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Nano-Immune-Engineering Approaches to Advance Cancer Immunotherapy: Lessons from Ultra-pH-Sensitive Nanoparticles

Published as part of the Accounts of Chemical Research special issue "Chemistry in Cancer Immunotheranostics".

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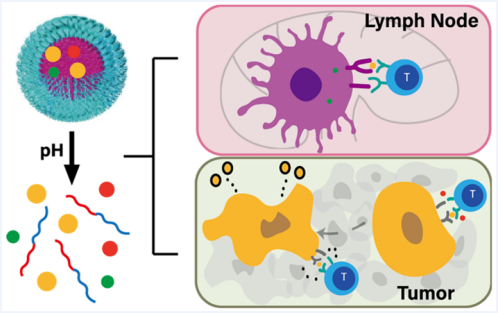
Cite This: *Acc. Chem. Res.* 2020, 53, 2546−2557 Read Online

CONSPECTUS: Immunotherapy has transformed the field of oncology and patient care. By leveraging the immune system of the host, immunostimulatory compounds exert a durable, personalized response against the patient's own tumor. Despite the clinical success, the overall response rate from current therapies (e.g., immune checkpoint inhibitors) remains low (∼20%) because tumors develop multiple resistance pathways at molecular, cellular, and microenvironmental levels. Unlike other oncologic therapies, harnessing antitumor immunity requires precise activation of a complex immunological system with multiple levels of regulation over its function. This requires the ability to exert control over immune cells in both intracellular compartments and various extracellular sites, such as the tumor microenvironment, in a spatiotemporally coordinated fashion.



The immune system has evolved to sense and respond to nano- and microparticulates (e.g., viruses, bacteria) as foreign pathogens. Through the versatile control of composition, size, shape, and surface properties of nanoparticles, nano-immune-engineering approaches are uniquely positioned to mount appropriate immune responses against cancer. This Account highlights the development and implementation of ultra-pH-sensitive (UPS) nanoparticles in cancer immunotherapy with an emphasis on nanoscale cooperativity. Nanocooperativity has been manifested in many biological systems and processes (e.g., protein allostery, biomolecular condensation), where the system can acquire emergent properties distinct from the sum of individual parts acting in isolation.

Using UPS nanoparticles as an example, we illustrate how all-or-nothing protonation cooperativity during micelle assembly/disassembly can be leveraged to augment the cancer-immunity cycle toward antitumor immunity. The cooperativity behavior enables instant and pH-triggered payload release and dose accumulation in acidic sites (e.g., endocytic organelles of antigen presenting cells, tumor microenvironment), intercepting specific immunological and tumor pathophysiological processes for therapy. These efforts include T cell activation in lymph nodes by coordinating cytosolic delivery of tumor antigens to dendritic cells with simultaneous activation of stimulator of interferon genes (STING), or tumor-targeted delivery of acidotic inhibitors to reprogram the tumor microenvironment and overcome T cell retardation. Each treatment strategy represents a nodal intervention in the cancer-immunity cycle, featuring the versatility of UPS nanoparticles. Overall, this Account aims to highlight nanoimmunology, an emerging cross field that exploits nanotechnology's unique synergy with immunology through nano-immune-engineering, for advancing cancer immunotherapy.

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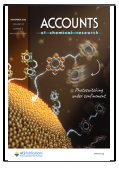
Received: July 22, 2020
Published: October 16, 2020



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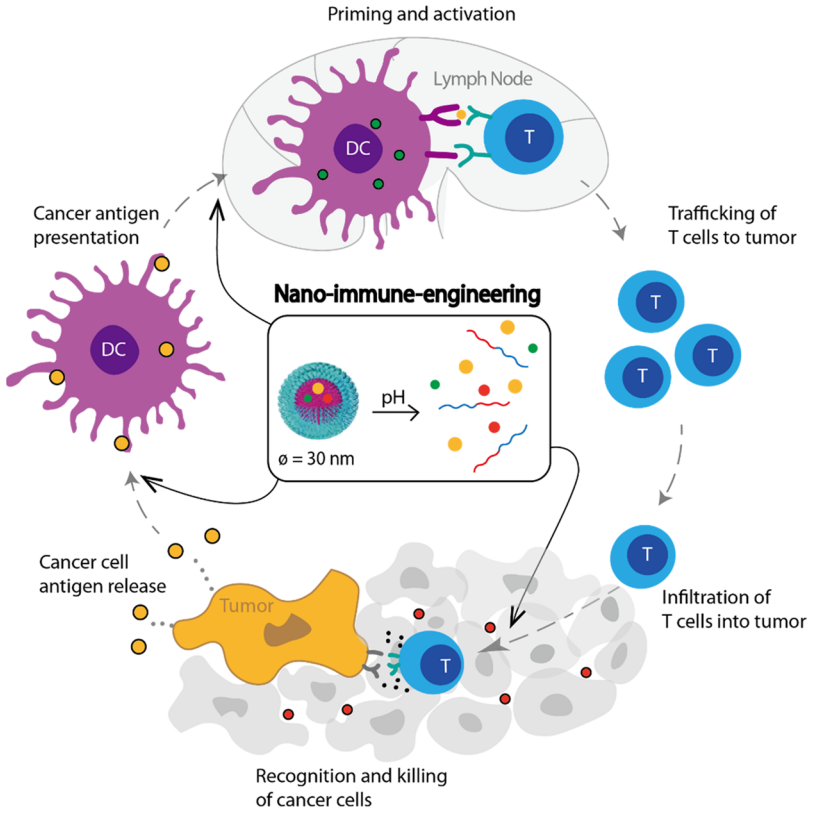

Figure 1. Nano-immune-engineering approaches to augment the cancer-immunity cycle. Ultra-pH-sensitive nanoparticles are loaded with distinct payloads depending on specific applications: tumor antigen (yellow), innate immune activator (green), or agent that reprograms tumor microenvironment (red). These molecules are targeted to the tumor microenvironment or the draining lymph nodes with precise spatiotemporal control, tilting the cancer-immunity balance toward antitumor immunity.

