



# Nanotechnology-enhanced immunotherapy for metastatic cancer

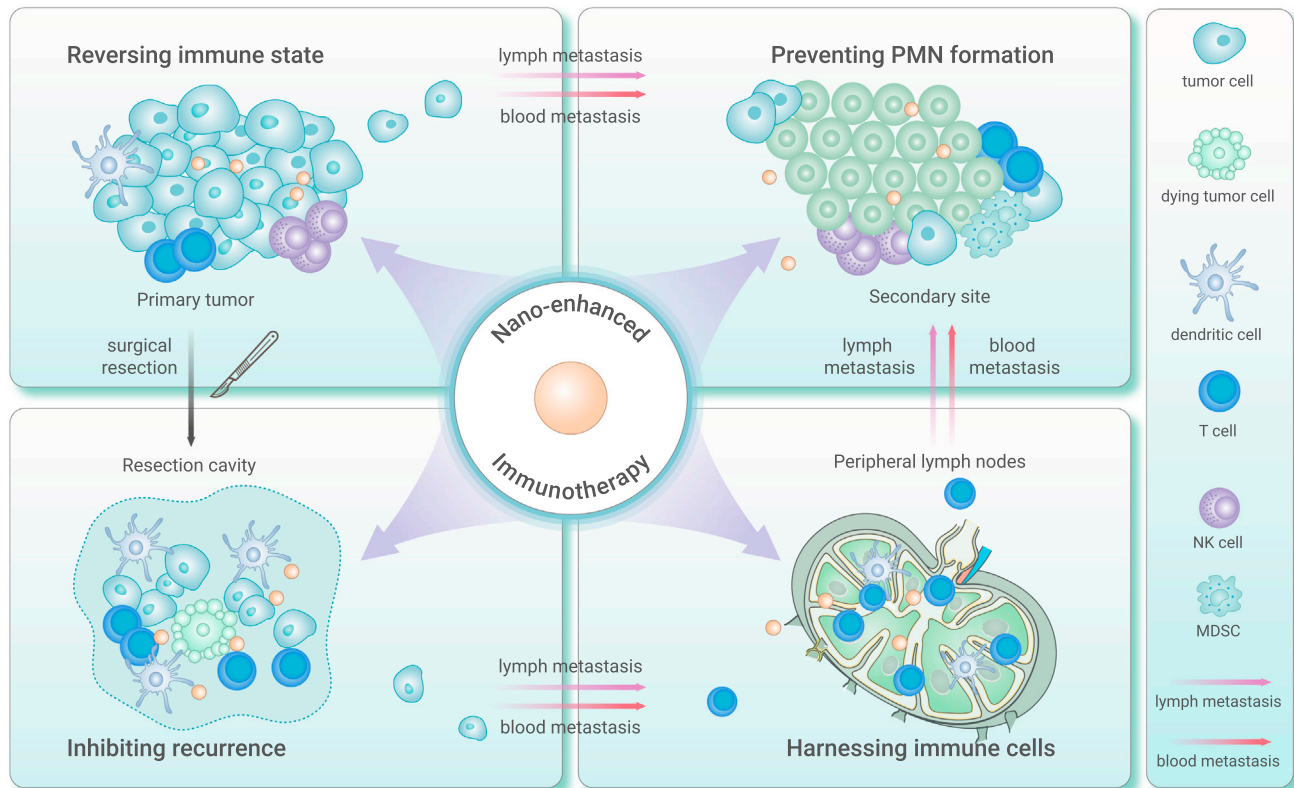
Peisen Zhang,<sup>1</sup> Junli Meng,<sup>1</sup> Yingying Li,<sup>1</sup> Chen Yang,<sup>1</sup> Yi Hou,<sup>2</sup> Wen Tang,<sup>3</sup> Kevin J. McHugh,<sup>4,\*</sup> and Lihong Jing<sup>1,\*</sup>

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## Graphical abstract



## Public summary

- The state of the art for nanotechnology-enabled cancer immunotherapy and the emerging concepts in nano-based immunomodulation are summarized
- The cutting-edge trends in nano-immunoengineering for metastatic cancers with an emphasis on different nano-immunotherapeutic strategies are highlighted
- Benefits, challenges, and opportunities of nanoscale immunomodulators and a forward-looking perspective on the innovative nanotechnology-based tools that may ultimately prove effective at eradicating metastatic diseases are presented



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**A vast majority of cancer deaths occur as a result of metastasis. Unfortunately, effective treatments for metastases are currently lacking due to the difficulty of selectively targeting these small, delocalized tumors distributed across a variety of organs. However, nanotechnology holds tremendous promise for improving immunotherapeutic outcomes in patients with metastatic cancer. In contrast to conventional cancer immunotherapies, rationally designed nanomaterials can trigger specific tumoricidal effects, thereby improving immune cell access to major sites of metastasis such as bone, lungs, and lymph nodes, optimizing antigen presentation, and inducing a persistent immune response. This paper reviews the cutting-edge trends in nano-immunoengineering for metastatic cancers with an emphasis on different nano-immunotherapeutic strategies. Specifically, it discusses directly reversing the immunological status of the primary tumor, harnessing the potential of peripheral immune cells, preventing the formation of a pre-metastatic niche, and inhibiting the tumor recurrence through postoperative immunotherapy. Finally, we describe the challenges facing the integration of nanoscale immunomodulators and provide a forward-looking perspective on the innovative nanotechnology-based tools that may ultimately prove effective at eradicating metastatic diseases.**

**Keywords:** metastatic cancer; nanomaterials; immunotherapy; tumor microenvironments; immunomodulators

## INTRODUCTION

Despite substantial efforts to develop superior therapies for malignant tumors, cancer remains the second leading cause of death worldwide. Eliminating distal tumors is particularly challenging, leading to high rates of recurrence.<sup>1–6</sup> Although surgical intervention, radiation therapy, and chemotherapy are frequently effective at prolonging the survival of patients with localized tumors, the prognosis for non-localized tumors is often poor. As a result, cancers that have spread to distal organs account for over 90% of cancer-related deaths.<sup>7</sup> Two of the biggest challenges in clinical cancer care are preventing metastasis from spreading in patients treated for localized disease and avoiding recurrence.<sup>8</sup> To overcome these challenges, we must develop better methods to kill tumor cells both broadly and completely.<sup>9,10</sup>

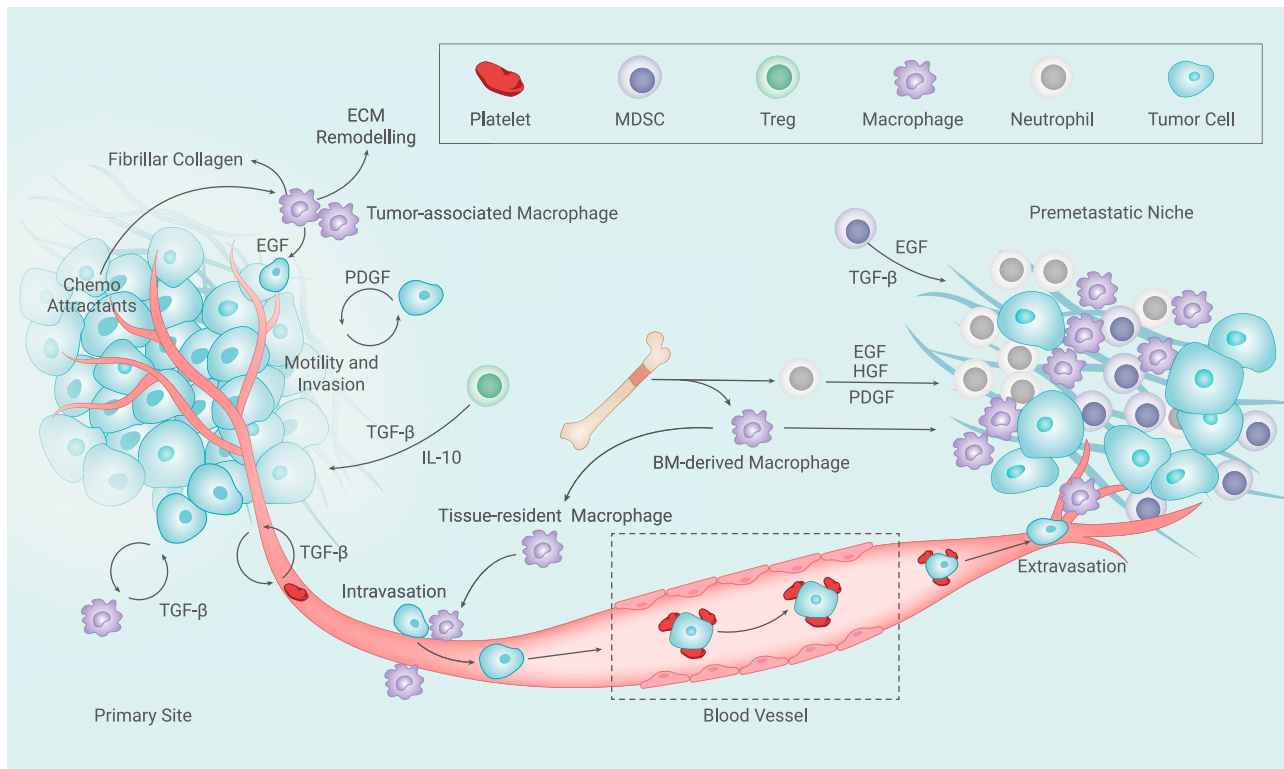
Unfortunately, the molecular and cellular mechanisms regulating metastasis remain elusive. It has been recognized that metastasis involves a complex interplay between cancer cells, immune cells, and stromal cells at both the primary tumor site and sites of metastases.<sup>11</sup> From an immunological perspective, immune evasion is the pivotal step in tumor progression, enabling tumor cells to escape typical immune surveillance using a diverse set of strategies.

In particular, intratumoral inflammation and the infiltration of several host immune cell types, mainly including tumor-associated macrophages (TAMs),

regulatory T cells (Tregs), and myeloid-derived suppressor cells (MDSCs), together with various cytokines and growth factors secreted by cancer cells or immune cells, are known to promote tumor growth and metastasis. These immune characteristics assimilated by tumors jointly promote the dissemination and colonization of tumor cells from the primary site to the pre-metastatic niche (PMN), as shown in Figure 1.<sup>12–15</sup> Accordingly, with a focus on counteracting the harmful immunomodulatory effects that solid tumors exert, immunotherapy for solid tumors aims to stimulate the innate immune system to generate systemic antitumor immunity that eliminates the primary tumor, destroys distant metastases, and prevents recurrence.<sup>16–21</sup> This therapeutic paradigm has experienced great success in the clinic. In particular, chimeric antigen receptor (CAR) T cell therapies<sup>22,23</sup> and immune checkpoint blockade, which blocks immune-inhibitory receptors, such as programmed cell death protein 1 (PD-1) or cytotoxic T lymphocyte antigen-4 (CTLA-4),<sup>24–26</sup> have been the most widely used. However, current immunotherapies have not been effective for all patients or all types of cancer due, in large part, to insufficient immune responses, especially when immunologically “cold” solid tumors create an immunosuppressive microenvironment that prevents the host immune system from attacking cancer cells.<sup>27,28</sup>

Among various attempts to enhance the immune response resulting from cancer immunotherapies, nanomaterials with unique immunogenicity and multiple immunomodulatory functions have achieved preliminary success.<sup>29–39</sup> With the potential to trigger different immune pathways, nanomaterials can be used to explore more aspects of tumor immunology, offering insights on how the immune system recognizes specific markers on tumor cells and on how immune cells interact with one another.<sup>40–50</sup> Compared with low-molecular-weight immunomodulators, nanoscale immunomodulators exhibit controllable pharmacokinetic behavior and the potential for enhanced immune activation through synergistic effects due to their unique size effects and co-loading capabilities resulting from the presence of multiple functional domains, which may be able to overcome barriers to effective immunotherapy for solid tumors.<sup>29,30,51</sup> A diverse set of nanomaterial-assisted tumor treatment strategies have been developed to amplify the benefits of cancer immunotherapy, which have not only boosted the response at the primary tumor site at the time of treatment but also elicited systemic and prolonged protective effects that prevent tumor metastasis and recurrence.<sup>3,34,52,53</sup> These successes signal the potential for the expanded use of nanotechnology in tumor treatment, especially for refractory and recurrent cancers.

