Biomaterials Science

ROYAL SOCIETY OF CHEMISTRY

REVIEW

View Article Online
View Journal | View Issue

Cite this: *Biomater. Sci.*, 2024, **12**, 3068

Non-viral vector-based genome editing for cancer immunotherapy

Tianxu Fang[a,b] and Guojun Chen *[a,b]

Published on 08 2024. Downloaded on 03.11.2024 5:08:38.

Received 24th February 2024,
Accepted 25th April 2024

DOI: 10.1039/d4bm00286e

rsc.li/biomaterials-science

Despite the exciting promise of cancer immunotherapy in the clinic, immune checkpoint blockade therapy and T cell-based therapies are often associated with low response rates, intrinsic and adaptive immune resistance, and systemic side effects. CRISPR-Cas-based genome editing appears to be an effective strategy to overcome these unmet clinical needs. As a safer delivery platform for the CRISPR-Cas system, non-viral nanoformulations have been recently explored to target tumor cells and immune cells, aiming to improve cancer immunotherapy on a gene level. In this review, we summarized the efforts of non-viral vector-based CRISPR-Cas-mediated genome editing in tumor cells and immune cells for cancer immunotherapy. Their design rationale and specific applications were highlighted.

1. Introduction

Cancer immunotherapy is a promising strategy with better anti-tumor efficacy than the traditional chemo- and radiotherapies.[1–4] The adaptive immune responses are to be boosted to remodel the tumor immunosuppressive microenvironment to inhibit tumor growth and defeat tumor recurrence.[5–7] Antigen presenting cells (APCs), including dendritic cells and macrophages, recognize the tumor antigens and present the antigens to adaptive immune cells, like T cells, which recognize and kill the tumor cells.[8–10] The delicate immune system relies on the expression and recognition of membrane proteins expressed on the tumor cells and immune cells, and the secreted cytokines and chemokines from these cells, which can all be the targets for the treatments to enhance the anti-tumor immunity by boosting the functioning of immune-active cells and inhibiting immunosuppressive cells.[11–15]

Immune checkpoint blockade and T cell-based therapies are commonly used in the clinic now.[16–21] Immune checkpoint inhibitors can block the negative immune regulators, such as programmed cell death 1 (PD-1) protein, programmed cell death ligand 1 (PD-L1), and cytotoxic T-lymphocyte associated protein 4 (CTLA-4), to reactivate the anti-tumor immune systems.[22–25] T cell-based therapies, including tumor infiltrating lymphocyte therapy, transgenic T cell receptor-T cell therapy, and chimeric antigen receptor (CAR)-T cell therapy, function primarily by increasing the number of tumor-specific T cells against tumor cells.[26–29] Although durable therapeutic efficacies of these therapies have been observed in a subset of cancer patients, the low response rate and the intrinsic and adaptive immune resistance in patients still remain.[30–38] Thus, novel immunotherapeutic strategies are urgently needed.

Genome editing appears to be an effective strategy for cancer immunotherapy since it could induce long-term effects in the context of gene expression of immunoregulatory proteins, cytokines, and chemokines on/from the cells to remodel anti-cancer immunity, which could be beneficial over conventional immunotherapeutic strategies, such as immune checkpoint antibodies, plasmids, messenger RNA, or small interfering RNA and gene therapies for cancer immunotherapies.[39–42] Compared with other conventional genome editing systems, such as zinc finger nucleases (ZFNs) and transcription activator-like nucleases (TALEN), the CRISPR-Cas-based approaches, an emerging technology which won the 2020 Nobel Prize in Chemistry, show unique advantages.[39,43–46] The CRISPR-Cas system, composed of Cas nuclease and guide RNA (gRNA),[47–49] can easily modify targeted genes by designing gRNAs.[50–54] The CRISPR-Cas technology also offers increased precision and efficiency with a reduced risk of off-target editing.[39,55–58] Additionally, various new CRISPR-Cas systems, such as prime-editing and base-editing, create new opportunities for precise gene corrections.[59–62]

The implementation of CRISPR-Cas technology can take the form of plasmid encoding both Cas and sgRNA, CRISPR mRNA/sgRNA, or ribonucleoprotein (RNP, the complex of Cas protein and sgRNA).[63–69] The plasmid DNA, RNA, and RNP all exhibit negative surface charges and low stability, leading to no to extremely low gene editing efficiency.[70–75] Therefore, the development of nano-systems for the delivery of CRISPR-Cas systems is urgently needed to elevate the intracellular delivery efficiency and broaden the applications of CRISPR-Cas technology.

Viral vectors, such as adeno-associated viruses (AAVs) and lentiviral vectors (LVs), have been commonly employed to load

[a]*Department of Biomedical Engineering, McGill University, Montreal, QC, H3G 0B1, Canada. E-mail: guojun.chen@mcgill.ca*
[b]*Rosalind & Morris Goodman Cancer Institute, McGill University, Montreal, QC, H3G 0B1, Canada*

 This journal is © The Royal Society of Chemistry 2024

and transport genome editing systems in cells efficiently.[76–80] However, viral vectors are associated with many drawbacks, including immunogenicity, insertional mutagenesis, toxicity, and packing limitations.[81–88] On the other hand, non-viral vectors like nanoparticles (NPs) are considered and investigated to overcome those limitations. Several unique NPs have been reported for CRISPR-Cas delivery, including polymeric, lipid, gold, and silicon nanoparticles.[75,86,89–95] These NPs exhibit chemical design flexibility, high loading capacity, high safety and stability, biocompatibility, and low immunogenicity.[71,94,96,97] In addition, NPs can be easily modified to target specific cells, making genome editing possible in a broad spectrum of cells in the complicated tumor microenvironment (TME) and immune systems.[94,98–100]

In this review, we reviewed the development of non-viral nanoformulations for the CRISPR-Cas system delivery in tumor cells and immune cells and highlighted their rationales and applications in cancer immunotherapy (Fig. 1). In tumor cells, gene editing targeted to the classical immune checkpoint, PD-L1, for enhanced T cell recognition and killing effect, and to other immune-related proteins for the promoted phagocytosis of tumor cells by macrophages and the preservation of vitality of T cells. As for gene editing in immune cells, we introduced the applications to macrophages, dendritic cells, and T cells. The goals of targeting macrophages lay in the induction of macrophage polarization from M2 to M1 phenotype, and the promotion of macrophage phagocytosis. The gene editing in dendritic cells enhanced the presentation of antigens to T cells, and in T cells strengthened the recognition of tumor cells for higher killing efficiency.

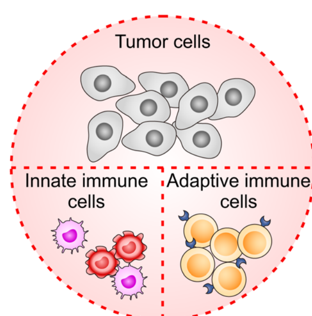
2. Genome editing targeting tumor cells

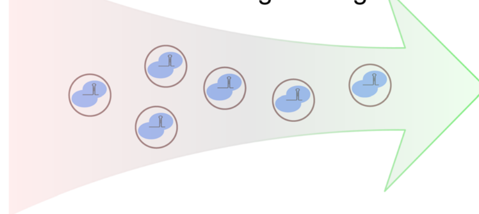
Tumors can escape immune surveillance through various mechanisms, in which the expression of immune checkpoints and the secretion of some immunomodulatory molecules have been widely investigated. For example, immune checkpoints which are expressed on tumor cell surfaces can interact with immune checkpoint receptors on immune cells to inhibit cell killing.[101–103] Tumor cells also secrete immunosuppressive molecules to inhibit the activation and infiltration of immunosupportive cells (*e.g.*, $CD4^+$ and $CD8^+$ T cells) and promote immunosuppressive cells (*e.g.*, regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs)).[104–109] Knockout of immune checkpoint expression and immunosuppressive molecule secretion in tumors using CRISPR-Cas tools could favor anti-tumor immunity (Table 1).

2.1. Targeting PD-L1

PD-L1 is one of the immune checkpoints that is often overexpressed on tumor cells, such as gastric cancer, ovarian cancer, melanoma, breast cancer, and colorectal cancer.[110–117] Binding PD-L1 and programmed cell death-1 (PD-1) on T cells will lead to impeding of the T cell-mediated anti-immune responses.[22,118–122] To this end, Lu *et al.*[123] utilized Ni^{2+}-modified mesoporous polydopamine (mPDA)-covering Fe_3O_4 NPs (termed FPP) to deliver Cas9 RNP targeting the PD-L1 gene (Fig. 2A). The magnetic targeting effect of the Fe_3O_4 NPs improved the accumulation of Cas9 RNP-loaded nanoparticles in tumor sites. Afterwards, near-infrared (NIR) light initiated the release of Cas9 RNP by disrupting the non-covalent bond between the metal ions and PDA. In B16F10 cells, FPP/RNP with NIR induced 42.1% of PD-L1 gene knockout. In the melanoma model *in vivo*, the gene knockout efficiency of FPP/RNP with NIR was about 25.1%, which translated to the best control of the tumor growth in this group. The treatment of FPP/RNP with NIR laser irradiation effectively reversed the immunosuppression of the TME and induced strong anti-tumor immune responses *in vivo*, including DC maturation, T cell activation and secretion of antibodies and cytokines.

Liu *et al.*[124] presented a mesoporous silica nanoparticle (MSN)-based virus-like nanoparticle (denoted as VLN) as a multifaceted nanoplatform to co-deliver the CRISPR/Cas9 system and small molecule drugs (Fig. 2B). The RNP was conjugated on the surface-thiolated MSN *via* glutathione (GSH)-responsive disulfide bonds. The CRISPR/Cas9 system with an





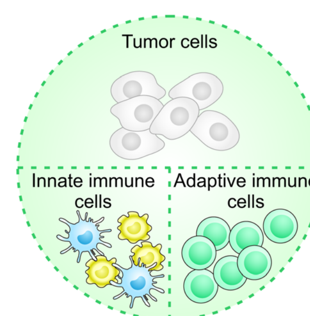


Fig. 1 Scheme of non-viral CRISPR-Cas-loaded vectors for cancer immunotherapy.

Published on 08 2024. Downloaded on 03.11.2024 5:08:38.

Table 1 Representative nanoformulation-mediated CRISPR-Cas9 delivery for genome editing targeting tumor cells

Target gene locus	CRISPR-Cas9 format	NP composition	Applications	Administration route	Ref.
PD-L1	RNP	Ni^{2+}-modified mesoporous PDA-covering Fe_3O_4 NPs	*In vivo* (B16F10 melanoma in C57BL/6 mice)	Intravenous injection	123
PD-L1	RNP	Mesoporous silica NP-based virus-like NPs	*In vivo* (B16F10 melanoma in C57BL/6 mice)	Intravenous injection	124
PD-L1; MHC-1	Plasmid	Polyplex of CRISPR plasmid DNA (pDNA) and thioketal-cross-linked polyethylenimine derivative, covered with glucose-modified poly(ethylene glycol)-*b*-polylysine	*In vivo* (B16F10 melanoma in C57BL/6 mice; B16F10 or 4T1 TNBC in BALB/c mice)	Intravenous injection	125
PD-L1	Plasmid	Au nanoparticle-loaded core–shell tecto-dendrimers (Au CSTDs), gold NPs entrapped in lactobionic acid (LA)-modified G5 PAMAM dendrimers, covered with PBA-conjugated G3 PAMAM dendrimers, complexed with Cas9-PD-L1 pDNA	*In vivo* (B16F10 melanoma in C57BL/6 mice)	Intratumoral injection	129
PD-L1; PVR	RNP	Sequence-defined oligo(ethylenamino) amide-based carriers modified with folic acid	*In vivo* (CT26 colorectal carcinoma in BALB/c mice)	Intratumoral injection	130
PD-L1	Plasmid	Polyplex of CRISPR/Cas13a pDNA and 4-(hydroxymethyl)phenylboronic acid (HPBA)-modified PEI, covered with *cis*-aconitic anhydride and sodium glucoheptonate dehydrate-modified PEG-*b*-polylysine	*In vivo* (B16F10 melanoma in C57BL/6 mice)	Intravenous injection	137
PD-L1	mRNA	LNP	*In vivo* (ID8-luc ovarian cancer in C57BL/6 mice; MYC-driven liver cancer in transgenic mice)	Local injection, intravenous injection	142
CD47	Plasmid	PEI-decorated gold nanoparticles	*In vivo* (B16F10 melanoma in C57BL/6 mice)	Peritumoral injection	154
CD47	Plasmid	Nanocomplex of plasmids and fluorinated polyethylenimine, coated with hyaluronic acid and TME-sensitive peptides	*In vivo* (B16F10 melanoma in C57BL/6 mice)	Intravenous injection	155
CD47	RNP	Cas9 RNP coated by *N*-(3-aminopropyl) methacrylamide (cationic monomer), methacrylate (anionic monomer), acrylated pheophorbide a (ICD monomer), acrylated PEG (APEG, blood-circulated monomer), folic acid modified APEG (tumor-targeted monomer), and *N*,*N*′-bis(acryloyl) cystamine (GSH-degradable crosslinker)	*In vivo* (B16F10 melanoma in C57BL/6 mice)	Intravenous injection	159
Ptpn2	Plasmid	Nanocomplex of plasmids, branched PEI, and iRGD peptides, assembled with 3-(carboxypropyl) triphenyl phosphonium bromide-PEI-Ce6	*In vivo* (B16F10 melanoma in C57BL/6 mice)	Intravenous injection	168
Ptpn2	Plasmid	DBCO decorated liposome	*In vivo* (B16F10 melanoma in C57BL/6 mice)	Intravenous injection	169
SLC43A2	Plasmid	Mn/Zn-ZIF-8 MOF	*In vivo* (4T1 TNBC in BALB/c mice)	Intravenous injection	172
HSP70; BAG3	Plasmid	LEGEND composed of poly(β-amino ester) PAE-C14, semiconducting polymer BDT-TQE, tumor-targeting PEGylated lipids DSPE-PEG-AEAA; FUGEND composed of PDA subunit, poly(β-amino ester) PAE-C14, PEGylated lipids DSPE-PEG-AEAA	*In vivo* (B16F10 melanoma, LLC lung cancer, Hepa1-6 liver cancer in C57BL/6 mice; A549 lung cancer cells in Nod/SCID/IL2RG$^{-/-}$ mice; PDX of liver cancer in B-NDG B2m/KO mice plus (B2m(v2) (mut/mut), B2m (−/−) IL-2rg (−/−)))	Intravenous injection	183

sgRNA targeting the PD-L1 encoding gene (denoted as sgPD-L1) was released in cytosol with high GSH present. VLN-sgPD-L1 decreased PD-L1 expression to 41.3% *in vitro* and exhibited 45.1% of PD-L1 knockout efficiency *in vivo* in B16F10 tumors. VLN-sgPD-L1 treatment significantly suppressed tumor growth and increased survival, as well as revitalized the exhausted T cells for enhanced anti-tumor immune activation.

