Review

Tumor immunotherapy and multi-mode therapies mediated by medical imaging of nanoprobes

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Abstract

Immunotherapy is an effective tumor treatment strategy that has several advantages over conventional methods such as surgery, radiotherapy and chemotherapy. Studies show that multifunctional nanoprobes can achieve multi-mode image-guided multiple tumor treatment modes. The tumor cells killed by chemotherapies or phototherapies release antigens that trigger an immune response and augment the effects of tumor immunotherapy. Thus, combining immunotherapy and multifunctional nanoprobes can achieve early cancer diagnosis and treatment. In this review, we have summarized the current research on the applications of multifunctional nanoprobes in image-guided immunotherapy. In addition, image-guided synergistic chemotherapy/photothermal therapy/photodynamic therapy and immunotherapy have also been discussed. Furthermore, the application potential and clinical prospects of multifunctional nanoprobes in combination with immunotherapy have been assessed.

Key words: nanoprobes, multi-mode imaging, immunotherapy, combination therapy

Introduction

Cancer incidence and mortality are rapidly increasing world-wide. Most cancers are usually detected at the advanced stages due to lack of effective screening and early diagnosis. In addition, the current therapeutic strategies against cancer have poor outcomes on account of their low efficacy and accuracy, considerable adverse effects, and the high tumor recurrence and metastasis rates. Thus, there is an urgent need to explore new safe and effective techniques of cancer diagnosis and treatment in order to improve patient survival [1].

Currently, malignant tumors are detected through biopsies and imaging. However, a biopsy is invasive, lacks sensitivity and specificity, and cannot be performed routinely due to limited pathological specimens. On the other hand, imaging methods are expensive and cannot detect tumors smaller than 1 cm in diameter [2,3]. Therefore, it is crucial to develop advanced imaging techniques for early diagnosis and monitoring of tumors. Nanoprobes, as novel kinds of contrast agents, have been extensively used in medical imaging and drug delivery given their size and optical properties, which translate to high resolution, sensitivity and other advantages [4-8]. The nanoprobes with imaging abilities are especially suitable for real-time imaging, which could greatly improve the accuracy of early tumor diagnosis and therapeutic monitoring [9,10]. In fact, several nanoprobes have been designed in recent years for the early diagnosis and monitoring of tumors [11].

The aim of cancer treatment is to eliminate the primary tumors, as well as the metastasizing cells to prevent recurrence. However, the complex tumor microenvironment (TME) greatly limits the efficacy of conventional anti-cancer treatment and inhibits complete tumor eradication [12]. Cancer immunotherapy is a new generation treatment modality that stimulates the host immune system to selectively kill cancer cells with fewer side effects on healthy tissues, and also promotes systemic immune surveillance that
can eliminate both primary and metastatic tumors. Compared to chemotherapy, surgical resection and radiotherapy, the anti-tumor immune cells activated by immunotherapeutic strategies can even kill circulating tumor cells (CTCs) and thus inhibit the formation of metastatic foci. Finally, immunotherapy can establish long-term immune memory to prevent tumor recurrence. Numerous clinical studies have demonstrated that immunotherapeutic agents such as cytokines [13], checkpoint blockers [14], anti-cancer nano-vaccines [15,16] and chimeric antigen receptor T-cells (CAR-T) can effectively treat advanced or metastatic tumors [17]. Several tumor immunotherapy drugs have been approved by the Food and Drug Administration of the United States for clinical use as well. However, clinical trials show that single immunotherapy cannot achieve complete tumor remission. For example, the response rate of programmed death receptor-1 (PD-1)/programmed death ligand-1 (PD-L1) checkpoint blockade is only 20%. This is largely attributed to the immunosuppressive TME and the high levels of PD-L1 expressed by the tumor cells, which enable immune escape and survival of the tumor cells at every stage of the immune response [18].

Nevertheless, multifunctional nanoprobes can obviate the above shortcomings [19,20] given their excellent biocompatibility, easy surface modification, and ability to deliver drugs to the target site and protect them against endogenous enzymes [21]. Studies show that nanoprobe-assisted radiotherapy, chemotherapy, photothermal therapy (PTT) and photodynamic therapy (PDT) can stimulate the immune system by inducing immunogenic cell death (ICD) [22,23]. When combined with immune checkpoint blockade (ICB), the localized therapies can activate tumor-specific immune response to target metastatic cancer cells, and induce immune memory to inhibit tumor recurrence [24,25]. Therefore, various therapeutic modalities can significantly augment the effects of immunotherapy against residual tumor cells.

The outcome of cancer treatment is assessed according to the response evaluation criteria in solid tumors (RECIST) by measuring the change in tumor volume and the generation of new tumors [26]. However, the infiltration of activated immune cells in the TME can increase tumor volume and delay the response to immunotherapy as per the conventional criteria [27,28]. In addition, routine imaging and histopathology cannot accurately predict the clinical response of tumor cells to immune agents [29-31]. Multifunctional nanoprobes with magnetic resonance, photoacoustic, fluorescence and other imaging elements can additionally achieve real-time and dynamic visualization of tumors and immune cells, which can not only elucidate the mechanisms of immunotherapy but also accurately monitor and predict patient response [32,33]. Furthermore, multi-modal imaging that combine the advantages of several imaging modes can increase the accuracy of tumor diagnosis and monitoring, and clarify the synergistic mechanisms of combination therapy [34].