In this review, we discuss the state-of-art and future prospects for nanomaterial-assisted immunotherapy in inhibiting tumor metastasis and recurrence, including changing immune activity within the primary tumor, activating the peripheral immune system, interrupting the PMN, and inhibiting recurrence *in situ* after surgical resection (Figure 2). The overarching goal of this review is to convey a comprehensive understanding of the latest achievements in nano-enabled cancer immunotherapy to deal with tumor



**Figure 1. The microenvironment promotes metastasis** Host immune cells, together with various cytokines and growth factors secreted by tumor cells, immune cells, and stromal cells, support the dissemination and colonization of tumor cells in the pre-metastatic niche. Note: this figure is modified from Quail and Joyce.<sup>15</sup>

metastasis and recurrence, which shows great promise for improving cancer survival.

### REVERSING THE IMMUNE STATUS OF THE PRIMARY TUMOR

Tumor development is a process that occurs due to a defect in host immunosurveillance caused by a series of cancerous escape mechanisms. In primary solid tumors, the immunogenicity of cancer cell neoantigens is too weak to effectively stimulate the immune response due to the immunosuppressed state of the tumor microenvironment.<sup>54–57</sup> Nanomaterials provide a new way to overcome this barrier to therapeutic efficacy. The accumulation of customized nanomaterials in primary tumors has been shown to alleviate the immunosuppressive effect by neutralizing the stealth properties of cancer cells, thereby enabling the immune system to recognize tumor neoantigens and kill tumor cells.<sup>30</sup> Furthermore, the activated immune system may also be able to continue to track and suppress the formation and growth of distant metastases.

### Remodeling the immunosuppressed tumor microenvironment

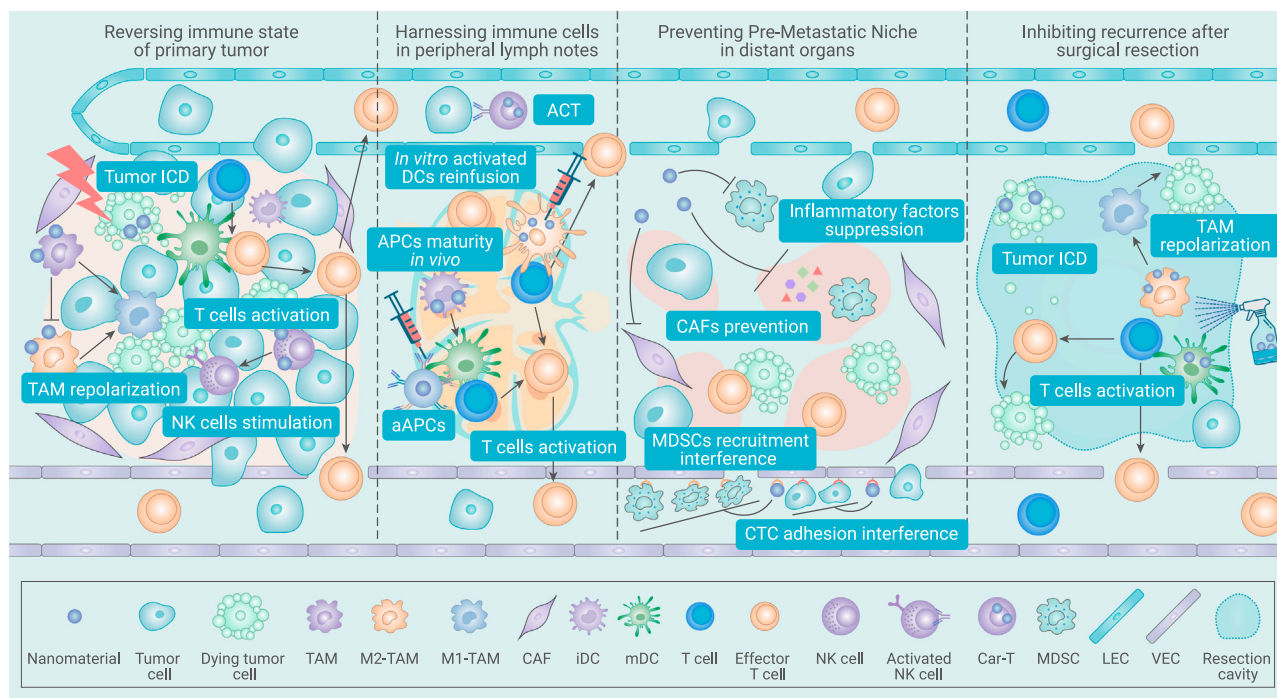
The characteristics of the tumor microenvironment play a major role in determining the success of cancer immunotherapies.<sup>58,59</sup> It is known that solid tumors can progressively develop several specific pathways to evade immune clearance, including through a microenvironmental remodeling process known as “cancer immunoeediting”. This process imposes selective pressures within the tumor microenvironment, promoting the aggravation of cancer. Within this immunoeedited environment, a variety of immune and non-immune cell types cause long-term inflammation and localized immune suppression, enabling malignant cells to split and mutate without detection and elimination by the host’s immune system.<sup>60,61</sup> Specifically, the intrinsic hyporesponsiveness or anergy of cytotoxic T lymphocytes (CTLs) impairs their ability to kill tumor cells while extrinsic immunosuppressive cell populations, such as Tregs and TAMs, simultaneously weaken the immune response. In addition, various inhibitory ligands, such as PD-L1, further depress immune function. Transforming growth factor  $\beta$  (TGF- $\beta$ )

can promote immature T cells to differentiate into Tregs and facilitate the spreading of tumor cells in advanced stages of cancer. Besides, the nutrient-catabolizing enzymes, such as indoleamine 2,3-dioxygenase (IDO) and stimulator of interferon genes (STING) agonist, interfere with the proliferation of effect T cells and help tumor evasion.

To break this rigid condition, nano-immunomodulators have been designed to directly target the immunosuppressive microenvironment, which can re-open the immune system *in situ* and inhibit tumor growth. Based on the migratory capacity of re-activated immune cells, the primary microenvironment-targeting nano-immunomodulators are also expected to prevent metastasis.

Macrophages represent one of the most abundant populations of immune cells in the tumor microenvironment, accounting for nearly half of tumor mass.<sup>40,62–65</sup> Although M1 macrophages can inhibit the growth of tumor cells by secreting NO and other cytotoxic factors, TAMs are mainly, unfortunately, M2 phenotype, which can promote tumorigenesis and malignancy.<sup>44,63,64,66–70</sup> Accordingly, repolarizing TAMs from M2 to M1 has been one of immunotherapeutic strategy. Very recently, Chen and coworkers constructed a programmable cellular vesicle to combat tumor recurrence and metastasis after surgery. The hybrid cell membrane nanovesicles (hNVs) can interact with circulating tumor cells (CTCs) in vascular lumens and accumulate at the resection site where they block the CD47-SIRP $\alpha$  interactions and repolarize TAMs from M2 to M1 to kill cancer cells. These nanovesicles can also promote the lethality of T cells against malignant cells through antigen presentation, significantly improving the survival rate of mice in a malignant melanoma model by reducing both locoregional relapse and distal spreading after surgery. In addition, when combined with a STING agonist, cGAMP-loaded hNVs successfully inhibited relapse after surgery in a poorly immunogenic triple-negative breast cancer model (Figure 3).<sup>71</sup>

In addition to TAMs, MDSCs are also involved in generating an immunosuppressive microenvironment. These cells can disable the immune function of T cells and natural killer (NK) cells. To reverse these effects, Shuai and coworkers designed a nanoregulator with MnO<sub>2</sub> particles and PI3K $\gamma$



**Figure 2. Overview of this article** Nano-enabled immunotherapy for inhibiting tumor metastasis and recurrence, including changing immune activity within the primary tumor, activating the peripheral immune system, interrupting the pre-metastasis niche, and conferring immunity to tumor cells to inhibit recurrence after surgery.

inhibitor IPI549 to alleviate hypoxia while downregulating the expression level of immunosuppressive PD-L1 molecules. In parallel, the nanoregulator can activate MDSCs to accelerate the polarization of TAMs toward the M1 phenotype and re-activated cytotoxic T cells to halt the proliferation of tumor cells, which further delayed recurrence and reduced the opportunity of CTCs to take root in the liver and lung due to the enhanced generation of cancer-suppressive memory T cells.<sup>72</sup> Chen et al. reported an anti-inflammatory nanofiber hydrogel through the self-assembly of steroid drugs for the local long-term delivery of anti-PD-L1, which inhibited both primary and distal tumors by activated T cells.<sup>73</sup>

Apart from influencing TAMs and MDSCs, NK cell activation is another strategy being explored to alleviate the immunosuppressive microenvironment. The immune function of NK cells as a specialized innate lymphoid cell population is independent of major histocompatibility complex (MHC)-mediated antigen presentation.<sup>74–77</sup> On the one hand, they can exert a natural cytotoxic function to fight both primary cancer cells and metastases by preventing cell proliferation, migration, and metastatic colonization.<sup>78,79</sup> On the other hand, NK cells can secrete a large number of cytokines to regulate the immune response and participate in a variety of downstream immune pathways, such as tumor necrosis factor alpha (TNF- $\alpha$ ), interferon- $\gamma$  (IFN- $\gamma$ ), and various interleukins.<sup>80,81</sup> Most importantly, compared with other immune cells, NK cells can differentiate abnormal cells from healthy cells and thus produce more specific antitumor cytotoxicity, which has been reported in many tumor models and experiments.<sup>75</sup> Therefore, NK cells have been recognized as an appealing target for cancer immunotherapy.<sup>82–84</sup>

Nano-assisted immunotherapy using several cytokines, such as interleukin-2 (IL-2) and IL-12, has been explored to prevent the progression and metastasis upon the activation of NK cells.<sup>85,86</sup> Irvine and coworkers developed a combination therapy system with agonistic anti-CD137 and IL-2 on the surface of PEGylated liposomes.<sup>35</sup> Through this liposome-based therapy, immune stimulators can quickly accumulate in tumors, thereby inducing the potent activation of NK cells and T cells, which in turn suppresses tumor progression in both the primary tumor site and metastatic lesions in the lungs in mice. Similar approaches of nanomaterials-assisted NK cell activation by cytokines have been extensively developed to treat metastatic cancers.<sup>87–89</sup> Apart from the cytokine-triggered NK cell activation,

some engineered nanomaterials can also activate NK cells through their intrinsic properties, which can increase the efficiency and reduce the costs of NK cell activation.<sup>90,91</sup> Apart from the direct activation of NK cells, nanoparticles can be also designed to improve the accumulation of NK cells in tumor sites to increase the therapeutic effect.<sup>92</sup>

