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Immunotherapy

Innate Immune Activation with Multifunctional Nanoparticles for Cancer Immunotherapy

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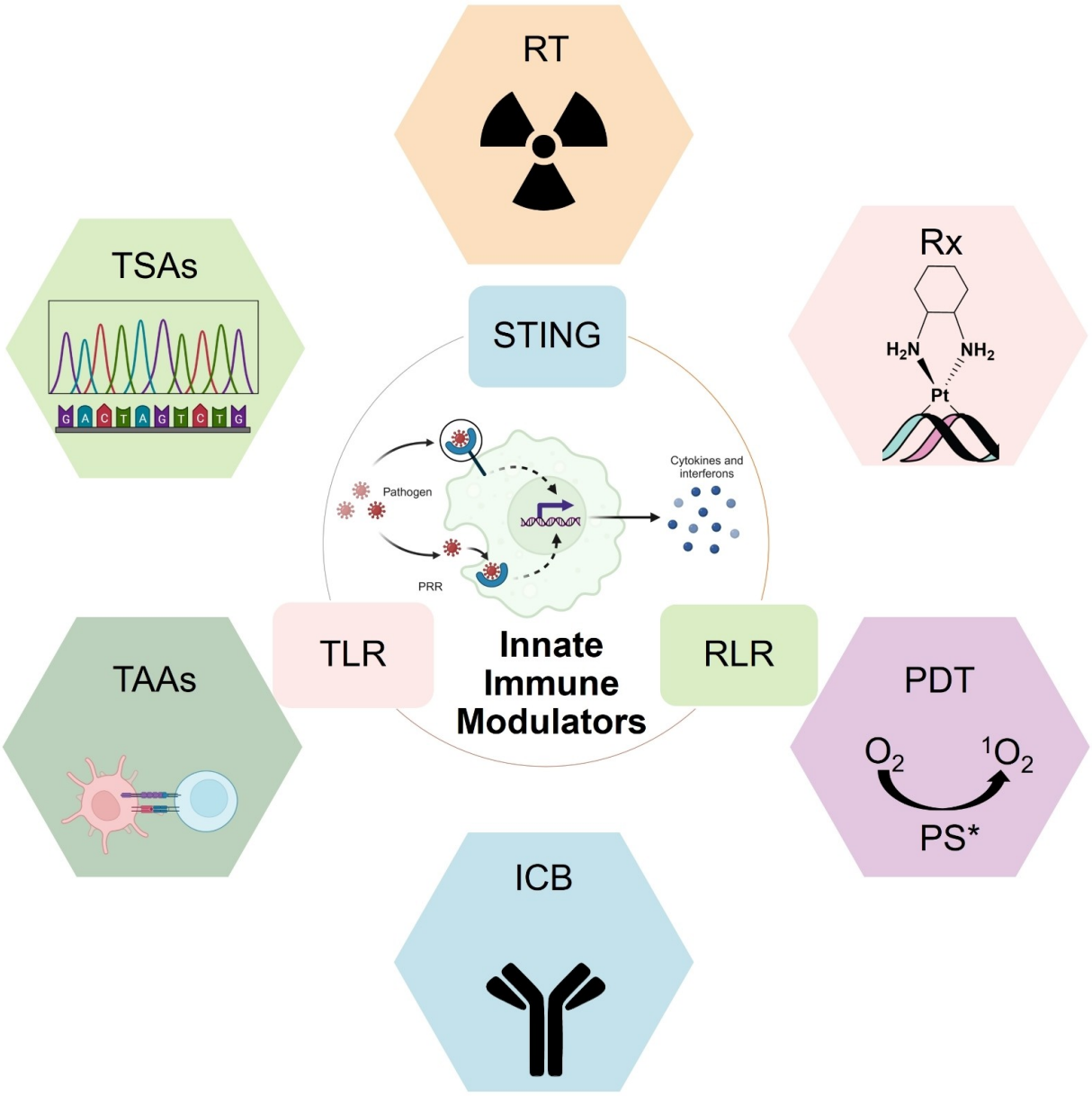
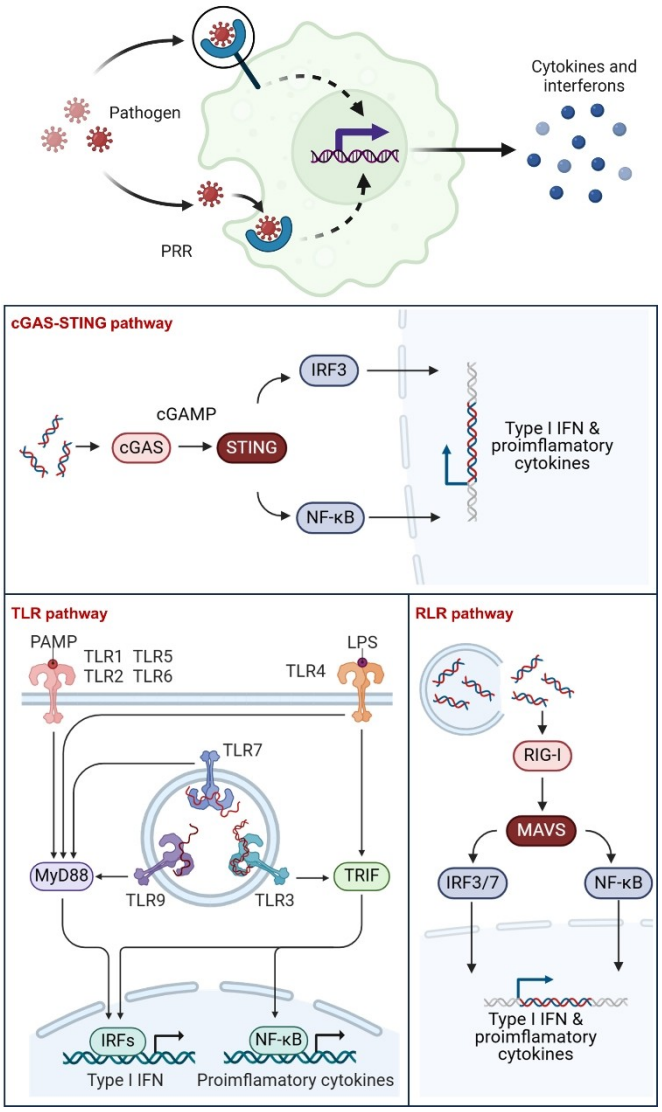


Abstract: Immune checkpoint blockade (ICB) has revolutionized the treatment of many cancers by leveraging the immune system to combat malignancies. However, its efficacy is limited by the immunosuppressive tumor microenvironment and other regulatory mechanisms of the immune system. Innate immune modulators (IIMs) provide potent immune activation to complement adaptive immune responses and help overcome resistance to ICB. This minireview provides an overview of IIMs and their roles in antitumor immune responses and summarizes recent advances in developing nanotechnology to enhance the delivery of IIMs to tumors for potentiating cancer immunotherapy and mitigating systemic toxicity. We discuss innovative nanoparticle platforms for the delivery of IIMs targeting the cyclic GMP-AMP synthase-stimulator of interferon genes pathway, the toll-like receptor pathway, and the retinoic acid-inducible gene I-like receptor pathway. We review the preliminary clinical readouts of representative IIM nanoptherapeutics and highlight the development of multifunctional nanoparticles for combination treatments of IIMs with conventional treatment mdoalities such as chemotherapy, radiotherapy, photodynmic therapy, and tumor antigens. Finally, we summarize the lessons learned from the existing systems, the challenges in the field, and future perspectives for this exciting field of nanotherapeutics for cancer immunotherapy.

1. Introduction

Immunotherapy has revolutionized cancer treatment by harnessing the immune system to kill cancer cells and has been combined with traditional therapies like surgery, chemotherapy, and radiotherapy to prolong patient survival.[1] Among cancer immunotherapies, immune checkpoint blockade (ICB) targeting cytotoxic T-lymphocyte associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed cell death ligand 1 (PD–L1) has provided a particularly successful strategy to activate systemic antitumor immunity by blocking immunosuppressive checkpoints.[2] ICB has provided significant benefits to melanoma and non-small cell lung cancer (NSCLC) patients but is mostly ineffective in the majority of tumor types with low T cell infiltration or PD–L1 expression.[3]

To increase tumor response to ICB, researchers have used innate immune modulators (IIMs) to activate immunostimulatory pathways and enhance tumor immunogenicity, which in turn improves antigen presentation by antigen-presenting cells (APCs).[4] IIMs are derived from pathogen-associated molecular patterns (PAMPs) that are commonly found on pathogens, allowing them to stimulate pattern recognition receptors (PRRs) in a similar way to actual pathogens (Scheme 1).[5] This mimicry activates innate immune responses, triggering signaling pathways that promote inflammation, cytokine production, and the recruitment of immune cells, which can also elicit adaptive immunity.[6] By mimicking these pathogen signals, IIMs are



Scheme 1. Schematic illustration of working mechanisms of IIMs. IIMs mimic PAMPs, initiating the pathogen pattern recognition response in innate immune cells and secreting cytokines and interferons. Commonly used IIMs activate the STING, TLR, or RLR pathways in host cells at various subcellular locations. The Scheme was created with BioRender.com.