Thus, multifunctional nanoprobes combining imaging and multimodal treatment can augment immunotherapy and achieve image-guided early diagnosis, precision treatment and real-time monitoring of tumors. In this review, the development and applications of multifunctional nanoprobes have been discussed in detail, with emphasis on image-guided and combination immunotherapy against cancer.

### Image-guided immunotherapy

Multifunctional nanoprobes have unique physical and chemical properties that allow high resolution imaging [35,36]. Some inorganic nanoparticles are effective contrast agents for various imaging modes, such as Fe3O4 nanoparticles for MRI and Au nanoparticles for computed tomography (CT) and photoacoustic imaging (PAI) [37,38]. Surface modification of these nanoparticles can additionally endow them with histocompatibility, stability and targeting capacity [39]. These nanoparticles can not only be used as contrast agents but also as immune-drug carriers that may overcome the limitations of immunotherapy (Table 1).

### Table 1. Image-guided tumor immunotherapy using multifunctional nanoprobes

| Immunotherapeutic agents | Delivery platform or modality | Imaging type | Model/Cell type | Refs |
|--------------------------|-----------------------------|-------------|----------------|------|
| Anti-EpCAM/anti-CD3 encoding MC.DNA T cells | Au NPs | MRI | HEK293/T/HUVEC/MCF-10A/C17.2 NSCs | [40] |
| Licor800 + PD-L1-mAb | Licer800 + PD-L1-mAb | CT/FI | Mouse melanoma | [41] |
| Anti-PD-L1-Ab | ErNPs + Phb QDs | SPECT/CT | Mouse breast cancer | [42] |
| IRDye800CW + Gd-DOTA + anti-PD-L1 | Cerasome NPs | Fl | Mouse colorectal cancer | [43] |
| Aza-BODIPY + Gd-chelating MR probe | Aza-BODIPY + Gd-chelating; MR probe | Fl/MRI | Mouse breast cancer; mouse colorectal cancer | [44] |
| IntraF/ ZnS QDs | PFD nanodroplets | Fl/MRI | RAW 264.7 cells;BMDCs | [45] |
| anti-PD-L1 | snowflake-like Au nanocarriers | CT | Mouse Prostate Cancer | [47] |
| CPG | Au NPs | CT | Mouse melanoma | [48] |
Figure 1. Schematic illustration of the preparation of polymeric-inorganic hybrid nanocomposites and the bispecific antibody-based immunotherapy. Adapted with permission from [50], copyright 2019 Elsevier Science Inc. BsAb: bispecific antibody; MC.DNA: minicircle DNA; PIHN: polymeric-inorganic hybrid nanocomposite; PEI: polyethyleneimine; SPIO: superparamagnetic Fe₃O₄.

Magnetic resonance imaging (MRI) is a non-invasive technique that uses a magnetic field to obtain nuclear magnetic resonance (NMR) of water protons in the tissue, resulting in three-dimensional images. In a strong magnetic field, hydrogen nuclei absorb resonant radio frequency pulses, and are subsequently excited by the nuclei to relax and return to their original low-energy state [31,49]. MRI contrast between tissues is produced by the different relaxation time of spin of the surrounding hydrogen nuclei, which depends on the tissue composition. The MR images of soft tissues are usually high-contrast given the high content of fat and water. Cai et al. [50] synthesized PEI-C₇-SPIO nano-micelles for MRI by coating superparamagnetic Fe₃O₄ (SPIO) with pH-sensitive N-(2-hydroxy)-cycloheximine and branched-chain polyethyleneimine (PEI). Minicircle DNA (MC.DNA) was then adsorbed on the nano-micelles through the surface positive charge to generate the polymeric-inorganic hybrid nanocomposite (PIHN) probes (Figure 1). The Fe₃O₄ nanoparticles functioned as the contrast agents for MRI, whereas the minicircle DNA was transfected into T cells and induced an anti-tumor immune response. The nitrogen/phosphate ratio (N/P) plays an important role in cytotoxicity and transfection efficiency [51]. The PIHN probes had low toxicity and resulted in >95% viable cells when the N/P was between 5 and 25. The transfection efficiency of the probes was the highest at the N/P of 25, resulting in maximum bispecific antibody expression. Thus, the PIHN probes have a broad application potential in cancer diagnosis and treatment given the excellent MRI ability of the Fe₃O₄ nanoparticles and enhanced T cell activation. In addition to MRI, magnetic particle imaging (MPI) is a more novel imaging technology that has not been used in clinical practice despite its potential. Unlike MRI, MPI requires magnetic nanoparticles (MNP) to create images with high spatial resolution in the sub-millimeter range, and can therefore detect MNP with high sensitivity [52].