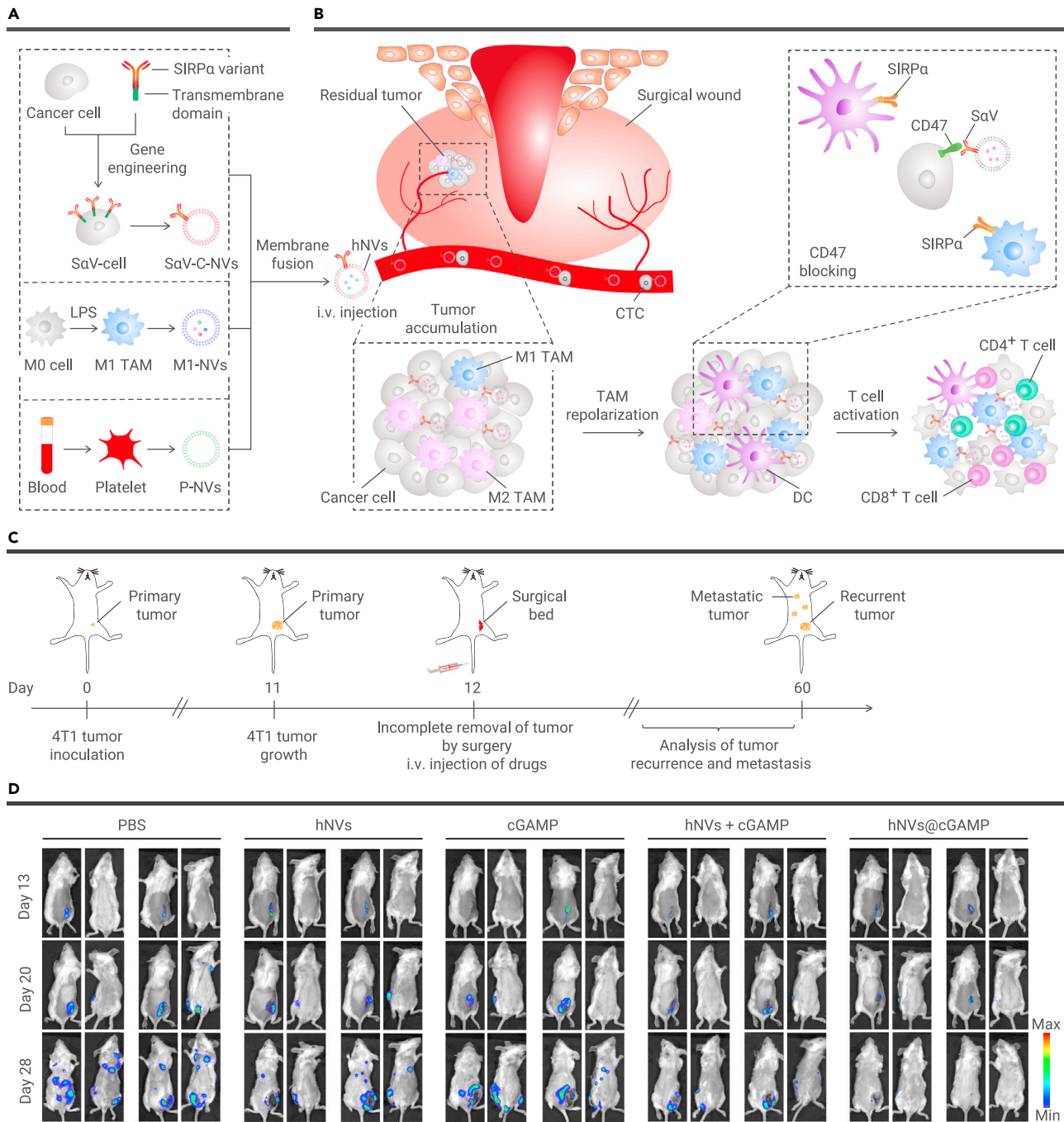
In addition to eliminating tumor cells through utilizing the exogenous nanomaterials to promote host NK cells, more sophisticated nanoparticles that can mimic the biological nature of NK have been also developed.<sup>93–96</sup> Aryal and coworkers designed a nanomaterial based on an NK cell membrane camouflage-fusion liposome, abbreviated as NKsome, which was used for targeted tumor immunotherapy to inhibit metastasis.<sup>97</sup> In comparison with the conventional nanomaterials, the biomimetic strategy significantly decreases the toxicity and thereby improves the safety of nano-assisted immunotherapy.

Overall, remodeling the immune status of the tumor microenvironment provides a feasible approach for locoregional cancer immunotherapy by alleviating the immunosuppressive effects on tumor-associated immune cells. More importantly, this strategy has the great potential to prevent distant tumor metastasis and recurrence *via* a systemic anticancer immune response.

### Activating immune cells through ICD

In recent years, research has revealed that conventional local therapeutic approaches, including locoregional thermal therapy, radiotherapy, or chemotherapy, not only destroy the primary tumor cells but induce the tumor immunogenic cell death (ICD).<sup>98,99</sup> As opposed to the traditional apoptosis, ICD involves a series of special stresses before cell death that can change the state of tumor cells from non-immunogenic to immunogenic. For example, calreticulin will be exposed on the surface of tumor cells, which can specifically bind with the CD91 receptor on dendritic cells (DCs) and promote the uptake of antigens. ATP released as a soluble mediator helps recruit monocytes and activates DCs. The release of high-mobility group box 1, which combines with antigen presenting cells (APCs) through various receptors, such as TLR 4, and facilitates antigen processing and presentation.<sup>99</sup> These molecular events consequently activate the antitumor immune responses, even exhibiting the ability to kill distant tumor cells out of the direct reach of treatment,<sup>100</sup> as shown in Figure 4. However, the immune responses stimulated



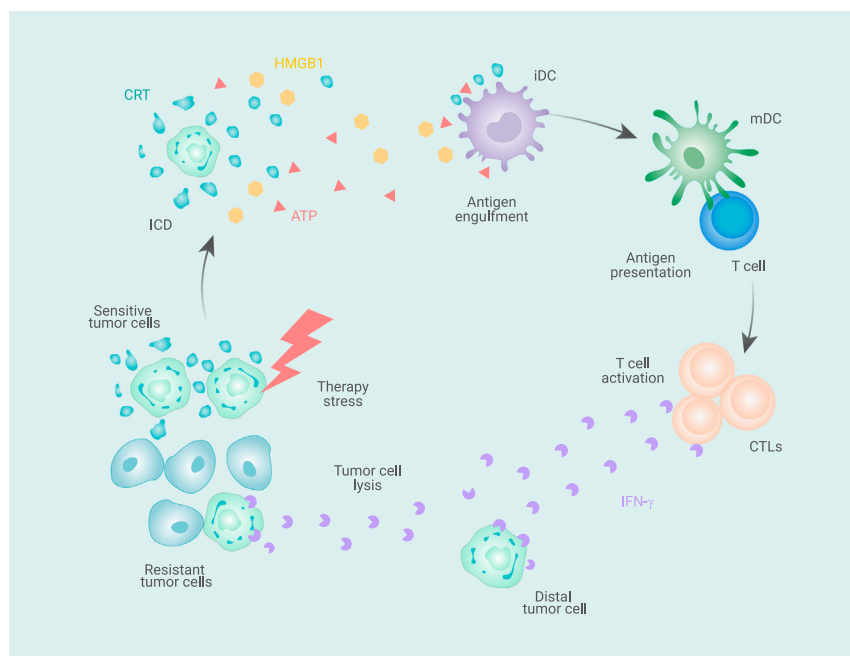


**Figure 3. Hybrid cellular membrane nanovesicles amplify macrophage immune responses against cancer recurrence and metastasis** (A) Schematic displaying hNVs consisting of engineered SaV-C-NVs, M1-NVs, and P-NVs. (B) Schematic displaying the interaction between hNVs and CTCs in the blood, accumulation in the postsurgical tumor bed, repolarization of TAMs toward an M1 phenotype, and blockage of the CD47-SIRP $\alpha$  “don’t eat me” pathway, which promotes the phagocytosis of cancer cells by macrophages and boosts antitumor T cell immunity. (C) Schematic displaying the treatment implemented in a mouse model of cancer recurrence after incomplete resection. (D) *In vivo* bioluminescence imaging of B16F10 tumor recurrence in different treatment groups. Reproduced with permission from Rao et al.<sup>71</sup> Copyright 2020 Nature Publishing Group.

by ICD are not usually strong enough to evoke a potent systemic effect in metastases or prevent tumor recurrence. Thus, nanomaterials have been designed to amplify the immune responses to conventional cancer therapeutics.<sup>32</sup>

Locoregional thermal treatment is the most commonly used approach to induce ICD.<sup>101,102</sup> For example, an artificial enzyme, i.e., Cu<sub>2-x</sub>Te that could resemble the similar catalysis function as glutathione oxidase (GSHO<sub>x</sub>) and peroxidase, and were used to catalyze immunotherapy. Owing to the NIR-II photothermal effect, the Cu<sub>2-x</sub>Te artificial enzyme catalyzed the cascade of

reactions that continuously elevated intratumoral oxidative stress and induced ICD, thereby eradicating the primary tumor. More importantly, this artificial enzyme continuously reversed the immunosuppressive state of the tumor microenvironment to stimulate systematic antitumor immunity, combat distal metastasis, and prevent recurrence. Moreover, effector memory T cells were largely generated after treatment to suppress tumor relapse.<sup>103</sup> In another study, Wang and coworkers employed systemically administered, erythrocyte membrane-coated 2D polypyrrole nanosheets as an NIR-II photothermal transducer, which resulted in synergistic



**Figure 4. Tumor ICD** As a result of endoplasmic reticulum (ER) stress and autophagy, dying cancer cells respond to therapeutic stress by exposing calreticulin (CRT) on their outer membrane at the pre-apoptotic stage and releasing ATP and the nuclear protein high-mobility group box 1 (HMGB1) during apoptosis. CRT, ATP, and HMGB1 bind to the receptor of immature dendritic cells (iDC), which facilitates the recruitment of iDCs into the tumor bed (stimulated by ATP), the engulfment of tumor antigens by iDCs (stimulated by CRT), and antigen presentation to T cells (stimulated by HMGB1). Activated CTLs will secrete IFN- $\gamma$ , which eventually leads to the inhibition of therapy-resistant tumor cells and distal tumor cells that therapeutics have not directly reached.

photothermal and immunological responses, favorable for preventing metastasis and prolonged survival in mice.<sup>104</sup> In addition to photothermal therapy, magnetothermal therapy is another feasible approach to induce ICD. Liang and coworkers designed novel ferrimagnetic iron oxide nanorings with a vortex domain that showed the ability to mediate mild magnetic hyperthermia leading to calreticulin expression in 4T1 breast tumor cells and promoting phagocytic uptake of tumor cells by immune cells. This mild thermotherapy can elicit large increases in CTLs infiltration in distal tumors and trigger immunotherapeutic effects by sensitizing tumor cells to PD-L1 blockade.<sup>105</sup>