Xing *et al.*[125] developed a pH/photo dual-activatable binary CRISPR nanomedicine (denoted as DBCN), where the polyplex of CRISPR plasmid DNA (pDNA that targets PD-L1) and thio-

Published on 08 2024. Downloaded on 03.11.2024 5:08:38.

This journal is © The Royal Society of Chemistry 2024

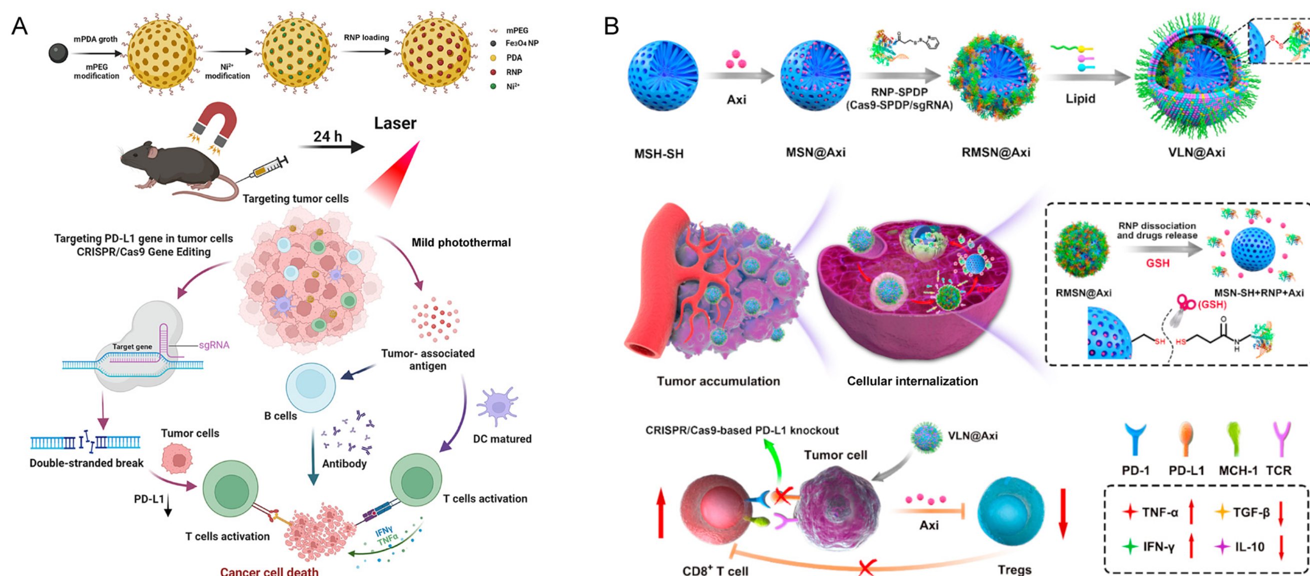


Fig. 2 Editing of the PD-L1 gene in tumor cells for cancer immunotherapies. (A) FPP preparation and RNP loading, and illustration of the multi-functional photothermal therapy and CRISPR/Cas9 genome-editing strategy for editing PD-L1 gene and remodeling the immunosuppressive tumor environment in *vivo.*[123] (B) Schematic illustration for the synthesis of Cas9-sgPD-L1-loaded VLN and the delivery process after intravenous injection, the suppression of Tregs and the activation of $CD8^+$ T cells by the Cas9-sgPD-L1-loaded VLN for efficient cancer immunotherapy.[124]

ketal (TK)-cross-linked polyethylenimine derivative (modified with phenylboronic acid (PBA) and photosensitizer pheophorbide a (Pha), TK-PPP) formed the core and glucose-modified poly(ethylene glycol)-*b*-polylysine (mPEG113-*b*-PLys25/Glu) formed the shell (Fig. 3A). The core was exposed because of the disassembly of the shell in the acidic TME. PBA groups on the TK-PPP/pDNA polyplex can then bind with sialic acid (SA) overexpressed on the tumor cells to enhance tumor accumulation and cellular internalization of the CRISPR components.[126] With tumor progression, MHC-1 expression on the tumor cell surface gradually decreases, resulting in insufficient tumor-associated antigen (TAA) presentation and immune evasion.[127,128] Thus, the up-regulation of MHC-1 expression is supposed to effectively restore TAA presentation on the surface of tumor cells. The pDNA respectively encoding the CRISPR-Cas9 system for disrupting PD-L1 and the aCRISPR-dCas9 system for up-regulating MHC-1 was co-loaded and co-delivered into B16F10-OVA cells. Both PD-L1 disruption and MHC-1 up-regulation were observed after treatment with DBCN under pH 6.5 with laser irradiation, initiating robust T cell-dependent antitumor immune responses to inhibit the growth of both primary and distant tumors. In B16F10 tumor-bearing mice, the DBCN (with laser) treatment significantly controlled the growth of primary tumors and prolonged the survival of tumor-bearing mice. Immunological analysis showed that DBCN (with laser) treatment promoted the intratumoral infiltration of $CD8^+$ T cells from 8.57% to 29.4% compared with PBS treatment. In addition, DBCN further triggered lasting and specific antitumor immune memory effects, which significantly prevented the recurrence and metastasis of malignant tumors. DBCN (with laser) treatment increased the fraction of effector memory T cells from 12.5% to 28.7% and central memory T cells from 18.7% to 32.8% compared with PBS treatment.

Liu *et al.*[129] constructed TME-responsive Au nanoparticle-loaded core–shell tecto dendrimers (Au CSTDs), composed of lactobionic acid (LA)-modified G5 poly(amidoamine) (PAMAM) dendrimer-entrapping Au NPs as the core and PBA-conjugated G3 PAMAM dendrimers assembled as the shell (*i.e.*, the vector), to electrostatically complex and deliver Cas9-PD-L1 pDNA (Cas9-PD-L1). In B16F10 cells, the vector/Cas9-PD-L1 group showed the lowest expression level of PD-L1 proteins. In the melanoma mouse model, the vector/Cas9-PD-L1 group displayed a significantly better suppression of tumor growth and more significant PL-L1 expression inhibition than PD-L1 antibody treatment. Vector/Cas9-PD-L1 treatment also led to the increased distribution of $CD4^+/CD8^+$ T cells, decreased immunosuppressive cell populations, and increased levels of tumor necrosis factor α (TNF-α), interferon γ (IFN-γ) and interleukin (IL)-6 cytokines.

Lin *et al.*[130] loaded Cas9/sgPD-L1 RNP and Cas9/sgPVR RNP in sequence-defined oligo(ethylenamino)amides (OAAs)-based carriers with folic acid (FolA) modification on the surface. The FolA mediated the Cas9 RNP delivery into tumor cells expressing folate receptor α (FRα).[131] The T cell immunoreceptor with immunoglobulin (Ig) and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT)/poliovirus receptor (PVR) is a newly identified immune checkpoint axis that emerged as a promising immunological target.[132–134] Blocking the TIGIT/PVR axis has been proved to enable the reversal of T cell exhaustion and enhance antitumor efficacy in diverse types of cancer.[132,135,136] A 54.4% dual knockout of PD-L1 and PVR *in vitro* and around 25% of dual knockout *in vivo* were detected in CT26 cells after treatment with FolA-PEG-nano-

Published on 08 2024. Downloaded on 03.11.2024 5:08:38.

Published on 08 2024. Downloaded on 03.11.2024 5:08:38.

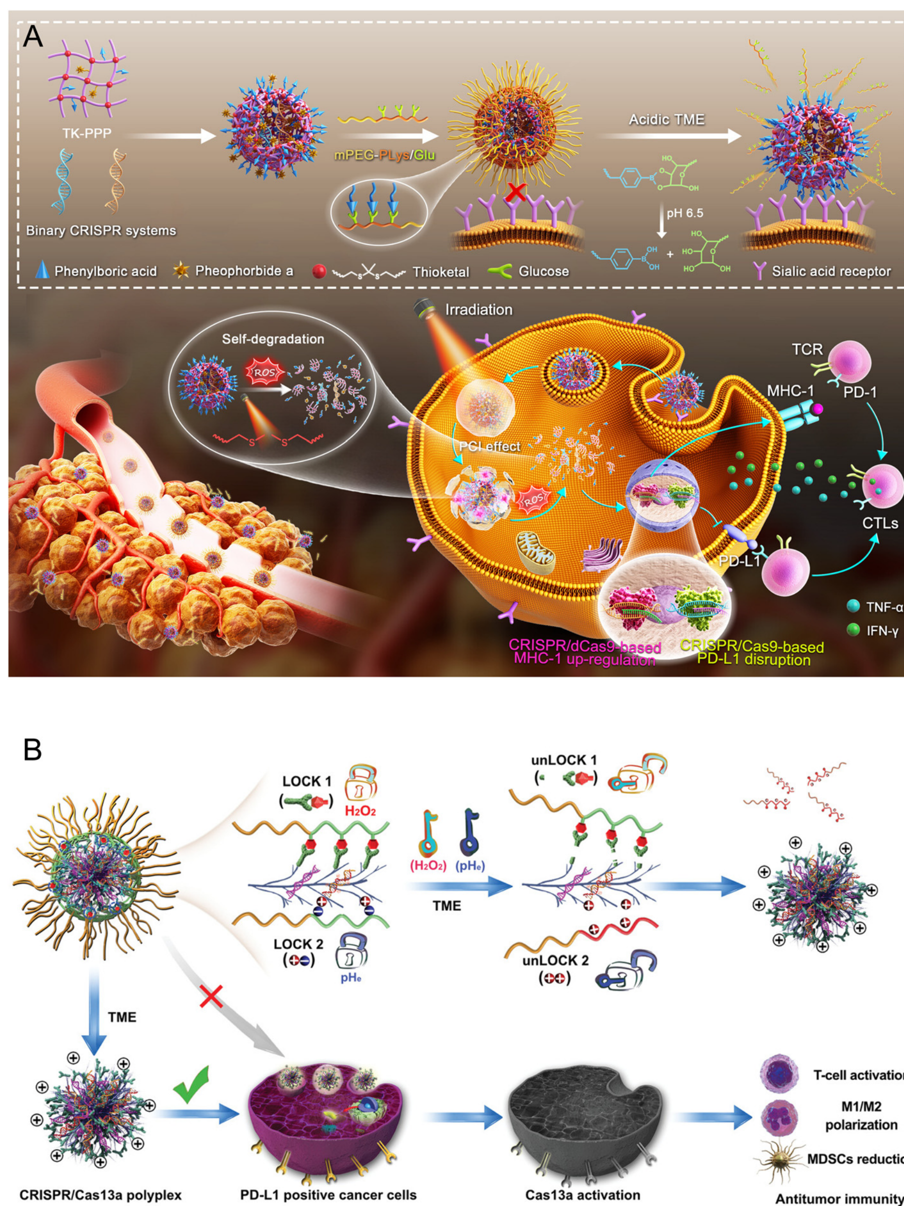


Fig. 3 Editing of the PD-L1 gene in tumor cells for cancer immunotherapies. (A) Preparation of DBCN, and the schematic illustration of DBCN delivering binary CRISPR systems to simultaneously correct the disturbance of MHC-1 and PD-L1 expression to remodel tumor immunogenicity for efficient cancer immunotherapy.[125] (B) Schematic illustration of DLNP for effective cancer immunotherapy. The CRISPR/Cas13a system can only be released from DLNP in a microenvironment with both a low pHe and high H_2O_2 concentration.[137]

carriers. FolA-PEG-nanocarriers exhibited delayed tumor growth and enhanced $CD8^+$ T cell infiltration in tumors.

Zhang *et al.*[137] also established a tumor-targeting dual-locking nanoparticle (DLNP) to deliver the CRISPR/Cas13a system (Fig. 3B). Cas13a, also as a nuclease, targets RNA instead of DNA (which is targeted by Cas9), realizing temporary but safe gene regulation.[138,139] The solid TME features a lower pH and higher ROS level, to which the DLNP was responsive to degrade into cationic polymers, facilitating the cellular internalization of the CRISPR/Cas13a system and gene editing activation.[140,141] The crRNA targeting the PD-L1 gene was complexed in the CRISPR/Cas13a system. In the B16F10 melanoma mouse model, the DLNP group showed a superior control of tumor growth, where 50% of mice survived to 36 days. DLNP also induced 6-fold higher $CD8^+$ T cell infiltration, 1.81-fold higher IFN-γ, and 3.84-fold higher TNF-α levels compared with the untreated group.

Zhang *et al.*[142] reported a lipid nanoparticle (LNP) to deliver focal adhesion kinase (FAK) siRNA (siFAK), Cas9 mRNA, and PD-L1 sgRNA. FAK is frequently overexpressed in tumors, and promotes the adhesion of tumor cells to the tumor stroma and the ECM.[143] The inhibition of FAK led to a decreased stiffness of the tumor ECM and enhanced infiltration of $CD8^+$ cytotoxic T cells.[144,145] In mice bearing ID8-luc xenograft ovarian

 This journal is © The Royal Society of Chemistry 2024

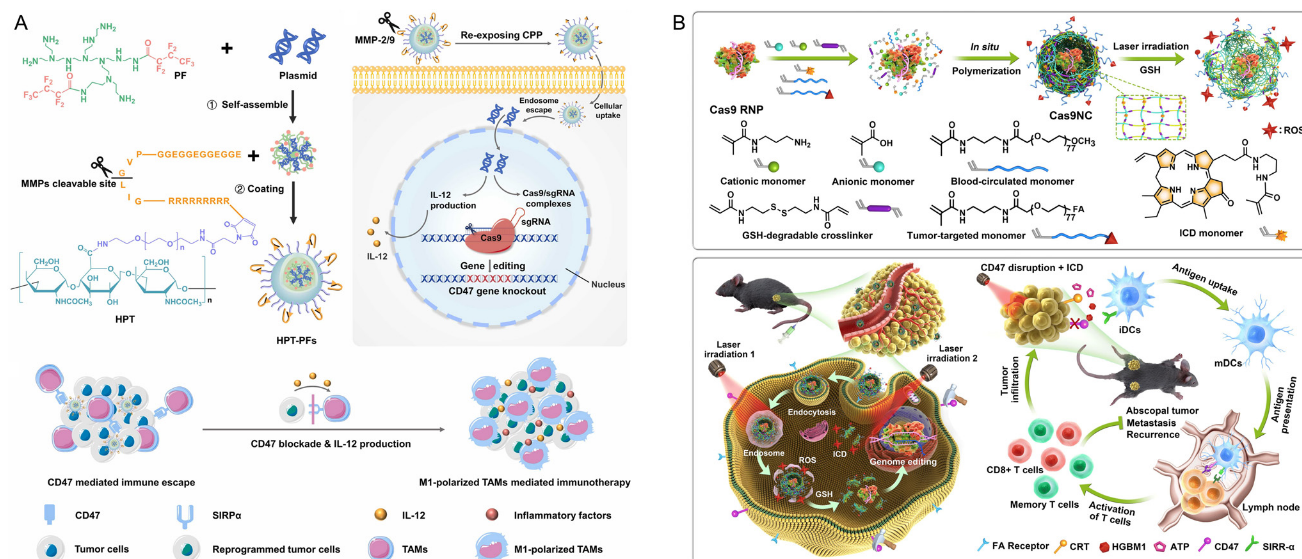


Fig. 4 Editing of the CD47 gene in tumor cells for cancer immunotherapies. (A) Schematic demonstration of HPT-PFs preparation and the nanoparticles' endosome escape, IL-12 production, Cas9/sgRNA complex expression, and CD47 gene knockout in cells. CD47 blockade combined with IL-12 production synergistically promoted the polarization of TAMs for eliciting macrophage-mediated immunotherapy.[155] (B) Schematic manifestation for Cas9NC fabrication and its degradation responding to GSH, and the delivery process of Cas9NC in cells and a simplified mechanism of Cas9NC-mediated antitumor immunity activation to inhibit the growth of both primary and abscopal tumors, preventing tumor recurrence and distant metastasis.[159]

Published on 08 2024. Downloaded on 03.11.2024 5:08:38.

tumors, siFAK + CRISPR-PD-L1-LNPs enhanced the infiltration of immune cells, especially T cells, which translated to the best anti-tumor effect. Similarly, the prolonged survival of mice and boosted infiltration of T cells and macrophages were discovered in the mouse model of liver cancer after siFAK + CRISPR-PD-L1-LNP treatment.