High-resolution imaging by multifunctional nanoprobes can also elucidate the mechanisms of immunotherapy [53,54] via real-time monitoring of immune cells in the TME and the biodistribution of immunomodulatory drugs at the target site [21,32]. Rinat et al. [55] labeled melanoma-specific T-cell receptor (TCR)-expressing T cells with Au nanoparticles (AuNPs) for CT image-guided therapy. AuNPs loading did not affect the T cells proliferation and cytokine secretion in vitro. Furthermore, the migration and accumulation of the AuNPs-loaded T cells at the tumor site was clearly observed by high-resolution CT. The functionalized T cells significantly reduced the tumor volume in the melanoma-bearing nude mice within 7 days post-injection, indicating excellent tumor-inhibitory effect. This study was the first to use real-time CT imaging to track labeled immune cells, and assess their biodistribution, migration and eventual fate, which provides new insights into the interaction between immune cells and tumor cells [55].

ICB is the most widely applied anti-tumor immunotherapeutic strategy. PD-L1 is an immunosuppressive molecule that inhibits T cell activation upon binding to PD-1 [56], and the PD-1/PD-L1 pathway is the major mechanism of immune tolerance. PD-L1 is a therapeutic target in breast cancer given that almost 20% of the patients express PD-L1 [57,58] and its high expression levels correlate...
with poor prognosis. Monoclonal antibodies (mAb) targeting PD-L1 have been approved by the US Food and Drug Administration (FDA) for the treatment of various cancers [59,60]. Several groups have imaged T cells and lymphoid tissues in mice using radioisotope-labeled PD-L1 and PD-1 antibodies [61,62]. However, despite the high affinity, strong antigen specificity and easy synthesis of radio-labeled antibodies, the prolonged circulation, high background signal and poor tumor penetration obviate imaging within 24 hours of tracer injection. On the other hand, fluorescence imaging (FI) has the advantages of rapid detection, high sensitivity and non-invasiveness. Samit et al. [63] coupled the near infrared (NIR) fluorescent dye Licor800 to PD-L1-mAb to detect the expression of PD-L1 on different breast cancer cell lines, and recorded strong fluorescence intensity for the PD-L1-positive MDA-MB-231 cells as opposed to the weak fluorescence for the PD-L1-negative SUM149 cells. Thus, NIR-PD-L1-mAb can detect PD-L1-expressing tumor cells with high specificity, and is a promising tool for the optical imaging of these tumors.

In recent years, the second near infrared (NIR-II, 1000-1700 nm) window has been used more frequently for in vivo imaging. Compared to routine FI that operates within the wavelength range of 400-900 nm, the longer wavelength of NIR-II reduces photon absorption and scattering, resulting in minimal tissue autofluorescence. In addition, NIR-II FI has deeper tissue penetration, and higher spatial and temporal resolution [64,65], which makes it ideal for deep tissue imaging [43,66]. Yeteng et al. [43] developed biocompatible cubic-phase (α-phase) erbium-based rare-earth nanoparticles (ErNPs) exhibiting bright down-conversion luminescence at ~1600 nm for dynamic imaging of cancer immunotherapy. The ErNPs functionalized with anti-PD-L1 antibodies imaged PD-L1 colon tumors in a mouse model with tumor to normal tissue signal ratio of almost 40. Since the outcome of ICB depends on the activation of tumor-specific cytotoxic T lymphocytes (CTLs) that infiltrate the tumor tissues and induce apoptosis of cancer cells [67,68], the PD-L1-targeting ErNPs were administered along with anti-CD8 α-mAb-labeled lead sulfide quantum dots (PbS QDs) to simultaneously locate CD8+/CTLs and tumor cells by luminescence at 1600 nm. The combination of the long-life luminescence (4.3 ms) of ErNPs and short-life luminescence (46 μs) of PbS QDs at the same emission wavelength further improved temporal resolution (Figure 2), and showed the presence of CD8+ CTLs in the TME and spleen, clearly indicating immune activation. At present, the proportion of PD-L1-expressing tumor cells and tumor infiltrating lymphocytes (TILs) after immunotherapy are detected by biopsies to predict treatment response. However, biopsies are invasive and often difficult to obtain from deeper tumor tissues, which can result in misdiagnosis. Bimolecular imaging by ErNPs and PbS QDs is a non-invasive alternative that can accurately track distribution of PD-L1 tumor cells and CTLs.

