 480–489.

[5] Colley, A.; Brauns, T.; Sluder, A. E.; Poznansky, M. C.; Gemechu, Y. Immunomodulatory drugs: A promising clinical ally for cancer immunotherapy. *Trends Mol. Med.* **2024**, *30*, 765–780.

[6] Propper, D. J.; Balkwill, F. R. Harnessing cytokines and chemokines for cancer therapy. *Nat. Rev. Clin. Oncol.* **2022**, *19*, 237–253.

[7] Dranoff, G. Cytokines in cancer pathogenesis and cancer therapy. *Nat. Rev. Cancer* **2004**, *4*, 11–22.

[8] Sharma, P.; Goswami, S.; Raychaudhuri, D.; Siddiqui, B. A.; Singh, P.; Nagarajan, A.; Liu, J. L.; Subudhi, S. K.; Poon, C.; Gant, K. L. et al. Immune checkpoint therapy-current perspectives and future directions. *Cell* **2023**, *186*, 1652–1669.

[9] Peng, L.; Sferruzza, G.; Yang, L. J.; Zhou, L. Q.; Chen, S. D. CAR-T and CAR-NK as cellular cancer immunotherapy for solid tumors. *Cell. Mol. Immunol.* **2024**, *21*, 1089–1108.

[10] Huang, X.; Williams, J. Z.; Chang, R.; Li, Z. B.; Burnett, C. E.; Hernandez-Lopez, R.; Setiady, I.; Gai, E.; Patterson, D. M.; Yu, W. et al. DNA scaffolds enable efficient and tunable functionalization of biomaterials for immune cell modulation. *Nat. Nanotechnol.* **2020**, *16*, 214–223.

[11] Yang, J. W.; Chen, Y. M.; Jing, Y.; Green, M. R.; Han, L. Advancing CAR T cell therapy through the use of multidimensional omics data. *Nat. Rev. Clin. Oncol.* **2023**, *20*, 211–228.

[12] Lin, M. J.; Svensson-Arvelund, J.; Lubitz, G. S.; Marabelle, A.; Melero, I.; Brown, B. D.; Brody, J. D. Cancer vaccines: The next immunotherapy frontier. *Nat. Cancer* **2022**, *3*, 911–926.

[13] Riley, R. S.; June, C. H.; Langer, R.; Mitchell, M. J. Delivery technologies for cancer immunotherapy. *Nat. Rev. Drug Discov.* **2019**, *18*, 175–196.

[14] Banchereau, J.; Palucka, A. K. Dendritic cells as therapeutic vaccines against cancer. *Nat. Rev. Immunol.* **2005**, *5*, 296–306.

[15] Zhu, G. Z.; Zhang, F. W.; Ni, Q. Q.; Niu, G.; Chen, X. Y. Efficient nanovaccine delivery in cancer immunotherapy. *ACS Nano* **2017**, *11*, 2387–2392.

[16] Karki, R.; Kanneganti, T. D. The 'cytokine storm': Molecular mechanisms and therapeutic prospects. *Trends Immunol.* **2021**, *42*, 681–705.

[17] Cabral, H.; Li, J. J.; Miyata, K.; Kataoka, K. Controlling the biodistribution and clearance of nanomedicines. *Nat. Rev. Bioeng.* **2023**, *2*, 214–232.

[18] Ma, W. J.; Zhan, Y. X.; Zhang, Y. X.; Mao, C. C.; Xie, X. P.; Lin, Y. F. The biological applications of DNA nanomaterials: Current challenges and future directions. *Signal Transduct. Target. Ther.* **2021**, *6*, 351.

[19] Lee, H.; Lytton-Jean, A. K. R.; Chen, Y.; Love, K. T.; Park, A. I.; Karagiannis, E. D.; Sehgal, A.; Querbes, W.; Zurenko, C. S.; Jayaraman, M. et al. Molecularly self-assembled nucleic acid nanoparticles for targeted *in vivo* siRNA delivery. *Nat. Nanotechnol.* **2012**, *7*, 389–393.