2.2. Targeting CD47

CD47 is a novel macrophage immune checkpoint expressed on cancer cells.[146–148] CD47 can react with signal regulatory protein-α (SIRP-α) on macrophages to help cancer cells to bypass phagocytosis.[149–153] Huang *et al.*[154] utilized PEI-decorated gold nanoparticles to load the CRISPR/Cas9 plasmid pH330/sgCD47, obtaining a nanosystem termed $AuP_{pH330/sgCD47}$. CD47 genomic disruption by the CRISPR/Cas9 plasmid was driven by a heat shock protein 70 (HSP70) promoter under NIR-II light. In *in vitro* experiments, the expression of CD47 protein on the surface of B16F10 cells was significantly downregulated in the $AuP_{pH330/sgCD47}$ (with NIR irradiation) group. $AuP_{pH330/sgCD47}$ (with NIR irradiation) triggered a powerful anticancer immune response, including M2-to-M1 phenotype macrophage polarization, $CD8^+$ T cell infiltration, and Treg consumption. An immune memory response was also elicited, with the increased ratio of effector memory T cells and central memory T cells in the spleen.

Lin *et al.*[155] prepared an environment-responsive gene delivery system (abbreviated as HPT-PFs) for CD47 blockade with IL-12 production in tumor cells (Fig. 4A). The plasmids encoding IL-12 and Cas9/sgRNA were complexed with fluorinated polyethylenimine to form the core. The core was further coated by hyaluronic acid (HA) together with TME-sensitive peptides (TMSP, composed of cell-penetrating peptides (CPP) and shielding peptides connected by a matrix metalloproteinases-2/9 (MMP-2/9)-cleavable peptide linker (PVGLIG)). MMPs are overexpressed in tumors and can cleave the PVGLIG linkers to expose the CPP.[156] The hyaluronic acid can interact with CD44 overexpressed on multiple tumor cells for active tumor targeting.[157,158] HPT-PFs treatment exhibited the effective control of tumor growth and decreased CD47 expression in a melanoma mouse model. The anti-tumor effect was further enhanced by the co-delivery of IL-12 plasmid, which promoted the polarization of macrophages from M2 to M1 phenotype.

Xing *et al.*[159] prepared a Cas9 RNP nanocapsule (Cas9NC) formed by coating CD47-disrupting Cas9 RNP with acrylated pheophorbide a (APPa, the photosensitizer for photodynamic therapy) and multiple functional and crosslinked monomers, which endowed the Cas9NC with excellent blood stability, tumor targeting, laser-triggered immunogenic cell death (ICD), GSH-responsive degradability, and protection from enzyme degradation (Fig. 4B). Cas9NC (with laser) treatment of B16F10 cells resulted in 62.2% CD47 disruption. Cas9NC intravenous injections into melanoma-bearing mice led to a significant decrease in CD47 expression, which can promote the phagocytosis of tumor cells by dendritic cells and facilitate dendritic cell maturation and $CD8^+$ T cell activation. The anti-tumor immune activation benefited the anti-metastatic efficiency of the Cas9NC treatments.

2.3. Targeting Ptpn2

Protein tyrosine phosphatase non-receptor type2 (Ptpn2) is a phosphatase involved in various signaling processes and it is

Published on 08 2024. Downloaded on 03.11.2024 5:08:38.

frequently mutated in multiple malignancies.[160–163] Deletion of Ptpn2 in tumor cells could elevate the immunotherapeutic efficacy by increasing the activated cytotoxic $CD8^+$ T cells and IFN-γ mediated effects.[164–167] Yang *et al.*[168] prepared a positively charged "core" (termed PR@CCP) co-loaded with a modified mitochondria-targeting chlorin e6 (TPP-PEI-Ce6) and the CRISPR-Cas9 system targeting the Ptpn2 gene (Cas9-Ptpn2) (Fig. 5A). The PR@CCP core was further coated with a negatively charged HA "shell" by electrostatic adsorption to obtain the nanosystem, named HPR@CCP. The HPR@Cas9-Ptpn2 significantly inhibited Ptpn2 expression and elevated the percentages of $CD3^+CD8^+$ T cells in tumor tissues (~35.29% com-

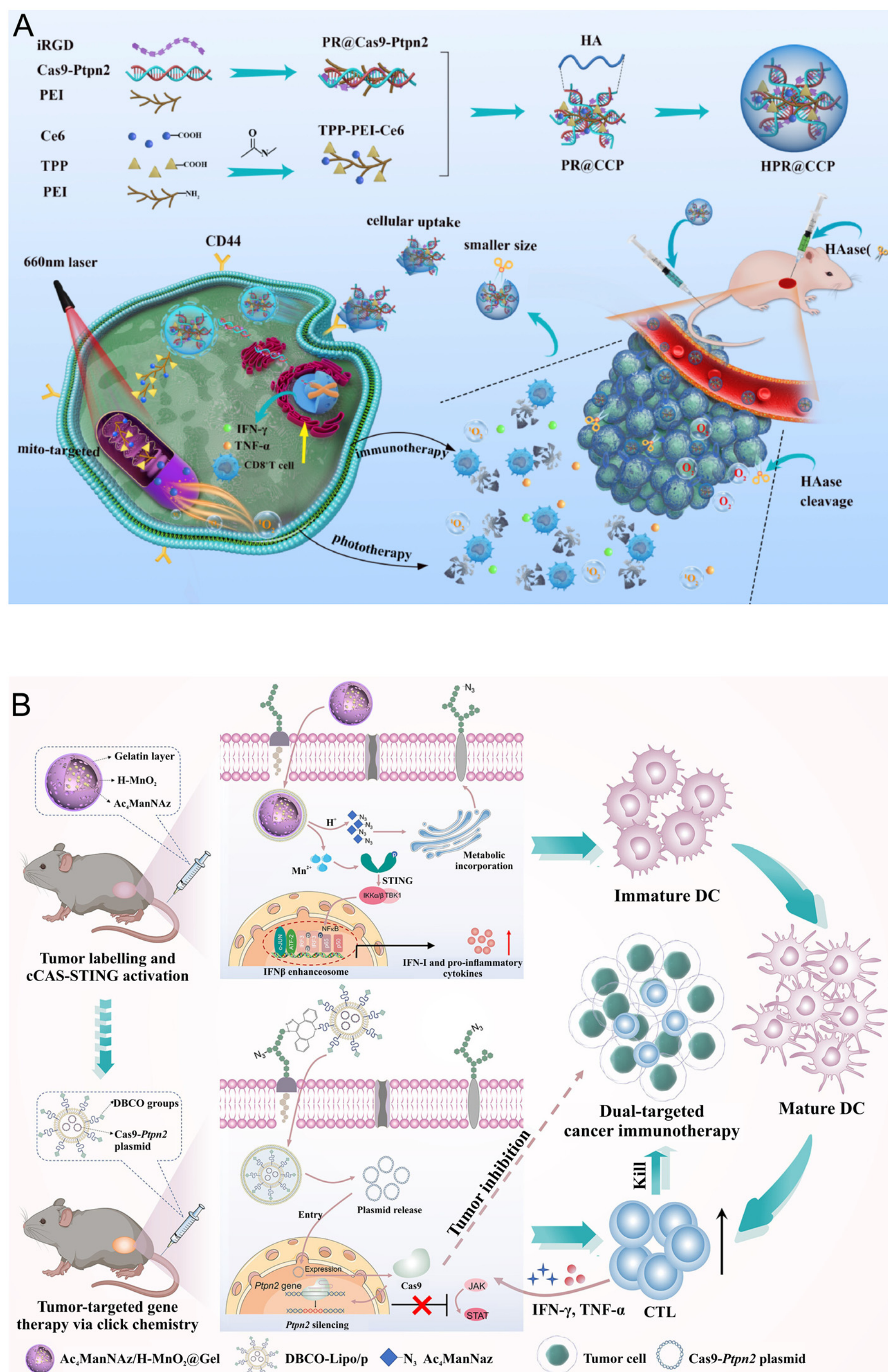


Fig. 5 Editing of the Ptpn2 gene in tumor cells for cancer immunotherapies. (A) The construction of HPR@CCP nanoparticles and their versatile application in cancer combined therapy.[168] (B) Process of the selective labeling of tumor tissues *via* metabolic engineering by $Ac_4ManNAz$/$H\text{-}MnO_2$@Gel, and the illustration of the targeted CRISPR/Cas9 system delivery and tumor-specific gene editing of Ptpn2 by DBCO-Lipo/p *via* biorthogonal click chemistry.[169]

 This journal is © The Royal Society of Chemistry 2024

Published on 08 2024. Downloaded on 03.11.2024 5:08:38.

pared with ~25.25% in the PBS group) and blood (~32.60% compared with ~26.57% in the PBS group).

Yang *et al.*[169] utilized the biorthogonal reaction to form a tumor-targeted CRISPR/Cas9 system delivery (Fig. 5B). Specifically, they developed an intelligent biodegradable hollow manganese dioxide (H-MnO_2) nanoplatform for tumor-specific labeling by *N*-azidoacetylmannosamine-tetraacylated ($Ac_4ManNAz$) through metabolic engineering. Then dibenzocyclooctyne (DBCO)-decorated liposome loading CRISPR/Cas9 plasmids (DBCO-Lipo/p) were used to disrupt the Ptpn2 gene in tumor cells through *in vivo* click chemistry. The combined treatment of $Ac_4ManNAz$/H-MnO_2Gel + DBCO-Lipo/p to B16F10 cells led to 28.2% of Ptpn2 gene disruption. In a melanoma mouse model, the $Ac_4ManNAz$/H-MnO_2Gel@DBCO-Lipo/p treatment group exhibited a gene editing efficiency of ~25% and attenuated tumor growth of 75%. Moreover, significantly elevated tumor-infiltrating $CD8^+$ T cells, transforming growth factor β (TGF-β), and IL-10 were observed, demonstrating that a robust anti-tumor immune response was effectively triggered.

2.4. Targeting SLC43A2

Methionine is a crucial protein for $CD8^+$ T cells because methionine deficiency can induce $CD8^+$ T cell death and dysfunction.[170] However, the acquisition of sufficient methionine by $CD8^+$ T cells can be interfered with by competitive methionine uptake in tumor cells through the high-expressing methionine transporter SLC43A2.[171] Therefore, downregulation of SLC43A2 expression on tumor cells could promote the survival and activity of $CD8^+$ T cells. Huang *et al.*[172] encapsulated the CRISPR/Cas9 plasmid targeting SLC43A2 into Mn/Zn bimetallic metal–organic framework (MOF) nanoparticles to obtain PMZH (Fig. 6A). PMZH treatment was able to disturb the expression of SLC43A2 in 4T1 cells *in vitro*, which led to improved survival of cytotoxic T cells in the coculture system. PMZH exhibited 85.47% of 4T1 breast cancer growth inhibition *in vivo*, and significantly enhanced $CD8^+$ T cell infiltration in tumors.

2.5. Targeting to HSP70

HSP70 and Bcl-2-associated athanogene 3 (BAG3) are highly expressed in various cancer types and their intracellular activities have a great impact on the survival of tumor cells.[173–175] HSP70 correlates with chemotherapy resistance, metastasis, tumor grade, and poor prognosis, while BAG3 is implicated in HSP70-related cell signaling.[176] The overexpression of HSP70 and its co-chaperone BAG3 exhibits an anti-apoptotic mechanism in tumor cells so that these tumor cells can protect themselves from various stresses (such as chemotherapy and hypoxia) and T-cell-mediated killing effects.[177–179] Besides, HSP70 and BAG3 also have the capacity to dynamically influence the TME, thereby fostering the advancement of cancer and treatment resistance, including T cell-based adoptive cell transfer.[180–182]To this end, Chen *et al.*[183] established a nanoformulation that can respond to near-infrared (NIR) light or focused ultrasound (FUS) to deliver the heat-inducible CRISPR-Cas9 system that targets the HSP70 and BAG3 genes in tumor cells (Fig. 6B). The light-enabled genome-editing nanodevice (LEGEND) included poly[[diisopropyl 4-(4,8-bis(heptan-3-yloxy) benzo[1,2-*b*:4,5-*b*′]dithiophen-2-yl)]-*alt*-*co*-[[1,2,5]thiadiazolo[3,4-*g*]quinoxaline-6,7-dicarboxylate]], a semiconducting polymer which can convert NIR light-to-heat, and a heat-triggered Cas9 plasmid that encoded both HSP70 sgRNA and BAG3 sgRNA driven by a heat-shock promoter. Thus, NIR-II can induce the expression of plasmid loaded in LEGEND. Compared with the non-pretreated B16F10 tumors, the LEGEND pretreatment led to the increasing of infiltrating tumor-infiltrating lymphocytes (TILs) by 85 fold, and a higher