Single-mode imaging of tumors is usually ineffective due to one or more limitations. For example, MRI has high spatial resolution but limited sensitivity, whereas FI has excellent sensitivity but lacks spatial and anatomical resolution [69,70]. Dual-mode imaging can significantly improve the efficiency of tumor diagnosis by compensating for the limitations of each mode [71,72]. Several multi-mode imaging methods have been developed in recent years to improve the accuracy of early cancer diagnosis [44,73]. Du et al. [44] developed the theranostic PD-L1-PCI-Gd nanoprobes that combined MRI/FI dual-mode imaging with PD-L1 targeting. The fluorescence intensity of PD-L1-targeted nanoparticles increased steadily in 4T1 tumors, and the final fluorescence intensity was almost 2-fold higher than that of the background. Similarly, MRI of 4T1 tumors showed higher signal intensity than that of the control group. The multifunctional dual-imaging nanoprobes can improve the accuracy of early tumor diagnosis with high sensitivity and spatial resolution. In addition, non-invasive imaging can also be used to monitor cell-based therapy and evaluate the immune microenvironment, which is critical to improve the efficacy of immunotherapy. Eun et al. [45] developed the MRI/NIR FI dual-imaging aza-BODIPY-based contrast agent (AB-BCA) nanoprobe by combining Gd-chelating MR probe and NIR fluorophore aza-BODIPY, which was able to trace macrophages and dendritic cells (DCs) in vivo (Figure 3). The probes were easily internalized by the phagocytic cells, and allowed continuous monitoring of the labeled cells through strong fluorescence and MRI signals without any obvious cytotoxicity.

Natural killer (NK) cells are innate immune cells that form the early defense barrier against pathogens and tumor cells [74,75]. Since exogenous substances are not easily internalized by non-phagocytic cells, it is difficult to label NK cells with imaging probes. Yong et al. [46] combined the MRI agent perfluorodecalin (PFD) with fluorescent InGaP/ZnS QDs to form PFD/[InGaP/ZnS QDs] dual-imaging nano-emulsions that were successfully internalized into immune cells such as macrophages, DCs and NK cells without any transfection reagent. The nanoprobe had almost no effect on cell viability and function, indicating potential applications in image-guided immunotherapy.
Figure 2. *In vivo* two-plex NIR-IIb molecular imaging of immune responses using ErNPs-aPDL1 and PbS-aCD8 at the same ~1,600 nm emission range. A. Schematic illustration outlining the experimental setup (left) to distinguish the PbS QD emission channel (right) by using an 808 nm CW laser. B. Schematic of the experimental setup (left) to differentiate the long-lived ErNP luminescence (right) from short-lived PbS QD fluorescence by using a 980 nm laser pulse. The insets show corresponding cross-sectional intensity profiles (blue color). C. Lifetime decays of ErNPs-aPDL1 and PbS-aCD8 in 1 x PBS solution. D. Absorption spectra of ErNPs-aPDL1 and PbS-aCD8. E. Emission spectra of ErNPs-aPDL1 and PbS-aCD8. The detection region is 1,500 - 1,700 nm. F. Two-plex molecular imaging (upper right) of a CT-26 tumor mouse at 24 h post intravenous injection of mixed ErNPs-aPDL1 (green color, upper left) and PbS-aCD8 (red color, lower left). Scale bar, 5 mm. The zoomed-in high-magnification two-plex image (lower right) outlines the CT-26 tumor with micrometer image resolution (scale bar, 500 μm). G. Corresponding two-plex rotation (−90º to +90º) imaging showing the in vivo bio-distribution of ErNPs-aPDL1 (green color) and PbS-aCD8 (red color) in the whole body. Scale bar, 5 mm. Similar results for n > 3 independent experiments. OD, optical density. Adapted with permission from [43], copyright 2019 Nature Publishing Group. ErNPs: cubic-phase (α-phase) erbium-based rare-earth nanoparticles; ErNPs-aPDL1: anti-PD-L1 mAb (atezolizumab) conjugated with ErNPs; PbS QDs: sulfide quantum dots; PbS-aCD8: anti-CD8α mAb (clone 2.43) conjugated with PbS QDs.

Figure 3. *In vivo* NIR and MRI tracking of the migration of AB-BCA-treated BMDCs to LNs in mice. BMDCs treated with or without AB-BCA were subcutaneously injected in both hind footpads at a density of 5 × 10⁶ cells/mouse. A. NIR fluorescence images of the whole body at 24 h after injection. B. NIR fluorescence images of the magnified thigh at 24 h after injection. C. NIR fluorescence images of the magnified thigh at 48 h after injection. D. MR images at 24 h after injection. Arrows indicated the LNs. Adapted with permission from [45], copyright 2016 Amer Chemical Soc.
Table 2. Multifunctional nanoprobes combined with immunotherapy guided by medical imaging