In addition to local thermal treatment, nano-assisted radiotherapy is another therapeutic strategy that can cause tumor ICD and potentially inhibit distal metastases. In fact, the abscopal effect of radiotherapy has already been observed in some clinical cases.<sup>106,107</sup> That is, radiotherapy cannot only kill the irradiated tumor cells but also the surrounding non-irradiated tumor cells, more formally known as the radiation-induced bystander effect (RIBE), which is triggered by the immune response to dead tumor cells.<sup>108</sup> However, this immune response may be insufficient to elicit an attack on distal metastases due to the immunosuppressive microenvironment in the primary tumor. Fortunately, the RIBE can be significantly amplified by the combination of nano-assisted immunotherapy. For example, Liu and coworkers developed a new radioisotope therapy through the combination of <sup>131</sup>I-Cat, a natural polysaccharide alginate, and synthetic cytosine phosphoguanosine (CpG). After intratumoral injection, the polysaccharide quickly formed hydrogels due to the presence of endogenous Ca<sup>2+</sup>, fixing <sup>131</sup>I-Cat at the tumor site, which enabled the complete elimination of the primary tumor with low-dose radiotherapy. Importantly, with the help of CpG, an immunostimulatory oligonucleotide, the systemic antitumor immune response was effectively triggered by the generation of tumor-associated antigens (TAAs) after locoregional radiotherapy of primary tumors, which successfully prevented metastasis and recurrence when combined with checkpoint blockade therapy.<sup>109</sup> Similarly, they designed core shell-structured nanoparticles based on poly(lactic-co-glycolic) acid (PLGA) shell encapsulating a water-soluble catalase that catalyzes the production of O<sub>2</sub> from H<sub>2</sub>O<sub>2</sub>. These nanoparticles significantly improved radiotherapy by mitigating hypoxia to provide a more hospitable environment to immune cells that can then detect TAAs that are present following radiotherapy-induced ICD. By loading Imiquimod (R837), an adjuvant, in the PLGA shell, a strong antitumor immune response was achieved, effectively inhibiting tumor metastasis and prolonging the survival of mice when combined with CTLA-4 (Figure 5).<sup>110</sup> In another

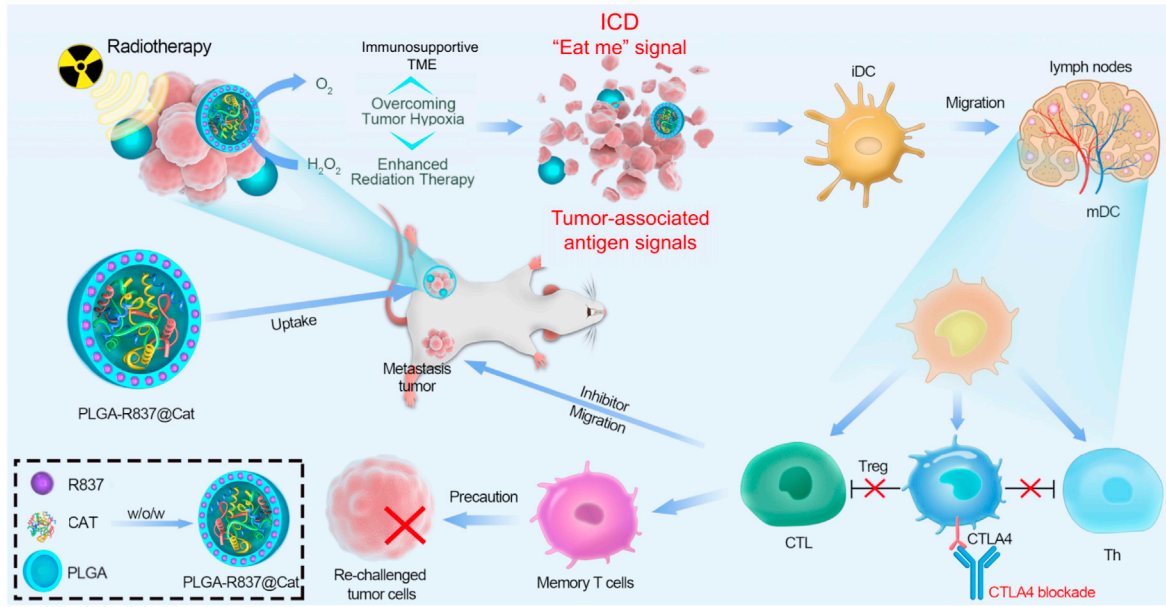
work, Lin and coworkers combined radiodynamic therapy with checkpoint blockade immunotherapy based on a nanoscale metal-organic framework. It cannot only kill primary tumor cells through X-rays but also trigger an abscopal effect to kill distal tumors in a mouse model of colorectal cancer.<sup>111</sup>

Several nano-assisted chemotherapeutic approaches can also induce a systemic immune response through the generation of TAAs when killing cancer cells, which can be employed to prevent metastasis when combined with immunotherapy. In previous studies, lots of conventional or clinically used chemotherapeutic drugs such as doxycycline (DOX), docetaxel, paclitaxel, and several mAb have been proven to elicit ICD process,<sup>112</sup> which has large potential to trigger a strong systemic immune response if combined with nanotechnology.<sup>113–115</sup> Recently, Chen and coworkers introduced a cocktail therapy including an extracellular matrix (ECM) destroyer as a component to improve immune check blockade (ICB) therapy and chemotherapy. This therapy strategy was realized by utilizing two different nanomaterial—DOX nanoparticles and nanoparticles containing plasmids encoding shPD-L1 and a hyaluronidase generator. These two nanoparticles could be stimulated by the acidic conditions at the tumor site. Through this nano-assisted cocktail immunotherapy, T cells were activated through DOX-triggered tumor ICD, while the immunosuppressive tumor microenvironment was remodeled to attain an immune-active phenotype. After treatment, the number of CD8<sup>+</sup> T cells was increased in the peripheral system, thus maintaining long-term immunological memory that inhibited cancer recurrence.<sup>116</sup> This cocktail strategy integrating multiple therapies may be an emerging trend because the antitumor immune response is a coherent process consisting of complex immunoreactions.

Interestingly, without the chemotherapeutic drugs, some nanomaterials can also achieve similar therapeutic effects owing to their intrinsic physical and chemical characteristics. For example, Zhang and coworkers developed phospholipid-coated Na<sub>2</sub>S<sub>2</sub>O<sub>8</sub> nanoparticles. Upon the decomposition triggered by the tumor microenvironment, these nanoparticles can produce toxic reactive oxygen species to kill cancer cells through inducing the ICD process and in turn trigger the systemic antitumor immunity, which can be potentially employed to inhibit tumor metastasis and recurrence.<sup>117</sup>

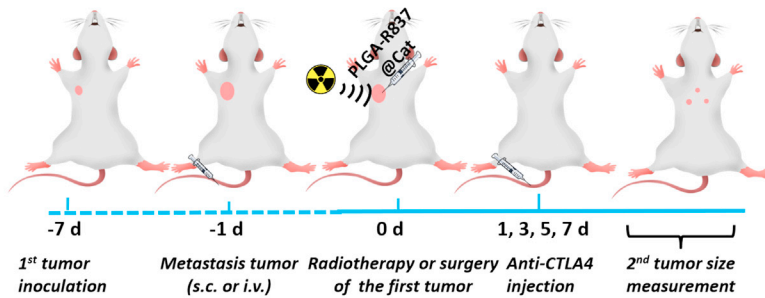
Nanomaterials can be designed to have the ability to not only remodel the immunosuppressive state in solid tumors but also directly kill tumors through ICD. Chen and Tao jointly synthesized triangular Te nanostars, known as GTe-RGD, which exhibited excellent radiotherapy-enhanced anti-PD-1 ICB therapeutic effects. In mouse breast cancer models, GTe-RGD nanostars not

A

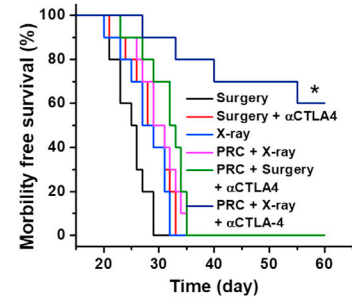


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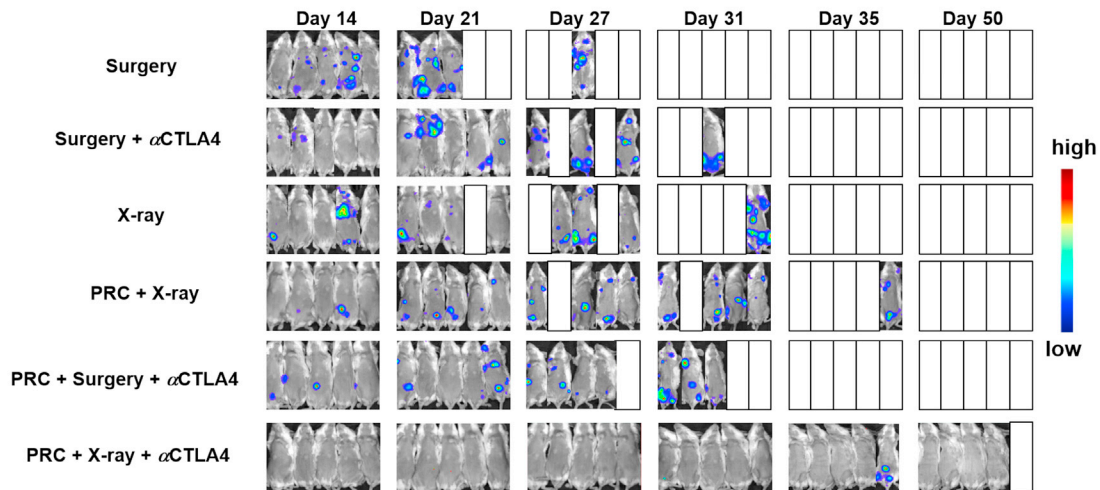
Orthotopic metastasis model



C



D



**Figure 5. Nanoparticle-enhanced radiotherapy to trigger robust cancer immunotherapy for metastasis prevention** (A) Antitumor immune response induced by PLGA-R837@Cat radiotherapy and checkpoint blockade to inhibit metastasis and recurrence. (B) Inhibition of tumor metastasis by radiotherapy with PLGA-R837@Cat plus αCTLA4 therapy in a 4T1 orthotopic breast tumor metastasis model. (C) Morbidity-free survival of different groups of mice with metastatic 4T1 tumors after various treatments. (\* P < 0.05). (D) *In vivo* bioluminescence images showing the spreading and growth of firefly luciferase-4T1 (fLuc-4T1) cancer cells in different groups of mice after eliminating their primary orthotopic tumors. Reproduced with permission from Chen et al.<sup>110</sup> Copyright 2019, Wiley-VCH.



only eliminated the *in situ* malignant tumor cells in combination with X-ray irradiation but also initiated a strong antitumor immune response by activating T cells with anti-PD-1, thereby successfully inhibiting the formation of distant tumors. This strategy improved the anticancer efficacy owing to the synergy between radiotherapy and nanomaterial-enhanced immunotherapy.<sup>118</sup>

These studies help to illustrate the enormous potential of combining tumor ICD with nano-assisted immunotherapy for the prevention of tumor growth and metastasis. In fact, this tumor-killing strategy is one of the most common paradigms exploited when combining immunotherapy with other antitumor approaches because it can overcome the weakness of individual immunotherapies. As a result, combination therapy presents a great opportunity for the clinical translation of nanomaterial-assisted immunotherapies.