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used in therapies to boost immune responses against infections, cancer, and other diseases.[4a] Despite their preclinical efficacy, IIMs have faced challenges in the clinic as they do not effectively sustain immune activation in tumors due to their suboptimal pharmacokinetics and poor tumor retention.[7] Furthermore, off-tumor immune activation by IIMs can induce massive release of cytokines, leading to cytokine release syndrome (CRS) and other undesirable side effects.[8] As a result, significant efforts have recently been devoted to the development of innovative nanoparticle (NP) platforms for enhanced delivery of IIMs to tumors and for integration with other cancer treatments to maximize the therapeutic potential.[9]

Different types of NPs, including polymeric NPs, liposomes, and inorganic nanoparticles, have been developed and optimized by fine-tuning their sizes, compositions, surface properties, and loading capacities for IIM delivery to tumors.[10] These optimizations have signficantly enhanced the delivery efficiency by stabilizing IIMs via encapsulation in nanoparticles, improving IIM pharmacokinetics, and reducing systemic toxicity and damage to healthy tissues. Over the past few years, nanoparticle platforms have further advanced to enable the co-delivery of mutiple therapeutics, allowing simultaneous administration of IIMs with traditional cancer treatments such as chemotherapy, radiotherapy (RT), photodynamic therapy (PDT), and tumor antigens.[11] These NP-based combination treatments enhance the precision and potency of antitumor immune responses. Recent preclinical studies have demonstrated that immumotherapeutic NPs can modulate the tumor microenvironment (TME), enhance immune cell infiltration, and transform "cold" tumors into "hot" tumors.[12] In this Minireview, we begin by exploring the key characteristics and design principles of nanoparticles for IIM delivery. We then provide an overview of nanoparticle platforms that combine IIMs with other cancer therapies, emphasizing their potential to enhance treatment precision and effectiveness in the growing field of nanoparticle-mediated cancer immunotherapy.

2. Innate Immune Modulators

IIMs are therapeutic agents designed to activate or regulate the body's innate immune response, which serves as the first line of defense against pathogens (Scheme 1).[4a] These modulators include a range of molecules targeting specific immune pathways, such as stimulator of interferon genes (STING) agonists, toll-like receptor (TLR) agonists, and retinoic acid-inducible gene I-like receptor (RLR) agonists.[13]

The cyclic GMP-AMP synthase (cGAS)-STING pathway, integral to the cytosolic DNA sensing mechanism, detects abnormal DNAs within cells—a common feature in viral infections and cancer.[14] Activation of cGAS leads to the production of cGAMP, which then activates the STING protein on the surface of endoplasmic reticulum.[15] STING activation triggers downstream signaling involving interferon regulatory factor 3 (IRF3) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB).[16] This results in the production of Type I interferons (IFNs) and proinflammatory cytokines, which enhance the immune response.[17] Consequently, type I IFN response stimulates dendritic cells, macrophages, and natural killer (NK) cells to facilitate adaptive immune responses by presenting tumor antigens to T cells and to produce pro-inflammatory cytokines like TNF-α and IL-6, which recruit and activate immune cells in the TME.[16,18]

TLR agonists activate immune cells by mimicking PAMPs and engaging well-characterized TLR pathways, which enhance the recognition of microbial components and trigger an immune response.[19] TLRs on the cell surface respond to extracellular pathogen components, such as lipoproteins (TLR1, 2, and 6), lipopolysaccharides (TLR4), and bacterial flagellin (TLR5).[20] Endosomal TLRs detect intracellular pathogen components, including double-stranded RNA (TLR3), single-stranded RNA (TLR7 and 8), and unmethylated CpG DNA (TLR9).[21] Upon activation, TLRs recruit adaptor proteins such as myeloid differentiation primary response protein 88 (MyD88) and TIR-domain-containing adapter-inducing interferon-β (TRIF), which in turn activate signaling molecules that trigger transcription factors like IRF and NF-κB.[22] In cancer

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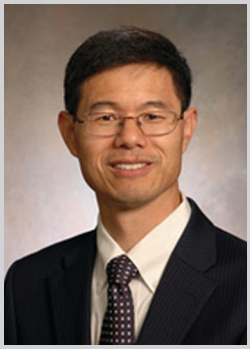

Wenbin Lin studied chemical physics at University of Science and Technology of China, received a Ph.D.in chemistry at University of Illinois at Urbana-Champaign, and carried out NSF postdoctoral research with Prof. Tobin J. Marks at Northwestern University. He is currently the James Franck Professor of Chemistry, Radiation and Cellular Oncology, and Ludwig Center for Metastasis Research at the University of Chicago. His group has pioneered the application of metal–organic frameworks in cancer therapy, bioimaging, earth-abundant metal catalysis, artificial photosynthesis, asymmetric catalysis, and second-order nonlinear optics. His startup company has five anticancer drug candidates under clinical investigation.

therapy, TLR agonists are used to stimulate these receptors to amplify the immune response against cancer cells. This is achieved through activating dendritic cells, promoting pro-inflammatory cytokine production, enhancing NK cell activity, modulating the TME, and synergizing with other treatments. Thus, TLR agonists provide a powerful tool in cancer treatment.[23]

RLR agonists detect viral RNA in the cytoplasm of cells to trigger antiviral signaling pathways that can also promote antitumor immunity.[24] Upon detecting viral RNA, RIG–I activates the adaptor protein MAVS (mitochondrial anti-viral-signaling protein), which triggers downstream signaling through IRF3/7 and NF-κB, similar to other immune pathways.[25] Activation of RLRs leads to immune responses that not only combat viral infections but also promotes antitumor immunity by producing type I IFNs and pro-inflammatory cytokines. These factors stimulate DCs and NK cells while indirectly activating cytotoxic T lymphocytes (CTLs).[26] RLR agonists promote inflammation, immune cell infiltration, and the modulation of the TME, making it more favorable for immune-mediated tumor clearance.

IIMs activate the function of critical immune cells, including NK cells, macrophages, and dendritic cells. NK cells are directly involved in the destruction of tumor cells, while macrophages contribute by phagocytizing tumor debris and presenting tumor antigens.[27] Dendritic cells play an essential role in antigen presentation, leading to the activation of T cells.[28] By enhancing the activity of these immune cells, IIMs effectively prime the immune system to efficiently recognize and eliminate cancer cells, bolstering the body's defense against tumor progression.

Although immune activations by IIMs share common outcomes, the differences in their inflammatory signatures and downstream immune effects are substantial. STING agonists predominantly stimulate type I interferon responses but may also trigger strong proinflammatory cytokines, posing a risk of systemic toxicities. TLR agonists display diverse inflammatory profiles, with TLR4 agonists typically producing more intense inflammatory responses than TLR7/8 agonists. RLR activation is highly effective in eliciting antiviral responses but may require additional modulation to mitigate off-target inflammation. Clinical translational of IIMs is complicated by the differences in PRR expression, signaling pathways, and immune cell compositions across species. For instance, variations in STING alleles between mice and humans significantly influence STING agonist potency, whereas differences in TLR expression across species impact the translational reliability of preclinical results.

3. Nanoparticles for IIM Delivery

The activation of immune cells by IIMs shall ideally occur only in the TME, where the activated immune cells can directly recognize and target tumor cells. However, most IIMs are hydrophilic nucleotides or hydrophobic small molecules. They have short blood circulation half-lives and do not efficiently accumulate in tumors. Selective activation of immune cells in tumors by IIMs remains a challenge due to their systemic effects to cause widespread immune activation and general toxicity. Advanced NP delivery systems offer a promising solution by encapsulating IIMs and enabling controlled release at the tumor site, which ensures local immune responses while minimizing systemic side effects.

Commonly used IIMs are divided into two groups: hydrophilic nucleotides and hydrophobic small molecules (Figure 1). Based on the molecular structures of IIMs, NPs can be precisely designed via introducing various functional groups to control the delivery of IIMs by leveraging the supramolecular interactions between NPs and IIMs, including electrostatic interactions, metal coordination, hydrogen bonding, hydrophobic interactions, and others.[29] Many nucleotide IIMs are hydrophilic macromolecules that generally cannot traverse lipid bilayers.[30] Liposomes are used to encapsulate these macromolecules and protect them

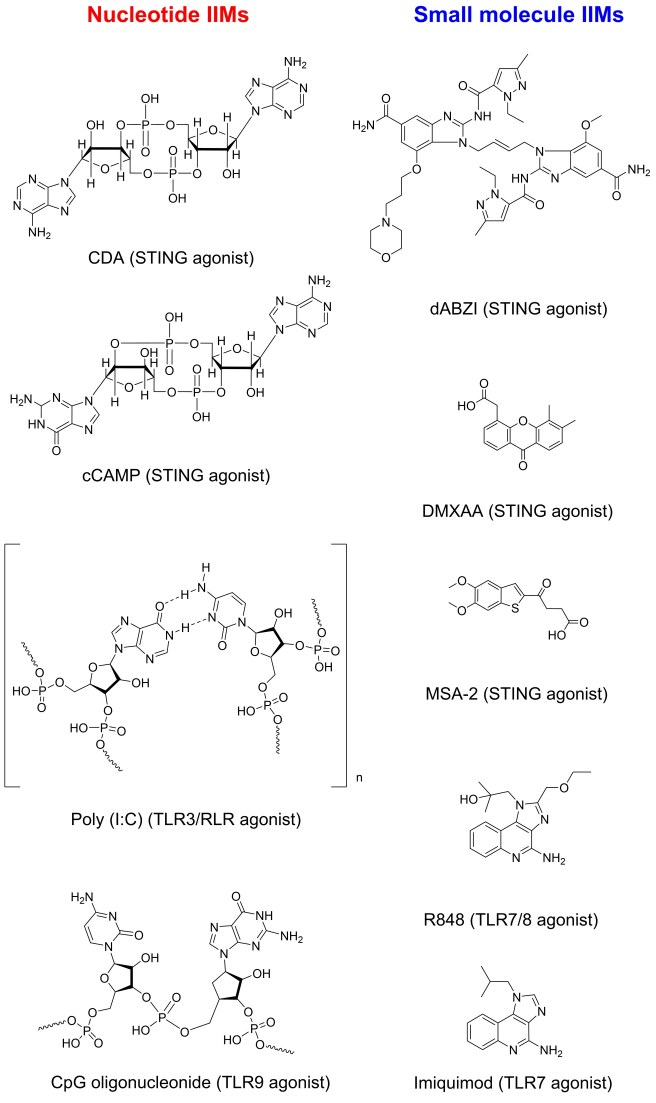

Figure 1. Examples of hydrophilic nucleotide and hydrophobic small molecule IIMs.