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INTRODUCTION

Tumor progression is a complex process defined by alterations in cancer genetics and proliferative cell phenotypes, which dynamically modulate the tumor microenvironment and the immune system.[5,6] Genetic mutations and uncontrolled cell growth often lead to expression of neoantigens or tumor-associated antigens, which are presented on major histocompatibility class (MHC) molecules on cancer cells and recognized as aberrant "nonself" signals.[7] Under immune surveillance, antigen presenting cells capture tumor antigens as well as danger associated molecular patterns, leading to effector T cell response and elimination of cancer cells.[8] However, this coordinated series of events, recognized as the cancer-immunity cycle (Figure 1),[9,10] is often disrupted by tumors and immune checkpoints,[11] changing tumors from an immune infiltrated (inflamed or "hot") to immune deserted ("cold") phenotype. Understanding tumor biology and its crosstalk with the immune system is paramount to designing a safe and personalized immunotherapy.

Multiple key factors contribute to tumor immune suppression and escape. First, recognition of cancer cells by the immune system can be impaired. While each patient's tumor is distinct, many cancers are characterized by loss or mutation of MHC molecules,[12] activation of negative regulatory pathways (checkpoints),[13] and disruption in homeostatic chemokine gradients.[14] Second, extracellular potassium ions,[15] acidic pH,[16] and hypoxia[17] in the tumor microenvironment directly inhibit T cell effector functions and proliferation. Tumor-infiltrating lymphocytes often have an exhausted phenotype and cannot control cancer progression. Third, various cell types in the tumor microenvironment exert immune suppressive effects. Myeloid derived suppressor cells, tumor associated macrophages, and regulatory T cells all inhibit cellular immunity against tumors. In many cases, tumors exploit intrinsic regulatory mechanisms to limit the strength of immune response. As such, robust antitumor immunity requires coordinated activation of both innate and adaptive immune pathways to prevent cancer from activating regulatory pathways that often present as a self-limiting process.

In this Account, we describe how nanotechnology is uniquely suited for eliciting antitumor immune responses while inhibiting tumor suppressive mechanisms, tilting the

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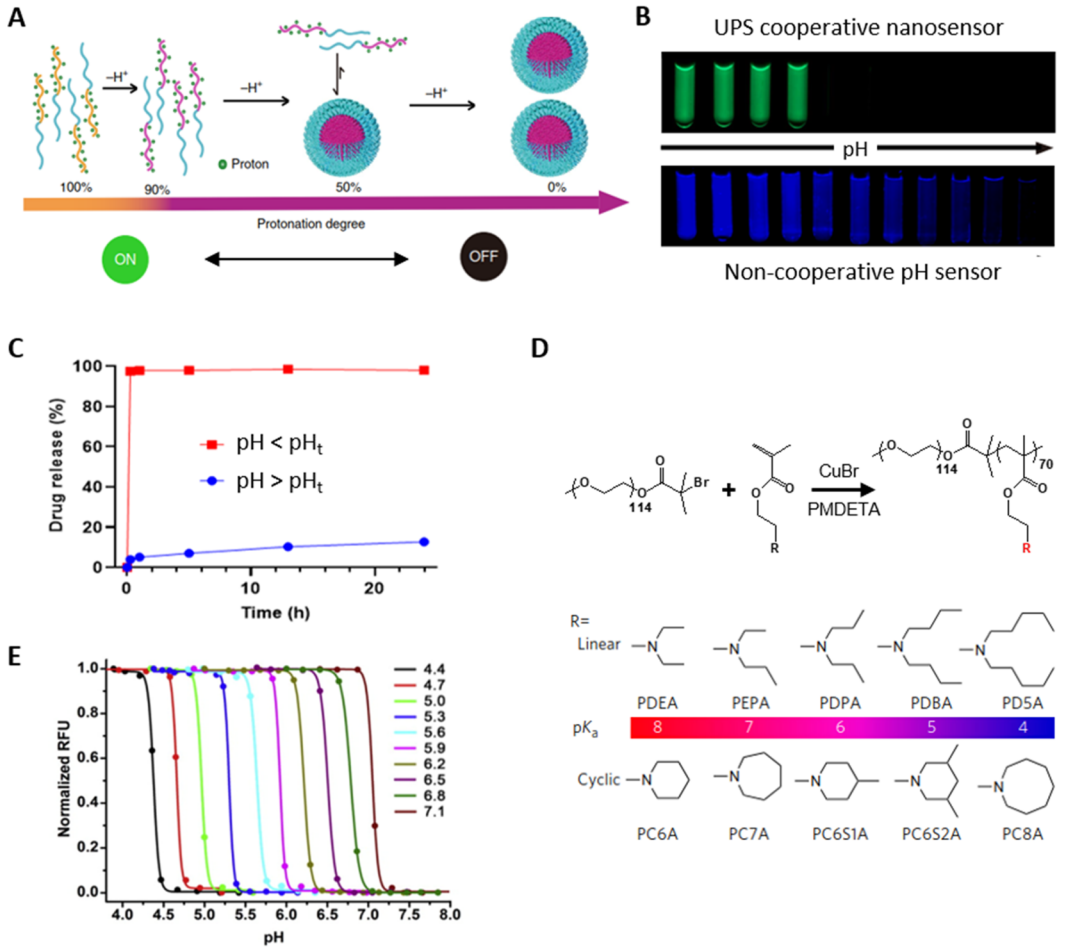

Figure 2. UPS nanoparticles exhibit cooperative response to environmental pH. (A) UPS NPs display an "all-or-nothing" cooperative deprotonation phenotype. (B) Binary on/off response is observed with UPS nanosensor (up), whereas a gradual response is shown with a small molecular pH sensor (Lysosensor Green, below) in response to the change of environmental pH. (C) UPS NPs display rapid payload release when the pH drops below the transition pH (pH_t). (D) Schematic illustration of the synthesis and chemical structures of the library of UPS polymers with finely tunable hydrophobicity and pK_a. PMDETA: *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine. (E) Representative library of UPS NPs with 0.3 pH increment covering the physiologic range of pH 4−7.4. Reproduced with permission from refs 1 and 27. Copyright 2016 Nature Publishing Group and 2014 American Chemical Society.

balance toward immune eradication of tumors. Recent preclinical studies from our lab and literature suggest that a nanoparticle-based approach improves safety and efficacy of cancer immunotherapy by selective targeting of lymphoid organs or the tumor microenvironment.[2,3,18−20] These nanoscale materials are adept at modulating immune responses because their nanoscale size mimics natural pathogens that the immune system has evolved to sense. Among these, pH-responsive polymers have been extensively studied as delivery systems targeting both the acidic tumor microenvironment and intracellular antigen processing organelles such as endosomes. For immunotherapy, various pH-responsive materials were developed to deliver immunotherapeutic agents to tumors (e.g., IL2,[21] IL12,[22] CpG,[23] and anti-PD1[24]), promoting immune effector cell infiltration and reduction of immunosuppressive factors. Nanocarriers can also escape endosomes in antigen presenting cells in response to the pH drop in these organelles, allowing cytosolic delivery of antigens[25] and nucleic acids,[26] which can then be cross-presented to generate a tumor specific cytotoxic T cell response. In this Account, we highlight the unique "all-or-nothing" protonation cooperativity of the ultra-pH-sensitive (UPS) nanoparticles over common pH-responsive polymers and how nanoscale cooperativity can be leveraged to augment different nodal points in the cancer-immunity cycle to advance cancer immunotherapy (Figure 1).