| Immunotherapeutic agents | Delivery platform or modality | Imaging type | Model | Refs |
|--------------------------|------------------------------|--------------|-------|------|
| Chemotherapeutic agents  |                              |              |       |      |
| NGC 919 + OXA            | Photosensitive NPs           | FI           | Mouse breast cancer | [82] |
| DOX + Cy7                | Melittin-RADA32 peptide hydrogel | FI     | Mouse breast cancer | [82] |
| DOX + Cpg                | Cpg self-crosslinking NPs    | Dual-FI      | Mouse melanoma     | [92] |
| SN38 + BLZ-945           | Au NPs                      | PAI          | Mouse breast cancer | [90] |
| DOX + SDF-1a             | Ag2Se QDs                   | FI           | Mouse breast cancer | [150] |
| Paclitaxel + IRDye800CW + PD-L1-PICl-Gd | Nanohybrid liposomal cerasome NPs | FI/MRI | Mouse breast cancer; mouse colon cancer | [44] |
| Photothermal immunotherapy |                       |              |       |      |
| Cpg self-crosslinked NPs | IR820-conjugated hydrogel   | Dual-FI      | Mouse melanoma     | [100]|
| Cpg                      | SIPO                        | PAI/MRI      | Mouse breast cancer | [112]|
| Resiquimod + CDs        | Polydopamine                | FI/MSOT      | Mouse breast cancer | [151]|
| Human CIK cells          | Au NR@SiO2                  | PAI          | Mouse gastric cancer | [152]|
| FNP/s/rGO-PgNPs          | FNP/s/rGO-PgNPs             | MRI          | Mouse breast cancer | [153]|
| anti-PD-L1 peptides      | AuI@Pt                     | PAI          | Mouse breast cancer | [136]|
| R837                     | Mesos-CuO heteronanocomposites | CTI/IRT/MRI | Mouse colon cancer | [154]|
| Photodynamic immunotherapy |                       |              |       |      |
| HPPH                     | Graphene oxide              | SPECT/CT     | Mouse breast cancer | [123]|
| Cpg                      | BPQD vesicles               | PAI          | Mouse breast cancer | [124]|
| siRNA                    | Positively charged hybrid micelles, FI | Mouse melanoma | [126]|
| NLC 919                  | A tumor-microenvironnement-sheddable prodrug | FI | Mouse colorectal cancer; mouse breast cancer | [129]|
| Anti-PD-L1 Ab + IRDie800CW + DOTA-66+ porphyrin | Cerasome NPs | FI/MRI | Mouse colorectal cancer | [155]|
| Cpg + C6                 | Mesoporous silica NPs       | PET          | Mouse colorectal cancer; mouse melanoma | [120]|
| CuMo5s/Au heterostructures | CuMo5s/Au heterostructures | PAI/CT    | Mouse cervical cancer | [156]|
| Photodynamic-thermothermal-immunotherapy | | | | |
| Carbon-silica nanocomposite | Carbon-silica nanocomposite | PAI | Mouse breast cancer; patient-derived xenograft model | [139]|
| C6 + CPG + Gd/Cy3        | Graphene QDs                | MRI/PAI     | Mouse breast cancer | [140]|
| Black phosphorus          | Black phosphorus            | PAI/PAI     | Mouse breast cancer | [157]|
| CPG + aPD-L1             | CuS                        | IRT          | Mouse breast cancer | [158]|
| Photothermal-chemotherapy-immunotherapy | | | | |
| IRDye800CW + polyPyrrole | CPT-conjugated HA shell     | PAI/PAI     | Mouse breast cancer | [134]|
| DOX + anti-PD-1          | AgolPDA                     | PAI/CT/IRT/MRI | Mouse breast cancer | [159]|
| anti-PD-1                | CuS                        | PAI/USI     | Mouse breast cancer | [160]|
| Photodynamic-thermothermal-immunotherapy | | | | |
| PTX + R837 + IR820       | Thermosensitive liposomal | FI | Mouse gastric carcinoma | [133]|
| ICG + PTX + CAT          | Au nanoshells               | IRT         | Mouse cervical cancer | [130]|

Tumor heterogeneity is a hallmark of cancer, and a major factor limiting therapeutic efficacy. Tumor cells with distinct genotypes co-exist in the same tumor, and significantly affect patient prognosis and treatment response. Therefore, different cancer patients respond inconsistently to the same treatment regimen [76,77], and monotherapies such as surgery, chemotherapy, radiotherapy, immunotherapy, PDT, and PTT cannot achieve complete tumor cessation. Therefore, combination therapies (Table 2) that can reduce some adverse effects of particular monotherapies and result in stronger anti-tumor effects through the complementary action of different treatment mechanisms are increasingly gaining precedence [78,79]. Studies show that chemotherapy with doxorubicin (DOX) or oxaliplatin (OXA) can trigger an immune response via ICD of tumor cells [22,80], which increases the surface expression of pro-apoptotic calreticulin (CRT), promotes the maturation of antigen-presenting cells (APCs) and increases accumulation of CTLs in the tumors [81,82]. In addition, PDT and PTT can also stimulate the immune system by inducing ICD [83,84]. The combination of these monotherapies with ICB can target the metastatic cancer cells and residual tumor cells, and promote expansion of memory immune cells to inhibit tumor recurrence. Therefore, chemotherapy, PDT or PTT combined with an immunoadjuvant can significantly augment the effects of immunotherapy [82].

Image-guided chemo-immunotherapy

Chemotherapy is not only limited by low tumor specificity and adverse effects, but also diminishes the anti-tumor immune response by inhibiting production of white blood cells in the bone marrow [85]. However, targeted chemotherapy can enhance the anti-tumor immune response by promoting the activation of tumor-associated DCs and the expansion of effector T cells, and augment the effects of immunotherapy [86,87]. In addition, multifunctional nanoparticles with chemotherapeutic drugs and imaging probes allows tumor-targeted drug delivery, real time monitoring of the nanoparticles and non-invasive controlled drug release at the target site [40,80]. Another advantage of image-guided, stimul-
responsive drug release is that the time window of treatment can be optimized [89,90].