## HARNESSING PERIPHERAL IMMUNE CELLS

Immune cells are located in peripheral immune organs, including lymph nodes, spleen, skin, and the vascular system.<sup>119</sup> Immune cells in peripheral immune organs often consist of mature lymphocytes, APCs, and monocytes, which play important roles in producing immune responses under the stimulation of foreign antigens.<sup>120</sup>

### Cancer vaccines targeting DCs

Among peripheral immune cells, DCs are the most potent APCs and can induce powerful antigen-specific CTL responses. Cancer vaccines based on DCs hold great potential for tumor prophylaxis and treatment,<sup>121–123</sup> which have been proven to effectively inhibit tumor metastasis and recurrence. Traditionally, the preparation of DC vaccines is a complicated process. DC precursors have to be isolated from patients, loaded with TAAs *in vitro*, and re-administered to the recipient. This technique has been used in the clinic to prevent metastasis of malignant tumors alone or in combination with other treatment methods. However, DC-based immunotherapy is still limited by an insufficient immune response, which makes it difficult to fully eradicate established solid tumors.<sup>124–127</sup>

Due to recent advances in nanotechnology, structures, such as liposomes, polymer nanoparticles, and inorganic nanoparticles, are able to be loaded with different components including small molecules, peptides, nucleic acids, and cell membranes. This enables the co-loading of antigen and adjuvant in nanovaccines, which ensures that these active ingredients are delivered simultaneously to the same APC. Furthermore, nanovaccines also prevent the rapid dissemination of components, such as antigen and adjuvant, into circulation and facilitate their efficient accumulation in draining lymph nodes.<sup>29,33</sup> Thus, vaccines based on nanoparticles may be valuable tools for augmenting the immune response and preventing tumor metastasis.<sup>33</sup> For example, Zhou et al. constructed an adjuvant/antigen co-delivery nanoplat-form by coating PLGA nanoparticles with phospholipid membranes. This nanovaccine can efficiently accumulate in lymph nodes and elicit an antigen-specific adaptive T cell response, which inhibited the metastasis of B16-OVA melanoma cells as demonstrated by the large reduction in the number of metastatic nodules.<sup>128</sup> In another example, an anti-metastatic nanovaccine was developed based on PLGA nanoparticles by encapsulating a novel TLR 7/8 bispecific agonists, denoted as 522NP. After intravenous administration, 522NPs entered the draining lymph node and activated DCs, which significantly enhanced subsequent CTL responses. The lung metastatic nodules in mice immunized with OVA+522NP decreased by approximately 75% more than control groups.<sup>50</sup>

Apart from classic polymer and liposome nanoplat-forms, inorganic nanoparticles are promising as vaccine delivery systems owing to their unique chemical and physical properties.<sup>129,130</sup> For example, Li et al. designed a nanovaccine with magnetic targeting capabilities by coating CpG-modified Fe<sub>3</sub>O<sub>4</sub> magnetic nanoclusters (MNCs) with anti-CD205-modified cancer cell membranes, denoted as A/M/C-MNCs (Figure 6).<sup>131</sup> This study took advantage of the superparamagnetism and magnetization of MNCs to achieve magnetic enrichment of vaccine in lymph nodes, which prolongs retention time to increase antigen captured by DCs. At the same time, the

camouflaged cancer cell membrane on the nanovaccine provided a reservoir of multiple neoantigens, enabling multi-antigenic immune responses. This combination of cell membrane-based nanovaccine with ICB may be a highly effective personalized antitumor therapy for inhibiting metastasis.<sup>131</sup> Luo et al. used Fe<sub>3</sub>O<sub>4</sub> nanoparticles as carriers for the antigen to inhibit metastasis of malignant melanoma. In this study, Fe<sub>3</sub>O<sub>4</sub> nanoparticles were able to serve as not only carriers but also immunopotentiators that synergistically stimulated DCs and activated macrophages (Figure 7).<sup>132</sup>

Due to the promising prospects of nanotechnology and immunotherapy for treating cancer metastasis, several novel and smart nanomaterials have been designed to enhance therapeutic efficacy, such as nanorobots. In 2018, Zhao and coworkers designed a DNA nanorobot to transport payloads and present them precisely in tumors through DNA origami. These nanorobots possessed the capability to serve as smart drug delivery systems that are responsive to molecular triggers and deliver thrombin precisely to the blood vessels of solid tumors, leading to intravascular thrombosis and consequently tumor necrosis.<sup>133</sup> The DNA origami scaffolds formed through complementary base pairing provided an elegant drug delivery platform to accurately control the number and the position of functional moieties and, in turn, impact both drug loading and stimulus-responsive behavior. Very recently, Ding and coworkers<sup>134</sup> developed a DNA-based cancer vaccine that was successfully transported to the tumor-draining lymph nodes and delivered tumor antigens to APCs to stimulate antitumor immune responses. The vaccine was designed by assembling an antigen peptide and two kinds of molecular adjuvants within a tubular DNA nanostructure. The pH-responsive DNA origami was unlocked within acidic endosomes, exposing the previously entrapped antigens and adjuvants that then bound to their receptors and promoted DC activation to initiate antigen presentation, thereby triggering T cell activation and cancer cell cytotoxicity. The strong, tumor-specific T cell immune response elicited by this DNA nanodevice vaccine subsequently led to the regression of tumors in mice and induced a long-term T cell immune memory response that strongly protected mice from tumor metastasis (Figure 8).<sup>134,135</sup>

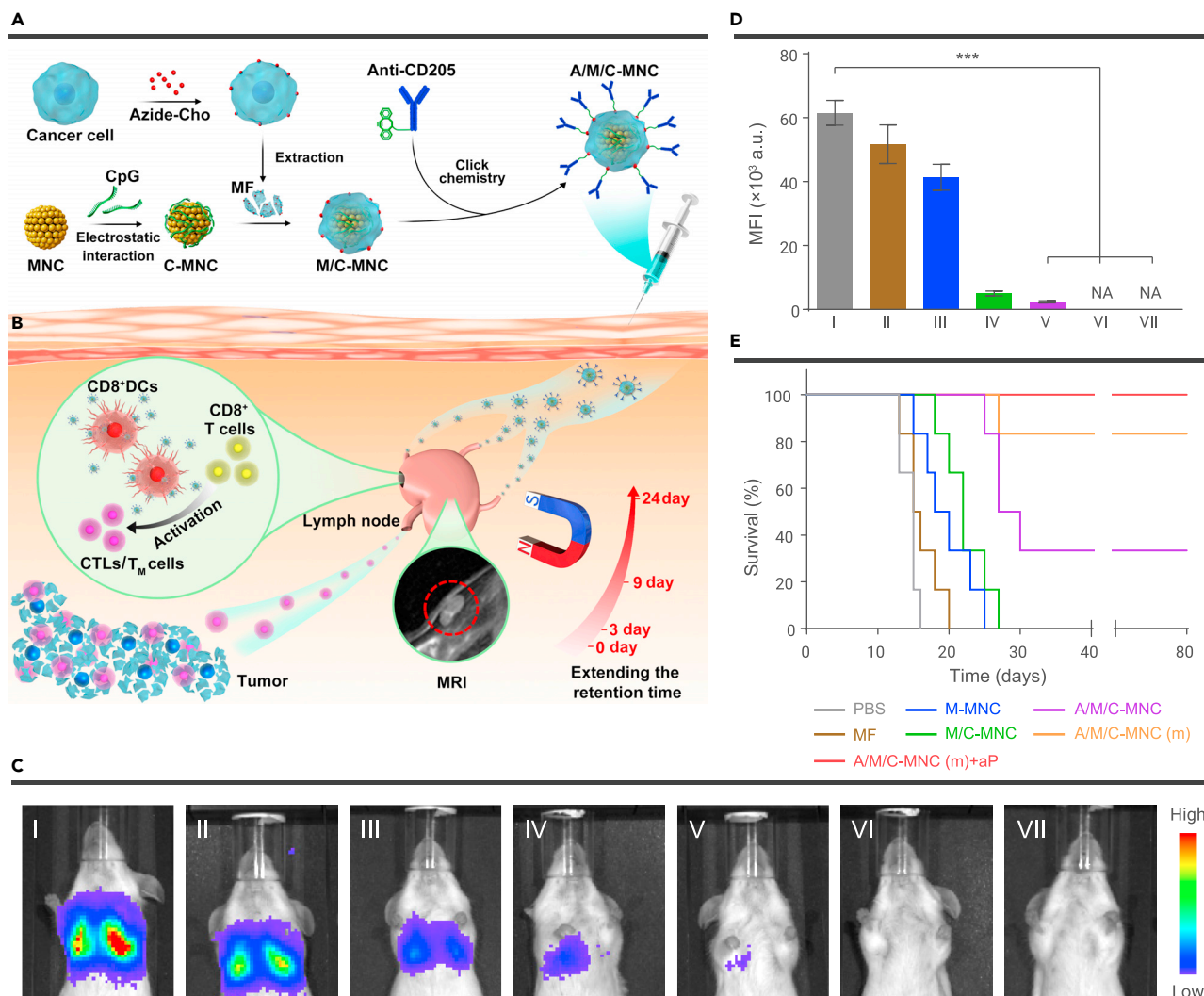
### Artificial immune cells mimics

Although autologous DC-based cancer vaccines were widely investigated by back-transfusing antigen-pulsed DCs into patients, this technology is costly and time-consuming. Micro- and nanomaterial-based artificial APCs (aAPCs) are designed to mimic the natural APCs by presenting important signal antigens to T cells and activate them for cancer inhibition.<sup>136,137</sup> To realize these antigen-presenting effects, aAPCs should include two parts on their surface: MHC-peptide complexes that can present cognate antigenic peptide to T cell receptors and co-stimulatory molecules that can bind to co-stimulatory receptors and activate T cells. Compared with natural DCs, aAPCs have a relatively defined composition and controllable biological behavior. Moreover, aAPCs can be applied for mass production, which could enable off-the-shelf vaccines.<sup>138</sup>

For example, Lu et al. developed an aAPC vaccine, in which microscale latex beads were coated with H-2Kb-Ig/pTRP2 dimeric complexes, anti-CD28 antibody, 4-1BB ligand, and CD83 molecules to expand CTLs from C57BL/6 splenocytes for adoptive cell transfer into a murine melanoma lung metastasis model. To assess the therapeutic efficacy against B16 pulmonary metastases, mice bearing B16 melanoma were intravenously injected with melanoma-specific CTLs stimulated by H-2Kb-Ig/pTRP2-aAPCs *ex vivo*. The results showed that H-2Kb-Ig/pTRP2-aAPC therapy achieved a significant reduction in pulmonary metastasis with only about 36 metastatic nodules in the lungs after treatment, whereas mice in the control groups developed approximately 330 pulmonary metastatic nodules.<sup>139</sup>

APC mimics are not the only immune cells that have been explored using nanomaterials. Neutrophils or Tregs, which also play crucial roles in the innate immune response to tumors, are two other types of immune cells that nanomaterials have been used to mimic with the goal of inhibiting tumor growth.<sup>140–142</sup> It can be expected that the novel biomimetic nanomaterials that can simulate more other types of tumor-related immune cells will be developed in future research.





**Figure 6. Engineering magnetosomes for high-performance cancer vaccination** (A) Fabrication process of A/M/C-MNC. (B) Schematic illustration of A/M/C-MNC-mediated cellular immune responses to elicit CTLs and memory T cells (TM cells) for cancer immunotherapy. (C) Bioluminescence images of hematogenous metastasis in lungs after intravenous inoculation of luciferase-expressing 4T1 (Luc-4T1) cells with different pretreatments: (I) PBS; (II) MF; (III) M-MNC; (IV) M/C-MNC; (V) A/M/C-MNC; (VI) A/M/C-MNC with magnetic retention; (VII) A/M/C-MNC with magnetic retention and anti-PD-1. (D) MFI statistics of lung metastasis in (C). (mean  $\pm$  SD,  $n = 6$ , \*\*\* $P < 0.01$ ) (E) Survival rate of mice in (C). Reproduced with permission from Li et al.<sup>131</sup> Copyright 2019, American Chemical Society.