against enzymatic degradation during systemic circulation.[31] Lipid nanoparticles (LNPs) containing cationic or ionizable lipids can also effectively encapsulate and stabilize negatively charged oligonucleotides.[32] Inorganic NPs, particularly those containing open metal sites, can coordinate to phosphate groups in nucleotides, which not only stabilizes the nucleotides but also enhances their pharmacokinetic profiles.[33] For small organic molecule IIMs, polymeric NPs are engineered with conjugation sites to enable intracellular release of the IIM payloads. Surface functionalization of polymeric NPs facilitates delivery across biological barriers.[34] Some NPs have been modified with hydrophobic pockets formed by supramolecular macrocycles.[35] Each of these NP platforms offers distinct advantages, such as improved loading capacity, controlled release of IIMs, and/or targeted delivery to immune cells; optimization of these features can lead to selective activation of immune cells in the TME.

3.1. STING Agonists

Most STING agonists are small molecules that can be categorized into CDN type or non-CDN type. Both types of STING agonists do not exhibit good tumor accumulation. NP delivery of CDN-type STING agonists usually relies on similar methods developed for oligonucleotide delivery, such as encapsulation by double emulsion, electrostatic stabilization with cation lipids, or coordination with metals. In 2019, Shae et al. designed the first polymersome nanoparticle (STING-NP) to enhance the cytosolic delivery of the endogenous STING ligand, cGAMP (Figure 2a).[36] STING-NP was engineered with an aqueous core to efficiently load cGAMP and a vesicle membrane formed from an amphiphilic di-block copolymer. The copolymer incorporated pH-sensitive, cationic 2-(diethylamino)ethyl methacrylate (DEAEMA) groups and hydrophobic butyl methacrylate (BMA) moieties to facilitate endosomal escape of cGAMP. Additionally, thiol-reactive pyridyl disulfide ethyl methacrylate (PDSMA) units were integrated into the polymer for in situ crosslinking of vesicle membrane chains through disulfide linkages. The PEGylated particles were surface-neutral with a size of ~80 nm at pH 7.4. Encapsulation of cGAMP in STING-NP dramatically enhanced its activity over free cGAMP, reducing the EC_{50} value from 31 μM to 67 nM in THP-1 monocyte cells, from 22 μM to 36 nM in RAW 264.7 macrophage cells, and from 55 μM to 230 nM in B16 melanoma cells with IFN-stimulated genes. Intratumoral administration of STING-NP significantly improved therapeutic outcomes in a B16F10 melanoma model, reducing tumor growth rate by 11-folds over cGAMP and extending median survival to 29 days from 11–12 days for control groups (Figure 2b, **2c**). Intravenous

injection of STING-NP also slowed the growth of subcutaneous B16F10 tumors and enhanced the efficacy of anti-PD-1 plus anti-CTLA-4 combination ICB therapy. Intravenous STING-NPs caused a mild, temporary weight loss in mice, with full recovery and no changes in serum alanine aminotransferase, bilirubin, or creatinine from PBS

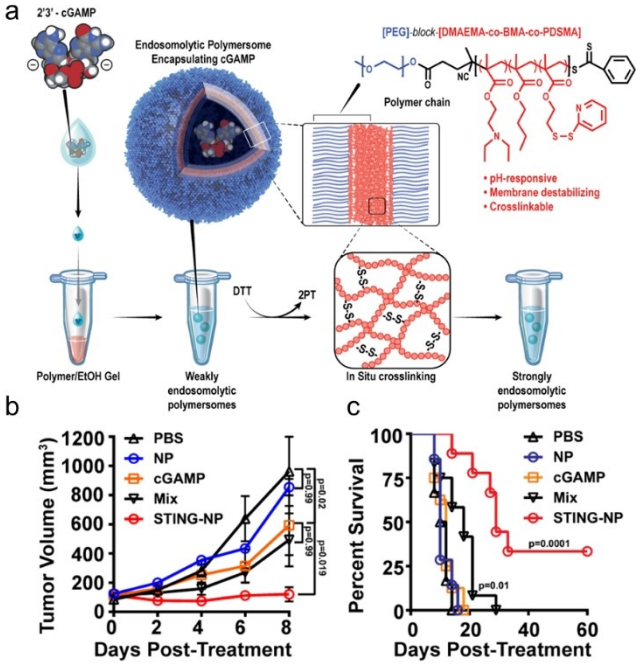

Figure 2. a) Scheme showing the structure of STING-NP and the strategy for improving intracellular delivery of cGAMP. cGAMP is encapsulated within endosomolytic polymersomes formed from pH-responsive diblock copolymers. b,c) Tumor growth and survival of mice after intratumoral administration of STING-NP and control treatments. Adapted from Ref. [36] with permission. Copyright 2019, Nature Publishing Group.

controls. The activity of STING-NP was also observed in resected human metastatic melanoma tissues, suggesting their potential to increase tumor immunogenicity in a clinical setting.

In 2022, Liu et al. developed a liposomal NP loaded with cyclic dinucleotide (LNP-CDN) to target and activate STING signaling in macrophages and DCs.[37] In LNP-CDN, CDN complexed with calcium phosphate was encapsulated in liposomes to enable pH-responsive release of CDN from endosomes into the cytosol, where it activates STING signaling by directly binding to STING proteins. LNP-CDN had a size of ~120 nm and a surface charge of −15 mV. Upon intrapleural administration, LNP-CDN induced significant transcriptional changes in malignant pleural effusion (MPE), transforming the immune "cold" MPE in both effusions and pleural tumors to an immunogenic phenotype. When combined with anti-PD–L1, LNP-CDN significantly reduced MPE volume, inhibited tumor growth in the pleural cavity and lung parenchyma, and prolonged survival in MPE-bearing mice. Notably, the immunological responses induced by LNP-CDN were also observed in clinical MPE samples, suggesting its potential for MPE immunotherapy in the clinic.

Lin and co-workers have developed nanoscale coordination polymers (NCPs) via coordination polymerization between Zn^{2+} ions and carboxylate- or phosphate-containing drugs.[29b,38] Compared to electrostatic interactions be-

tween lipids and CDN STING agonists, strong coordination between Zn^{2+} ions and CDNs provides more stable NPs with a superior delivery efficiency. Yang et al. synthesized ZnCDA, a robust tumor-targeted STING agonist NP, by encapsulating CDA in the NCP core through coordination with Zn^{2+} ions (Figure 3).[39] NCP particles consist of a biocompatible zinc-phosphate core encased in a lipid bilayer and are capable of delivering both hydrophilic and hydrophobic drugs in a stimuli-responsive fashion.[38b,c] ZnCDA had a Z-averaged diameter of 111.8 nm with a PDI of 0.12 and a CDA loading of 2.63 wt %. Intravenously administered ZnCDA extended CDA circulation with a 3.8-fold longer half-life than a liposome formulation with encapsulated CDA (LipoCDA, Figure 3b). A single dose of ZnCDA efficiently targeted tumors and mediated strong antitumor effects in various preclinical cancer models, leaving all mice with MC38 subcutaneous tumors and the majority (5/7) of mice with MC38 liver metastases tumor-free (Figure 3c). It was further elucidated that ZnCDA preferentially targeted tumor-associated macrophages (TAMs) to modulate antigen processing and presentation and prime an antitumor T-cell response (Figure 3d). ZnCDA overcame anti-PD–L1 resistance to control the growth of "cold" Panc02-SIY tumors and extend mouse survival for >20 days. When combined with irradiation, ZnCDA eradicated tumors in 40 % Panc02-SIY tumor-bearing mice 40 days post-treatment. This study elucidated a novel mechanism underlying the antitumor immune responses mediated by ZnCDA and offered a promising therapeutic approach for the treatment of clinically intractable tumors.

For non-CDN type STING agonists, many studies have examined their conjugation to NPs via tumor cleavable linkers.[40] Sheehy et al. developed SAPCon, a STING-activating polymer–drug conjugate platform designed to enhance circulation time and promote passive tumor accumulation.[41] This system utilizes strain-promoted azide–alkyne cycloaddition to link a dimeric amidobenzimidazole (diABZI) STING prodrug to hydrophilic polymer chains of poly(dimethylacrylamide-co-azido-ethylmethacrylate) via a cathepsin B-responsive linker. When administered intravenously, SAPCon demonstrated prolonged circulation with a serum elimination half-life of ~4.4 h, and enhanced accumulation at orthotopic EO771 tumor sites with ~4-fold increase of drug deposition over the normal contralateral mammary fat pad. SAPCon was taken up by tumor-associated myeloid cells and promoted an immunogenic TME which was characterized by higher levels of activated macrophages and DCs, as well as improved CTL infiltration. As a result, SAPCon significantly reduced tumor sizes, and rendered 37.5 % of mice tumor free rate in an orthotopic E0771 breast cancer model.