Bing et al. [82] synthesized a light-induced immunotherapy nano-drug (LINC) consisting of photosensitizer pheophorbide A (PPa), redox-responsive heterodimer of indoleamine 2-dioxygenase 3-dioxygenase 1 (IDO-1) inhibitor NLG919 (PN), and light-activatable prodrug oxaliplatin (OXA) (Figure 4). LINC accumulated at the tumor site and emitted NIR fluorescence signals following intravenous injection in mice. Furthermore, the first FI-guided NIR laser irradiation induced the production of reactive oxygen species (ROS) and cleavage of polyethylene glycol (PEG) canopy, which significantly increased the retention and penetration of LINC into the deeper tumor layers, resulting in 2.5-fold stronger fluorescence intensity compared to the non-irradiated group 24 h post-injection. After the second NIR laser irradiation, LINC promoted anti-tumor immunogenicity by promoting infiltration of CTLs in the tumor. In addition, NLG919 reversed the immunosuppressive TME by activating IDO-1. Taken together, LINC inhibited tumor growth and lung metastasis by chemo-immunotherapy and induced continuous immune memory to prevent tumor recurrence. The targeted accumulation and retention of LINC was monitored by in vivo FI and PAI. The latter also showed that LINC was mainly distributed in the peripheral regions of the tumor in the non-irradiated mice, whereas laser irradiation increased LINC diffusion into the deeper tumor region within 2 h of injection.

Despite the aforementioned advantages of nanoplatforms, the pathophysiological barriers of solid tumors limit the accumulation of drug-loaded nanoparticles, which in turn lowers therapeutic efficacy. Multifunctional nanoprobes that can release the chemotherapeutic drug in a sustained manner at the target site can significantly improve the treatment efficiency and reduce damage to normal tissues [91]. Jin et al. [92] synthesized melittin-RADA 32 hybrid polypeptide hydrogel and loaded it with the antineoplastic drug DOX. The melittin-RADA32-DOX (MRD) nanogel was further functionalized with curcumin, paclitaxel and optical contrast agents such as Cy7 and indocyanine green, with a loading rate close to 100%. The hydrogel showed excellent biocompatibility and controlled release of melittin and DOX, which inhibited melanoma growth in a mouse model by 95% after a single injection with minimal toxicity. The antigens released by the dying tumor cells were engulfed and presented by DCs in the draining lymph nodes, resulting in the activation of specific CTLs that killed the residual tumor cells. The distribution and breakdown of the hydrogels were monitored by the NIR fluorescent dye Cy7 (Cyanine7), which has a molecular weight similar to that of DOX. Xia et al. [93] synthesized multifunctional FI nanoprobes by loading DOX into CpG self-crosslinked hydrogel nanoparticles. Co-stimulation by DOX and the immunogen CpG reversed the immunosuppressive TME and induced a stronger

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**Figure 4. Schematic illustration of NIR light-inducible LINC for self-amplified drug delivery and combination immunotherapy.** Arrows indicated the LNs. Adapted with permission from [82], copyright 2019 Wiley-VCH Verlag Gmbh. CTLs: cytotoxic T lymphocytes; IDO-1: indoleamine 2-dioxygenase 3-dioxygenase 1; ICD: immunogenic cell death; LINC: light-induced immunotherapy nano-drug; OXA: oxaliplatin; PN: indoleamine 2-dioxygenase 3-dioxygenase 1 (IDO-1) inhibitor NLG919; GHS: glutathione; PPa: photosensitizer pheophorbide A.
immune response, thus achieving efficient chemoinmunotherapy. In addition, compared to the direct hydrogel-mediated delivery of DOX, the CpG self-crosslinked hydrogel nanoparticles achieved more sustained immunostimulatory effect. F1 confirmed the targeted delivery of the nanohydrogel, which significantly reduced any adverse effects of the loaded drugs.

Blocking monocyte recruitment and the subsequent activation of tumor-associated macrophages (TAMs) via inhibition of CSF-1/CSF-1R is a novel immunotherapeutic strategy [94,95]. The small molecule BLZ-945 specifically inhibits CSF-1R phosphorylation and blocks CSF-1-mediated signal transduction in TAMs, leading to apoptosis and improving CTC infiltration in the tumor [96,97]. However, free BLZ-945 has a poor tumor inhibition effect, which necessitates dual-responsive nano-delivery systems to drug delivery [98-100]. Rong et al. [90] loaded BLZ-945 into self-assembled AuNNP@PEG/PSN38VP probes synthesized by grafting AuNNPs with polyethylene glycol (PEG) and pH/GSH (glutathione)-responsive 7-ethyl-10-hydroxycamptothecin (SN38). The AuNNP@SN38/BLZ-945Ve particles dissociated into the hydrophilic AuNNP@PEG/PSN38VP in the acidic TME and released BLZ-945. The smaller AuNNP@PEG/G/PSN38VP penetrated into the deeper tumor regions and released the SN38 prodrug in the reductive environment, leading to tumor cell apoptosis. The strong plasmonic coupling between AuNNP significantly enhanced the localized surface plasmon resonance (LSPR) absorbance in the NIR-II window, and the resulting PA signals enabled high contrast PAI for monitoring drug release and treatment (Figure 5). The multifunctional nanoprobes achieved PAI-guided simultaneous chemo-immunotherapy to inhibit the growth of primary and metastatic tumors, and prolonged the survival of tumor-bearing mice. Thus, AuNNP@SN38/BLZ-945Ve is an excellent dual-responsive nanoplatform with broad application prospects in drug delivery and bioimaging.