### Adoptive cell therapy

Adoptive cell therapy (ACT) has emerged as a promising technology for the treatment of cancer. ACT is a highly personalized therapy involving the administration of immune cells with direct anticancer bioactivity to the tumor-bearing host.<sup>143–145</sup> In both clinical practice and laboratory studies, ACT employing naturally generated tumor-active lymphocytes mediates durable tumor elimination by targeting somatic mutations characteristic to an individual's cancer.<sup>146–148</sup>

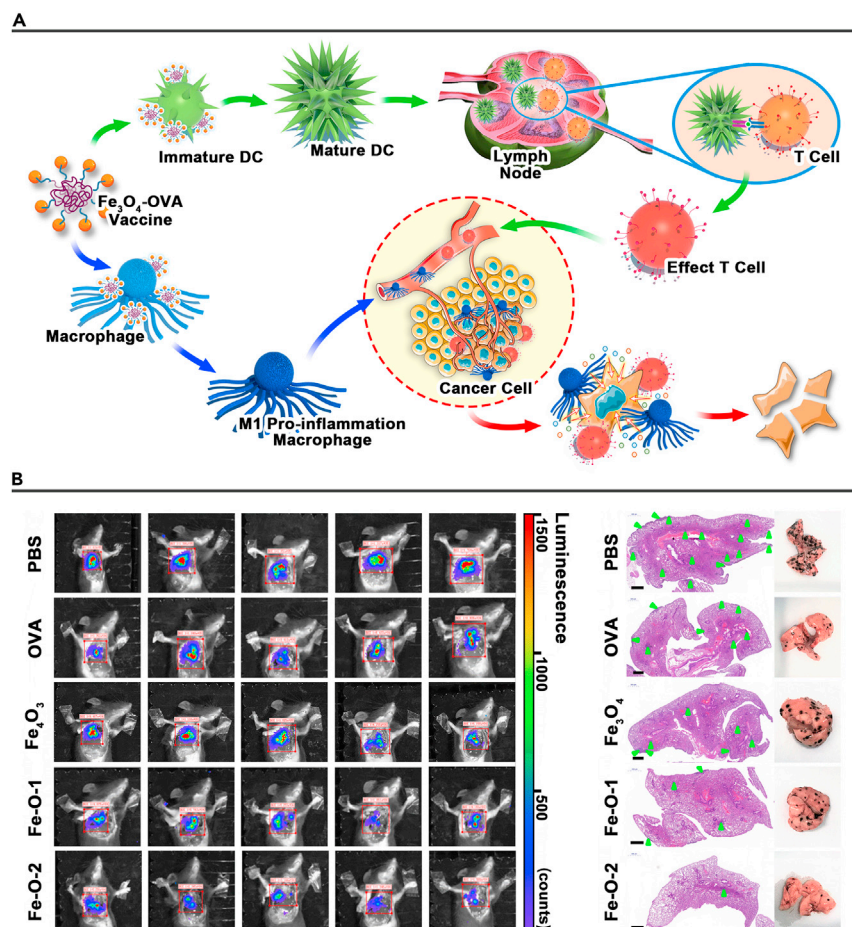
Adoptive T cell transfer is one of the major ACT approaches for cancer. In this strategy, separated autologous tumor-specific T cells are stimulated and expanded *ex vivo* and reinfused into the patient to elicit potent antigen-specific antitumor CTL responses.<sup>149</sup> Adoptive T cells are also an effective approach to inhibit tumor metastasis since the transferred cells can actively target the secondary tumor sites and kill metastatic tumor cells.<sup>150</sup> The use of nanotechnology to effectively engineer lymphocytes to express T cell receptors or chimeric antigen receptors has further expanded the successful use of ACT for cancer therapy.<sup>151–153</sup> For example, Irvine and coworkers demonstrated that decorating the surface of T cells with cytokine- or drug-loaded nanoparticles can significantly improve the efficacy of ACT.<sup>153,154</sup> They also used nanogel backpacks to load the protein drugs on the T cells. This strategy disproportionately increased the number of T cells present in

tumors, which improved the safety of their ACT treatment.<sup>155</sup> Very recently, Cao and coworkers employed PEGylated dendrimer-entrapped gold nanoparticles (Au DENPs) as non-viral vectors for delivering CpG to mature bone marrow derived cells (BMDCs) to stimulate T cells for adoptive cancer immunotherapy. This strategy using genetically engineered BMDCs based on nanotechnology induced an adaptive immune response and immune memory of T cells, inhibiting tumor metastasis and preventing recurrence.<sup>156</sup>

In addition to T cells, platelets also have been employed in ACT. They can spontaneously accumulate in the wound area due to their intrinsic properties. Inspired by this ability, Gu and coworkers conjugated anti-PD-L1 on the surface of platelets. These adoptive platelets can successfully target the surgical wound after tumor resection and release anti-PD-L1 through platelet-derived microparticles following platelet activation *in situ*. This strategy was shown to successfully eliminate residual tumor cells and also prevent cancer recurrence.<sup>157</sup>

### INTERFERING WITH THE FORMATION OF THE PMN

The primary tumors need to transform the microenvironment of distant organs to create a favorable condition for CTCs, known as the PMN. In this phenomenon, primary tumor cells first secrete soluble components, such as extracellular vesicles (EVs), at a potential site of metastasis and mediate



**Figure 7. Nano-immunopotentiators Fe<sub>3</sub>O<sub>4</sub>-OVA promote cancer immunotherapy for preventing lung metastasis of melanoma** (A) Schematic illustration of the Fe<sub>3</sub>O<sub>4</sub>-OVA vaccination strategy. The Fe<sub>3</sub>O<sub>4</sub>-OVA vaccine composed of ultra-small Fe<sub>3</sub>O<sub>4</sub> nanoparticles and OVA not only improved DC maturation and T cell activation but also showed a positive effect on macrophage activation, resulting in improved outcomes.

(B) *In vivo* bioluminescence imaging of the B16-OVA-luc lung metastasis in control and treated groups after 13 days of treatment (left), together with the representative images of lungs stained with H&E from mice intravenously injected with B16-OVA tumor cells and different treatments (right). Green arrows indicate the tumor areas. Scale bars, 500 μm. Inset: representative photographs of lungs from each group after 13 days of treatment.

Reproduced with permission from Luo et al.<sup>132</sup> Copyright 2019, Elsevier.

the microenvironment of this region by transferring small nucleic acid fragments to the normal cells. The primary tumor-derived inflammatory factors can simultaneously recruit the suppressive immune cells including MDSCs, TAMs, or Tregs, which further secrete chemokines and cytokines to support PMN formation.<sup>158</sup> Accordingly, interfering with the formation of PMN represents an opportunity to prevent the colonization of tumor cells. Nevertheless, there is still a clear lack of effective strategies for nano-immunotherapeutic targeting of cells involved in PMN formation process. Further exploration and development are still required for the biochemical characterization of PMN formation and the design of PMN-targeted nanomedicines as well.

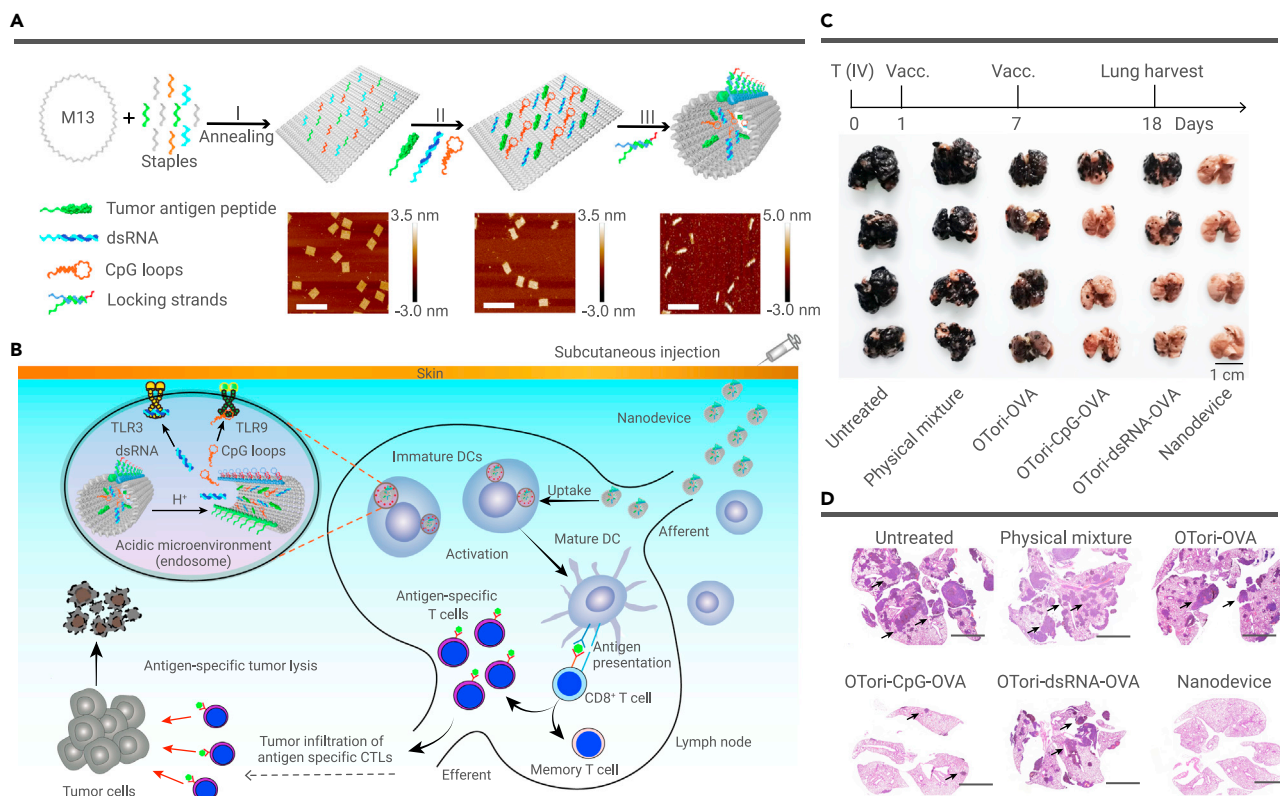
### Remodeling the molecular components of the PMN

The PMN can support the extravasation, anchorage, survival, proliferation, and immune evasion of CTC through changing the vascular state of tissues.<sup>159–162</sup> Moreover, PMN also shows inflammation and stromal reprogramming, which is also a crucial process for CTC colonization and survival.<sup>158</sup> To prevent these processes, researchers have found molecules that can remodel the PMN components to inhibit metastasis.<sup>163,164</sup> Lysyl oxidase is an enzyme overexpressed in tumors and the PMN to help tumor cell colonization by remodeling the ECM.<sup>165–167</sup> In addition to isolated small molecules, nanodrug formulations against molecular components in the PMN have also begun to emerge. Jiang et al. designed metformin and docosahexaenoic acid hybrid micelles as an anti-inflammatory treatment for the PMN. Micelles were coated with fucoidan to target the P-selectin-rich lung PMN for better PMN targeting ability. These nanodrugs reduced the adhesion between CTCs and endothelial cells and reversed the abnormal expression of inflammatory molecules, including fibronectin, matrix metalloproteinase-9 (MMP-9), and S100A9 in the PMN, therefore exhibiting an inhibitory effect on metastasis.<sup>168</sup>