[20] Jani, M. S.; Veetil, A. T.; Krishnan, Y. Precision immunomodulation with synthetic nucleic acid technologies. *Nat. Rev. Mater.* **2019**, *4*, 451–458.

[21] Iinuma, R.; Ke, Y. G.; Jungmann, R.; Schlichthaerle, T.; Woehrstein, J. B.; Yin, P. Polyhedra self-assembled from DNA tripods and characterized with 3D DNA-PAINT. *Science* **2014**, *344*, 65–69.

[22] Goodman, R. P.; Schaap, I. A. T.; Tardin, C. F.; Erben, C. M.; Berry, R. M.; Schmidt, C. F.; Turberfield, A. J. Rapid chiral assembly of rigid DNA building blocks for molecular nanofabrication. *Science* **2005**, *310*, 1661–1665.

[23] Chu, Z. Y.; Wang, W. N.; Zheng, W.; Fu, W. Y.; Wang, Y. J.; Wang, H.; Qian, H. S. Biomaterials with cancer cell-specific cytotoxicity: Challenges and perspectives. *Chem. Soc. Rev.* **2024**, *53*, 8847–8877.

[24] Dai, Y. L.; Xu, C.; Sun, X. L.; Chen, X. Y. Nanoparticle design strategies for enhanced anticancer therapy by exploiting the tumour microenvironment. *Chem. Soc. Rev.* **2017**, *46*, 3830–3852.

[25] Xie, S. T.; Sun, W. D.; Fu, T.; Liu, X. S.; Chen, P.; Qiu, L. P.; Qu, F. L.; Tan, W. H. Aptamer-based targeted delivery of functional nucleic acids. *J. Am. Chem. Soc.* **2023**, *145*, 7677–7691.

[26] Li, F.; Song, N. C.; Dong, Y. H.; Li, S.; Li, L. H.; Liu, Y. J.; Li, Z. M.; Yang, D. Y. A proton-activatable DNA-based nanosystem enables co-delivery of CRISPR/Cas9 and DNAzyme for combined gene therapy. *Angew. Chem., Int. Ed.* **2022**, *61*, e202116569.

[27] Guo, X. C.; Li, F.; Liu, C. X.; Zhu, Y.; Xiao, N. N.; Gu, Z.; Luo, D.; Jiang, J. H.; Yang, D. Y. Construction of organelle-like architecture by dynamic DNA assembly in living cells. *Angew. Chem., Int. Ed.* **2020**, *59*, 20651–20658.

[28] Sun, W. J.; Jiang, T. Y.; Lu, Y.; Reiff, M.; Mo, R.; Gu, Z. Cocoon-like self-degradable DNA nanoclew for anticancer drug delivery. *J. Am. Chem. Soc.* **2014**, *136*, 14722–14725.

[29] Li, F.; Liu, Y. J.; Dong, Y. H.; Chu, Y. W.; Song, N. C.; Yang, D. Y. Dynamic assembly of DNA nanostructures in living cells for mitochondrial interference. *J. Am. Chem. Soc.* **2022**, *144*, 4667–4677.

[30] Qian, R. C.; Zhou, Z. R.; Guo, W. J.; Wu, Y. T.; Yang, Z. L.; Lu, Y. Cell surface engineering using DNAzymes: Metal ion mediated control of cell-cell interactions. *J. Am. Chem. Soc.* **2021**, *143*, 5737–5744.

[31] Lv, Z. Y.; Huang, M. X.; Yang, J.; Li, P. R.; Chang, L. L.; Tang, Q. Y.; Chen, X. J.; Wang, S. Q.; Yao, C.; Liu, P. F. et al. A smart DNA-based nanosystem containing ribosome-regulating siRNA for enhanced mRNA transfection. *Adv. Mater.* **2023**, *35*, 2300823.