UPS NANOPARTICLE LIBRARY AND COOPERATIVE PH RESPONSE

A sharp pH response of UPS nanoparticles is driven by self-assembly of multiple polymer chains (~800 polymers per particle) into a nanoscopic core−shell micelle structure.[1,27] Each block copolymer consists of a poly(ethylene glycol) (PEG) hydrophilic segment and an ionizable poly(methyl methacrylate)-based segment with tertiary amine side chains. In high pH environments, the tertiary amines are neutral and form the hydrophobic core, while hydrophilic PEG segments form the shell, yielding micelles 30−50 nm in size. When exposed to a phase transition pH (pH_t) below a particular threshold, the tertiary amines become protonated and cationic, favoring the solubilization of each polymer chain with subsequent micelle disassembly. Distinct from conventional pH responsive materials such as polyethylenimine (PEI), polylysine, or chitosan that exhibit a gradual protonation during acidification, UPS nanoparticles display an "all-or-

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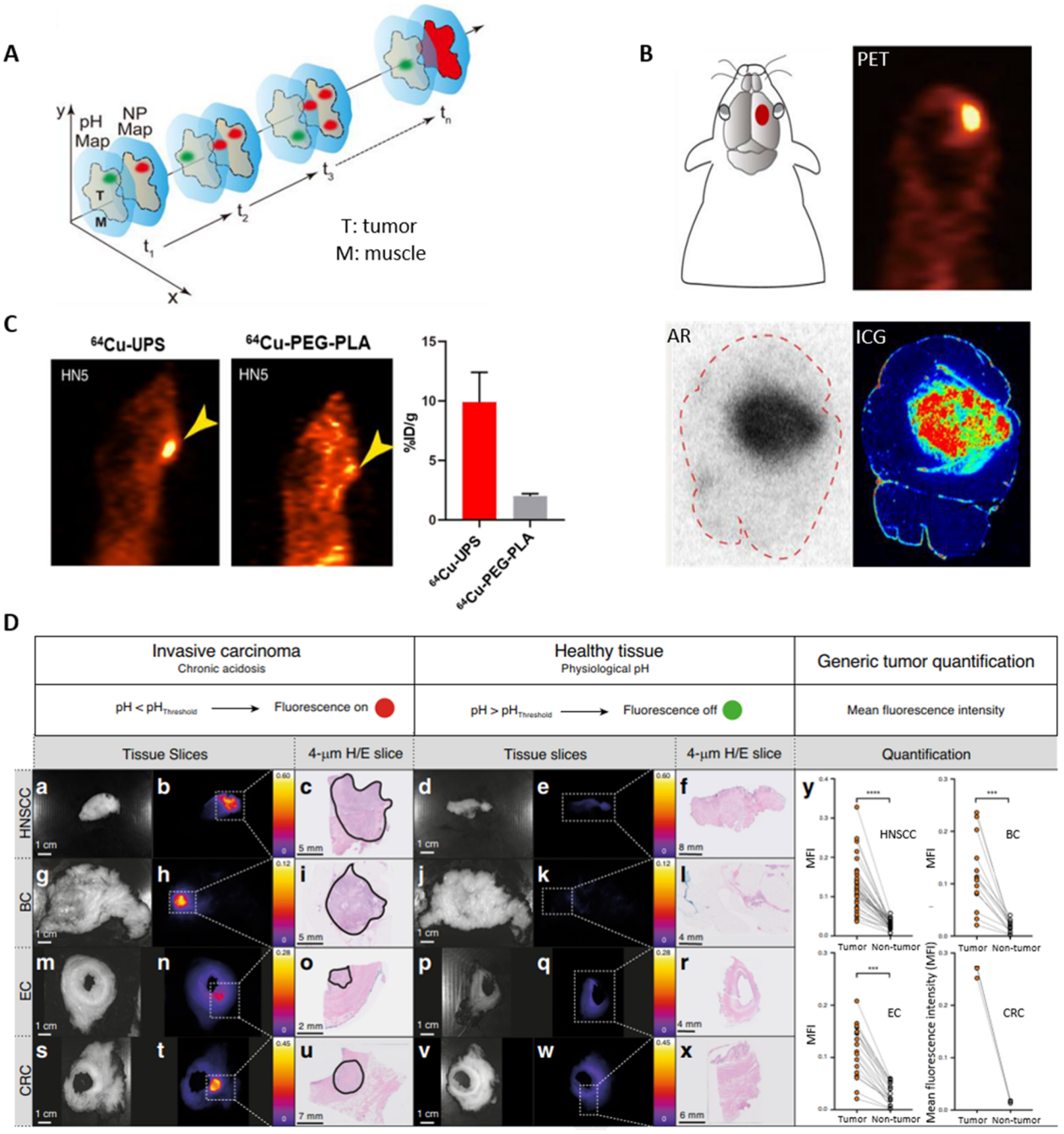

Figure 3. Capture and integration of UPS nanoparticles in acidic tumor microenvironment. (A) Dissociated polycationic polymers accumulate in tumors over time yielding a binary tumor map over the normal muscle background. (B) UPS polymers conjugated with either ^{64}Cu (PET image in top-right panel and autoradiography in bottom-left panel) or indocyanine green (fluorescent image, bottom-right panel) showed conspicuous tumor detection after intravenous injection. (C) UPS NPs achieve 4-fold higher tumor accumulation compared to non-pH-sensitive PEG−PLA micelles in a HN5 mouse tumor model. (D) Clinical evaluation of UPS NPs shows a sharp demarcated fluorescent signal in tumor over normal tissue irrespective of the tumor type after intravenous injection in human patients (HNSCC, head and neck squamous cell cancer; BC, breast cancer; EC, esophageal cancer; CRC, colorectal cancer). Reproduced with permission from refs 29 and 30. Copyright 2020 Nature Publishing Group.

nothing" response across a narrow pH range (<0.3 pH unit).[1] Along the pH titration coordinate, the tertiary amine residues are either electrostatically neutral in the micelle state or highly protonated in the unimer state, without a variable intermediate charge state in each polymer chain (Figure 2A).