### Inhibiting MDSCs

Among the immunosuppressive cells in the PMN, MDSCs play a key role in the formation of the PMN, which can suppress the activity of CD8<sup>+</sup> T cells.<sup>169–172</sup> Therefore, inhibiting MDSC recruitment is an effective way to prevent metastasis.<sup>173</sup> Nanotechnology has been applied to interfere with the early recruitment of MDSCs as well. Low-molecular-weight heparin and tocopherol succinate were used to self-assemble into micellar nanoparticles (LT NPs). The former inhibited P-selectin/PSGL-1-mediated granulocytic myeloid-derived suppressor cell (g-MDSCs) extravasation through competitive binding, while the latter impaired the expression of MMP-9 in g-MDSCs. Furthermore, by loading a chemotherapeutic drug and an immunopotentiator, the micellar nanoparticles enhanced the nonspecific immune response, activated the specific immune response of invariant NK T cells, and inhibited postoperative metastasis and recurrence (Figure 9).<sup>174</sup> In addition, the depletion of MDSCs in PMN is another approach to inhibit metastasis. Ni et al. reported that hafnium-DBP (5,15-di(p-benzoato) porphyrin), a nanoscale metal-organic layer, in combination with αPD-L1 exhibited outstanding antitumor activity and anti-metastatic effects in an orthotopic breast cancer lung metastasis model. Further investigation indicated that the anti-metastatic effect came from a reduction of both monocytic MDSCs (mMDSCs) and granulocytic MDSCs in the lungs, as well as mMDSC reduction in the primary tumor.<sup>175</sup>

There are many other cells involved in the formation of the PMN in addition to MDSCs. For example, stromal cells, such as fibroblasts, macrophages, and leukocytes, can remodel the ECM, thereby promoting the adhesion of bone marrow-derived cells or tumor cells.<sup>176</sup> There are four reviews recommended for readers who would like more information about the cell types involved in PMN formation.<sup>12,158,177,178</sup> However, there has yet to be an effective strategy employing nano-immunotherapy against these cells. Nano-immunotherapy



**Figure 8. DNA nanodevice-based vaccine for cancer metastasis immunotherapy** (A) Schematic illustration of the construction of the tumor antigen peptide/CpG loop/dsRNA-co-loaded robotic nanostructure by DNA origami. Images of the DNA origami rectangles with capture strands, robotic structures in the open state, and robotic structures in the locked state are shown. Scale bars, 200 nm. (B) Utilization of the DNA nanodevices for efficient cancer immunotherapy. (C) Mice were intravenously inoculated with B16-OVA tumor cells on day 0 and immunized on days 1 and 7 with the DNA nanodevice vaccine or given control formulations. The lungs were harvested on day 18 post-inoculation and imaged to count the metastatic nodules. (D) H&E staining of lungs collected from the treated mice. Scale bars, 3 mm. Reproduced with permission from Liu et al.<sup>134</sup> Copyright 2021, Nature Publishing Group.

strategies can be further explored for these cells to better interfere with the formation of the PMN in future studies.

### Blocking oncogenic EVs

EVs are important messengers for primary tumor cells and stromal cells that remodel the microenvironment of remote organs. Interfering with EVs can block the signals conveyed by the primary tumor, potentially for therapeutic gain. Tumor EVs are divided into four categories according to different diameters: exosomes, microvesicles, apoptotic bodies, and oncosomes, among which exosomes have been the most widely studied.<sup>179</sup> Exosomes can transport proteins, nucleic acids, and lipids to distal organs, resulting in immunosuppression, vascular leakiness, and ECM remodeling of the PMN.<sup>180,181</sup> A promising strategy to interfere with exosomes is the direct elimination of exosomes in circulation. Xie et al. innovatively proposed to tow exosomes into the small intestine through the hepatobiliary metabolic pathway of nanoparticles. Specifically, they designed epidermal growth factor receptor-targeting aptamers functionalized on positively charged mesoporous silica nanoparticles to recognize and bind negatively charged exosomes in the blood. *In vivo* experiments demonstrated that these nanomaterials effectively increased the distribution of exosomes in the liver and small intestine.<sup>182</sup>

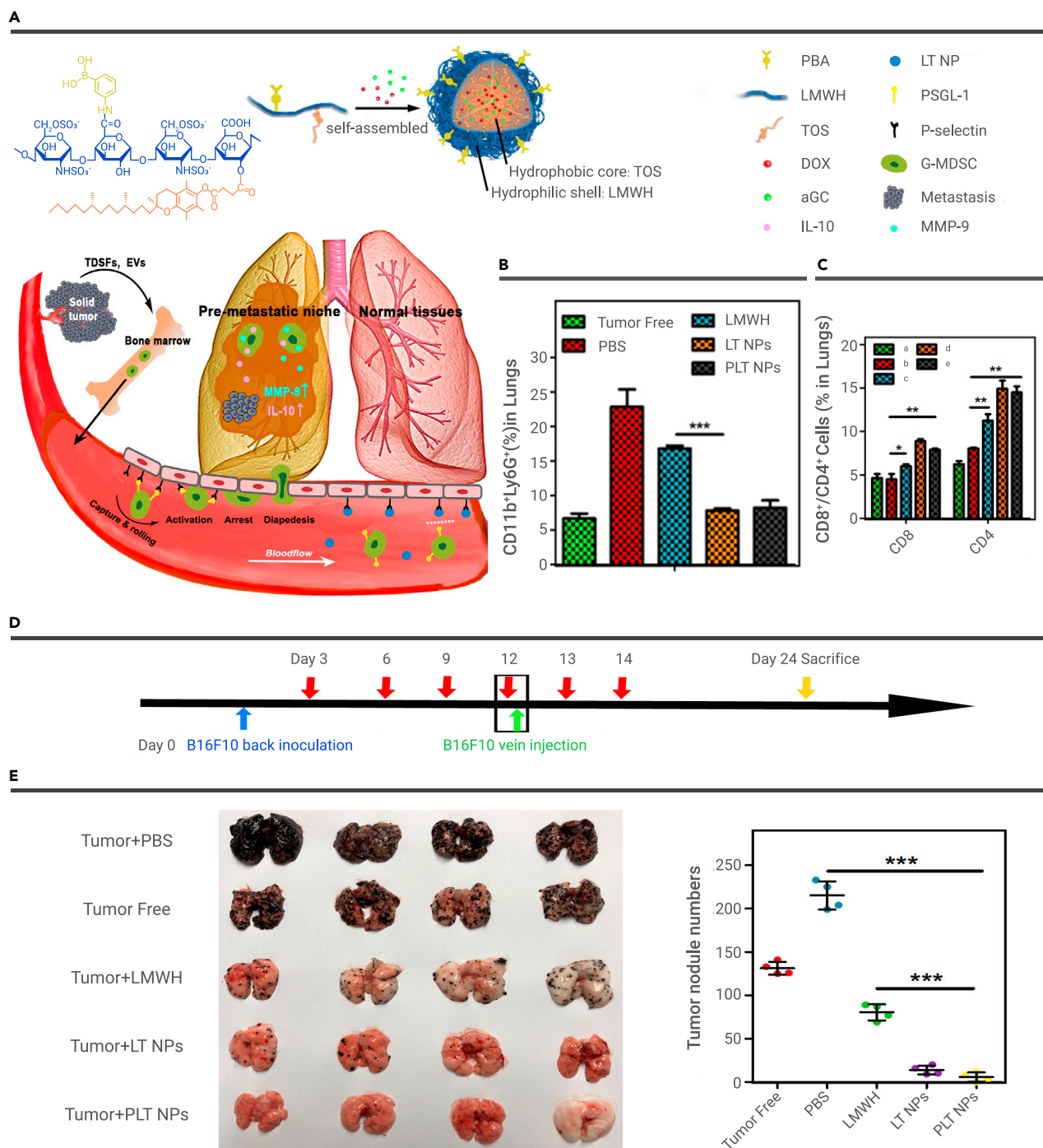
Overall, small-molecule drugs have been the major focus of immunotherapies that aim to inhibit the formation of the PMN to date. However, there are several examples of nano-immunotherapy aimed at PMN inhibition. The advantages of nanomaterials, such as long blood circulation time, customizable response release, and the ability to integrate PMN inhibition and other therapies, make nanomaterials potentially useful across a broad range of applications. As the formation mechanism of the PMN has not been extensively studied, the further development of nano-immunodrugs for PMN inhibition

will benefit greatly from the discovery of new components and pathways in PMN formation.

### POSTOPERATIVE IMMUNOTHERAPY FOR RECURRENCE INHIBITION

In the clinic, surgery remains the preferred treatment for many early-stage solid tumors. Even though advanced imaging technologies have been developed for distinguishing the boundary of the tumor to make resection more precise, the complete removal of the solid tumor is often still exceptionally challenging.<sup>183–188</sup> Infiltrating carcinoma cells that are difficult to clean up by resection may cause the regrowth of a tumor at the resection site or a distant site. Therefore, postoperative neoadjuvant therapy is regarded as an indispensable part of cancer treatment. To address recurrence, various nano-immunoregulators have been developed for use after surgical resection. Nanomaterials that can be administered directly on surgical wounds are preferable due to their safety and ease of use. The Gu group developed a nano-formulation that can be sprayed into the tumor resection cavity, which represents a good approach for nanomaterial-assisted postoperative immunotherapy. After spraying, the immunotherapeutic fibrin gel can be formed *in situ* and gradually release anti-CD47 antibody to induce macrophage phagocytosis of tumor cells through blockade of the CD47 and SIRP $\alpha$  interaction. This nano-formulation can effectively inhibit tumor recurrence both locally and distantly after surgery (Figure 10).<sup>189</sup> In another work, Wang and co-workers developed a nanomedicine-assembled hydrogel for postsurgical tumor treatment. Their system, composed of a hydrogel loaded with thermal-responsive curcumin-loaded polymer nanoparticles, covered the entire surgical bed at the primary tumor site and displayed spatiotemporal control over the delivery of cognate nanomedicines and encapsulated nanovaccines. This nanomaterial successfully boosted the ICD of the residual tumor