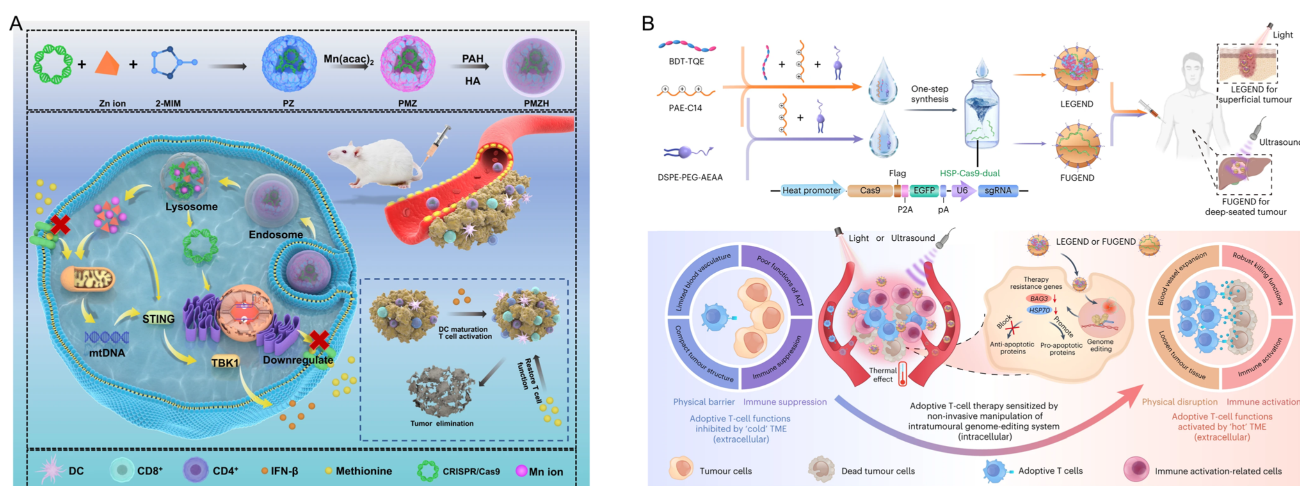


Fig. 6 (A) Editing of the SLC43A2 gene in tumor cells for cancer immunotherapies. Schematic illustration of PMZH nanoplatform formation and PMZH for methionine metabolism regulation, nutritional metal ion therapy, and immune stimulation.[172] (B) Editing of the HSP70 gene in tumor cells for cancer immunotherapies. Schematic demonstration of the synthesis of LEGEND or FUGEND, and LEGEND- or FUGEND-mediated intracellular genome editing of tumor cells mediated by LEGEND or FUGEND and TME modulation to promote the adoptive T cell efficacy synergistically.[183]

Published on 08 2024. Downloaded on 03.11.2024 5:08:38.

proliferation rate (97.6%) than that in the non-pretreated tumors (13.6%). The notable anti-tumor therapeutic efficacy of LEGEND-sensitized adoptive TIL (LEGEND + TIL) was seen in B16F10 melanoma, and mouse models xenografted with A549 lung tumor and Hepa1-6 hepatocellular carcinoma cells. LEGEND pretreatment also led to more CAR-T cell infiltration in A549 primary and distant tumors, showing effective tumor growth control. In the humanized patient-derived xenograft (PDX) mouse model, LEGEND-sensitized adoptive CAR-T-cell therapy greatly enhanced the CAR-T cell infiltration into the PDX tumor, which was 6.5-fold higher than the group with CAR-T. FUS-enabled genome-editing nanodevice (FUGEND) included the sonosensitive 1,2-distearoyl-*sn-glycero*-3-phosphoethanolamine-polyethylene glycol (DSPE-PEG). FUS can precisely generate hyperthermia in local deep tissues that light cannot reach. Hence, FUGEND can be applied to treat deep orthotopic tumors. Under ultrasound, the FUGEND system succeeded in promoting TIL proliferation and infiltration in orthotopic hepatocellular carcinoma.

3. Genome editing targeting innate immune cells

Innate immune cells include macrophages, dendritic cells, neutrophils, and NK cells.[184,185] Genome editing in these innate immune cells has potential to induce activation, polarization, or other immune activation-oriented transformation for an enhanced effect of killing tumor cells. In this section, we will introduce CRISPR system delivery *via* non-viral nanocarriers for genome editing in innate immune cells (mainly macrophages) for cancer immunotherapy (Table 2).

3.1. Targeting macrophages

Macrophages exhibit phagocytosis of tumor cells, degradation of dead cells and debris, and modulation of inflammatory processes.[185–189] There are two phenotypes of macrophage, where M1 macrophages are usually considered anti-tumoral, pro-inflammatory and immune-activating, whereas M2 phenotype macrophages are related to tumor-associated, anti-inflammatory, and immunosuppressive functions.[190–192] Therefore, promotion of the recognition of macrophages of tumor cells and the polarization of macrophage phenotypes from M2 to M1 in tumors can be beneficial for cancer treatment, which can be realized by leveraging genome editing systems. Generally, to enhance the genome editing efficiency in macrophages, non-viral vectors are modified with ligands that can bind to receptors expressed on macrophages, such as macrophage galactose-type lectin and retinoid X receptor beta.[193,194]

Zhao *et al.*[195] developed *E. coli* protoplast-derived functionalized nanovesicles (NVs) to encapsulate Cas9-sgRNA RNP targeting Pik3cg (also known as PI-3 kinase gamma (PI3Kγ), a critical modulator of macrophage phenotype) (Fig. 7A). The plasmids which encoded sgPik3cg and Cas9 were transformed into *E. coli*, so the derived NVs exhibited high loading efficiency of the Cas9-sgPik3cg complex. Inactivation of macrophage PI3Kγ led to suppression of the tumor growth by inducing immune activation of the macrophages, and promoted an immunostimulatory transcriptional program that restores $CD8^+$ T cell activation and cytotoxicity.[196,197] The NVs were also modified with a pH-responsive PEG-conjugated phospholipid derivative of 1,2-distearoyl-*sn-glycero*-3-phosphorylethanolamine (DSPE)-hydrazone bond-PEG2000 (DHP) and a TAM-targeted phospholipid derivative of DSPE-galactosamine (DGA) to obtain sgPik3cg-DHP/DGA-NVs, for dual targeting acts towards TAMs. sgPik3cg-DHP/DGA-NV treatment of TAMs *in vitro*

Table 2 Representative nanoformulations for CRISPR-Cas9 delivery for genome editing targeting immune cells

Target gene locus	Target immune cell	CRISPR-Cas9 format	NP composition	Applications	Administration route	Ref.
PI3Kγ	Macrophages	RNP	Nanovesicles derived from *E. coli* protoplasts and functionalized with a pH-responsive PEG-conjugated phospholipid derivative of DSPE-hydrazone bond-PEG2000 and a TAM-targeted phospholipid derivative of DSPE-galactosamine	*In vivo* (4T1 TNBC in BALB/c mice; MC38 colorectal cancer in C57BL/6J mice)	Intravenous injection	195
PI3Kγ	Macrophages	Plasmid	Exosomes externally engineered with a macrophage-targeting peptide CRVLRSGSC	*In vivo* (LLC lung cancer in C57BL/6 mice)	Intravenous injection	198
SIRP-α	Macrophages	RNP	Cationic arginine-coated gold nanoparticles	*In vitro* (RAW264.7 macrophages)	—	200
RICTOR	Macrophages	RNP	Liposomes composed of Lipoid S100 and DOTAP	*In vivo* (4T1 TNBC in BALB/c mice)	Intratumoral injection	201
YTHDF1	Dendritic cells	Plasmid	Polymer-LNP (1,2-dioleoyl-3-trimethylammonium-propane and PEG-PLGA) coated with attenuated *Salmonella*-derived OMVs	*In vivo* (MC38 colorectal cancer in C57BL/6 mice)	Intravenous injection	210
PD-1	T cells	RNP	Liposomes	*In vitro* (primary T cells from peripheral blood of human)	—	243

 This journal is © The Royal Society of Chemistry 2024

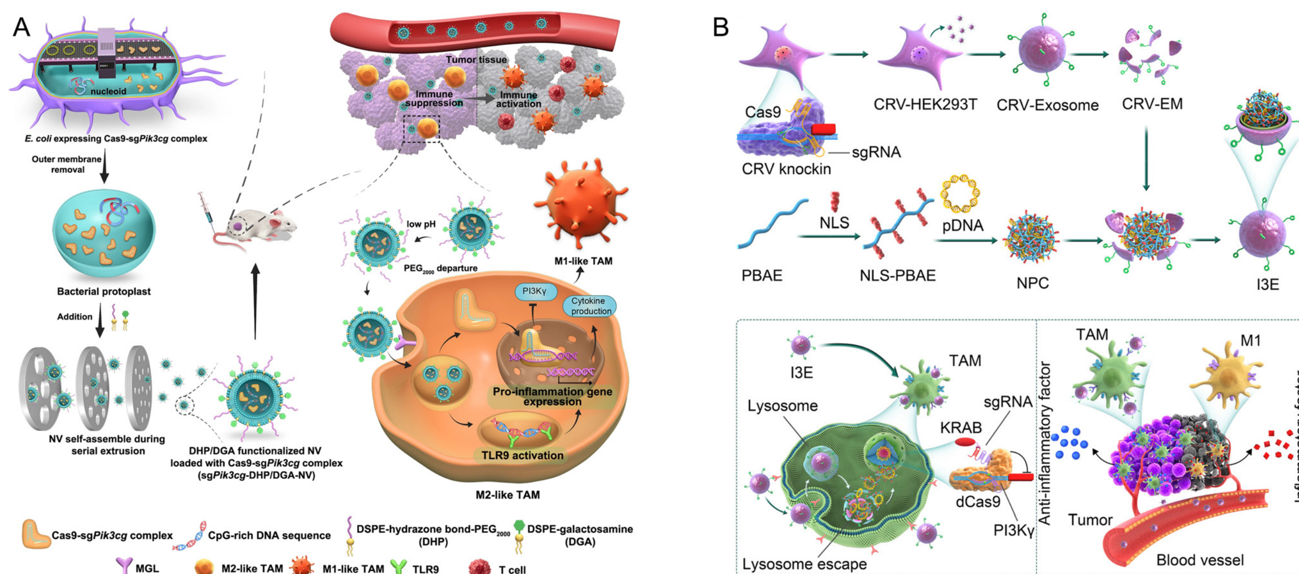


Fig. 7 Genome editing in macrophages for cancer immunotherapies. (A) Schematic illustration of sgPik3cg-DHP/DGA-NVs for TAM-targeting genome editing for anti-tumor efficacy enhancement.[195] (B) The construction of I3E, the mechanism of M1 polarization, and the awakening of the "hot" tumor-immunity.[198]

Published on 08 2024. Downloaded on 03.11.2024 5:08:38.

resulted in the significant downregulation of PI3Kγ and macrophage polarization. In the 4T1 breast cancer mouse model, intravenous sgPik3cg-DHP/DGA-NVs treatment exhibited effective macrophage M2-to-M1 polarization and the following TME remodeling from an immunosuppressive "cold" state to an immune active "hot" state.

Zhang *et al.*[198] constructed CRVLRSGSC (CRV) peptide-functionalized exosomes to encapsulate the dCas9-KRAB-sgPI3Kγ-encoded plasmid (Fig. 7B). The CRV peptide can selectively home to tumor tissues and target macrophages.[194] Additionally, the nuclease-dead mutants of Cas9 (dCas9) fused with a transcription repressor Krüppel-associated box (KRAB) were used for repressing the gene expression with higher efficiency and lower off-target effects.[199] The engineered exosomes remodeled the microenvironment with more stimulated M1 macrophages and T cells and decreased percentages of M2 macrophages and MDSCs, leading to the inhibited growth of tumors.

Ray *et al.*[200] established that cationic arginine-coated gold nanoparticles (ArgNPs) can deliver the CRISPR-Cas9 system for knocking out the signal regulatory protein α (SIRP-α) gene in macrophages. The interaction of SIRP-α on macrophages and CD47 on tumor cells sends a "don't eat me" signal, and the elimination of this signal favors the phagocytosis of cancer cells by (−) SIRP-α macrophage. A peptide tag containing glutamic acids (E20-tag) was inserted at the N-terminus of Cas9 protein for efficient cytosolic delivery, and a nuclear localization signal tag was appended at the C terminus to enhance nuclear accumulation. In *in vitro* experiments, SIRP-α gene knockout exhibited the efficiency of ∼27% in RAW264.7 cells after 48 h incubation, and the SIRP-α knockout RAW264.7 cells revealed a fourfold increase in phagocytosis of cocultured human osteosarcoma cells (U2OS) compared with the un-edited RAW264.7 cells.

Leonard *et al.*[201] used liposomes loaded with CRISPR complex (crRNA and Cas12a) to target rapamycin-insensitive companion of mammalian target of rapamycin (RICTOR), a rapamycin-insensitive companion of mTOR (mammalian target of rapamycin). The deletion of the RICTOR gene in macrophages induces M1 macrophage polarization, because mTORC2 signaling regulates the generation of M2 macrophages.[202,203] The Cas12a system is also a member of the CRISPR-associated nucleases that binds and cleaves DNA targets.[204,205] CRISPR-RICTOR-liposome successfully knocked down RICTOR expression, leading to M2 macrophage polarization into M1 macrophages in an *in vitro* 3D TME formed by the co-culture of breast cancer spheres and M2 macrophages.

3.2. Targeting dendritic cells

Dendritic cells are professional antigen-presenting cells that can initiate antigen-specific adaptive immune responses for T cell activation in anti-tumor immunity.[206–208] Specifically, dendritic cells recognize the antigens and become maturate, then present the antigens to T cells to trigger the immune system.[209] These important steps for anti-tumor immune activation are anticipated to be enhanced through genome editing. Li *et al.*[210] utilized the outer membrane vesicles (OMVs) of bacteria to load CRISPR/Cas9 plasmid (termed BNMs) (Fig. 8). OMVs preserve various bacteria-derived pathogen-associated molecular patterns (PAMPs), which can be processed and presented by dendritic cells.[211,212] The CRISPR/Cas9 plasmid was against YTHDF1, whose downregulation in dendritic cells can promote the cross-presentation of tumor antigens and the cross-priming of $CD8^+$ T cells.[213] pCas9/gYthdf1 plasmid transfection *in vitro* in DC2.4 cells resulted in

Published on 08 2024. Downloaded on 03.11.2024 5:08:38.