When conjugated to a fluorescence reporter, UPS nanoparticle response to pH displays a binary output (Figure 2B).[1] In the micelle state (pH > pH_t), homoFRET-induced quenching of fluorophores abolishes the fluorescent signals. Below the pH transition, micelles dissociate into unimers with full recovery of fluorescence. Quantitative analysis from a modified allosteric model reveals that the UPS nanoparticles have a large Hill coefficient (n_H = 51) compared to conventional pH responsive materials (e.g., PEI with n_H < 1). This micellization-driven nanoscale cooperativity allows an instantaneous release of therapeutic payloads. For example, a

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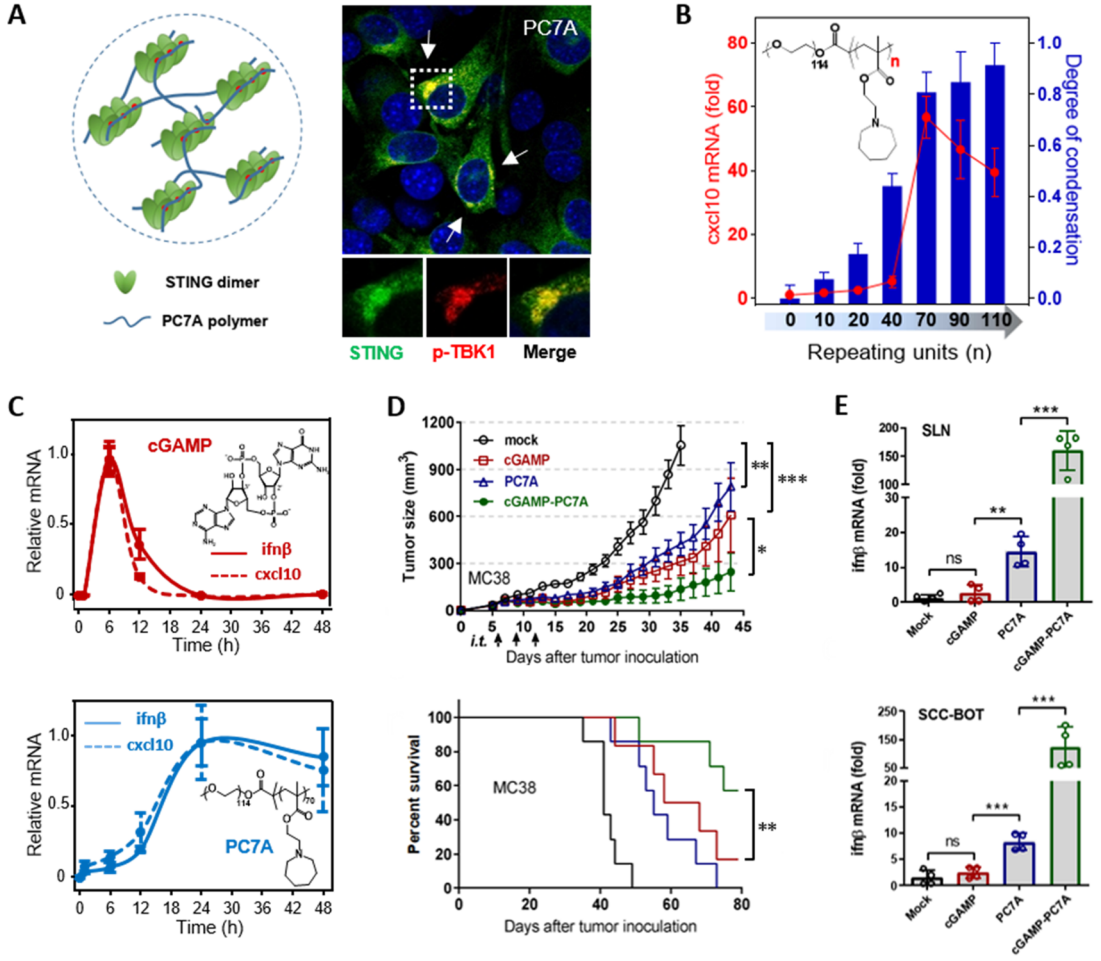

Figure 4. PC7A polymer serves as an immunomodulator through STING pathway. (A) PC7A drives STING oligomerization and biomolecular condensation through polyvalent interactions. (B) STING activation correlates with the PC7A length, with maximal cxcl10 expression induced by PC7A(70). (C) PC7A shows slower but prolonged STING activation compared to cGAMP. (D) cGAMP-PC7A NP confers a synergistic antitumor immune response in MC38 tumor-bearing mouse. (E) cGAMP-PC7A NP shows robust STING activation in freshly resected human tissues (SLN, sentinel lymph node; SCC-BOT, squamous cell carcinoma from the base of tongue).

monocarboxylate transporter 1 inhibitor releases into solution in response to the low environmental pH (Figure 2C, also see its ability to reprogram the tumor microenvironment to overcome T cell suppression below).[3]

Investigation on the molecular structure of the UPS nanoparticles demonstrated a linear correlation between the pH_t value and the hydrophobicity of the tertiary amine segment (the partition coefficient, log *P*, quantifies the hydrophobicity of the repeating unit). By selecting two monomers with different hydrophobicity, we synthesized a library of UPS nanoparticles with finely tunable pH_t values (Figure 2D,E).[27] In a proof of concept, we constructed a barcode pH nanosensor with three fluorescent reporters, each encoding a pH transition (6.9, 6.1, and 5.3). The nanosensor allowed digitization of luminal pH of endosomes and lysosomes at a single organelle resolution and further identified mutant KRAS as the driver for accelerated lysosome acidification in cancer cells.[28] The UPS library offers a valuable tool to target a variety of physiological and pathological processes involving pH regulation.