[32] Li, J.; Zheng, C.; Cansiz, S.; Wu, C. C.; Xu, J. H.; Cui, C.; Liu, Y.; Hou, W. J.; Wang, Y. Y.; Zhang, L. Q. et al. Self-assembly of DNA nanohydrogels with controllable size and stimuli-responsive property for targeted gene regulation therapy. *J. Am. Chem. Soc.* **2015**, *137*, 1412–1415.

[33] Wang, Z. R.; Song, L. L.; Liu, Q.; Tian, R.; Shang, Y. X.; Liu, F. S.; Liu, S. L.; Zhao, S.; Han, Z. H.; Sun, J. S. et al. A tubular DNA nanodevice as a siRNA/chemo-drug co-delivery vehicle for combined cancer therapy. *Angew. Chem., Int. Ed.* **2021**, *60*, 2594–2598.

[34] Li, Q.; Liu, L. F.; Mao, D. K.; Yu, Y. Y.; Li, W. L.; Zhao, X. F.; Mao, C. D. ATP-triggered, allosteric self-assembly of DNA nanostructures. *J. Am. Chem. Soc.* **2020**, *142*, 665–668.

[35] Song, P.; Ye, D. K.; Zuo, X. L.; Li, J.; Wang, J. B.; Liu, H. J.; Hwang, M. T.; Chao, J.; Su, S.; Wang, L. H. et al. DNA hydrogel with aptamer-toehold-based recognition, cloaking, and decloaking of circulating tumor cells for live cell analysis. *Nano Lett.* **2017**, *17*, 5193–5198.

[36] Gong, H. S.; Zhang, Y. H.; Xue, Y.; Fang, B. W.; Li, Y. T.; Zhu, X. D.; Du, Y.; Peng, P. NETosis-inspired cell surface-constrained framework nucleic acids traps (FNATs) for cascaded extracellular recognition and cellular behavior modulation. *Angew. Chem., Int. Ed.* **2024**, *63*, e202319908.

[37] Wang, J.; Li, Y. Y.; Nie, G. J. Multifunctional biomolecule nanostructures for cancer therapy. *Nat. Rev. Mater.* **2021**, *6*, 766–783.

[38] Wang, C.; Sun, W. J.; Wright, G.; Wang, A. Z.; Gu, Z. Inflammation-triggered cancer immunotherapy by programmed delivery of CpG and anti-PD1 antibody. *Adv. Mater.* **2016**, *28*, 8912–8920.

[39] Liu, M. T.; Hao, L. Y.; Zhao, D.; Li, J. J.; Lin, Y. F. Self-assembled immunostimulatory tetrahedral framework nucleic acid vehicles for tumor chemo-immunotherapy. *ACS Appl. Mater. Interfaces* **2022**, *14*, 38506–38514.

[40] Wu, Y. N.; Li, Q. Y.; Shim, G.; Oh, Y. K. Melanin-loaded CpG DNA hydrogel for modulation of tumor immune microenvironment. *J. Control. Release* **2021**, *330*, 540–553.

[41] Yao, C.; Zhu, C. X.; Tang, J. P.; Ou, J. H.; Zhang, R.; Yang, D. Y. T lymphocyte-captured DNA network for localized immunotherapy. *J. Am. Chem. Soc.* **2021**, *143*, 19330–19340.

[42] Douglas, S. M.; Bachelet, I.; Church, G. M. A logic-gated nanorobot for targeted transport of molecular payloads. *Science* **2012**, *335*, 831–834.

[43] Jiang, Q.; Liu, S. L.; Liu, J. B.; Wang, Z. G.; Ding, B. Q. Rationally designed DNA-origami nanomaterials for drug delivery *in vivo*. *Adv. Mater.* **2019**, *31*, 1804785.

[44] Xie, Y. X.; Li, H. S.; Xu, L.; Zou, H. B.; Wang, X. G.; He, X. Z.; Tang, Q. Y.; Zhou, Y.; Zhao, X.; Chen, X. J. et al. DNA nanoclusters combined with one-shot radiotherapy augment cancer immunotherapy efficiency. *Adv. Mater.* **2023**, *35*, 2208546.