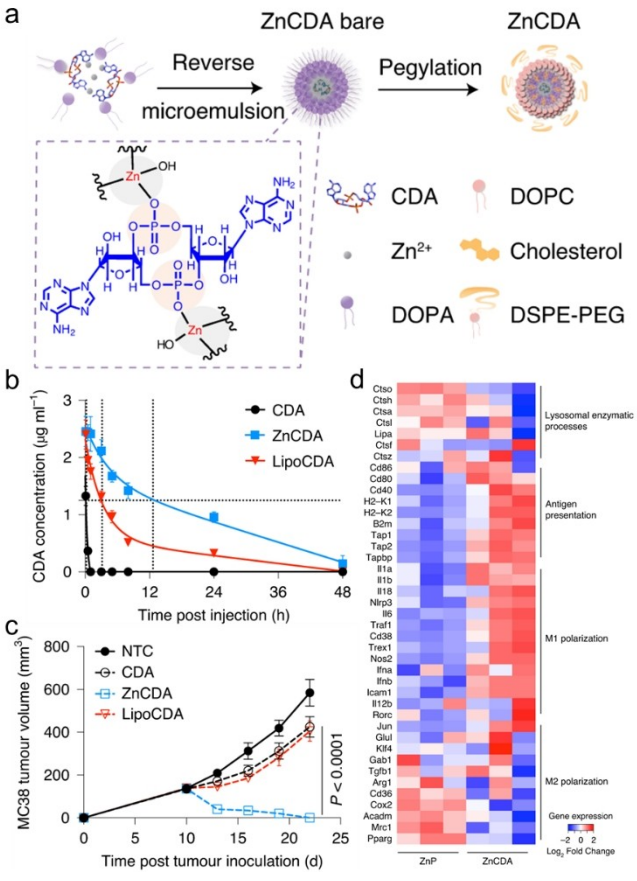

Figure 3. a) Preparation and characterization of ZnCDA. b) Pharmacokinetics of free CDA, LipoCDA and ZnCDA. LipoCDA, lipid NP fomulated CDA. c) Anti-tumour effects of free CDA, LipoCDA and ZnCDA on MC38 tumors in C57BL/6 mice. NTC, non-treated control. d) Heatmap comparing genes related to lysosomal enzymatic processes, antigen presentation, and M1 and M2 polarization post ZnP or ZnCDA treatment. Adapted from Ref. [39] with permission. Copyright 2022, Nature Publishing Group.

3.2. TLR Agonists

Depending on the molecular structures of TLR agonists, diverse NP platforms have been used to enhance their delivery to tumors. For example, as TLR3 and TLR9 agonists are typically hydrophilic nucleotides, they can be effectively encapsulated within NPs through metal coordination or electrostatic interactions. In 2022, Li et al. developed pIC@NCP by crosslinking the phosphate groups of Poly(I:C) with non-toxic Zn^{2+} ions and encapsulating the complex within a lipid bilayer for enhanced pharmacokinetics and tumor accumulation.[42] pIC@NCP had a Z-averaged diameter of 100.9 nm and a Poly(I:C) loading of 4.74 wt %. pIC@NCP specifically targeted immune cells, achieving >12-fold higher cellular uptake than tumor cells. pIC@NCP specifically localized in the endo/lysosomes with a colocalization factor (Pearson R's value) of 0.92 between Lysotracker and pIC@NCP. Systemically administered pIC@NCP showed significant tumor targeting effects and tumor growth inhibition (TGI) of 78.7 % in a CT26 subcutaneous mouse model; the TGI further increased to 85.8 % for its combination treatment with α-PD–L1. In the spontaneous prostate cancer TRAMP mouse model, the combination of pIC@NCP and anti-PD–L1 effectively suppressed tumor growth in the prostate and seminal vesicles,

with immunohistochemistry analysis revealing increased $CD8^+$ T cell infiltration in such a "cold" tumor model.

TLR agonists have also been loaded into NPs through a base pairing approach. DNA origami serve as a promising platform for loading CpG oligonucleotides by incorporating anti-sense sequences into the nanostructures.[43] In 2022, Comberlato demonstrated that precise nanoscale spatial arrangement of CpG motifs on DNA origami NPs enhanced TLR9 activation in RAW 264.7 macrophages (Figure 4).[44] The CpG molecules were positioned with a 7 nm spacing, corresponding to crystal structure of the dimeric form of the TLR9-CpG complex (PDB ID: 5zln). Disk-like DNA origami of ~60 nm in size was synthesized and used for CpG delivery based on the optimal cellular uptake. DNA origami stapled with CpG positioned at 7 nm spacing significantly enhanced immune activation over another DNA origami system with a 38 nm CpG spacing, showing greater upregulation of CD83 and CD40 surface markers and increased IL-6 release. It is interesting to find the significant effect of spatial control on TL9 activation, but this finding was not further studied in vivo.

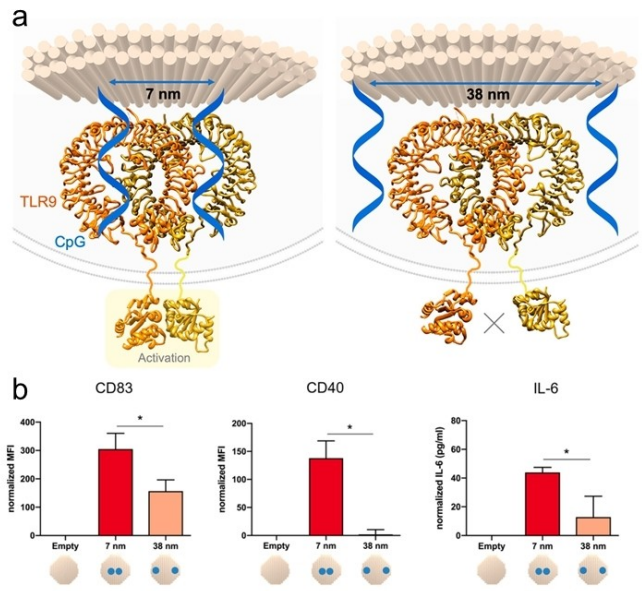

Figure 4. a) Spatial control of CpG molecules on DNA origami disks with CpG molecules spaced at 7 nm (left) and 38 nm (right) to observe TLR9 activation effects. b) Activation assays in RAW 264.7 cells with Cy5-labeled disks carrying CpG pairs at varying distances via analyzing CD83 and CD40 expression in the Cy5-positive population by flow cytometry. Adapted from Ref. [44] with permission. Copyright 2022, American Chemical Society.

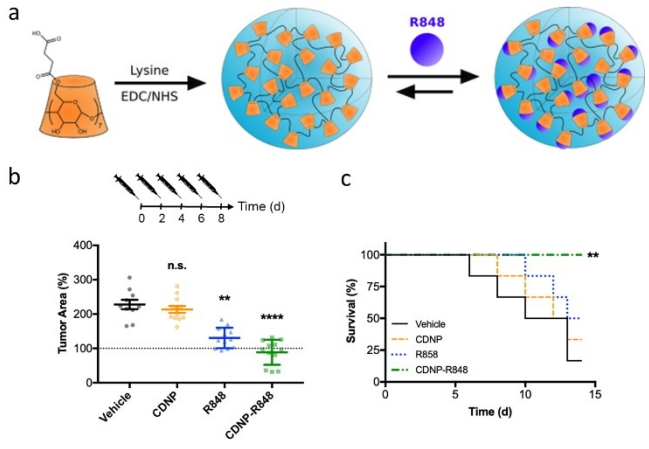

Figure 5. a) Schematic of CDNP synthesis via lysine crosslinking of succinyl-β-cyclodextrin (orange), followed by drug loading through guest–host complexation with R848 (blue). b) Tumour area at day eight after the first CDNP–R848 treatment. c) Survival following the start of CDNP–R848 treatment. Adapted from Ref. [45] with permission. Copyright 2018, Nature Publishing Group.

In contrast to TLR3 and TLR9 agonists, TLR7 and TLR8 agonists are typically hydrophobic small organic molecules. They are commonly loaded into the hydrophobic cavities in NPs or covalently linked to NPs via cleavable linkers for controlled release at the tumor site. Rodell et al. used β-cyclodextrin (CD) as a supramolecular drug reservoir for R848, a TLR7/8 agonist, through hydrophobic host–guest interaction (Figure 5).[45] CDs can form water-soluble inclusion complexes with many poorly soluble drugs, and have been used to improve solubilization and enable affinity-based delivery with NP formulations.[46] CDNP–R848 NPs of ~30 nm in size were synthesized via amide bond formation between succinyl-β-CD and L-lysine in aqueous conditions to achieve high drug loading (10.39 wt %) at a 1.1:1 CD-to-R848 ratio. Time-lapse confocal microscopy revealed a vascular half-life of 62.5 min, while fluorescence imaging at 24 h showed highest CDNP accumulation in tumors (94.9 % ID/g) and lymph nodes (93.0 %ID/g), with lower retention in the liver (78.4 %ID/g) and spleen (35.6 ID/g). CDNPs cleared from the vasculature and accumulated in TAMs in tumors. CDNP–R848 shifted the TAMs towards an M1 phenotype, controlling tumor growth with complete regression in 2 out of 7 MC38 tumor-bearing mice and protecting the cured mice against tumor rechallenge.