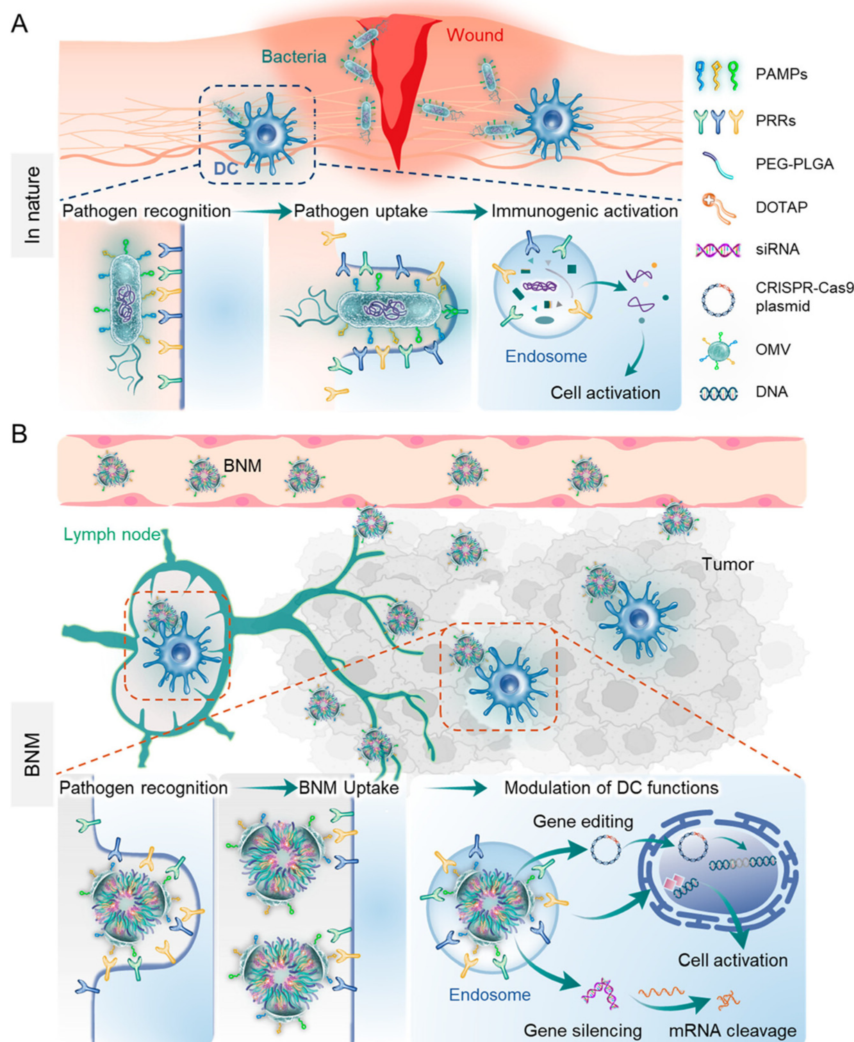


Fig. 8 Genome editing in dendritic cells for cancer immunotherapies. BNMs specifically target dendritic cells for gene silencing and editing *via* pathogen recognition.[210] (A) In nature, dendritic cells sense and internalize invading pathogens by recognizing various PAMPs, and then digest phagocytosed bacteria into nucleic acids and bacterial antigens to initiate anti-pathogen immunity. (B) The concept of pathogen recognition-driven dendritic cell-specific targeting for functional manipulation. BNMs containing various PAMPs derived from bacteria were prepared for dendritic cell targeting. The various PAMPs of BNMs facilitate the recognition and internalization of BNMs by dendritic cells through the interaction of PAMPs and pattern recognition receptors (PRRs). After endosome escape and dissociation, the siRNA or CRISPR-Cas9 system from BNMs can modulate dendritic cell functions *via* gene silencing or gene editing. Moreover, the PAMPs of BNMs can stimulate dendritic cell activation *via* the ligation of PRRs, which is a vital factor for initiating effective antitumor immunity.

a ~30% decrease of the Ythdf1 mRNA level. In MC38 tumor-bearing mice, $BNM_{pCas9/gYthdf1}$ treatment showed 97.72% tumor inhibition compared with the PBS group. In both lymph nodes and spleens after $BNM_{pCas9/gYthdf1}$ treatment, the numbers of activated $CD8^+$ T cells and memory $CD8^+$ T cells increased sharply, demonstrating the successful activation of $CD8^+$ T cell-mediated antitumor immune cells by Ythdf1 knockout in dendritic cells.

3.3. Targeting other innate immune cells

The activated neutrophils provide signals for the activation and maturation of macrophages and dendritic cells.[214–216] Thus, neutrophils play a crucial role in regulating immunity during inflammatory conditions.[217–220] The CRISPR-Cas9 system has been documented for editing in neutrophils *via* non-viral vectors to treat diabetes.[221] Although currently there is no example of non-viral vector-based genome editing in other innate immune cells by the CRISPR-Cas9 system for cancer immunotherapies, successful delivery of the CRISPR-Cas9 system to these innate immune cells holds promise for cancer immunotherapy in the future. For example, natural killer (NK) cells play an essential role in anti-tumor immunity as the front line in immune surveillance.[222,223] However, NK cells are usually dysfunctional in the tumor

 This journal is © The Royal Society of Chemistry 2024

microenvironment.[224] The CRISPR-Cas9 system was applied by electroporation to downregulate the expression of immune checkpoints (*e.g.*, NKG2A) expressed on NK cells for enhanced tumor cell killing efficiency, whereas the employment of non-viral vectors to deliver the CRISPR-Cas system into NK cells for genome editing is still expected.[225] In addition, eosinophils display a potent anti-tumorigenic effect.[226] Basophils may exhibit anti- or pro-tumorigenic effects, which may be related to the environment and the stage of tumorigenesis.[227] MDSCs are immunosuppressive cells, which inhibit the activation of T cells in tumors.[228,229]

4. Genome editing targeting adaptive immune cells

T cells and B cells are the lymphocytes that the adaptive immune system heavily relies on.[230] Genome editing in these adaptive immune cells is expected to promote their activation, proliferation, recruitment and infiltration in tumors for activated anti-tumor immunity. In this section, non-viral vector-enabled genome editing in adaptive immune cells (mainly T cells) was introduced (Table 2). Many adaptive immune cells, particularly T cells, are known to be hard to transfect.[231] Thus, several strategies were applied to improve the transfection efficiency *via* non-vectors to T cells, including the application of cell-penetrating peptide to form nanocomplexes, conjugation of T cell targeting ligands, and optimization of the phospholipid and PEG-lipid components in LNPs.[232–234]

4.1. Targeting T cells

$CD4^+$ and $CD8^+$ T cells are essential T lymphocytes in anti-tumor immunity.[235,236] $CD4^+$ T cells as the helper T cells can assist the activation of $CD8^+$ T cells, B cells, and other immune cells.[237,238] $CD8^+$ T cells as the cytotoxic T cells are responsible for killing tumor cells.[239] The activation and infiltration of T cells and their recognition of tumor cells are crucial in T cell-mediated cancer immunotherapy, and these steps can also be the targets for genome editing.[240–242] Lu *et al.*[243] loaded RNP in liposomes to knock out the PD-1 gene in T cells. PD-1 knockout T cells showed a significantly higher ability to proliferate, secrete pro-inflammatory cytokine IFN-γ, and kill HepG2 cells than un-edited T cells *in vitro*. In HepG2 xenografts in mice, edited T cells induced apoptosis of HepG2 cells, leading to significantly suppressed tumor growth and improved mouse survival.

4.2. Targeting other adaptive immune cells

B cells include short- or long-lived plasma cells, germinal center cells, and memory cells.[244] Although currently a non-viral vector-based CRISPR delivery system has not been applied in genome editing in B cells for cancer immunotherapy, the lipid nanoparticle-mediated delivery of CRISPR pDNA downregulated the number of B cells *in vivo* for rheumatoid arthritis therapy.[245] The successful genome in B cells could also provide possibilities for cancer immunotherapies.

5. Conclusions and outlook

Non-viral vectors have emerged as the next-generation delivery system for gene editing systems due to their excellent loading capacity, chemical versatility, and biocompatibility when compared with viral vectors, despite relatively low transfection efficiency in the targeted tissues after administration *in vivo*. As summarized in this review, utilizing the CRISPR-Cas system to boost cancer immunotherapy has been a promising combination. Non-viral vectors to deliver the CRISPR-Cas system in tumor cells and immune cells have been recently explored, proving the potential in future pre-clinical research. Currently, the immune checkpoints on tumor cells and immune cells (*e.g.*, PD-1 on T cells, PD-L1 on tumor cells) are the common and straightforward targets for non-viral vector-based genome editing for cancer immunotherapy. Besides, some other immune-related genes are also considered as genome editing targets, because their high expression in tumor cells or immune cells can lead to immune cell dysfunction, such as compromised antigen presentation and phagocytosis by immune cells, and macrophage M2-to-M1 polarization disability. Given the fact that these gene expressions in tumors interfere with active anti-tumor immunity, using the CRISPR/Cas system to knock out these genes for immunity retrieval is beneficial.

While there is no CRISPR-Cas9 genome editing approved for cancer immunotherapies for clinical applications, it is encouraging that the U.S. Food and Drug Administration (FDA) approved the first CRISPR/Cas9 therapy for treating sickle cell disease in December 2023.[246] Clinical trials of the CRISPR-Cas system for cancer therapy have been initiated for targeting leukemia, lymphoma, breast cancer, lung cancer, and other hematologic malignancies and solid tumors, while the employment of non-viral vectors for CRISPR-Cas system delivery for cancer treatments is rare in these clinical trials,[42,247,248] which is believed will be seen in the near future.

Despite the great promise, several crucial challenges should be considered and tackled to make non-viral vector-based genome editing more applicable for cancer immunotherapy. First, the safety of the compositions of nanocarriers, including their immunogenicity and biocompatibility, needs to be further ensured. Although lipids, polymers, and inorganic materials were employed to develop the CRISPR-Cas delivery system for successful genome editing and cancer immunotherapy promotion, the FDA-approved materials composition should be taken into more consideration for *in vivo* genome editing *via* CRISPR delivery in clinical study. Second, the gene editing efficiency in immune cells could be further improved, which is because targeting immune cells is still challenging, despite the accumulative knowledge about nanoparticles targeting tumor cells. Only a few cases focus on the *in vivo* targeting of nanocarriers to immune cells rather than *in vitro* induction. Especially for T cells and B cells, the delivery of CRISPR-Cas systems into these cells is even more difficult, because they are not actively phagocytic cells. Yet, considering the fact that T cells and B cells play important roles in the

Published on 08 2024. Downloaded on 03.11.2024 5:08:38.

adaptive anti-immune system and are promising targets for cancer immunotherapy, more effort is expected to validate the targeted delivery of CRISPR based on non-viral carriers to T and B cells. Additionally, non-viral vector-based gene editing in cancer and immune cells can be further combined with other immunotherapeutic strategies (*e.g.*, immune checkpoint blockade and adoptive T cell therapy) or other immune-activating therapies (*e.g.*, radiotherapy, photothermal therapy, photodynamic therapy) for a further enhanced anti-tumor immunotherapeutic efficacy.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by grants from the NSERC Discovery Program (RGPIN-2021-02669, to G.C.), NOVA-FRQNT/NSERC Grant (2023-NOVA-314056 & ALLRP 571574-21, to G.C.), and the start-up package of McGill University (to G.C.). T. F. would also like to acknowledge the Rolande and Marcel Gosselin Graduate Studentship, Dr Victor KS Lui Studentship, Charlotte and Leo Karassik Foundation Oncology Ph.D. Fellowship from the Rosalind & Morris Goodman Cancer Institute as well as the BME recruitment award.

Published on 08 2024. Downloaded on 03.11.2024 5:08:38.

References

1 Z. Liu, M. Shi, Y. Ren, H. Xu, S. Weng, W. Ning, X. Ge, L. Liu, C. Guo, M. Duo, L. Li, J. Li and X. Han, *Mol. Cancer*, 2023, **22**, 35.
2 S. Tan, D. Li and X. Zhu, *Biomed. Pharmacother.*, 2020, **124**, 109821.
3 S. Farkona, E. P. Diamandis and I. M. Blasutig, *BMC Med.*, 2016, **14**, 73.
4 K. Yi, H. Kong, Y. H. Lao, D. Li, R. L. Mintz, T. Fang, G. Chen, Y. Tao, M. Li and J. Ding, *Adv. Mater.*, 2023, e2300665, DOI: **10.1002/adma.202300665**.
5 L. Mauge, M. Terme, E. Tartour and D. Helley, *Front. Oncol.*, 2014, **4**, 61.
6 A. Labani-Motlagh, M. Ashja-Mahdavi and A. Loskog, *Front. Immunol.*, 2020, **11**, 940.
7 N. Xie, G. Shen, W. Gao, Z. Huang, C. Huang and L. Fu, *Signal Transduction Targeted Ther.*, 2023, **8**, 9.
8 J. Park, L. Wang and P. C. Ho, *Oncogenesis*, 2022, **11**, 62.
9 A. Del Prete, V. Salvi, A. Soriani, M. Laffranchi, F. Sozio, D. Bosisio and S. Sozzani, *Cell. Mol. Immunol.*, 2023, **20**, 432–447.
10 S. Y. Wu, T. Fu, Y. Z. Jiang and Z. M. Shao, *Mol. Cancer*, 2020, **19**, 120.
11 S. Zhao, L. Zhang, S. Xiang, Y. Hu, Z. Wu and J. Shen, *Front. Immunol.*, 2022, **13**, 791006.
12 Y. Liu and G. Zeng, *J. Immunother.*, 2012, **35**, 299–308.
13 D. Li and M. Wu, *Signal Transduction Targeted Ther.*, 2021, **6**, 291.
14 P. Lacy, *Front. Immunol.*, 2015, **6**, 190.
15 G. Arango Duque and A. Descoteaux, *Front. Immunol.*, 2014, **5**, 491.
16 C. N. Baxevanis, *Cancers*, 2023, **15**, 881.
17 V. Debien, A. De Caluwe, X. Wang, M. Piccart-Gebhart, V. K. Tuohy, E. Romano and L. Buisseret, *npj Breast Cancer*, 2023, **9**, 7.
18 A. Ribas and J. D. Wolchok, *Science*, 2018, **359**, 1350–1355.
19 O. Melaiu, V. Lucarini, R. Giovannoni, D. Fruci and F. Gemignani, *Semin. Cancer Biol.*, 2022, **79**, 18–43.
20 E. Baulu, C. Gardet, N. Chuvin and S. Depil, *Sci. Adv.*, 2023, **9**, eadf3700.
21 A. Lahiri, A. Maji, P. D. Potdar, N. Singh, P. Parikh, B. Bisht, A. Mukherjee and M. K. Paul, *Mol. Cancer*, 2023, **22**, 40.
22 D. M. Pardoll, *Nat. Rev. Cancer*, 2012, **12**, 252–264.
23 A. Mpakali and E. Stratikos, *Cancers*, 2021, **13**, 134.
24 Q. Li, Y. Zhou, W. He, X. Ren, M. Zhang, Y. Jiang, Z. Zhou and Y. Luan, *J. Controlled Release*, 2021, **338**, 33–45.
25 L. Barrueto, F. Caminero, L. Cash, C. Makris, P. Lamichhane and R. R. Deshmukh, *Transl. Oncol.*, 2020, **13**, 100738.
26 J. Pazhani, S. Jayaraman and V. P. Veeraraghavan, *Int. J. Surg.*, 2023, **109**, 230–231.
27 A. Titov, E. Zmievskaya, I. Ganeeva, A. Valiullina, A. Petukhov, A. Rakhmatullina, R. Miftakhova, M. Fainshtein, A. Rizvanov and E. Bulatov, *Cancers*, 2021, **13**, 743.
28 M. Hiltensperger and A. M. Krackhardt, *Front. Immunol.*, 2023, **14**, 1121030.
29 C. J. Kirk, D. Hartigan-O'Connor and J. J. Mulé, *Cancer Res.*, 2001, **61**, 8794–8802.
30 D. A. Wainwright, A. L. Chang, M. Dey, I. V. Balyasnikova, C. K. Kim, A. Tobias, Y. Cheng, J. W. Kim, J. Qiao, L. Zhang, Y. Han and M. S. Lesniak, *Clin. Cancer Res.*, 2014, **20**, 5290–5301.
31 V. P. Balachandran, G. L. Beatty and S. K. Dougan, *Gastroenterology*, 2019, **156**, 2056–2072.
32 D. Ciardiello, P. P. Vitiello, C. Cardone, G. Martini, T. Troiani, E. Martinelli and F. Ciardiello, *Cancer Treat. Rev.*, 2019, **76**, 22–32.
33 X. Lu, J. W. Horner, E. Paul, X. Shang, P. Troncoso, P. Deng, S. Jiang, Q. Chang, D. J. Spring, P. Sharma, J. A. Zebala, D. Y. Maeda, Y. A. Wang and R. A. DePinho, *Nature*, 2017, **543**, 728–732.
34 N. N. Shah and T. J. Fry, *Nat. Rev. Clin Oncol.*, 2019, **16**, 372–385.
35 R. C. Sterner and R. M. Sterner, *Blood Cancer J.*, 2021, **11**, 69.
36 S. Kelderman, T. N. Schumacher and J. B. Haanen, *Mol. Oncol.*, 2014, **8**, 1132–1139.
37 C. M. Jackson, J. Choi and M. Lim, *Nat. Immunol.*, 2019, **20**, 1100–1109.
38 A. Kalbasi and A. Ribas, *Nat. Rev. Immunol.*, 2020, **20**, 25–39.