TUMOR TARGETING BY UPS NANOPARTICLES THROUGH CAPTURE AND INTEGRATION

Biological signals, such as tumor acidosis, are perpetually changing in space and time. The spatiotemporal heterogeneity creates challenges in cancer diagnosis and therapy. To overcome these challenges, UPS nanoparticles improve precision in cancer detection and dose accumulation through a "capture and integration" mechanism (Figure 3A).[29] To illustrate the concept, a positron-emitting radionuclide (^{64}Cu) encoded $UPS_{6.9}$ nanoparticle (^{64}Cu-UPS) was developed to investigate polymer dose accumulation throughout a solid tumor. Polymer distribution in the tumor and surrounding tissues was quantified over time via positron emission tomography (PET), autoradiography, and fluorescence imaging (Figure 3B). Transient secretion of acids by the cancer cells in different tumor subregions triggered UPS micelle disassembly, with the polycationic unimers irreversibly arrested in that microarea for a full capture. Intact micelles are cleared from normal tissues by blood perfusion, resulting in a high tumor over normal tissue contrast. Through capture and integration, the UPS micelles demonstrate superior tumor-targeted dose accumulation over conventional non-pH-sensitive micelles after intravenous injection (Figure 3C).

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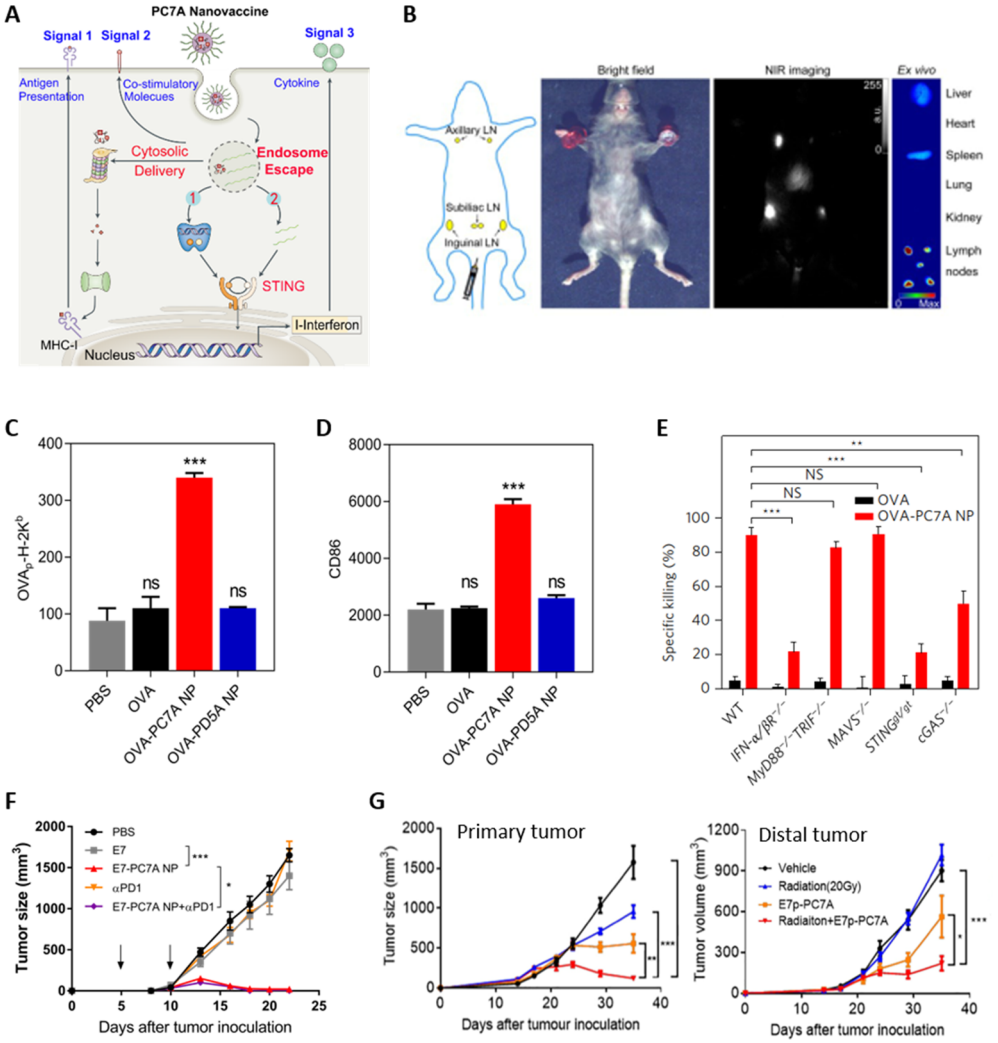

Figure 5. PC7A nanoparticle vaccine induces robust T cell activation for cancer immunotherapy. (A) PC7A NP provides all three signals (antigen delivery, co-stimulatory signals, and innate stimulation through the STING pathway) for generation of cytotoxic T cells. (B) ICG-labeled PC7A NPs accumulate in lymphoid organs after subcutaneous injection. (C) OVA-loaded PC7A nanovaccine promotes antigen presentation on H-2K^b in BMDCs. (D) OVA-PC7A nanovaccine increases expression of co-stimulator CD86 on CD8α^+ DCs in inguinal lymph nodes 24 h after injection. (E) OVA-PC7A nanovaccine generates specific CTL responses through STING-IFN pathway. (F) PC7A nanovaccine induces robust antitumor immunity against TC-1 tumors. (G) PC7A nanovaccine synergizes with radiation and achieves tumor growth inhibition in both primary and distal tumors. Reproduced with permission from refs 2 and 47. Copyright 2017 Nature Publishing Group and 2019 Elsevier B.V.

An indocyanine green (ICG)-conjugated UPS micelle, ONM-100, was investigated in a phase I clinical trial for real-time imaging of tumor margins.[30] In patients with four distinct cancer types (head and neck squamous cell carcinoma, breast cancer, esophageal cancer, and colon cancer), the trial met its primary end points of safety and imaging efficacy. Each tumor type demonstrated fluorescence intensity *in situ*. Validation of ONM-100 by histopathology revealed a demarcated fluorescent signal across tumor margins (Figure 3D). The clinical success of UPS micelles for intraoperative imaging of tumors highlights the effectiveness of the nanoengineering approach to amplify tumor acidic pH signals for cancer detection and dose accumulation.