3.3. RLR Agonists

As RLR agonists function via detecting cytosolic RNAs, they need to traverse to the cytoplasm after cellular uptake, which presents an additional challenge for their application in cancer immunotherapy. NP-based RLR agonists need to be delivered to tumor cells and released into the cytoplasm via endosomal escape after cellular uptake.[47] To address this, researchers have used properly designed lipids, such as cationic and ionizable lipids, to facilitate endosomal escape of RLR agonists. Wang-Bishop et al. developed and evaluated LNPs for the delivery of 3p-modified stem-loop RNAs (SLRs), which function as 5'-triphosphate RNA (3pRNA) agonists of RIG–I (Figure 6).[48] Encapsulation of SLRs into LNPs produced surface charge-neutral SLR-LNPs of ~100 nm in size at ~100 % RNA encapsulation efficiency. The immunostimulatory activity of SLR-LNP was assessed in IFN–I reporter cell lines, which showed dose-dependent RIG–I activation with EC_{50} values of 1–10 nM in

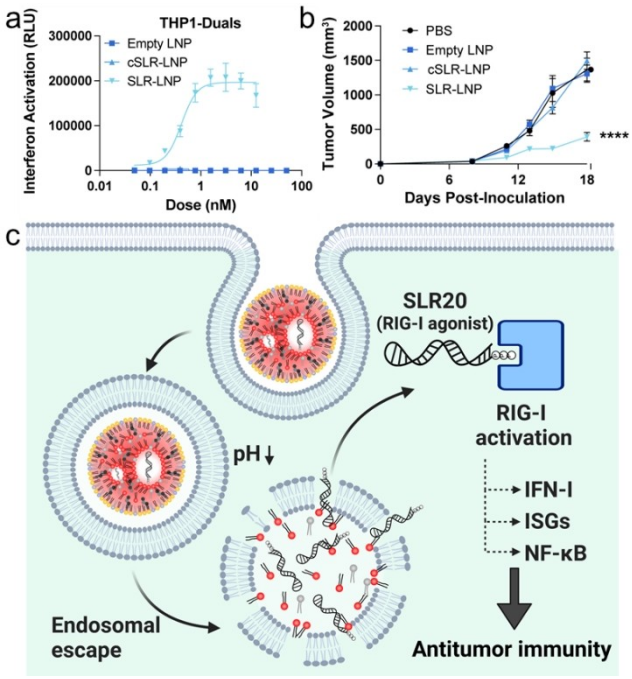

Figure 6. a) Type-I IFN (IFN–I) dose-response curves for LNP formulations of SLRs in THP1 cells with an incorporated IFN regulatory factor (IRF)-inducible reporter. b) Tumor growth curves of B16F10 tumor-bearing mice after treatment with SLR-LNP. c) Proposed mechanism for RIG–I activation by SLR-LNP. Adapted from Ref. [48] with permission. Copyright 2024, American Chemical Society.

vitro. These SLR-LNPs were administered to mice through intratumoral and intravenous routes, leading to RIG–I activation in the TME and inhibition of tumor growth in mouse models of poorly immunogenic B16F10 melanoma and EO771 breast cancer. Although mice experienced ~5 % temporary weight loss shortly after treatment, the therapeutic regimen was generally well tolerated. Systemic SLR-LNP administration further reprogrammed the TME to enhance T cell infiltration, boosting ICB response in EO771 breast tumors and reducing lung metastases in a B16F10 melanoma model.

3.4. NP formulations of IIMs in Clinical Trials

Building on promising preclinical results, several nanoformulations of IIMs have advanced to clinical trials and shown promising outcomes (Table 1). The majority of these nanoformulations utilized TLR or RLR agonists. For example, BO-112 is a nanocomplex of double-stranded synthetic RNA TLR3 agonist with polyethyleneimine (PEI) that was designed to mimic a viral infection. In a Phase I trial, intratumorally administered BO-112 elevated immune gene expression in 46 % patients and increased circulating immune cells in 88 % patients.[49] In a phase II trial of 40 patients with advanced melanoma, BO-112 combined with the anit-PD-1 antibody pembrolizumab achieved a 25 % objective response rate (ORR), including 3 complete responses (CRs) and 7 partial responses (PRs), and 44 % stable disease. The combination treatment was well tolerated with 79 % patients experiencing mostly mild BO-112-related adverse effects (AEs).[50]

Two TLR9 agonist nanoformulations, CMP-001 (Vidutolimod) and AST-008 (Cavrotolimod), demonstrated promising clinical outcomes when they were combined with ICB. CMP-001 is a virus-like particle incorporating a TLR9 agonist CpG oligonucleotide. CMP-001 plus the PD-1 antibody nivolumab was tested in 30 stage III melanoma patients.[51] After 7 weekly doses, 70 % patients showed pathologic responses, including 50 % pathologic CR and 20 % pathologic PR. Pathologic responses correlated with increased intratumoral plasmacytoid dendritic cells (pDCs) and $CD8^+$ T cells. Grade 3/4 infusion-related AEs occurred in 3 patients, and 2 of them discontinued CMP-001 treatment. AST-008 is a spherical nucleic acid NP containing densely packed, radially arranged TLR9 agonist CpG oligonucleotides. In a Phase 1b trial of AST-008 plus pembrolizumab or nivolumab, no dose-limiting toxicities (DLTs) or serious AEs were observed in 20 patients with solid tumors,.[52] Among 19 evaluable patients, the ORR was 21 % with four responders showing durable responses over 52 weeks.

EG-70 is chitosan nanoparticle delivering an IL-12 plasmid and two RIG–I agonists, VA1 and eRNA11a.[53] In 19 non-muscle invasive bladder cancer patients treated with

Table 1: Representative IIM nanoformulations in clinical trials.

Drugs & NCT numbers	Target	Formulation (administration route)	Phase	Results
BO-112 NCT04570332 NCT02828098	TLR3	PEI nanoplex of Poly(I:C) (i.t.)	I/II	6/13 immune gene expressions and 14/16 circulating immune cell increases;[49] BO-112 plus pembrolizumab: 25 % ORR and 44 % stable disease[50]
CMP-001 (Vidutolimod) NCT03618641	TLR9	Virus-like particles of CpG (i.t.)	II	CMP-001 plus nivolumab: 70 % pathologic ORR, 3 Grade 3/4 AEs (2 discontinued)[51]
AST-008 (Cavrotolimod) NCT03684785	TLR9	Spherical nucleic acids formulation of CpG (i.t.)	I/II	No DLTs; 21 % ORR with > 52-week responses in 2 pts; 3/4 responders progressed on prior PD-1 blockade[52]
EG-70 NCT04752722	RLR	Chitosan particles of an IL-12 plasmid and 2 RIG–I agonists (intravesical)	I/II	19 patients treated: No DLTs, 67 % CR, durable CRs observed at all dose levels[54]

intravesical EG-70, no DLTs were observed and 67 % patients achieved CR after one cycle.[54] Durable CRs were observed across all dose levels in Bacillus Calmette-Guérin (BCG)-unresponsive non-muscle invasive bladder cancer.

Nanoformulations of STING agonists such as exoSTING (CDK-002) have also been translated to the clinic (NCT04592484), but no clinical readouts are available yet. IIM nanoformulations currently tested in clinical trials are all administered intratumorally or intravesically. Local administration was adopted to avoid severe systemic cytokine elevation which can lead to fatal cytokine release syndrome.[55] Repeated weekly administrations of these nanoformulations are needed to maintain therapeutic responses, which presents a significant drawback. To date, no nanoformulations of IIMs have advanced beyond phase II clinical trials.

4. Multifunctional NPs of IIMs for Enhanced Antitumor Effects

In the cancer-immunity cycle, tumor antigens released in the TME are internalized by APCs and presented to naïve T cells, which mature into CTLs.[56] Integrating NP-delivered IIMs into this cycle has significantly improved therapeutic outcomes as they play crucial roles in reprogramming the TME, increasing tumor infiltration by immune cells, and enhancing the cytotoxic response. However, as immune activation by IIMs is not specific to tumors, monotherapeutic IIMs may not provide sufficient immune activation in the TME without triggering the undesired systemic immune response. As an example, a phase I clinical study of XMT-2056, antibody drug conjugate of the STING agonist diABZI, was terminated after a therapy-related death in the first cohort of patients.[57] This safety incident underscores the unpredictable nature of a systemically administered IIM and highlights the need to develop other strategies to enhance the antitumor effects of IIMs. One strategy is to combine IIMs with other treatments that can enhance the presentation of tumor antigens to immune cells, which can activate both innate and adaptive immune responses to enhance tumor specificity and reduce potential side effects from the IIMs.

Tumor antigens are antigenic molecules that are produced by tumor cells and recognized by the immune system, particularly T cells.[58] They are classified into two main categories: tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs) which include neoantigens.[59] TAAs can be released through immunogenic cell death (ICD), accompanied by the release of DAMPs and pro-inflammatory cytokines.[60] These factors facilitate the presentation of TAAs to adaptive immune cells, triggering an antigen-specific immune response against a wide range of solid tumors. In ideal scenarios, ICD can be harnessed to enhance immune stimulatory effects or reverse immune suppressive effects for the activation, proliferation, and tumor infiltration of T cells. Significant efforts have recently been devoted to developing multifunctional NPs of IIMs that can synergize tumor antigen release and presentation with IIMs to generate in situ "cancer vaccines." Over the past few years, many NP platforms have been developed to broaden and enhance immunotherapy by combining IIMs with ICD-inducing treatment modalities such as chemotherapy, RT, PDT, and tumor antigens.