This journal is © The Royal Society of Chemistry 2024

Published on 08 2024. Downloaded on 03.11.2024 5:08:38.

39 H. Li, Y. Yang, W. Hong, M. Huang, M. Wu and X. Zhao, *Signal Transduction Targeted Ther.*, 2020, **5**, 1.
40 J. C. Carpenter and G. A.-O. Lignani, *Neurotherapeutics*, 2021, **18**, 1515–1523.
41 K. Liu, J. J. Cui, Y. Zhan, Q. Y. Ouyang, Q. S. Lu, D. H. Yang, X. P. Li and J. Y. Yin, *Mol. Cancer*, 2022, **21**, 98.
42 Z. Xu, Q. Wang, H. Zhong, Y. Jiang, X. Shi, B. Yuan, N. Yu, S. Zhang, X. Yuan, S. Guo and Y. Yang, *Exploration*, 2022, **2**, 20210081.
43 Editorial, *Nat. Mater.*, 2021, **20**, 1.
44 H. Zhu, C. Li and C. Gao, *Nat. Rev. Mol. Cell Biol.*, 2020, **21**, 661–677.
45 S. Q. Tsai and J. K. Joung, *Nat. Rev. Genet.*, 2016, **17**, 300–312.
46 T. Fang, X. Cao, M. Ibnat and G. Chen, *J. Nanobiotechnol.*, 2022, **20**, 354.
47 M. Adli, *Nat. Commun.*, 2018, **9**, 1911.
48 T. Li, Y. Yang, H. Qi, W. Cui, L. Zhang, X. Fu, X. He, M. Liu, P. F. Li and T. Yu, *Signal Transduction Targeted Ther.*, 2023, **8**, 36.
49 M. Chavez, X. Chen, P. B. Finn and L. S. Qi, *Nat. Rev. Nephrol.*, 2023, **19**, 9–22.
50 A. Hendel, R. O. Bak, J. T. Clark, A. B. Kennedy, D. E. Ryan, S. Roy, I. Steinfeld, B. D. Lunstad, R. J. Kaiser, A. B. Wilkens, R. Bacchetta, A. Tsalenko, D. Dellinger, L. Bruhn and M. H. Porteus, *Nat. Biotechnol.*, 2015, **33**, 985–989.
51 C. A. Lino, J. C. Harper, J. P. Carney and J. A. Timlin, *Drug Delivery*, 2018, **25**, 1234–1257.
52 R. M. Gupta and K. Musunuru, *J. Clin. Invest.*, 2014, **124**, 4154–4161.
53 D. J. Segal, B. Dreier, R. R. Beerli and C. F. Barbas, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 2758–2763.
54 N. E. Sanjana, L. Cong, Y. Zhou, M. M. Cunniff, G. Feng and F. Zhang, *Nat. Protoc.*, 2012, **7**, 171–192.
55 K. Yoshimi and T. Mashimo, *J. Hum. Genet.*, 2018, **63**, 115–123.
56 Z. WareJoncas, J. M. Campbell, G. Martinez-Galvez, W. A. C. Gendron, M. A. Barry, P. C. Harris, C. R. Sussman and S. C. Ekker, *Nat. Rev. Nephrol.*, 2018, **14**, 663–677.
57 L. Cong, F. A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, P. D. Hsu, X. Wu, W. Jiang, L. A. Marraffini and F. Zhang, *Science*, 2013, **339**, 819–823.
58 P. Ma, Q. Wang, X. Luo, L. Mao, Z. Wang, E. Ye, X. J. Loh, Z. Li and Y. L. Wu, *Biomater. Sci.*, 2023, **11**, 5078–5094.
59 A. V. Anzalone, P. B. Randolph, J. R. Davis, A. A. Sousa, L. W. Koblan, J. M. Levy, P. J. Chen, C. Wilson, G. A. Newby, A. Raguram and D. R. Liu, *Nature*, 2019, **576**, 149–157.
60 P. J. Chen and D. R. Liu, *Nat. Rev. Genet.*, 2023, **24**, 161–177.
61 N. M. Gaudelli, A. C. Komor, H. A. Rees, M. S. Packer, A. H. Badran, D. I. Bryson and D. R. Liu, *Nature*, 2017, **551**, 464–471.
62 E. M. Porto, A. C. Komor, I. M. Slaymaker and G. W. Yeo, *Nat. Rev. Drug Discovery*, 2020, **19**, 839–859.
63 D. Collias, E. Vialetto, J. Yu, K. Co, E. D. H. Almasi, A. S. Ruttiger, T. Achmedov, T. Strowig and C. L. Beisel, *Nat. Commun.*, 2023, **14**, 680.
64 J. K. Lee, E. Jeong, J. Lee, M. Jung, E. Shin, Y. H. Kim, K. Lee, I. Jung, D. Kim, S. Kim and J. S. Kim, *Nat. Commun.*, 2018, **9**, 3048.
65 C. D. Sago, M. P. Lokugamage, K. Paunovska, D. A. Vanover, C. M. Monaco, N. N. Shah, M. Gamboa Castro, S. E. Anderson, T. G. Rudoltz, G. N. Lando, P. Munnilal Tiwari, J. L. Kirschman, N. Willett, Y. C. Jang, P. J. Santangelo, A. V. Bryksin and J. E. Dahlman, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**, E9944–E9952.
66 C. Xu, Z. Lu, Y. Luo, Y. Liu, Z. Cao, S. Shen, H. Li, J. Liu, K. Chen, Z. Chen, X. Yang, Z. Gu and J. Wang, *Nat. Commun.*, 2018, **9**, 4092.
67 T. Wei, Q. Cheng, Y. L. Min, E. N. Olson and D. J. Siegwart, *Nat. Commun.*, 2020, **11**, 3232.
68 D. Chaverra-Rodriguez, V. M. Macias, G. L. Hughes, S. Pujhari, Y. Suzuki, D. R. Peterson, D. Kim, S. McKeand and J. L. Rasgon, *Nat. Commun.*, 2018, **9**, 3008.
69 N. Rouatbi, T. McGlynn and K. T. Al-Jamal, *Biomater. Sci.*, 2022, **10**, 3410–3432.
70 Y. Lin, E. Wagner and U. Lachelt, *Biomater. Sci.*, 2022, **10**, 1166–1192.
71 J. O'Keeffe Ahern, I. Lara-Saez, D. Zhou, R. Murillas, J. Bonafont, A. Mencia, M. Garcia, D. Manzanares, J. Lynch, R. Foley, Q. Xu, A. Sigen, F. Larcher and W. Wang, *Gene Ther.*, 2022, **29**, 157–170.
72 D. R. Jacobson and O. A. Saleh, *Nucleic Acids Res.*, 2017, **45**, 1596–1605.
73 S. Zhen and X. Li, *Cancer Gene Ther.*, 2020, **27**, 515–527.
74 C. Zhuo, J. Zhang, J. H. Lee, J. Jiao, D. Cheng, L. Liu, H. W. Kim, Y. Tao and M. Li, *Signal Transduction Targeted Ther.*, 2021, **6**, 238.
75 G. Chen, A. A. Abdeen, Y. Wang, P. K. Shahi, S. Robertson, R. Xie, M. Suzuki, B. R. Pattnaik, K. Saha and S. Gong, *Nat. Nanotechnol.*, 2019, **14**, 974–980.
76 A. P. Chandrasekaran, M. Song, K. S. Kim and S. Ramakrishna, *Prog. Mol. Biol. Transl. Sci.*, 2018, **159**, 157–176.
77 D. Wang, P. W. L. Tai and G. Gao, *Nat. Rev. Drug Discovery*, 2019, **18**, 358–378.
78 C. Li and R. J. Samulski, *Nat. Rev. Genet.*, 2020, **21**, 255–272.
79 M. Humbel, M. Ramosaj, V. Zimmer, S. Regio, L. Aeby, S. Moser, A. Boizot, M. Sipion, M. Rey and N. Deglon, *Gene Ther.*, 2021, **28**, 75–88.
80 S. E. Ahmadi, M. Soleymani, F. Shahriyary, M. R. Amirzargar, M. Ofoghi, M. D. Fattahi and M. Safa, *Cancer Gene Ther.*, 2023, 1–19, DOI: **10.1038/s41417-023-00597-z**.
81 C. E. Nelson, C. L. Duvall, A. Prokop, C. A. Gersbach and J. M. Davidson, in *Principles of Tissue Engineering*, ed. R. Lanza, R. Langer, J. P. Vacanti and A. Atala, Academic Press, 5th edn, 2020, pp. 519–554. DOI: **10.1016/B978-0-12-818422-6.00030-7**.

Published on 08 2024. Downloaded on 03.11.2024 5:08:38.

82 F. Uddin, C. M. Rudin and T. Sen, *Front. Oncol.*, 2020, **10**, 1387.
83 M. Ramamoorth and A. Narvekar, *J. Clin. Diagn. Res.*, 2015, **9**, GE01-06.
84 T. Travieso, J. Li, S. Mahesh, J. Mello and M. Blasi, *npj Vaccines*, 2022, **7**, 75.
85 K. J. Ewer, T. Lambe, C. S. Rollier, A. J. Spencer, A. V. Hill and L. Dorrell, *Curr. Opin. Immunol.*, 2016, **41**, 47–54.
86 C. F. Xu, G. J. Chen, Y. L. Luo, Y. Zhang, G. Zhao, Z. D. Lu, A. Czarna, Z. Gu and J. Wang, *Adv. Drug Delivery Rev.*, 2021, **168**, 3–29.
87 Z. Iqbal, K. Rehman, J. Xia, M. Shabbir, M. Zaman, Y. Liang and L. Duan, *Biomater. Sci.*, 2023, **11**, 3762–3783.
88 Y. Ye, X. Zhang, F. Xie, B. Xu, P. Xie, T. Yang, Q. Shi, C. Y. Zhang, Y. Zhang, J. Chen, X. Jiang and J. Li, *Biomater. Sci.*, 2020, **8**, 2966–2976.
89 Q. Zheng, W. Wang, Y. Zhou, J. Mo, X. Chang, Z. Zha and L. Zha, *Biomater. Sci.*, 2023, **11**, 5361–5389.
90 R. Mout, M. Ray, Y. W. Lee, F. Scaletti and V. M. Rotello, *Bioconjugate Chem.*, 2017, **28**, 880–884.
91 Y. Wang, B. Ma, A. A. Abdeen, G. Chen, R. Xie, K. Saha and S. Gong, *ACS Appl. Mater. Interfaces*, 2018, **10**, 31915–31927.
92 Z. Glass, Y. Li and Q. Xu, *Nat. Biomed. Eng.*, 2017, **1**, 854–855.
93 H. X. Wang, M. Li, C. M. Lee, S. Chakraborty, H. W. Kim, G. Bao and K. W. Leong, *Chem. Rev.*, 2017, **117**, 9874–9906.
94 T. Wei, Q. Cheng, L. Farbiak, D. G. Anderson, R. Langer and D. J. Siegwart, *ACS Nano*, 2020, **14**, 9243–9262.
95 G. Chen, B. Ma, Y. Wang and S. Gong, *ACS Appl. Mater. Interfaces*, 2018, **10**, 18515–18523.
96 J. Eoh and L. Gu, *Biomater. Sci.*, 2019, **7**, 1240–1261.
97 H. Zu and D. Gao, *AAPS J.*, 2021, **23**, 78.
98 M. J. Mitchell, M. M. Billingsley, R. M. Haley, M. E. Wechsler, N. A. Peppas and R. Langer, *Nat. Rev. Drug Discovery*, 2021, **20**, 101–124.
99 A. Cifuentes-Rius, A. Desai, D. Yuen, A. P. R. Johnston and N. H. Voelcker, *Nat. Nanotechnol.*, 2021, **16**, 37–46.
100 Z. Zhao, A. Ukidve, J. Kim and S. Mitragotri, *Cell*, 2020, **181**, 151–167.
101 R. Ephraim, S. Fraser, K. Nurgali and V. Apostolopoulos, *Cancers*, 2022, **14**, 3788.
102 X. Cai, H. Zhan, Y. Ye, J. Yang, M. Zhang, J. Li and Y. Zhuang, *Front. Genet.*, 2021, **12**, 785153.
103 A. D. Waldman, J. M. Fritz and M. J. Lenardo, *Nat. Rev. Immunol.*, 2020, **20**, 651–668.
104 H. Gonzalez, C. Hagerling and Z. A.-O. Werb.
105 P. H. Pandya, M. E. Murray, K. E. Pollok and J. L. Renbarger, *J. Immunol. Res.*, 2016, **2016**, 4273943.
106 G. P. Dunn, C. M. Koebel and R. D. Schreiber, *Nat. Rev. Immunol.*, 2006, **6**, 836–848.
107 C. H. Patel, R. D. Leone, M. R. Horton and J. D. Powell, *Nat. Rev. Drug Discovery*, 2019, **18**, 669–688.
108 C. N. Baxevanis, S. P. Fortis and S. A. Perez, *Semin. Cancer Biol.*, 2021, **72**, 76–89.
109 Y. Tie, F. Tang, Y. Q. Wei and X. W. Wei, *J. Hematol. Oncol.*, 2022, **15**, 61.
110 J. H. Cha, L. C. Chan, C. W. Li, J. L. Hsu and M. C. Hung, *Mol. Cell*, 2019, **76**, 359–370.
111 E. I. Buchbinder and A. Desai, *Am. J. Clin. Oncol.*, 2016, **39**, 98–106.
112 T. Powles, J. Walker, J. Andrew Williams and J. Bellmunt, *Cancer Treat. Rev.*, 2020, **82**, 101925.
113 X. Wang, F. Teng, L. Kong and J. Yu, *OncoTargets Ther.*, 2016, **9**, 5023–5039.
114 C. J. Li, L. T. Lin, M. F. Hou and P. Y. Chu, *Oncol. Rep.*, 2021, **45**, 5–12.
115 J. Yang, M. Dong, Y. Shui, Y. Zhang, Z. Zhang, Y. Mi, X. Zuo, L. Jiang, K. Liu, Z. Liu, X. Gu and Y. Shi, *Cancer Cell Int.*, 2020, **20**, 96.
116 N. Yaghoubi, A. Soltani, K. Ghazvini, S. M. Hassanian and S. I. Hashemy, *Biomed. Pharmacother.*, 2019, **110**, 312–318.
117 W. J. Cheng, L. C. Chen, H. O. Ho, H. L. Lin and M. T. Sheu, *Int. J. Nanomed.*, 2018, **13**, 7079–7094.
118 A. H. Sharpe and K. E. Pauken, *Nat. Rev. Immunol.*, 2018, **18**, 153–167.
119 F. K. Dermani, P. Samadi, G. Rahmani, A. K. Kohlan and R. Najafi, *J. Cell Physiol.*, 2019, **234**, 1313–1325.
120 C. Sun, R. Mezzadra and T. N. Schumacher, *Immunity*, 2018, **48**, 434–452.
121 X. He and C. Xu, *Cell Res.*, 2020, **30**, 660–669.
122 H. Deng, S. Tan, X. Gao, C. Zou, C. Xu, K. Tu, Q. Song, F. Fan, W. Huang and Z. Zhang, *Acta Pharm. Sin. B*, 2020, **10**, 358–373.
123 Y. Lu, F. Wu, Y. Xu, C. He, S. Luo and X. Sun, *J. Controlled Release*, 2023, **354**, 57–68.
124 Q. Liu, C. Wang, Y. Zheng, Y. Zhao, Y. Wang, J. Hao, X. Zhao, K. Yi, L. Shi, C. Kang and Y. Liu, *Biomaterials*, 2020, **258**, 120275.
125 Y. Xing, J. Yang, Y. Wang, C. Wang, Z. Pan, F. L. Liu, Y. Liu and Q. Liu, *ACS Nano*, 2023, **17**, 5713–5726.
126 S. Deshayes, H. Cabral, T. Ishii, Y. Miura, S. Kobayashi, T. Yamashita, A. Matsumoto, Y. Miyahara, N. Nishiyama and K. Kataoka, *J. Am. Chem. Soc.*, 2013, **135**, 15501–15507.
127 J. M. M. Levy, C. G. Towers and A. Thorburn, *Nat. Rev. Cancer*, 2017, **17**, 528–542.
128 J. Zhou, G. Wang, Y. Chen, H. Wang, Y. Hua and Z. Cai, *J. Cell. Mol. Med.*, 2019, **23**, 4854–4865.
129 J. Liu, G. Li, H. Guo, C. Ni, Y. Gao, X. Cao, J. Xia, X. Shi and R. Guo, *ACS Appl. Mater. Interfaces*, 2023, **15**, 12809–12821.
130 Y. Lin, U. Wilk, J. Pohmerer, E. Horterer, M. Hohn, X. Luo, H. Mai, E. Wagner and U. Lachelt, *Small*, 2023, **19**, e2205318.
131 P. M. Klein, S. Kern, D. J. Lee, J. Schmaus, M. Hohn, J. Gorges, U. Kazmaier and E. Wagner, *Biomaterials*, 2018, **178**, 630–642.
132 E. Y. Chiang and I. Mellman, *J. Immunother. Cancer*, 2022, **10**, e004711.
133 H. Stamm, F. Klingler, E. M. Grossjohann, J. Muschhammer, E. Vettorazzi, M. Heuser, U. Mock,