[45] Hopfner, K. P.; Hornung, V. Molecular mechanisms and cellular functions of cGAS-STING signalling. *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 501–521.

[46] Zhang, L. P.; Wang, Y. Q.; Karges, J.; Tang, D. S.; Zhang, H. C.; Zou, K. X.; Song, J.; Xiao, H. H. Tetrahedral DNA nanostructure with interferon stimulatory DNA delivers highly potent toxins and activates the cGAS-STING pathway for robust chemotherapy and immunotherapy. *Adv. Mater.* **2022**, *35*, 2210267.

[47] Guo, W. J.; Gao, H. B.; Li, H.; Ge, S. H.; Zhang, F. F.; Wang, L. Y.; Shi, H. J.; Han, A. J. Self-assembly of a multifunction DNA tetrahedron for effective delivery of aptamer PL1 and *Pcsk9* siRNA potentiate immune checkpoint therapy for colorectal cancer. *ACS Appl. Mater. Interfaces* **2022**, *14*, 31634–31644.

[48] Tan, J.; Li, H.; Hu, X. X.; Abdullah, R.; Xie, S. T.; Zhang, L. L.; Zhao, M. M.; Luo, Q.; Li, Y. Z.; Sun, Z. J. et al. Size-tunable assemblies based on ferrocene-containing DNA polymers for spatially uniform penetration. *Chem* **2019**, *5*, 1775–1792.

[49] Fan, D. H.; Cao, Y. K.; Cao, M. Q.; Wang, Y. J.; Cao, Y. L.; Gong, T. Nanomedicine in cancer therapy. *Signal Transduct. Target. Ther.* **2023**, *8*, 293.

[50] Rothemund, P. W. K. Folding DNA to create nanoscale shapes and patterns. *Nature* **2006**, *440*, 297–302.

[51] Hu, X. X.; Chi, H. L.; Fu, X. Y.; Chen, J. L.; Dong, L. Y.; Jiang, S. Q.; Li, Y. Y.; Chen, J. Y.; Cheng, M.; Min, Q. H. et al. Tunable multivalent aptamer-based DNA nanostructures to regulate multiheteroreceptor-mediated tumor recognition. *J. Am. Chem. Soc.* **2024**, *146*, 2514–2523.

[52] Liu, S. L.; Jiang, Q.; Zhao, X.; Zhao, R. F.; Wang, Y. N.; Wang, Y. M.; Liu, J. B.; Shang, Y. X.; Zhao, S.; Wu, T. T. et al. A DNA nanodevice-based vaccine for cancer immunotherapy. *Nat. Mater.* **2021**, *20*, 421–430.

[53] Wamhoff, E. C.; Ronsard, L.; Feldman, J.; Knappe, G. A.; Hauser, B. M.; Romanov, A.; Case, J. B.; Sanapala, S.; Lam, E. C.; Denis, K. J. S. et al. Enhancing antibody responses by multivalent antigen display on thymus-independent DNA origami scaffolds. *Nat. Commun.* **2024**, *15*, 795.

[54] Sun, Y. Y.; Sun, J. J.; Xiao, M. S.; Lai, W.; Li, L.; Fan, C. H.; Pei, H. DNA origami-based artificial antigen-presenting cells for adoptive T cell therapy. *Sci. Adv.* **2022**, *8*, eadd1106.

[55] Teplensky, M. H.; Evangelopoulos, M.; Dittmar, J. W.; Forsyth, C. M.; Sinegra, A. J.; Wang, S. Y.; Mirkin, C. A. Multi-antigen spherical nucleic acid cancer vaccines. *Nat. Biomed. Eng.* **2023**, *7*, 911–927.