Image-guided PTT and immunotherapy

Compared to conventional anti-cancer treatments, PTT has certain advantages like high specificity, minimal invasiveness and precise spatiotemporal selectivity [101]. Several nanoparticles have been developed in recent years that integrate contrast agents and photoabsorbers into a single platform for simultaneous diagnosis and PTT [102,103]. Image-guided PTT can effectively ablate primary tumors with little damage to surrounding tissues, but has a poor therapeutic effect on metastatic tumors. Nevertheless, the antigens released from photothermally ablated tumor cells can trigger systemic anti-tumor immunity. The combination of PTT and immunotherapy can synergize their therapeutic effects on distant primary tumors and metastatic cancer cells, and enhance anti-cancer immunity with less invasiveness and shorter course [101]. Local hyperthermia also trigger the release of immunomodulatory molecules [29,104], upregulate MHC-I, MHC-II, CD80 and CD86 on DCs, and increase their migration to draining lymph nodes, thereby enhancing the ability of DCs to present antigens to T cells. In addition, local hyperthermia increases blood flow and vascular permeability to promote infiltration of T cells into the tumor, which further augments the anti-tumor immune response and delays tumor growth [105]. However, lymphocyte infiltration into the deeper layers of solid tumors is inadequate due to several immune escape mechanisms, which can only be obviated by reversing immunosuppression and inducing stronger ICD.

Yinchu et al. [106] achieved more uniform and deeper ICD in solid tumors with PTT using NIR-II, resulting in stronger innate and adaptive immune responses to control tumor growth and prevent metastasis. Photothermal conversion was mediated by Au nanoparticles self-assembled on fluid liposomes, and exhibited similar composition, structure and conversion efficiency, but differed in terms of absorption in the red light, NIR-I and NIR-II window. One study showed that PTT using wavelengths of all regions induced ICD and released damage associated molecular patterns (DAMPs) in vitro [107], although only NIR-II PTT released DAMPs from the deeper tumor regions in vivo and completely inhibited tumor growth in most mice by triggering both innate and adaptive immune responses.

Adjuvants are drugs or bioactive substances that enhance immune response to antigens, and have also been tested in tumor immunotherapy [108,109]. Hydrogels are highly suitable drug carriers given their excellent good biocompatibility and slow drug release [110]. Cytosine-phosphate-guanine (CpG) oligodeoxynucleotides are potent immunostimulants that have proved to be effective as adjuvants in tumor immunotherapy [111]. Dong et al. [108] synthesized CpG nanoparticles (CpG NPs) by crosslinking CpG and PEI in the presence of genipin and conjugated them with indocyanine green (IR820)-loaded hydrogels for combined PTT and immunotherapy against melanoma. IR820 induced tumor antigen release via the photothermal effect, and CpG augmented the ensuing anti-tumor immune response. In addition, the distinct fluorescence signals of genipin and IR820 hydrogels could be individually monitored in vivo for 12 days, which revealed that the
The photothermal action of IR820 hydrogel not only accelerated the degradation of CpG NPs but also enhanced their persistence to achieve an effective immune response. Yuan et al. [112] designed magneto-responsive multifunctional nanoprobes (MINPs) loaded with CpG and SPIO for PA/MR dual-mode imaging-guided PTT and immunotherapy (Figure 6). MINPs functioned as MRI contrast agents and enabled targeted accumulation of SPIO and CpG in the tumors under an external magnetic field, thus achieving accurate dual-mode imaging. The photothermal effect of MINPs effectively destroyed the primary tumor and released tumor-associated antigens, thereby acting as an “autologous tumor vaccine”. The adjuvant action of CpG further enhanced the effect of immunotherapy to achieve a more effective systemic treatment effect than PTT or immunotherapy alone.