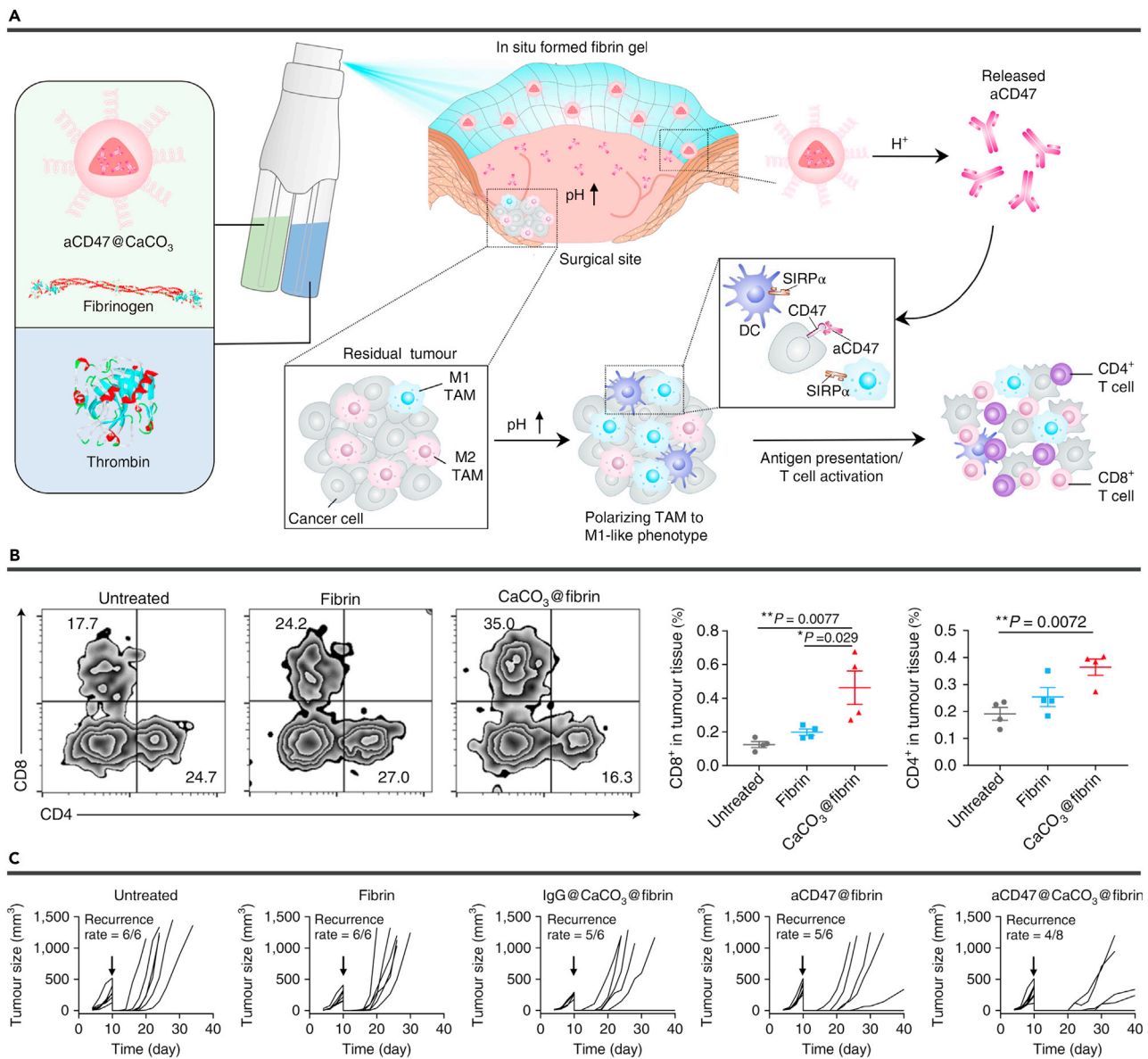
**Figure 9. Self-delivery of micellar nanoparticles prevent the PMN formation** (A) Schematic illustration of PLT/DOX/ $\alpha$ GC nanoparticles and the mechanism of LT NPs interference of g-MDSCs recruitment. (B) The percent of g-MDSCs (CD11b<sup>+</sup> ly6g<sup>+</sup> cells) in the lungs of healthy mice and B16F10 melanoma-bearing mice after different treatments (PBS, LMWH, LT, PLT). (means  $\pm$  SD,  $n = 3$ ,  $***P < 0.001$ ) (C) The percentage of cytotoxic T lymphocytes (CD8<sup>+</sup> T cells) and CD4<sup>+</sup> T helper cells in the lungs of healthy mice and B16F10 melanoma-bearing mice after different treatments. (means  $\pm$  SD,  $n = 3$ ,  $*P < 0.05$ ,  $**P < 0.01$ ) (D) Schematic diagram of the establishment of tumors and treatment process. (E) Photographs of the harvested lungs (left) and numbers of pulmonary nodules (right) from B16F10 melanoma-bearing mice receiving different treatments. PLT, phenylboronic acid (PBA)-low-molecular-weight heparin (LMWH)-tocopherol succinate (TOS); DOX, doxorubicin;  $\alpha$ -GC,  $\alpha$ -galactosylceramide; LT NPs, LMWH-TOS nanoparticles. (means  $\pm$  SD,  $n = 4$ ,  $***P < 0.001$ ) Reproduced with permission from Long et al.<sup>174</sup> Copyright 2020, American Chemical Society.

cells and led to an enhancement of tumor immunogenicity through the generation of neoantigen-specific T cells.<sup>190</sup> Similarly, Lim and coworkers designed an engineered 3D scaffold immune niche loaded with an immunosuppressive drug, gemcitabine, and an immunostimulatory cancer vaccine to prevent tumor relapse after surgery. The local peritumoral implantation of this immune niche served as a postsurgical treatment that stimulated systemic antitumor immunity, inhibited tumor recurrence at the surgical site,

and prevented distant lung metastases in an advanced-stage primary 4T1 breast tumor murine model.<sup>191</sup>

Nanomaterial-assisted postoperative immunotherapy possesses a greater advantage for preventing long-term recurrence in tumors growing in special locations where resecting additional tissue is highly undesirable, such as brain tumors that possess poor prognosis and high recurrence rates owing to residual cancer cells in the brain.<sup>192</sup> Jiang and coworkers presented





**Figure 10. In situ sprayed bioresponsive immunotherapeutic gel for postsurgical cancer treatment** (A) Schematic of *in situ* sprayed bioresponsive immunotherapeutic gel containing aCD47@CaCO<sub>3</sub> NPs within the post-surgery tumor bed. (B) Flow cytometric analysis gating on CD3<sup>+</sup> cells (left) and absolute quantification (right) of CD8<sup>+</sup> and CD4<sup>+</sup> T cells in the tumor (\**P* < 0.05, \*\*\**P* < 0.001). (C) Individual tumor growth kinetics in different groups. Reproduced with permission from Chen et al.<sup>185</sup> Copyright 2019, Nature Publishing Group.

an injectable self-fabricating oligopeptide hydrogel system that promotes the tumor-specific immune response after glioblastoma surgical resection, which effectively prevents the recurrence of the brain tumors in mice. The hydrogel serves as the drug reservoir for the co-delivery of CXC chemokine ligand 10 (CXCL10) and tumor-homing immune nanoregulator (THINR). After being administered in the surgical cavity, the precursor solution formed a hydrogel and released its cargo, which consisted of mitoxantrone and small interfering RNA-targeting IDO (siIDO1), over time. The liberalized THINR targeted residual tumor cells remaining after primary tumor resection that infiltrated the brain parenchyma. Mitoxantrone and siIDO1 were released after the acidic decomposition of internalized nanocarriers and exerted an immunomodulatory effect on tumor cells, which in turn activated circulating T cells and relieved the immunosuppression of Tregs. Activated T cells were then recruited to the brain by CXCL10 to attack residual tumor cells.<sup>193</sup>

In comparison with systemic administration, the locoregional administration of nanomaterials has the potential to reduce adverse effects by limiting organ exposure while serving to enhance the drug concentration within the

affected area.<sup>188</sup> Due to the activation of tumor-specific immunity and the formation of long-term immune memory, locoregional nanomaterial-assisted postoperative immunotherapy is a feasible strategy to prevent tumor recurrence after surgical resection, which can compensate for incomplete resection.

## SUMMARY AND FUTURE PERSPECTIVES

In this review, we have summarized the emerging concepts in nanomaterial-enabled immunomodulation, including changing the immunosuppressive state of the primary tumor, activating the peripheral immune system, preventing the formation of the PMN, and the *in situ* suppression of tumor recurrence after surgery. Nano-immunomodulators cannot only effectively eliminate the primary tumor but also exhibit superior inhibitory effects on distal metastases and prevent recurrence. For the first strategy, altering the immunosuppressive state of the primary tumor with the aid of nanomaterials by either relieving the immunosuppressive microenvironment or inducing ICD of cancer cells, could effectively expose the body's immune

system to tumor neoantigens, resulting in a systemic tumor-specific immune response initiating an abscopal effect on tumor metastases. Based on the results of preclinical trials, current immunotherapies are most effective when treating small, premalignant lesions in the body or tumors that retain immune activity, rather than directly attacking solid tumors that have established a protective immunosuppressive microenvironment. Therefore, for solid tumors, it may be a more reasonable and promising strategy to first use conventional therapies such as thermal therapy, chemotherapy, or radiotherapy to eliminate the vast majority of cancer cells and induce ICD. This can be then combined with nanomaterial-assisted immunotherapy to further eliminate residual cancer cells and prevent metastasis and recurrence. As for the second strategy—activating cells of the peripheral immune system to promote their migration to both the primary tumor and distal metastasis—nanovaccines appear to be useful tools that can ensure a highly specific tumor immune response and potentially lead to the eradication of cancer cells from the body. However, due to the complex mechanisms by which cancer cells avoid immune surveillance, it is difficult to completely eradicate solid tumors by only partially activating the body's immune system. Instead, combining this strategy with ICB therapy may be able to reverse the immunosuppressive microenvironment to further amplify the antitumor immune response. With respect to the third strategy—remodeling the immune microenvironment of the PMN—nano-immunomodulators can suppress tumor-associated immunosuppressive cells and reduce the likelihood of tumor metastasis. However, a deep understanding of the PMN is still lacking, which limits the field's ability to create nanodrugs that leverage the biological and biochemical mechanisms that might otherwise provide good therapeutic targets. In addition, it is difficult for this strategy to cover all potential sites of tumor metastasis. With the cultivation of additional understanding, preventing the development of the pre-migration niche may become a viable clinical strategy for preventing tumor metastasis. In the final approach, postoperative immunotherapy is introduced for long-term inhibition of tumor recurrence. Considering the advantages of *in situ* administered nanomaterials (i.e., low toxicity and ease of application), postsurgical adjuvant immunotherapy is highly clinically relevant in the field of oncology. Although several immunomodulators have been shown to cause adverse effects, such as anemia and thrombocytopenia, when delivered systemically in clinical trials, the locoregional administration of these agents may be a feasible alternative to reduce toxicity.

Due to the poor response rates of single cancer immunotherapies, it stands to reason that immunotherapy regimens will continue to evolve from monotherapies to combination therapies that include multiple agents, such as immune checkpoint inhibitors, such as anti-PD-1 or anti-CTLA-4, chemotherapy, anti-angiogenic agents, and kinase inhibitors.<sup>194</sup> Nanomaterials can be employed as a multi-functional platform to complement the deficiency of immunotherapies in various pathways. More immunotherapy combinations, artificial immune cells, and rapidly emerging nanomaterials, such as nanorobots, will continue to be developed with the goal of improving cancer treatment. Ultimately, their clinical impact will be determined by their ability to treat metastatic disease, as borne out in ongoing and future preclinical and clinical studies. Through the synergistic combination of therapeutic effects, multi-agent immunotherapies will effectively amplify the potency and duration of immune responses against cancer.

Although great progress has been made in nanomaterial-assisted tumor immunotherapy, several challenges remain to be solved before these approaches can be translated to the clinic. First, the large-scale, reproducible production of nano-immunomodulators suitable for *in vivo* applications remains difficult. The synthesis of nanomaterials, loading with immunoactive substances, and the purification of the final synthetic nano-immunomodulators make reproducibility and quality control very challenging, especially at an industrial production scale. Second, the limited biocompatibility of some nanomaterials, which has already hindered the clinical translation of nanomedicines, must be overcome. To facilitate clinical translation, both maximizing therapeutic efficacy and minimizing adverse side effects should be considered. According to previous studies, the findings in relation to the nano-bio interface and the studies on protein corona have greatly promoted

the clinical translation of nanomedicine.<sup>195–197</sup> In future research, the mechanisms of interaction between nanomaterials and *in vivo* biomolecules should be further revealed, thereby proposing more strategies for nano-immunomodulators with reliable safety profiles. Third, the murine immune system is obviously different from that of humans; thus, more complex animal models, such as non-human primate models, are needed to evaluate whether the significant effects achieved in rodent models are likely to be reproduced in humans, which will also help to determine precise administration regimens. Nevertheless, there is still reason to expect that nanomaterial-assisted immunotherapies may one day serve as effective treatments for patients with metastatic tumors.

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## AUTHOR CONTRIBUTIONS

P.Z. designed the review under the guidance of L.H.J. and K.J.M. P.Z., J.M., Y.L., and C.Y. organized the review and prepared the manuscript. Y.H., W.T., L.H.J., and K.J.M. revised the manuscript. All authors provided critical conceptual input and critically revised the review.

## DECLARATION OF INTERESTS

The authors declare no competing interest.

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