[56] Sun, L. L.; Shen, F. Y.; Xiong, Z. J.; Chao, Y.; Fan, C. H.; Liu, Z. Nanoscale precise editing of multiple immune stimulating ligands on DNA origami for T cell activation and cell-based cancer immunotherapy. *CCS Chem.* **2024**, *6*, 719–732.

[57] Zeng, Y. C.; Young, O. J.; Wintersinger, C. M.; Anastassacos, F. M.; MacDonald, J. I.; Isinelli, G.; Dellacherie, M. O.; Sobral, M.; Bai, H. Q.; Graveline, A. R. et al. Fine tuning of CpG spatial distribution with DNA origami for improved cancer vaccination. *Nat. Nanotechnol.* **2024**, *19*, 1055–1065.

[58] Min, H. Y.; Lee, H. Y. Molecular targeted therapy for anticancer treatment. *Exp. Mol. Med.* **2022**, *54*, 1670–1694.

[59] Zhou, J. H.; Rossi, J. Aptamers as targeted therapeutics: Current potential and challenges. *Nat. Rev. Drug Discov.* **2017**, *16*, 181–202.

[60] Li, L.; Xu, S. J.; Yan, H.; Li, X. W.; Yazd, H. S.; Li, X.; Huang, T.; Cui, C.; Jiang, J. H.; Tan, W. H. Nucleic acid aptamers for molecular diagnostics and therapeutics: Advances and perspectives. *Angew. Chem., Int. Ed.* **2021**, *60*, 2221–2231.

[61] Soundararajan, S.; Chen, W. W.; Spicer, E. K.; Courtenay-Luck, N.; Fernandes, D. J. The nucleolin targeting aptamer AS1411 destabilizes *Bcl-2* messenger RNA in human breast cancer cells. *Cancer Res.* **2008**, *68*, 2358–2365.

[62] Lv, Z. Y.; Li, Z. M.; Zou, S. L.; Li, P. R.; Song, N. C.; Zhang, R.; Xu, M. D.; Liu, M. X.; Li, F. Q.; Li, J. R. et al. A smart DNA nanoassembly containing multivalent aptamers enables controlled delivery of CRISPR/Cas9 for cancer immunotherapy. *Adv. Funct. Mater.* **2024**, *34*, 2311069.

[63] Ali, M. M.; Li, F.; Zhang, Z. Q.; Zhang, K. X.; Kang, D. K.; Ankrum, J. A.; Le, X. C.; Zhao, W. A. Rolling circle amplification: A versatile tool for chemical biology, materials science and medicine. *Chem. Soc. Rev.* **2014**, *43*, 3324–3341.

[64] Wang, H.; Yang, C. P.; Wu, T. T.; Fan, J.; Zhu, H. Y.; Liu, J. B.; Ding, B. Q. A highly tumor-permeating DNA nanoplatform for efficient remodeling of immunosuppressive tumor microenvironments. *Angew. Chem., Int. Ed.*, in press, DOI: 10.1002/anie.202412804.

[65] He, X.; Xu, C. Q. Immune checkpoint signaling and cancer immunotherapy. *Cell Res.* **2020**, *30*, 660–669.

[66] Sharma, P.; Allison, J. P. The future of immune checkpoint therapy. *Science* **2015**, *348*, 56–61.

[67] Sun, Q.; Hong, Z. Y.; Zhang, C.; Wang, L. L.; Han, Z. Q.; Ma, D. Immune checkpoint therapy for solid tumours: Clinical dilemmas and future trends. *Signal Transduct. Target. Ther.* **2023**, *8*, 320.

[68] Wolchok, J. D.; Kluger, H.; Callahan, M. K.; Postow, M. A.; Rizvi, N. A.; Lesokhin, A. M.; Segal, N. H.; Ariyan, C. E.; Gordon, R. A.; Reed, K. et al. Nivolumab plus ipilimumab in advanced melanoma. *N. Engl. J. Med.* **2013**, *369*, 122–133.