4.1. Combination with Chemotherapeutics

Increasing evidence shows that certain chemotherapeutics, such as oxaliplatin[61] and doxorubicin,[62] can induce ICD to lead to a pro-inflammatory and immunogenic TME. Several regimens of chemotherapy and ICB combinations have been approved to treat patients with non-small lung cancer, early triple-negative breast cancer, endometrial cancer, gastric cancer and small lung cancer.[63] NP-mediated chemotherapy has been reported to enhance ICD, thereby improving the antitumor effects of free chemotherapeutics. The proinflammatory TME induced by IIMs may also enhance the efficacy of chemotherapeutics in the tumors.[64]

Jiang et al. reported a bifunctional NCP, OX/GA, by encapsulating chemotherapeutic oxaliplatin (OX) and cGAMP (GA) via coordination polymerization of their phosphate groups with Zn^{2+} ions (Figure 7).[65] OX/GA protected cGAMP from enzymatic degradation and significantly increased its plasma half-life from to 16.33 h from 0.29 h for free cGAMP. With significantly improved pharmacokinetics, OX/GA disrupted tumor vasculature via host STING activation to increase the intratumoral deposition of OX by ~5-fold over oxaliplatin-NCP. This co-delivery strategy overcame the enhanced permeability and retention (EPR) limitation for nanomedicines. OX/GA induced ICD in tumor cells and STING activation in innate immune cells to enhance TAA presentation, with a 2-fold higher IFN-γ secreting spot-forming cells than GA-NCP monotherapy by ELISpot analysis. OX/GA demonstrated exceptional antitumor effects, with over 95 % tumor growth inhibition and high cure rates in subcutaneous CT26 and MC38 colon cancer, orthotopic Panc02 pancreatic cancer, spontaneous TRAMP prostate cancer mouse models. When combined with anti-PD–L1, OX/GA achieved 90.0 % TGI, extended median survival to 58.8 days from 38.5 days for PBS in immunogenic "cold" Panc02 tumor model. The therapeutic regimen demonstrated safety across all in vivo studies, with no noticeable decrease in body weight observed in mice. This study showed that IIMs in bifunctional NPs could be used to disrupt tumor vasculatures and enhance chemotherapeutic deposition in tumors, which in turn led to much enhanced ICD for immune activation and antitumor effects.

4.2. Combination with Radiotherapy

RT is an important cancer treatment in the clinic and elicits its antitumor effects by causing DNA double-strand breaks in cancer cells. The advent of ICB generated significant amounts of excitement among radiation oncologists in extending the local effects of RT to systemic tumor

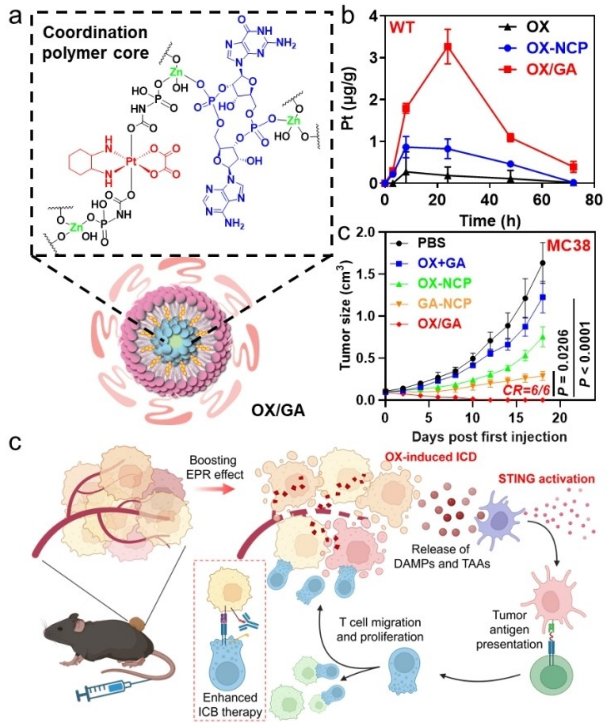

Figure 7. a) Schematic illustration of OX/GA NCP with a coordination polymer core of an oxaliplatin prodrug and cGAMP. b) Pt levels in MC38 tumors in C57BL/6 mice post-OX/GA injection. c) Tumor growth of MC38 tumor-bearing C57BL/6 mice after five does of OX/GA. d) Proposed mechanism of OX/GA-based chemo-immunotherapy: OX/GA disrupts vasculature for enhanced drug delivery, induces ICD, activates STING, releases DAMPs and TAAs, repolarizes macrophages, and primes cold tumors for anti-PD–L1 treatment. Adapted from Ref. [65] with permission. Copyright 2024, American Association for the Advancement of Science.

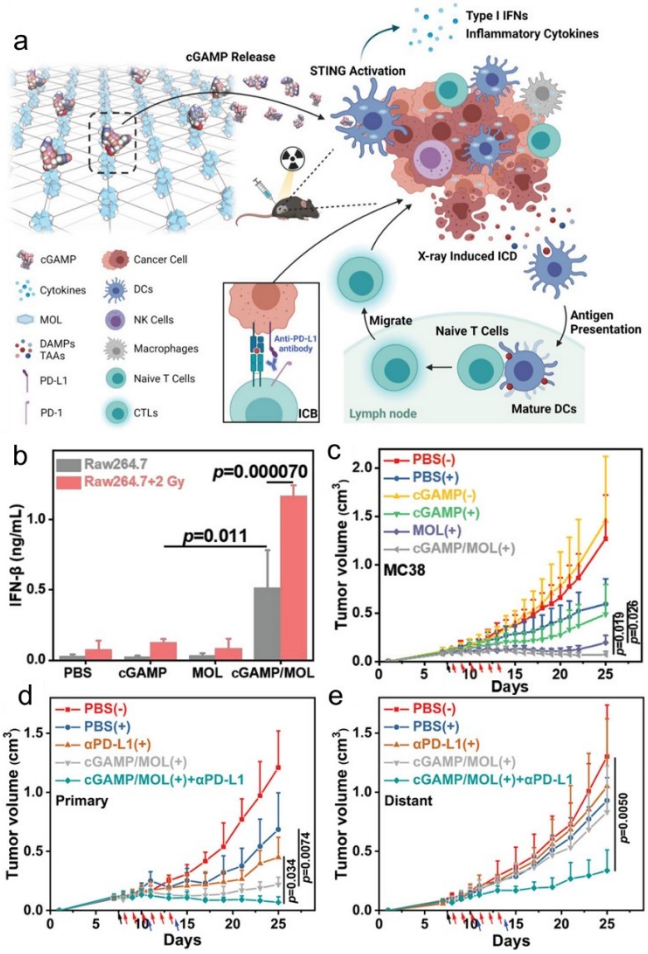

Figure 8. a) Schematic illustration of synergistic radiosensitization and immune activation by cGAMP/MOL. cGAMP/MOL induces ICD to release TAAs and DAMPs, sustains STING activation in APCs to secrete type I IFN and cytokines for leukocyte recruitment. APCs activate T cells in lymph nodes while αPD–L1 reverses immune suppression to enhance antitumor CTL responses. b) Secretion levels of IFN-β by cGAMP/MOL(+)-treated Raw264.7 cells. c) Tumor growth curves of subcutaneous MC38-bearing C57BL/6 mouse model. d,e) Tumor growth curves of treated primary tumors and untreated distant tumors on the bilateral subcutaneous MC38 bearing C57BL/6 mouse model The black arrow indicates intratumoral injection, red arrows indicate X-ray irradiation, and blue arrows indicate intraperitoneal injection of αPD–L1. Adapted from Ref. [71] with permission. Copyright 2022, Wiley-VCH.

control.[66] Several hundreds of clinical trials were launched to combine RT with ICB, but no survival benefits have been observed for these combination treatments in randomized, controlled clinical trials.[67] Preclinical studies have shown that RT induces ICD in situ, as evidenced by the surface exposure of calreticulin (CRT) and high mobility group box 1 (HMGB-1).[68] This process can also release DAMPs and TAAs and DAMPs, which can potentially further stimulate immune cells.