NANOPARTICLES AS IMMUNE ADJUVANTS

Conventional nanomaterials often act as inert carriers, which improve drug solubility and stability during blood circulation. To intercept the cancer-immunity cycle, it is often desirable to introduce immunological functions to the nanoparticle carriers. A synthetic polymer in the UPS library, PC7A, illustrates the ability to activate an innate immune pathway, the stimulator of interferon genes (STING). STING plays a central role in stimulating a multifaceted type I interferon response that promotes the maturation and migration of dendritic cells (DCs), priming cytotoxic T lymphocytes and nature killer cells against infection or cancer.[31,32] STING has recently emerged as an important target in cancer immunotherapy. However, therapeutic attempts using small molecule STING agonists have been hampered by enzymatic degradation as well as perfusion loss after intratumoral injection, leading to elevated systemic toxicity.[33]

Isothermal calorimetry studies showed that PC7A polymer has a strong binding affinity to STING (dissociation constant K_d = 72 nM). Each polymer chain consists of multiple binding units, allowing PC7A polymer to multimerize several STING molecules in a biomolecular condensate for activation (Figure 4A).[4] The pH$_t$ of PC7A is 6.9, which permits the polymer to form intact micelles at physiological pH (7.4), endocytose into

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Acc. Chem. Res. 2020, 53, 2546−2557

cells, disassemble into unimers during endosomal maturation, disrupt endosomal membranes, and reach intracellular STING molecules.[34] Polymers with more repeating units exhibited stronger capability to induce STING condensation, which demonstrates the importance of multivalency in the immune response (Figure 4B). A boost in cxcl10 mRNA expression (an interferon stimulated gene downstream of STING) was observed after increasing the number of repeating units of PC7A polymer from 10 to 70; further elongation of the valency (e.g., 110) reduced STING activation.

Compared to natural cGAMP ligand, PC7A polymer prolongs STING activation (Figure 4C). When primed by cGAMP, activated STING rapidly peaked at 6 h after stimulation, followed by fast degradation and immune silence after 24 h. In contrast, PC7A generated a durable STING activation profile, with sustained expression of interferon-stimulated genes (ifn-β and cxcl10) over 48 h. Given the importance of prolonged proinflammatory signals in antitumor immunity, we theorize that a combination of the two STING agonists would allow for an optimal time window for DC maturation and T cell priming (normally requires 1−2 days).[35,36] Indeed, treatment with cGAMP-loaded PC7A nanoparticle (cGAMP-PC7A NP) caused potent tumor growth inhibition after intratumoral administration in mice (Figure 4D). The combination strategy displayed translational potential as indicated by the large increase in STING activity in resected human tumors and lymph nodes *ex vivo* (Figure 4E). Further treatment with cGAMP-PC7A NPs with anti-PD-1 conferred a notably stronger protection in the MC38 colon carcinoma model, with 100% of mice remaining tumor-free after 80 days. Besides cancer application, cGAMP-PC7A NPs also demonstrated effective inhibition of HIV-1 replication in patient peripheral blood mononuclear cells through activation of STING-type I interferon pathway.[37]

NANOPARTICLE DRAINAGE TO LYMPH NODES FOR T CELL ACTIVATION

Lymph nodes are secondary lymphoid organs which sample particulates draining from the interstitial fluids through lymphatic vessels. A network of lymph node-resident antigen presenting cells engulf these particulates and search for pathogen-associated molecular patterns or danger-associated molecular patterns. Upon activation, antigen presenting cells become mature to prime lymphocytes, triggering adaptive immune responses including generation of antigen-specific effector T cells as well as memory cells. Direct delivery of nanoparticle vaccines to the lymph nodes can overcome scarce tumor antigen release while coordinating antigen delivery with innate activation to boost an antigen-specific cytotoxic T lymphocyte response. However, it is a challenging task to achieve the spatiotemporal coordination of precise antigen transportation and immune activation at the tissue and cellular levels.[38]

Nanoparticles are an ideal vaccine choice that can protect antigen molecules from enzymatic degradation, enable lymph node accumulation, and facilitate DC uptake. Nanoparticles less than 50 nm in size show an optimal effect in lymph node drainage.[39,40] Additionally, shape,[41] surface charge,[42] hydrophobicity,[43] and composition of the particle[44] also influence lymphatic drainage and phagocytosis. Recently, we reported a minimalist nanovaccine,[2] comprising a physical mixture of tumor antigens encapsulated inside the PC7A nanoparticles, which generated a robust T cell response with low systemic toxicity (Figure 5A). Through subcutaneous injection in a mouse model, the antigen-loaded nanoparticles (29 nm in diameter) transported to peripheral lymph nodes within 24 h, with no significant accumulation at other organs (Figure 5B). The UPS micelle has a high PEGylated density on its surface, which is known to facilitate lymphatic drainage after subcutaneous injection.[45] This shell stabilizes encapsulated antigen during lymphatic trafficking (pH 7.4) and enables precise release of antigen upon exposure to acidic endocytic vesicles (pH 5.0−6.0). Specifically, antigen uptake increased by 30-fold compared to the soluble antigen control in lymph node-resident CD8α^+ DC cells, which are known to be critical for the induction of cytotoxic T cell response.

T cell activation is driven by an immunological synapse between DCs and naive T cells. DC−T cell priming requires spatiotemporal orchestration of three signals occurring simultaneously at the DC and T cell interface. First, cytosolic delivery of tumor antigens in the DCs is important to allow antigen processing and presentation by the MHC-I molecule on the cell surface (signal 1). Lack of cytosolic delivery may leave antigens inside endosomes, which readily process into the MHC-II pathway for a humoral response. Our work with mouse bone marrow-derived DCs showed that nanoparticle-encapsulated ovalbumin increased and sustained antigen presentation via the MHC class I complex to a much higher degree than soluble ovalbumin (Figure 5C). The induction of co-stimulatory molecules (CD80/CD86) on antigen presenting cells is equally important (signal 2, Figure 5D). Lack of signal 2 can lead to immune resistance or T cell apoptosis. Lastly, proinflammatory cytokine production (signal 3) by PC7A NPs through the STING pathway completes the requirement for DC−T cell priming, as illustrated by the failure of PC7A NPs in generating antitumor immunity in STING$^{gt/gt}$ or IFN-$\alpha/\beta R^{-/-}$ mice (Figure 5E). With the optimal spatiotemporal control, the PC7A nanovaccine primed robust antigen-specific CD8$^+$ T cells and enabled efficient tumor growth inhibition with prolonged survival in preclinical animal studies (Figure 5F). The tumor-free mice also showed robust immunity against newly inoculated cancer cells, indicating the generation of immune memory response.