 This journal is © The Royal Society of Chemistry 2024

Published on 08 2024. Downloaded on 03.11.2024 5:08:38.

F. Thol, G. Vohwinkel, E. Latuske, C. Bokemeyer, R. Kischel, C. Dos Santos, S. Stienen, M. Friedrich, M. Lutteropp, D. Nagorsen, J. Wellbrock and W. Fiedler, *Oncogene*, 2018, **37**, 5269–5280.
134 C. Yue, S. Gao, S. Li, Z. Xing, H. Qian, Y. Hu, W. Wang and C. Hua, *Front. Immunol.*, 2022, **13**, 911919.
135 J. Yeo, M. Ko, D. H. Lee, Y. Park and H. S. Jin, *Pharmaceuticals*, 2021, **14**, 200.
136 H. Stamm, L. Oliveira-Ferrer, E. M. Grossjohann, J. Muschhammer, V. Thaden, F. Brauneck, R. Kischel, V. Muller, C. Bokemeyer, W. Fiedler and J. Wellbrock, *OncoImmunology*, 2019, **8**, e1674605.
137 Z. Zhang, Q. Wang, Q. Liu, Y. Zheng, C. Zheng, K. Yi, Y. Zhao, Y. Gu, Y. Wang, C. Wang, X. Zhao, L. Shi, C. Kang and Y. Liu, *Adv. Mater.*, 2019, **31**, e1905751.
138 J. Tao, D. E. Bauer and R. Chiarle, *Nat. Commun.*, 2023, **14**, 212.
139 F. Bigini, S. H. Lee, Y. J. Sun, Y. Sun and V. B. Mahajan, *Vision Res.*, 2023, **213**, 108317.
140 M. Damaghi, N. K. Tafreshi, M. C. Lloyd, R. Sprung, V. Estrella, J. W. Wojtkowiak, D. L. Morse, J. M. Koomen, M. M. Bui, R. A. Gatenby and R. J. Gillies, *Nat. Commun.*, 2015, **6**, 8752.
141 B. Perillo, M. Di Donato, A. Pezone, E. Di Zazzo, P. Giovannelli, G. Galasso, G. Castoria and A. Migliaccio, *Exp. Mol. Med.*, 2020, **52**, 192–203.
142 D. Zhang, G. Wang, X. Yu, T. Wei, L. Farbiak, L. T. Johnson, A. M. Taylor, J. Xu, Y. Hong, H. Zhu and D. J. Siegwart, *Nat. Nanotechnol.*, 2022, **17**, 777–787.
143 M. S. Bauer, F. Baumann, C. Daday, P. Redondo, E. Durner, M. A. Jobst, L. F. Milles, D. Mercadante, D. A. Pippig, H. E. Gaub, F. Grater and D. Lietha, *Proc. Natl. Acad. Sci. U. S. A.*, 2019, **116**, 6766–6774.
144 H. Jiang, S. Hegde, B. L. Knolhoff, Y. Zhu, J. M. Herndon, M. A. Meyer, T. M. Nywening, W. G. Hawkins, I. M. Shapiro, D. T. Weaver, J. A. Pachter, A. Wang-Gillam and D. G. DeNardo, *Nat. Med.*, 2016, **22**, 851–860.
145 A. Serrels, T. Lund, B. Serrels, A. Byron, R. C. McPherson, A. von Kriegsheim, L. Gomez-Cuadrado, M. Canel, M. Muir, J. E. Ring, E. Maniati, A. H. Sims, J. A. Pachter, V. G. Brunton, N. Gilbert, S. M. Anderton, R. J. Nibbs and M. C. Frame, *Cell*, 2015, **163**, 160–173.
146 M. P. Chao, C. H. Takimoto, D. D. Feng, K. McKenna, P. Gip, J. Liu, J. P. Volkmer, I. L. Weissman and R. Majeti, *Front. Oncol.*, 2019, **9**, 1380.
147 R. H. Vonderheide, *Nat. Med.*, 2015, **21**, 1122–1123.
148 Y. Liu, Y. Wang, Y. Yang, L. Weng, Q. Wu, J. Zhang, P. Zhao, L. Fang, Y. Shi and P. Wang, *Signal Transduction Targeted Ther.*, 2023, **8**, 104.
149 Z. Bian, L. Shi, K. Kidder, K. Zen, C. Garnett-Benson and Y. Liu, *Nat. Commun.*, 2021, **12**, 3229.
150 M. Zhang, G. Hutter, S. A. Kahn, T. D. Azad, S. Gholamin, C. Y. Xu, J. Liu, A. S. Achrol, C. Richard, P. Sommerkamp, M. K. Schoen, M. N. McCracken, R. Majeti, I. Weissman, S. S. Mitra and S. H. Cheshier, *PLoS One*, 2016, **11**, e0153550.
151 M. A. Morrissey, N. Kern and R. D. Vale, *Immunity*, 2020, **53**, 290–302.
152 Y. Kojima, J. P. Volkmer, K. McKenna, M. Civelek, A. J. Lusis, C. L. Miller, D. Direnzo, V. Nanda, J. Ye, A. J. Connolly, E. E. Schadt, T. Quertermous, P. Betancur, L. Maegdefessel, L. P. Matic, U. Hedin, I. L. Weissman and N. J. Leeper, *Nature*, 2016, **536**, 86–90.
153 M. Feng, W. Jiang, B. Y. S. Kim, C. C. Zhang, Y. X. Fu and I. L. Weissman, *Nat. Rev. Cancer*, 2019, **19**, 568–586.
154 Q. Huang, M. Su, L. Zhao, Z. Zhang, Y. Zhang, X. Yang and J. Wang, *Nano Today*, 2023, **50**, 101857.
155 M. Lin, Z. Yang, Y. Yang, Y. Peng, J. Li, Y. Du, Q. Sun, D. Gao, Q. Yuan, Y. Zhou, X. Chen and X. Qi, *Nano Today*, 2022, **42**, 101359.
156 F. Ren, R. Tang, X. Zhang, W. M. Madushi, D. Luo, Y. Dang, Z. Li, K. Wei and G. Chen, *PLoS One*, 2015, **10**, e0135544.
157 K. Mikecz, F. R. Brennan, J. H. Kim and T. T. Glant, *Nat. Med.*, 1995, **1**, 558–563.
158 P. Kesharwani, R. Chadar, A. Sheikh, W. Y. Rizg and A. Y. Safhi, *Front. Pharmacol.*, 2021, **12**, 800481.
159 Y. Xing, J. Yang, C. Wang, Z. Kang, Z. Pan, J. Tang, F. Li, X. Wang, X.-m. Meng, Z. Cheng, Y. Liu and Q. Liu, *Chem. Eng. J.*, 2023, **474**, 145796.
160 J. Wei, L. Long, W. Zheng, Y. Dhungana, S. A. Lim, C. Guy, Y. Wang, Y. D. Wang, C. Qian, B. Xu, A. Kc, J. Saravia, H. Huang, J. Yu, J. G. Doench, T. L. Geiger and H. Chi, *Nature*, 2019, **576**, 471–476.
161 T. Xia, X. M. Yi, X. Wu, J. Shang and H. B. Shu, *Proc. Natl. Acad. Sci. U. S. A.*, 2019, **116**, 20063–20069.
162 P. F. Wang, H. Q. Cai, C. B. Zhang, Y. M. Li, X. Liu, J. H. Wan, T. Jiang, S. W. Li and C. X. Yan, *J. Neuroinflammation*, 2018, **15**, 145.
163 Z. Huang, M. Liu, D. Li, Y. Tan, R. Zhang, Z. Xia, P. Wang, B. Jiao, P. Liu and R. Ren, *J. Biol. Chem.*, 2020, **295**, 18343–18354.
164 R. T. Manguso, H. W. Pope, M. D. Zimmer, F. D. Brown, K. B. Yates, B. C. Miller, N. B. Collins, K. Bi, M. W. LaFleur, V. R. Juneja, S. A. Weiss, J. Lo, D. E. Fisher, D. Miao, E. Van Allen, D. E. Root, A. H. Sharpe, J. G. Doench and W. N. Haining, *Nature*, 2017, **547**, 413–418.
165 M. W. LaFleur, T. H. Nguyen, M. A. Coxe, B. C. Miller, K. B. Yates, J. E. Gillis, D. R. Sen, E. F. Gaudiano, R. Al Abosy, G. J. Freeman, W. N. Haining and A. H. Sharpe, *Nat. Immunol.*, 2019, **20**, 1335–1347.
166 M. W. LaFleur, T. H. Nguyen, M. A. Coxe, K. B. Yates, J. D. Trombley, S. A. Weiss, F. D. Brown, J. E. Gillis, D. J. Coxe, J. G. Doench, W. N. Haining and A. H. Sharpe, *Nat. Commun.*, 2019, **10**, 1668.
167 F. Wiede, K. H. Lu, X. Du, S. Liang, K. Hochheiser, G. T. Dodd, P. K. Goh, C. Kearney, D. Meyran, P. A. Beavis, M. A. Henderson, S. L. Park, J. Waithman, S. Zhang, Z. Y. Zhang, J. Oliaro, T. Gebhardt, P. K. Darcy and T. Tiganis, *EMBO J.*, 2020, **39**, e103637.
168 C. Yang, Y. Fu, C. Huang, D. Hu, K. Zhou, Y. Hao, B. Chu, Y. Yang and Z. Qian, *Biomaterials*, 2020, **255**, 120194.

Published on 08 2024. Downloaded on 03.11.2024 5:08:38.