[69] Guo, M. Y.; Zhang, X.; Liu, J.; Gao, F. N.; Zhang, X. L.; Hu, X. H.; Li, B.; Zhang, X.; Zhou, H. G.; Bai, R. et al. Few-layer bismuthene for checkpoint knockdown enhanced cancer immunotherapy with rapid clearance and sequentially triggered one-for-all strategy. *ACS Nano* **2020**, *14*, 15700–15713.

[70] Lu, Q. L.; Chen, R. Y.; Du, S. Y.; Chen, C.; Pan, Y. C.; Luan, X. W.; Yang, J. J.; Zeng, F.; He, B. S.; Han, X. et al. Activation of the cGAS-STING pathway combined with CRISPR-Cas9 gene editing triggering long-term immunotherapy. *Biomaterials* **2022**, *291*, 121871.

[71] Cotton, A. D.; Nguyen, D. P.; Gramespacher, J. A.; Seiple, I. B.; Wells, J. A. Development of antibody-based PROTACs for the degradation of the cell-surface immune checkpoint protein PD-L1. *J. Am. Chem. Soc.* **2021**, *143*, 593–598.

[72] Su, W.; Tan, M. X.; Wang, Z. H.; Zhang, J.; Huang, W. P.; Song, H. H.; Wang, X. Y.; Ran, H. T.; Gao, Y. F.; Nie, G. J. et al. Targeted degradation of PD-L1 and activation of the STING pathway by carbon-dot-based PROTACs for cancer immunotherapy. *Angew. Chem., Int. Ed.* **2023**, *62*, e202218128.

[73] Sun, Y.; Mo, L. T.; Hu, X. X.; Yu, D.; Xie, S. T.; Li, J. L.; Zhao, Z. L.; Fang, X. H.; Ye, M.; Qiu, L. P. et al. Bispecific aptamer-based recognition-then-conjugation strategy for PD1/PDL1 axis blockade and enhanced immunotherapy. *ACS Nano* **2022**, *16*, 21129–21138.

[74] Zhang, R.; Lv, Z. Y.; Chang, L. L.; Wang, J.; Tang, J. P.; Wang, Z.

Q.; Li, S. Q.; Guo, J. F.; Yao, C.; Yang, D. Y. A responsive DNA hydrogel containing poly-aptamers as dual-target inhibitors for localized cancer immunotherapy. *Adv. Funct. Mater.* **2024**, *34*, 2401563.

[75] Li, Y. Q.; Liu, X. L.; Yu, L.; Huang, X.; Wang, X.; Han, D.; Yang, Y.; Liu, Z. Covalent LYTAC enabled by DNA aptamers for immune checkpoint degradation therapy. *J. Am. Chem. Soc.* **2023**, *145*, 24506–24521.

[76] Bi, S. Y.; Chen, W.; Fang, Y. Y.; Shen, J. Y.; Zhang, Q.; Guo, H. Q.; Ju, H. X.; Liu, Y. Cancer cell-selective PD-L1 inhibition via a DNA safety catch to enhance immunotherapy specificity. *Angew. Chem., Int. Ed.* **2024**, *63*, e202402522.

[77] Shi, J. J.; Kantoff, P. W.; Wooster, R.; Farokhzad, O. C. Cancer nanomedicine: Progress, challenges and opportunities. *Nat. Rev. Cancer* **2017**, *17*, 20–37.

[78] Hou, J. J.; Zhu, S. T.; Zhao, Z. W.; Shen, J. L.; Chao, J.; Shi, J. Y.; Li, J.; Wang, L. H.; Ge, Z. L.; Li, Q. Programming cell communications with pH-responsive DNA nanodevices. *Chem. Commun.* **2021**, *57*, 4536–4539.

[79] Li, F.; Yu, W. T.; Zhang, J. J.; Dong, Y. H.; Ding, X. H.; Ruan, X. H.; Gu, Z.; Yang, D. Y. Spatiotemporally programmable cascade hybridization of hairpin DNA in polymeric nanoframework for precise siRNA delivery. *Nat. Commun.* **2021**, *12*, 1138.