Radiotherapy is a widely used clinical regimen against solid tumors. Increasing evidence shows the therapeutic effect of radiotherapy largely depends on DC-mediated cross-priming of CD8$^+$ T cells through the type I interferon pathways.[46] We investigated the synergy of STING-activating nanovaccine combined with local ionizing radiation of primary tumors.[47] PC7A nanovaccine initiated a systemic cancer-specific T cell response, while radiation offered local STING stimulation for enhanced T cell infiltration into tumors. The combination regimen showed a synergistic effect in treating both primary and distal tumors (Figure 5G).

TARGETING ACIDIC TUMOR MICROENVIRONMENT TO OVERCOME IMMUNE SUPPRESSION

Cancer cells rewire their metabolism and generate an immunosuppressive tumor microenvironment (TME) to support cell survival, proliferation, and metastasis.[48] A common feature of this deregulated energy metabolism is upregulated glucose uptake and aerobic glycolysis, also known as the Warburg effect.[49] This leads to the rapid production and exportation of lactic acids into the tumor microenvironment. Accumulation of lactic acids results in decrease in extracellular

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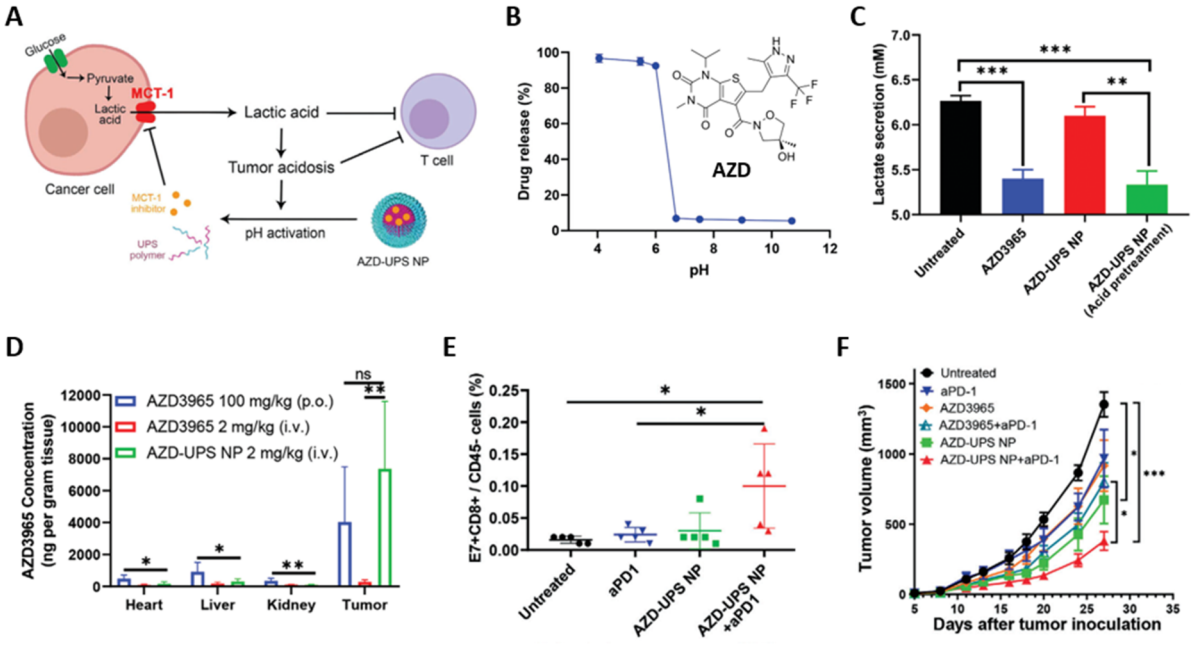

Figure 6. AZD-UPS nanoparticles target and reprogram the tumor microenvironment for improved checkpoint therapy. (A) Schematic of tumor-targeted delivery of AZD by UPS NPs and the subsequent inhibition of tumor acidosis for enhanced T cell immunity. (B) AZD-UPS NPs display binary drug release profile across the micelle transition pH (6.1) of the polymer. (C) AZD-UPS NPs reduce lactate secretion after acid pretreatment *in vitro*. (D) UPS delivery of AZD allows higher tumor accumulation and lower distribution in pivotal organs at 50-fold lower dose compared to oral administration of the free drug. (E) Combination of AZD-UPS NPs and anti-PD-1 increases tumor infiltration and antigen specific $CD8^+$ T cells. (F) AZD-UPS NPs synergize with anti-PD-1 in immune protection against TC-1 tumors. Reproduced with permission from ref 3. Copyright 2020 Wiley.

pH and retardation of a variety of immune cells against solid tumors.[16,50] Therefore, agents to reverse this metabolic process have the potential to normalize the tumor microenvironment toward immune elimination of tumors.

AZD3965 is a small molecule drug developed by AstraZeneca that reduces lactic acid export from cancer cells by inhibiting monocarboxylate transporter 1 (MCT-1). Clinical evaluation of an oral formulation of AZD3965 shows dose-limiting toxicities, particularly in the heart and eye tissues.[51] UPS nanoparticles are used to target tumor acidic pH for drug release and dose accumulation (Figures 2C and 3A). Furthermore, delivery of AZD drug by UPS nanoparticles offers a negative feedback strategy to selectively deliver MCT1 inhibitors to cancer cells with high metabolic activity and acidosis, achieving "on-demand" delivery with expanded therapeutic window (Figure 6A).[3]

A thermodynamically stable nanodrug with 3% drug loading was prepared by a microfluidic-based method. AZD molecules were encapsulated in the micelles with negligible release at pH 7.4 (mimicking blood pH) and underwent a rapid release below pH 6.1 within minutes (Figures 2C and 6B). This pH-triggered drug release profile corresponds to the phase transition point of the UPS (PDPA) polymer at pH 6.1, the apparent pK_a of the specific polymer. AZD−UPS nanodrug reduced lactate secretion after pretreatment with low-pH buffers and generated minimal effect in the physiological pH environment (Figure 6C). In addition, UPS nanoparticles improved the pharmacokinetics and biodistribution of AZD drugs *in vivo*. Animal studies demonstrated that the nanoparticles achieved higher accumulation in tumors even at a 50-fold lower dose than the free drug (Figure 6D). Immune profiling of tumor samples revealed significantly increased infiltration of antigen-specific $CD8^+$ T cells (Figure 6E) and down-regulation of exhaustive biomarkers ($PD1^+Tim3^+$) on these T cells. Antitumor studies in preclinical tumor models showed that AZD−UPS nanodrug significantly augmented the therapeutic efficacy of immune checkpoint therapy by anti-PD-1 (Figure 6F). More importantly, the nanoparticles effectively reduced the nonspecific distribution in healthy organs (e.g., heart) and abrogated the off-target toxicity (e.g., normal cardiac troponin levels) compared to oral formulation. Taken together, these studies demonstrate that AZD−UPS nanodrug offers a safe and effective strategy to reprogram the tumor suppressive environment for improved cancer immunotherapy.