To address the insufficient immune activation by RT, Lin and co-workers developed nanoscale metal–organic frameworks (MOFs) to not only enhance reactive active oxygen species (ROS) generation but also induce more immunogenic TMEs via a unique radiotherapy-radiodynamic therapy (RT-RDT) mechanism.[69] A MOF candidate, RiMO-301, has shown promising antitumor efficacy in a phase 1 clinical trial[70] and is currently tested in combination with pembrolizumab in a phase 2 trial on recurrent and metastatic head and neck cancer patients (NCT05838729). In 2022, Luo et al. developed two-dimensional (2D) nanoscale metal–organic layers (MOLs) to integrate RT and immunotherapy (Figure 8).[71] The MOL was constructed from Hf_{12}-oxo clusters as secondary building units (SBUs) and a porphyrin-based bridging ligands, and further conjugated with cGAMP on the SBUs to afford cGAMP/MOL. This 2D structure maximizes surface area to achieve potent radiosensitization through the RT-RDT process and enhanced ROS diffusion. cGAMP/MOL sustained the release of cGAMP in tumors via a phosphate concentration gradient, resulting in stronger STING activation than cGAMP. cGAMP/MOL plus X-ray irradiation [denoted cGAMP/MOL(+)] significantly inhibited MC38 proliferation and triggered type I IFN and cytokine secretion, with significant IFN-β release in macrophages over 24 hours and in DCs over 72 hours. The synergistic effects of RT-RDT and STING activation effectively regressed local tumors and

activated the tumor immune environment, achieving TGI of 99.7 % for CT26 and 96.4 % for MC38 tumors with 4 of 6 CT26-bearing mice being tumor-free. The treatment also induced immune memory to prevent tumor regrowth upon tumor rechallenge in cured mice (Figure 8c). cGAMP/MOL-(+) enhanced TAA presentation to T cells, with more than 10-fold higher IFN-γ secreting spot-forming cells over PBS-(+). The combination of cGAMP/MOL(+) with anti-PD–L1 demonstrated improved distant tumor control with a TGI of 70.6 % and an abscopal effect in a bilateral tumor model, which extended the cGAMP/MOL(+) treatment from local synergy to a systemic anticancer immune response (Figures 8e,**f**). With stable body weight and minimal abnormalities in major organs, this treatment was safe to the mice. More recently, Luo et al. reported a cGAMP-conjugated MOF that elicited strong anticancer efficacy by forming immune cell-rich nodules (artificial leukocytoid structures) and transforming them into immunostimulatory hotspots with RT.[72]

4.3. Combination with Photodynamic Therapy

PDT uses light and photosensitizers to induce ICD of tumor cells by generating highly cytotoxic ROS. PDT efficiently elicits tumor immunogenicity by inducing CRT exposure and releasing TAAs. As most photosensitizers are highly conjugated molecules which tend to aggregate in aqueous media, NPs have been extensively used to overcome the solubility limitation. NPs also enhanced photosensitizer delivery to tumors, thus minimizing damage to normal tissues, including skin photosensitivity. Combination of PDT with innate immune activation by IIMs is expected to amplify the antitumor immune responses.

In 2023, Jiang et al. developed Ce6/R848 NCP particles for the co-delivery of the photosensitizer Ce6 and cholesterol-conjugated R848 (Chol-R848) for simultaneous PDT-induced ICD and TLR7/8 activation in tumors (Figure 9).[73] Ce6 was polymerized with Zn ions in the NCP core, which reduced aggregation-induced quenching and enhanced PDT efficiency. R848 was conjugated to cholesterol via a tumor-activatable, enzyme-responsive succinate linker and stably incorporated into the NCP shell. Ce6/R848 improved the pharmacokinetics by increasing blood circulation half-lives of Ce6 and R848 to 19.7 h and 10.8 h from 5.1 h to 0.7 h, respectively. As a result, Ce6/R848 enhanced tumor accumulation by 1.4-folds over free drugs and reduced systemic toxicity. Ce6-mediated PDT induced ICD in the TME to release DAMPs and TAAs, while the delivered R848 acted as a PAMP to activate APCs for TAA presentation to T cells (Figure 9b). Ce6/R848 plus light irradiation regressed MC38 colorectal tumors, achieving a 50 % cure rate and 99.4 % TGI (Figure 9c), and significantly prolonged mouse survival in a metastatic 4T1 tumor model.

Xu et al. developed photoactivatable nanoagonists based on a boron dipyrromethene polymer with linked R848 TLR7/8 agonists to facilitate light-triggered ROS generation and release, synergistic photoimmunotherapy, adaptive immunity, and fibrosis reduction for pancreatic ductal adenocarcinoma treatment.[74] Chen et al. reported cancer cell membrane-coated NPs (CCMV/LTNPs) as a platform that integrates PDT, a TLR7 agonist, and tumor antigens, for effective tumor cell destruction.[75] However, due to the limited penetration depth of light, photo-immunotherapy is likely to be limited to the treatment of superficial lesions.

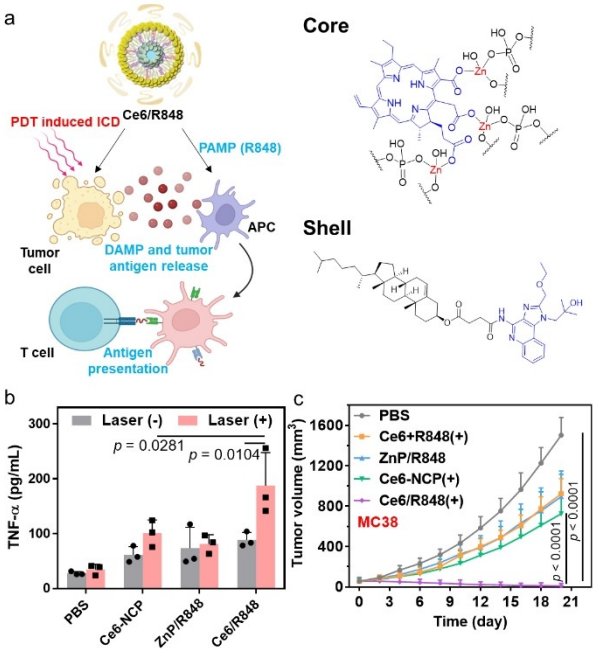

Figure 9. a) Schematic illustration of core–shell Ce6/R848 NCP. Ce6/R848(+) induced ICD of tumor cells to release TAAs and DAMPs, which synergized with TLR7/8 activation by R848 to stimulate APCs for antigen presentation to T cells. b) TNF-α secretion from BMDCs after culturing with the supernatants of treated MC38 cells. c) Tumor growth curves of MC38 tumor-bearing C57BL/6 mice. Adapted from Ref. [73] with permission. Copyright 2023, Elsevier.

4.4. Combination with Tumor Antigens as Cancer Vaccines

TAAs are overexpressed in cancer cells and some normal cells.[76] In contrast, TSAs, including neoantigens, are unique to cancer cells and arise from mutations during cancer development, making them highly specific to the tumor.[77] TSAs, such as those arising from point mutations, gene fusions, or other genetic alterations, can drive precise antitumor immune responses. Integrating TSAs with IIMs enhances tumor-specific antigen presentation, improving the immune system's ability to target cancer cells. NP-based co-delivery further increases TSA presentation and enhances the precision of interactions with APCs, leading to more accurate antitumor immune responses.

LNP mRNA cancer vaccines have offered a groundbreaking platform for leveraging TSAs, such as neoantigens, to achieve precise and effective cancer immunotherapy.[78] Neoantigens, arising from point mutations unique to cancer cells, are exclusively expressed by tumors, reducing off-target effects and the risk of autoimmunity. LNP-based

systems deliver mRNA encoding these neoantigens alongside IIMs, enhancing antigen presentation to APCs. This approach amplifies the specificity and potency of the immune response, enabling personalized and highly effective cancer treatments.[79] A prime example is Moderna's investigational mRNA-based cancer vaccine, mRNA-4157/V940, designed to encode up to 34 neoantigens derived from the unique mutational profile of an individual's tumor.[80] In a Phase IIb trial involving resectable melanoma, the combination of mRNA-4157/V940 and pembrolizumab demonstrated a 49 % reduction in the risk of recurrence or death compared to pembrolizumab alone. These exciting results have pushed the vaccine into Phase III clinical trials, signaling its potential as a transformative therapy in immunotherapy of melanoma.

In 2017, Luo et al. reported a nanovaccine comprising a tumor antigen and a synthetic polymeric PC7 A NP.[81] PC7 A NP efficiently delivered tumor antigens to the cytosol of APCs in lymph nodes, while PC7 A activated the STING pathway while bypassing TLR and MAVS signaling. PC7 A NP was loaded with ovalbumin (OVA) as a model antigen to form the OVA-PC7 A NP with a diameter of 20–50 nm. PC7 A-mediated delivery significantly enhanced OVA accumulation in CD8α$^+$ DCs, CD8α$^-$ DCs, and macrophages in lymph nodes (LNs), with a 29-fold increase in OVA-positive CD8α$^+$ DCs compared to the OVA control. OVA-PC7 A NP increased OVA epitope (SIINFEKL)-specific CD8$^+$ T cells by 15-folds over the OVA-only group. This nanovaccine demonstrated significant inhibition of tumor growth in mouse models of B16-OVA melanoma, MC38 colon cancer, and HPV E6/E7-associated TC-1 lung cancer. The combination of the nanovaccine and anti-PD-1 resulted in 100 % survival over 60 days in the TC-1 tumor model and complete tumor rejection upon rechallenge in tumor-free mice, suggestive of the induction of durable antitumor immune memory.

In 2017 Kuai et al. used high-density lipoprotein (HDL)-mimicking nanodiscs to deliver antigenic (Ag) peptides and CpG to lymphoid organs and sustain antigen presentation on dendritic cells.[82] The HDL nanodisc was formulated using lipids such as DMPC and ApoA1-mimetic peptides such as 22 A to afford clear suspensions. The nanodisc vaccine was then synthesized by incorporating DOPE-PDP (a pyridyldithio lipid linker) into HDL nanodiscs, which allowed surface decoration with cysteine-modified Ag peptides (e.g., $OVA_{257–264}$, Adgpk) via disulfide exchange and non-covalent insertion of cholesterol-conjugated CpG (Cho-CpG). The nanodisc vaccine exhibited a uniform disc-like morphology with an average diameter of 10.5 nm and a PDI of 0.20, and showed a robust CTL response with a 47-fold increase in neoantigen-specific CTL frequencies compared to a soluble vaccine. The nanodisc vaccine generated broad-spectrum T-cell responses to suppress tumor growth, and when combined with anti-PD-1 and anti-CTLA-4, completely eradicated MC38 colon and B16F10 melanoma tumors.