[80] Li, F.; Lv, Z. Y.; Zhang, X.; Dong, Y. H.; Ding, X. H.; Li, Z. M.; Li, S.; Yao, C.; Yang, D. Y. Supramolecular self-assembled DNA nanosystem for synergistic chemical and gene regulations on cancer cells. *Angew. Chem., Int. Ed.* **2021**, *60*, 25557–25566.

[81] Yang, S.; Wu, J. L.; Wang, Z. Y.; Cheng, Y.; Zhang, R.; Yao, C.; Yang, D. Y. A smart DNA hydrogel enables synergistic immunotherapy and photodynamic therapy of melanoma. *Angew. Chem., Int. Ed.* **2024**, *63*, e202319073.

[82] Song, N. C.; Chu, Y. W.; Li, S.; Fan, X. T.; Tang, J. P.; Li, H. J.; Tao, R. Y.; Li, F. Q.; Li, J. R.; Yang, D. Y. et al. A dual-enzyme-responsive DNA-based nanoframework enables controlled co-delivery of CRISPR-Cas9 and antisense oligodeoxynucleotide for synergistic gene therapy. *Adv. Funct. Mater.* **2023**, *33*, 2306634.

[83] Chen, Z. G.; Yang, S.; Zhao, Z. Y.; Feng, L.; Sheng, J.; Deng, R. J.; Wang, B. P.; He, Y.; Luo, D.; Chen, M. et al. Smart tumor cell-derived DNA nano-tree assembly for on-demand macrophages reprogramming. *Adv. Sci.* **2024**, *11*, 2307188.

[84] Hu, Y. W.; Gao, S. J.; Lu, H. F.; Tan, S. S.; Chen, F.; Ke, Y. J.; Ying, J. Y. A self-immolative DNA nanogel vaccine toward cancer immunotherapy. *Nano Lett.* **2023**, *23*, 9778–9787.

[85] Li, S. P.; Jiang, Q.; Liu, S. L.; Zhang, Y. L.; Tian, Y. H.; Song, C.; Wang, J.; Zou, Y. G.; Anderson, G. J.; Han, J. Y. et al. A DNA nanorobot functions as a cancer therapeutic in response to a molecular trigger *in vivo*. *Nat. Biotechnol.* **2018**, *36*, 258–264.

[86] Zhang, P. H.; Gao, D.; An, K. L.; Shen, Q.; Wang, C.; Zhang, Y. C.; Pan, X. S.; Chen, X. G.; Lyv, Y. F.; Cui, C. et al. A programmable polymer library that enables the construction of stimuli-responsive nanocarriers containing logic gates. *Nat. Chem.* **2020**, *12*, 381–390.

[87] Harris, A. L. Hypoxia-a key regulatory factor in tumour growth. *Nat. Rev. Cancer* **2002**, *2*, 38–47.

[88] Webb, B. A.; Chimenti, M.; Jacobson, M. P.; Barber, D. L. Dysregulated pH: A perfect storm for cancer progression. *Nat. Rev. Cancer* **2011**, *11*, 671–677.

[89] Fischer, A.; Ehrlich, A.; Plotkin, Y.; Ouyang, Y.; Asulin, K.; Konstantinos, I.; Fan, C. H.; Nahmias, Y.; Willner, I. Stimuli-responsive hydrogel microcapsules harnessing the COVID-19 immune response for cancer therapeutics. *Angew. Chem., Int. Ed.* **2023**, *62*, e202311590.

[90] Sun, L. L.; Shen, F. Y.; Tian, L. L.; Tao, H. Q.; Xiong, Z. J.; Xu, J.; Liu, Z. ATP-responsive smart hydrogel releasing immune adjuvant synchronized with repeated chemotherapy or radiotherapy to boost antitumor immunity. *Adv. Mater.* **2021**, *33*, 2007910.