CHALLENGES OF UPS NANOPARTICLES FOR CLINICAL DEVELOPMENT

Despite the therapeutic promise, we foresee several potential challenges in the development of UPS nanotherapeutics for cancer immunotherapy. First, intravenous injection of UPS nanoparticles still leads to a majority of the dose accumulating in the liver.[29] The nonspecific uptake limits drug selection to candidates that do not activate liver-specific immune pathways. This is exemplified by the choice of the MCT-1 inhibitor for intravenous nanodrug development, where lactic acid exportation is known to suppress T cell activity in tumors but not in liver tissues. Preclinical study shows that AZD−UPS nanodrugs did not lead to elevated liver enzyme secretion or immune-related toxicity.[3] On the other hand, broad immune activating drugs such as IL-2 or STING agonists may not be suitable for intravenous UPS therapeutic development. For these agents, alternative routes of administration may be necessary. A comprehensive understanding of the biodistribution of UPS nanoparticles from different modes of administration and their effects on the immune system is necessary to design the optimal dose, schedule, and administrative route.

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Acc. Chem. Res. 2020, 53, 2546−2557

Second, current UPS polymers are synthesized from nondegradable poly(methyl methacrylate) backbones. For imaging applications such as ONM-100 where the payload (i.e., ICG dye) is nontoxic and a single injection is administered, the safety profile of the polymeric drug is favorable.[30] For therapeutic applications where repeated injections are necessary, a biodegradable UPS polymer may be desirable to avoid persistent immune activation and autoimmunity. Lastly, chemistry, manufacturing, and control (CMC) processes will be more challenging for UPS as well as other nanotherapeutics compared to small molecule and antibody-based immune drugs. Polymer-related complexity such as molecule weight distribution, polydispersity of nanoparticles, and reproducibility of formulations will add to the production and regulatory costs and present additional barriers for clinical translation. These challenges are not insurmountable but will take expertise beyond academic laboratories to overcome.

SUMMARY AND FUTURE PERSPECTIVES

Immunotherapy has transformed cancer care with curable outcomes in a subset of patient populations. Despite the early success, the overall response rate remains low for the majority of patients, especially in late stage cancer patients. For any immunotherapeutic, achieving a balance between antitumor efficacy and autoimmunity is challenging because of the complexity and regulatory entanglements between the tumor and immune systems. The need to continually program the antitumor immune response to an evolving cancer requires an ability to exert multiple levels of control over the immune system. This often demands spatiotemporal orchestration of the specific type and density of immune cells as well as coordination of proinflammatory pathways and factors to overcome tumor immune evasion.

Nano-immune-engineering brings together two diverse fields in nanotechnology and immunology to intercept and reprogram the cancer-immunity cycle toward immune elimination of tumors (Figure 1). First, unlike conventional single agent therapy, nanoparticles are modular in nature, consisting of multiple components in self-assembly. Distinct classes of molecules (e.g., tumor antigen and immune modulators) can be co-delivered simultaneously, exerting therapeutic synergy over a single agent alone. This is exemplified in the STING-activating nanoparticle vaccine, which coordinates cytosolic delivery of tumor antigens to lymph node-resident DCs with STING-type I interferon activation, triggering robust cancer-specific T cell immunity with minimal systemic cytokine expression.[2,47] Second, nanoparticles can be tailored with unique physical properties including size, charge, and surface chemistry, allowing specific tissue or tumor targeting capabilities. A recent example of controlling surface hydrophobicity and charge properties to achieve selective organ targeting (SORT) nanoparticles opens up opportunities for tissue-specific delivery of therapeutic payloads including gene editing.[52] Finally, nanoparticles often display cooperative behaviors absent in unimolecular sensors or drugs. As a whole, the system acquires emergent properties that are different from adding the functions of individual parts in isolation. For example, UPS nanoparticles depart from a gradual pH response to a binary on/off phenotype due to nanoscale phase separation. The resulting pH threshold response improves the precision of tumor imaging (Figure 3) and drug delivery (Figure 6).

Moving forward, we anticipate several opportunities in nano-immune-engineering that might add value to the existing efforts in cancer immunotherapy. First, integrated theranostic nanoplatforms that synergize diagnostic imaging with therapeutic response will be valuable to simultaneously probe and perturb the cancer-immunity cycle.[53,54] Judicious incorporation of imaging modalities that can predict and monitor therapeutic outcomes may be particularly useful to "co-evolve" therapeutics in the dynamic crosstalk between the tumor and immune systems. Second, bioinformatic, network-based analysis has greatly impacted our understanding of tumor immune physiology[55,56] and further generated unprecedented insights on the intrinsic tumor immune resistant pathways at the molecular, cellular, and microenvironmental levels.[57] These large scale computations will be valuable to provide a holistic understanding on how spatiotemporally orchestrated perturbations by nanotherapeutics will impact tumor growth and immune evasion, which can accelerate clinical development. Finally, creation of nanotechnology−immunology hybrids with synergistic cross plays may overcome the deficiency of each modality alone. Recent work on an amphiphile-ligand vaccine to boost CAR T cells with broad cancer specificity[58] and CD47-encoded nanoconstructs to evade liver and spleen[59] are great examples of integrated therapeutics. Cooperativity and feedback controls (either positive or negative) are fundamental organization principles of biology to achieve signal over noise and robustness in action. Nanoimmunology approaches that incorporate nature's design with engineering ingenuity have great potential to improve the safety and efficacy of cancer immunotherapy.

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J.G. initiated the outline. S.L. wrote the first draft. Z.T.B., B.D.S., and J.G. revised the Account. The authors give approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest(s): B.D.S. and J.G. are founders and scientific advisors for OncoNano Medicine, Inc.

https://dx.doi.org/10.1021/acs.accounts.0c00475
Acc. Chem. Res. 2020, 53, 2546−2557

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■ ACKNOWLEDGMENTS

We appreciate the funding support from the National Institutes of Health (U54CA244719, R01CA216839, U01CA218422). The lab is also supported by the Mendelson-Young Endowment in Cancer Therapeutics.

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