169 J. Yang, K. Yang, S. Du, W. Luo, C. Wang, H. Liu, K. Liu, Z. Zhang, Y. Gao, X. Han and Y. Song, *Angew. Chem., Int. Ed.*, 2023, **62**, e202306863.
170 D. G. Roy, J. Chen, V. Mamane, E. H. Ma, B. M. Muhire, R. D. Sheldon, T. Shorstova, R. Koning, R. M. Johnson, E. Esaulova, K. S. Williams, S. Hayes, M. Steadman, B. Samborska, A. Swain, A. Daigneault, V. Chubukov, T. P. Roddy, W. Foulkes, J. A. Pospisilik, M. C. Bourgeois-Daigneault, M. N. Artyomov, M. Witcher, C. M. Krawczyk, C. Larochelle and R. G. Jones, *Cell Metab.*, 2020, **31**, 250–266.
171 P. M. Gubser and A. Kallies, *Immunol. Cell Biol.*, 2020, **98**, 623–625.
172 Y. Huang, G. Qin, T. Cui, C. Zhao, J. Ren and X. Qu, *Nat. Commun.*, 2023, **14**, 4647.
173 A. Rosati, A. Basile, R. D'Auria, M. d'Avenia, M. De Marco, A. Falco, M. Festa, L. Guerriero, V. Iorio, R. Parente, M. Pascale, L. Marzullo, R. Franco, C. Arra, A. Barbieri, D. Rea, G. Menichini, M. Hahne, M. Bijlsma, D. Barcaroli, G. Sala, F. F. di Mola, P. di Sebastiano, J. Todoric, L. Antonucci, V. Corvest, A. Jawhari, M. A. Firpo, D. A. Tuveson, M. Capunzo, M. Karin, V. De Laurenzi and M. C. Turco, *Nat. Commun.*, 2015, **6**, 8695.
174 L. Marzullo, M. C. Turco and M. De Marco, *Biochim Biophys Acta Gen Subj*, 2020, **1864**, 129628.
175 M. F. Romano, M. Festa, G. Pagliuca, R. Lerose, R. Bisogni, F. Chiurazzi, G. Storti, S. Volpe, S. Venuta, M. C. Turco and A. Leone, *Cell Death Differ.*, 2003, **10**, 383–385.
176 T. A. Colvin, V. L. Gabai, J. Gong, S. K. Calderwood, H. Li, S. Gummuluru, O. N. Matchuk, S. G. Smirnova, N. V. Orlova, I. A. Zamulaeva, M. Garcia-Marcos, X. Li, Z. T. Young, J. N. Rauch, J. E. Gestwicki, S. Takayama and M. Y. Sherman, *Cancer Res.*, 2014, **74**, 4731–4740.
177 A. Rosati, V. Graziano, V. De Laurenzi, M. Pascale and M. C. Turco, *Cell Death Dis.*, 2011, **2**, e141.
178 B. K. Wang, X. F. Yu, J. H. Wang, Z. B. Li, P. H. Li, H. Wang, L. Song, P. K. Chu and C. Li, *Biomaterials*, 2016, **78**, 27–39.
179 J. Joung, P. C. Kirchgatterer, A. Singh, J. H. Cho, S. P. Nety, R. C. Larson, R. K. Macrae, R. Deasy, Y. Y. Tseng, M. V. Maus and F. Zhang, *Nat. Commun.*, 2022, **13**, 1606.
180 L. Avinery, V. Gahramanov, A. Hesin and M. Y. Sherman, *Cancers*, 2022, **14**, 4168.
181 M. De Marco, A. Falco, R. Iaccarino, A. Raffone, A. Mollo, M. Guida, A. Rosati, M. Chetta, G. Genovese, F. De Caro, M. Capunzo, M. C. Turco, V. N. Uversky and L. Marzullo, *Br. J. Cancer*, 2021, **125**, 789–797.
182 M. De Marco, M. C. Turco and L. Marzullo, *Trends Cancer*, 2020, **6**, 985–988.
183 X. Chen, S. Wang, Y. Chen, H. Xin, S. Zhang, D. Wu, Y. Xue, M. Zha, H. Li, K. Li, Z. Gu, W. Wei and Y. Ping, *Nat. Nanotechnol.*, 2023, **18**, 933–944.
184 E. B. Okeke and J. E. Uzonna, *Front. Immunol.*, 2019, **10**, 680.
185 C. J. Burrell, C. R. Howard and F. A. Murphy, in *Fenner and White's Medical Virology*, 2017, pp. 57–64. DOI: 10.1016/b978-0-12-375156-0.00005-9.
186 A. Hochreiter-Hufford and K. S. Ravichandran, *Cold Spring Harbor Perspect. Biol.*, 2013, **5**, a008748.
187 P. Mehrotra and K. S. Ravichandran, *Nat. Rev. Drug Discovery*, 2022, **21**, 601–620.
188 S. Arandjelovic and K. S. Ravichandran, *Nat. Immunol.*, 2015, **16**, 907–917.
189 C. Varol, A. Mildner and S. Jung, *Annu. Rev. Immunol.*, 2015, **33**, 643–675.
190 S. Kadomoto, K. Izumi and A. Mizokami, *Int. J. Mol. Sci.*, 2022, **23**, 144.
191 J. Liu, X. Geng, J. Hou and G. Wu, *Cancer Cell Int.*, 2021, **21**, 389.
192 Z. Duan and Y. Luo, *Signal Transduction Targeted Ther.*, 2021, **6**, 127.
193 A. Gabba, A. Bogucka, J. G. Luz, A. Diniz, H. Coelho, F. Corzana, F. J. Canada, F. Marcelo, P. V. Murphy and G. Birrane, *Biochemistry*, 2021, **60**, 1327–1336.
194 T. Tang, Y. Wei, J. Kang, Z. G. She, D. Kim, M. J. Sailor, E. Ruoslahti and H. B. Pang, *J. Controlled Release*, 2019, **301**, 42–53.
195 M. Zhao, X. Cheng, P. Shao, Y. Dong, Y. Wu, L. Xiao, Z. Cui, X. Sun, C. Gao, J. Chen, Z. Huang and J. Zhang, *Nat. Commun.*, 2024, **15**, 950.
196 M. M. Kaneda, K. S. Messer, N. Ralainirina, H. Li, C. J. Leem, S. Gorjestani, G. Woo, A. V. Nguyen, C. C. Figueiredo, P. Foubert, M. C. Schmid, M. Pink, D. G. Winkler, M. Rausch, V. J. Palombella, J. Kutok, K. McGovern, K. A. Frazer, X. Wu, M. Karin, R. Sasik, E. E. Cohen and J. A. Varner, *Nature*, 2016, **539**, 437–442.
197 X. Xiang, J. Wang, D. Lu and X. Xu, *Signal Transduction Targeted Ther.*, 2021, **6**, 75.
198 L. Zhang, Y. Lin, S. Li, X. Guan and X. Jiang, *Angew. Chem., Int. Ed.*, 2023, **62**, e202217089.
199 L. S. Qi, M. H. Larson, L. A. Gilbert, J. A. Doudna, J. S. Weissman, A. P. Arkin and W. A. Lim, *Cell*, 2013, **152**, 1173–1183.
200 M. Ray, Y. W. Lee, J. Hardie, R. Mout, G. Yesilbag Tonga, M. E. Farkas and V. M. Rotello, *Bioconjugate Chem.*, 2018, **29**, 445–450.
201 F. Leonard, L. T. Curtis, A. R. Hamed, C. Zhang, E. Chau, D. Sieving, B. Godin and H. B. Frieboes, *Cancer Immunol. Immunother.*, 2020, **69**, 731–744.
202 R. W. Hallowell, S. L. Collins, J. M. Craig, Y. Zhang, M. Oh, P. B. Illei, Y. Chan-Li, C. L. Vigeland, W. Mitzner, A. L. Scott, J. D. Powell and M. R. Horton, *Nat. Commun.*, 2017, **8**, 14208.
203 W. T. Festuccia, P. Pouliot, I. Bakan, D. M. Sabatini and M. Laplante, *PLoS One*, 2014, **9**, e95432.
204 D. C. Swarts and M. Jinek, *Wiley Interdiscip. Rev.: RNA*, 2018, **9**, e1481.
205 A. R. Krysler, C. R. Cromwell, T. Tu, J. Jovel and B. P. Hubbard, *Nat. Commun.*, 2022, **13**, 1617.

 This journal is © The Royal Society of Chemistry 2024

Published on 08 2024. Downloaded on 03.11.2024 5:08:38.

206 F. Alfei, P. C. Ho and W. L. Lo, *Oncogene*, 2021, **40**, 5253–5261.
207 R. M. Steinman, *Annu. Rev. Immunol.*, 1991, **9**, 271–296.
208 C. Fu and A. Jiang, *Front. Immunol.*, 2018, **9**, 3059.
209 Y. Wang, Y. Xiang, V. W. Xin, X. W. Wang, X. C. Peng, X. Q. Liu, D. Wang, N. Li, J. T. Cheng, Y. N. Lyv, S. Z. Cui, Z. Ma, Q. Zhang and H. W. Xin, *J. Hematol. Oncol.*, 2020, **13**, 107.
210 M. Li, H. Zhou, N. Wu, W. Deng, W. Dong, X. Sun, H. Liu, Z. Tian and Y. Wang, *Nano Lett.*, 2023, **23**, 2733–2742.
211 M. G. Sartorio, E. J. Pardue, M. F. Feldman and M. F. Haurat, *Annu. Rev. Microbiol.*, 2021, **75**, 609–630.
212 K. Cheng, R. Zhao, Y. Li, Y. Qi, Y. Wang, Y. Zhang, H. Qin, Y. Qin, L. Chen, C. Li, J. Liang, Y. Li, J. Xu, X. Han, G. J. Anderson, J. Shi, L. Ren, X. Zhao and G. Nie, *Nat. Commun.*, 2021, **12**, 2041.
213 D. Han, J. Liu, C. Chen, L. Dong, Y. Liu, R. Chang, X. Huang, Y. Liu, J. Wang, U. Dougherty, M. B. Bissonnette, B. Shen, R. R. Weichselbaum, M. M. Xu and C. He, *Nature*, 2019, **566**, 270–274.
214 I. Puga, M. Cols and A. Cerutti, *Inmunologia*, 2013, **32**, 25–34.
215 J. A. Marwick, R. Mills, O. Kay, K. Michail, J. Stephen, A. G. Rossi, I. Dransfield and N. Hirani, *Cell Death Dis.*, 2018, **9**, 665.
216 M. Yi, T. Li, M. Niu, Q. Mei, B. Zhao, Q. Chu, Z. Dai and K. Wu, *Mol. Cancer*, 2023, **22**, 187.
217 E. Mortaz, S. D. Alipoor, I. M. Adcock, S. Mumby and L. Koenderman, *Front. Immunol.*, 2018, **9**, 2171.
218 C. Rosales, *Front. Physiol.*, 2018, **9**, 113.
219 V. Kumar and A. Sharma, *Int. Immunopharmacol.*, 2010, **10**, 1325–1334.
220 A. Herrero-Cervera, O. Soehnlein and E. Kenne, *Cell. Mol. Immunol.*, 2022, **19**, 177–191.
221 Y. Liu, Z. T. Cao, C. F. Xu, Z. D. Lu, Y. L. Luo and J. Wang, *Biomaterials*, 2018, **172**, 92–104.
222 T. J. Laskowski, A. Biederstadt and K. Rezvani, *Nat. Rev. Cancer*, 2022, **22**, 557–575.
223 E. Vivier, E. Tomasello, M. Baratin, T. Walzer and S. Ugolini, *Nat. Immunol.*, 2008, **9**, 503–510.
224 J. Bi and Z. Tian, *Front. Immunol.*, 2017, **8**, 760.
225 T. Bexte, J. Alzubi, L. M. Reindl, P. Wendel, R. Schubert, E. Salzmann-Manrique, I. von Metzler, T. Cathomen and E. Ullrich, *OncoImmunology*, 2022, **11**, 2081415.
226 S. Ghaffari and N. Rezaei, *J. Transl. Med.*, 2023, **21**, 551.
227 G. Marone, A. R. Gambardella, F. Mattei, J. Mancini, G. Schiavoni and G. Varricchi, in *Tumor Microenvironment: Hematopoietic Cells – Part A*, ed. A. Birbrair, Springer International Publishing, Cham, 2020, pp. 21–34. DOI: 10.1007/978-3-030-35723-8_2.
228 M. K. Srivastava, P. Sinha, V. K. Clements, P. Rodriguez and S. Ostrand-Rosenberg, *Cancer Res.*, 2010, **70**, 68–77.
229 M. Rowlands, F. Segal and D. Hartl, *Front. Immunol.*, 2021, **12**, 697405.
230 J. S. Marshall, R. Warrington, W. Watson and H. L. Kim, *Allergy, Asthma, Clin. Immunol.*, 2018, **14**, 49.
231 I. Rahimmanesh, M. Totonchi and H. Khanahmad, *Res. Pharm. Sci.*, 2020, **15**, 437–446.
232 D. V. Foss, J. J. Muldoon, D. N. Nguyen, D. Carr, S. U. Sahu, J. M. Hunsinger, S. K. Wyman, N. Krishnappa, R. Mendonsa, E. V. Schanzer, B. R. Shy, V. S. Vykunta, V. Allain, Z. Li, A. Marson, J. Eyquem and R. C. Wilson, *Nat. Biomed. Eng.*, 2023, **7**, 647–660.
233 A. Kheirolomoom, A. J. Kare, E. S. Ingham, R. Paulmurugan, E. R. Robinson, M. Baikoghli, M. Inayathullah, J. W. Seo, J. Wang, B. Z. Fite, B. Wu, S. K. Tumbale, M. N. Raie, R. H. Cheng, L. Nichols, A. D. Borowsky and K. W. Ferrara, *Biomaterials*, 2022, **281**, 121339.
234 H. Tanaka, R. Miyama, Y. Sakurai, S. Tamagawa, Y. Nakai, K. Tange, H. Yoshioka and H. Akita, *Pharmaceutics*, 2021, **13**, 2097.
235 V. Appay, R. A. van Lier, F. Sallusto and M. Roederer, *Cytometry, Part A*, 2008, **73**, 975–983.
236 D. N. Nguyen, T. L. Roth, P. J. Li, P. A. Chen, R. Apathy, M. R. Mamedov, L. T. Vo, V. R. Tobin, D. Goodman, E. Shifrut, J. A. Bluestone, J. M. Puck, F. C. Szoka and A. Marson, *Nat. Biotechnol.*, 2020, **38**, 44–49.
237 S. L. Swain, K. K. McKinstry and T. M. Strutt, *Nat. Rev. Immunol.*, 2012, **12**, 136–148.
238 P. Castiglioni, M. Gerloni, X. Cortez-Gonzalez and M. Zanetti, *Eur. J. Immunol.*, 2005, **35**, 1360–1370.
239 A. Durgeau, Y. Virk, S. Corgnac and F. Mami-Chouaib, *Front. Immunol.*, 2018, **9**, 14.
240 M. Wang, H. Y. Yin, B. Fau-Wang, R.-F. Wang Hy Fau-Wang and R. F. Wang, *Front. Immunol.*, 2018, **9**, 14.
241 B. Weigelin and P. Friedl, *Trends Cancer*, 2022, **8**, 980–987.
242 M. J. W. Sim and P. D. Sun, *Front. Immunol.*, 2022, **13**, 833017.
243 S. Lu, N. Yang, J. He, W. Gong, Z. Lai, L. Xie, L. Tao, C. Xu, H. Wang, G. Zhang, H. Cao, C. Zhou, L. Zhong and Y. Zhao, *J. Biomed. Nanotechnol.*, 2019, **15**, 593–601.
244 J. G. Cyster and C. D. C. Allen, *Cell*, 2019, **177**, 524–540.
245 M. Li, Y.-N. Fan, Z.-Y. Chen, Y.-L. Luo, Y.-C. Wang, Z.-X. Lian, C.-F. Xu and J. Wang, *Nano Res.*, 2018, **11**, 6270–6282.
246 M. Senior, *Nat. Biotechnol.*, 2024, **42**, 355–361.
247 H. Kong, E. Ju, K. Yi, W. Xu, Y. H. Lao, D. Cheng, Q. Zhang, Y. Tao, M. Li and J. Ding, *Adv. Sci.*, 2021, **8**, e2102051.
248 S. Zhang, Y. Wang, D. Mao, Y. Wang, H. Zhang, Y. Pan, Y. Wang, S. Teng and P. Huang, *Front. Med.*, 2023, **10**, 1292452.