In 2024, Zeng et al. developed a square-block DNA origami nanovaccine, DoriVac, with fine-tuned CpG oligonucleotide spacing (Figure 10).[83] In vitro and in vivo studies

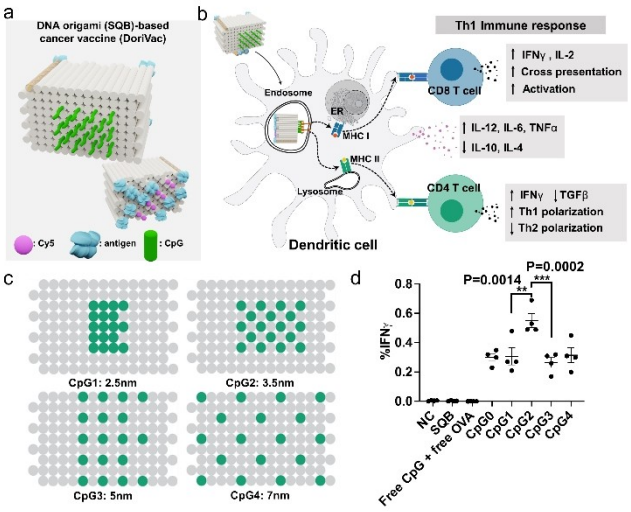

Figure 10. a) DNA origami cancer vaccine (DoriVac) with CpG (green) as an adjuvant, OVA protein (blue) as an antigen, and Cy5 dye (pink) as a tracer. b) Schematic of DoriVac co-delivering antigen and adjuvant at optimal spacing to enhance Th1 immune response. c) Modification sites and CpG array on SQB DNA origami, showing varied CpG spacing across four DoriVac versions (CpG1–4). d) IFNγ expression in human pDCs treated with different vaccine groups. Adapted from Ref. [83] with permission. Copyright 2024, Nature Publishing Group.

revealed that CpG motifs spaced at 3.5 nm in the DNA origami structure induced a strong Th1 immune polarization. DoriVac was constructed by attaching antigen proteins or peptides using SMCC linkage or DBCO-azide click chemistry and formed monodispersed structures of 35.0×22.5×27.0 nm^3 in size. DoriVac showed minimal aggregation and enhanced DC activation, antigen cross-presentation, CD8$^+$ T-cell activation, Th1-polarized CD4$^+$ T-cell responses, and NK cell activation. DoriVac predominantly accumulated in the nearest draining LNs with minimal presence in other LNs, persisted there for at least 48 hours with high antigen intensity, and was largely cleared via the liver and kidneys within two days. The enhanced immune activation inhibited tumor growth and prolonged the median survival from 18 days to 26 days in a B16-OVA melanoma model. Furthermore, the vaccine demonstrated strong synergy with anti-PD–L1, achieving 80 % tumor regression in the B16-OVA melanoma model with no tumor regrowth upon rechallenge. In EG7-OVA lymphoma models, the vaccine completely eradicated tumors while inducing long-lasting T-cell memory.

5. Conclusions and Future Perspectives

ICB has revolutionized the treatment of several cancers by reinvigorating the immune system to recognize and attack malignancies. However, ICB monotherapy has only provided clinical benefits to cancer patients with high mutational burden and T-cell pre-infiltration to their tumors, including melanoma and non-small cell lung cancer. Innate

immune activation provides a potential solution to overcome the immunosuppressive TME and significantly enhance the therapeutic efficacy of ICB. Due to the risk of systemic immune activation by IIMs to lead to potentially fatal cytokine release syndrome, nanoformulations of IIMs have been developed to enhance the delivery of IIMs to tumors. As IIMs have diverse chemical structures with vastly different physicochemical properties, many different NP platforms have been used to deliver IIMs to target the cGAS-STING, TLR, and RLR pathways and shown exciting preclinical results. Importantly, several nanotherapeutics targeting these pathways have already been translated into the clinic, and available clinical readouts have shown promising antitumor effects. When combined with ICB, nanoformulations of IIMs have provided durable treatment responses to some cancer patients. However, current nanoformulations of IIMs need to be intratumorally or intravesically injected on a weekly basis to sustain the immune activation, which has significantly limited the clinical utility of the first generation of IIM nanotherapeutics.

Multifunctional nanoparticles have been designed to enhance the antitumor effects of IIM nanotherapeutics by combining IIMs with chemotherapy, radiosensitizers, photosensitizers, or tumor antigens. NPs with both IIMs and chemotherapeutics were designed to provide a more immunogenic TME for potential combination therapy with ICB. This research is highly clinically relevant as several chemotherapy and ICB combinations have been approved to treat patients with non-small lung cancer, early triple-negative breast cancer, endometrial cancer, gastric cancer and small lung cancer. Multifunctional NPs of IIMs and chemotherapeutics have the potential to enhance treatment responses and reduce general toxicity over the clinically approved chemotherapy and ICB combinations.

Despite initial excitement from radiation oncologists and immunologists on RT and ICB combination treatments, recent randomized, controlled clinical trials have not demonstrated survival benefits for cancer patients. Preclinical results indicate that combining nanoradiosensitizers and IIMs in the same particles not only enhances tumor control of RT but also activates the tumor immune microenvironment. To date, no clinical data is yet available to validate the preclinical findings. Multifunctional NPs of IIMs and photosensitizers provide another interesting approach to activate the tumor immune microenvironment due to the immunogenic nature of PDT. However, the shallow penetration depth of light will likely limit this photo-immunotherapy treatment to superficial lesions.

Multifunctional NPs with both IIMs and tumor antigens represent one of the most exciting strategies for cancer immunotherapy as LNP mRNA cancer vaccines have entered registrational trial for stage III, resectable melanoma. Other NP platforms are explored for developing vaccines to target many tumor types. We expect the development of more robust and versatile cancer vaccines by combining IIMs and tumor antigens in innovative NPs.

The field of IIM nanotherapeutics have significantly advanced in the past decade. To facilitate clinical translation, further improvements of IIM nanotherapeutics are needed. First, NPs need to improve pharmacokinetics and biodistribution to increase their tumor accumulation. Second, NPs should have built-in controlled release properties to ensure precise IIM delivery and release in tumors. Third, NPs can combine therapeutic agents with IIMs to achieve synergistic effects, enhancing efficacy while reducing IIM doses to reduce systemic toxicity. Finally, the use of biodegradable and biocompatible materials in NPs can facilitates clearance to minimize long-term toxicities. Additionally, the doses and dose schedules of IIMs need optimization to maximize their therapeutic potential while minimizing systemic toxicity. The timing and frequency of IIM administration are important for sustaining immune activation without triggering adverse effects. As the efficacy of IIMs is significantly influenced by the TME, which often imposes immunosuppressive barriers and hinders therapeutic outcomes, novel strategies are needed to modulate the TME to promote immune infiltration and activity for significant enhancement of IIM therapeutic efficacy. Another important consideration is the potential for tumors to develop resistance to IIM-based treatments. Combination therapies and adaptive treatment strategies are needed to overcome resistance mechanisms, such as upregulation of immunosuppressive cytokines, loss of antigen presentation, or adaptation to immune pressure.

Multifunctional NPs with IIM and chemotherapy, radiosensitizer, and tumor vaccine combinations have shown exciting preclinical results. Early clinical readouts of LNP mRNA cancer vaccines have been exciting. The clinical utility of these combination treatments can be further enhanced by increasing tumor-specificity and controlled release ability of NP systems. Limiting off-target effects and ensuring consistent drug delivery across diverse tumor types present significant challenges. Scaling and reproducibility of these multifunctional nanotherapeutics are essential for their clinical translation and regulatory approval. In-depth investigations of the mechanisms of action of IIMs in the TME can facilitate the rational design of next-generation multifunctional IIM nanotherapeutics with enhanced antitumor responses and reduced general toxicity. Close collaborations among synthetic chemists, nanotechnologists, cancer biologists, immunologists, and clinicians are needed to move the exciting field of IIM nanotherapeutics forward to benefit more cancer patients.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: cancer immunotherapy · innate immunity · nanoparticles · antigen presentation · tumor microenvironment

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Minireview

Immunotherapy

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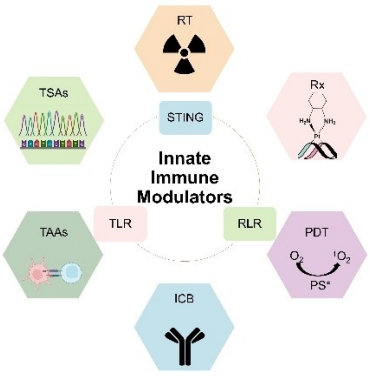
Innate Immune Activation with Multifunctional Nanoparticles for Cancer Immunotherapy



This minireview discusses the roles of innate immune modulators (IIMs) in cancer therapy and their ability to enhance the antitumor effects of immune checkpoint blockade and summarizes recent progress in the development of IIM nanotherapeutics to target cGAS-STING, TLR, and RLR pathways. Multifunctional nanoparticles combining IIMs and chemotherapy, radiotherapy, photodynamic therapy, or tumor vaccines are discussed next, followed by brief summaries of the lessons learned, current challenges, and future perspectives in optimizing IIM nanotherapeutics for cancer immunotherapy.