[91] Wang, D. Y.; Liu, J. W.; Duan, J.; Yi, H.; Liu, J. J.; Song, H. W.; Zhang, Z. Z.; Shi, J. J.; Zhang, K. X. Enrichment and sensing tumor cells by embedded immunomodulatory DNA hydrogel to inhibit postoperative tumor recurrence. *Nat. Commun.* **2023**, *14*, 4511.

[92] Velusamy, A.; Sharma, R.; Rashid, S. A.; Ogasawara, H.; Salaita, K. DNA mechanocapsules for programmable piconewton responsive drug delivery. *Nat. Commun.* **2024**, *15*, 704.

[93] Rajasooriya, T.; Ogasawara, H.; Dong, Y. X.; Mancuso, J. N.; Salaita, K. Force-triggered self-destructive hydrogels. *Adv. Mater.* **2023**, *35*, 2305544.

[94] Ma, Y. H.; Zhu, Y.; Wu, H.; He, Y.; Zhang, Q.; Huang, Q. L.; Wang, Z. M.; Xing, H.; Qiu, L. P.; Tan, W. H. Domain-targeted membrane partitioning of specific proteins with DNA nanodevices. *J. Am. Chem. Soc.* **2024**, *146*, 7640–7648.

[95] Comberlato, A.; Koga, M. M.; Nüssing, S.; Parish, I. A.; Bastings, M. M. C. Spatially controlled activation of toll-like receptor 9 with DNA-based nanomaterials. *Nano Lett.* **2022**, *22*, 2506–2513.

[96] Sun, L. L.; Shen, F. Y.; Xu, J.; Han, X.; Fan, C. H.; Liu, Z. DNA-edited ligand positioning on red blood cells to enable optimized T cell activation for adoptive immunotherapy. *Angew. Chem., Int. Ed.* **2020**, *59*, 14842–14853.

[97] Shi, P.; Wang, X. L.; Davis, B.; Coyne, J.; Dong, C.; Reynolds, J.; Wang, Y. *In situ* synthesis of an aptamer-based polyvalent antibody mimic on the cell surface for enhanced interactions between immune and cancer cells. *Angew. Chem. , Int. Ed.* **2020**, *59*, 11892–11897.

[98] Tang, R.; Fu, Y. H.; Gong, B.; Fan, Y. Y.; Wang, H. H.; Huang, Y.; Nie, Z.; Wei, P. A chimeric conjugate of antibody and programmable DNA nanoassembly smartly activates T cells for precise cancer cell targeting. *Angew. Chem., Int. Ed.* **2022**, *61*, e202205902.

[99] Du, Y. L.; Lyu, Y. F.; Lin, J.; Ma, C. R.; Zhang, Q.; Zhang, Y. T.; Qiu, L. P.; Tan, W. H. Membrane-anchored DNA nanojunctions enable closer antigen-presenting cell-T-cell contact in elevated T-cell receptor triggering. *Nat. Nanotechnol.* **2023**, *18*, 818–827.

[100] Li, F.; Sun, X. L.; Yang, J.; Ren, J.; Huang, M. X.; Wang, S. Q.; Yang, D. Y. A thermal and enzymatic dual-stimuli responsive DNA-based nanomachine for controlled mRNA delivery. *Adv. Sci.* **2023**, *10*, 2204905.

[101] Liu, Q.; Xing, H.; Xiong, M. Y.; Zhang, X. B. Specifically editing cancer sialoglycans for enhanced immunotherapy *in vivo* through aptamer-enzyme chimeras. *Angew. Chem., Int. Ed.*, in press, DOI: 10.1002/anie.202414327.

[102] Guo, Y. F.; Huang, Y.; Liu, M. X.; Liu, J. J.; Liu, J. F.; Yang, D. Y. Evaluation of pharmacokinetics, immunogenicity, and immunotoxicity of DNA tetrahedral and DNA polymeric nanostructures. *Small Methods*, in press, DOI: 10.1002/smtd.202401007.