Figure 5. In vivo PAI-guided monitoring of BLZ-945 and drug release. A. PA images of MCF-7 tumor-bearing Balb/c nude mice treated with AuNNP@SN38/BLZ-945 Ve at different time points post-injection. B. PA images of MCF-7 tumor-bearing Balb/c nude mice treated with AuNNP Ve at different time points post-injection. C. The PA spectra of the tumors treated with AuNNP@SN38/BLZ-945 Ve at different time points in the photograph of tumor bearing mice. D. The PA spectra of the tumors treated with AuNNP Ve at different time points in the photograph of tumor bearing mice. Adapted with permission from [90], copyright 2020 Amer Chemical Soc.
The efficacy of ICB-based cancer immunotherapy depends primarily on the expression of PD-L1 in tumor tissues and the recruitment of TILs. The immunologically “cold” tumors with poor lymphocyte infiltration do not respond to immune checkpoint inhibitors [113]. Although mild PTT (about 45 °C) can function as an adjuvant in immunotherapy, it can also up-regulate heat shock proteins (HSPs), indoleamine 2,3-dioxygenase (IDO) and PD-L1 on the tumor cells, which aid in immune escape. Liping et al. [114] proposed a combination of mild PTT and anti-PD-L1 antibody to reprogram the “cold” TME and sensitize the tumors to ICB, thereby achieving photothermal immunotherapy. Yan et al. [113] developed photothermal CpG nanotherapy (PCN) to modulate the TME using mild heat (Figure 7). Ovalbumin (OVA) was assembled with Au nanorods through free mercaptan and disulfide...
bonds, and CpG was selectively integrated through interaction with OVA and coordination effect of terminal thiol group with Au nanorod. The multifunctional nanoprobes exhibited good biocompatibility and biodispersion, and significantly increased apoptosis and necrosis of tumor cells under light stimulation. The latter released tumor antigens, which promoted anti-tumor immunotherapy in coordination with CpG. Local mild hyperthermia further augmented the immune response, thereby promoting the activation/maturation of innate immune cells. Numerous CD8+ T lymphocytes and high IL-6 levels were detected in the light-treated PCN group. In addition, mild hyperthermia down-regulated the expression of several immunosuppressive genes (SNCG, IDO2 and Csf3) in the CD8+ T cells, which significantly improved the infiltration, proliferation and activation of CTLs. These results suggest that local PTT-induced immune responses are usually weak and limited by the immunosuppressive TME, which can be overcome by including an immune adjuvant. Thus, a combination of mild PTT and immunotherapy can synergistically eliminate primary tumors, suppress metastasis and prevent tumor recurrence [25,115].

However, most lasers can only target the superficial tumors, which limits the hyperthermic effect in the deeper tumor tissues. Magnetic hyperthermia (MHT) of solid tumors can be achieved through a remote-controlled alternating magnetic field (AMF), which can penetrate deeper tissues more safely and efficiently compared to light and other sources of heat. Magnetic iron oxide nanoparticles (MIONs) repeatedly rearrange the magnetic moments of the ions under AMF, likely due to the internal spin shifting from one direction to another (Néel relaxation) or the physical rotation of the particles (Brownian relaxation). In both cases, this reorientation leads to the conversion of electromagnetic energy into thermal energy, making MIONs controllable heat sources [101]. The heat generated by the interaction of MIONs with magnetic fields induces apoptosis or necrosis in the tumors, which in turn stimulate an immune response [116]. Maintaining T cell activity after activating the immune response is also the key to obtaining antitumor immunity. Combining checkpoint inhibitors with hyperthermia to avoid immune escape has been confirmed to provide significantly improved antitumor immunity [117]. MHT can overcome poor penetration of PTT into deeper tumor tissues, and should be explored as an alternative for combined PTT and immunotherapy.

Image-guided photodynamic and immunotherapy

PDT is a non-invasive tumor ablation method that relies on cytotoxic ROS production by photosensitizers (PSs), eventually leading to the apoptosis and necrosis of tumor cells [118]. However, traditional PSs have poor targeting ability and water solubility, and are easily destabilized in vivo. In addition, PDT is also ineffective against the deep-seated and metastatic tumors due to limited tissue penetration of the laser sources and the hypoxic microenvironment, thus precluding its widespread application. Nano-PDT platforms that deliver PSs using targeted nanoprobes can not only improve the stability, biocompatibility and accessibility of PSs, but also increase therapeutic efficacy and reduce side effects [119]. On the other hand, PDT-induced tumor cell death releases DAMPs that include multiple tumor-specific antigens, which are then presented by the DCs and other APCs to the CD4+ and CD8+ T cells [120,121], thereby eliciting an anti-tumor immune response. Image-guided PDT can improve its therapeutic efficacy by elucidating the tumor size and location, and the optimal treatment time [122].

Yu et al. [123] synthesized graphene oxide nanoprobes loaded with the PS photochlor (HPPH) and conjugated to integrin αvβ6-trargeting peptides for tumor-specific PDT. The probe was also labeled with radionuclide and optical dyes, which enabled non-invasive monitoring of in-situ and distal tumor infiltration by CD8+ T cells after PDT by single photon emission computed tomography (SPECT) and NIR FI (Figure 8), thus confirming the key role of CD8+ T cells in PDT-mediated immunotherapy. In addition, the nanoprobes effectively inhibited tumor metastasis by direct killing of tumor cells in situ and turning them into “autologous vaccines” that can activate the anti-tumor immune response and generate long-term immune memory.

However, PDT alone can achieve very limited activation of innate and adaptive immune responses, which is inadequate for distant tumors. Immuno-adjuvants such as CpG can enhance photodynamic immunotherapy by inducing DC activation and promoting the secretion of pro-inflammatory IL-12p70 and TNF-α [123]. Zhi et al. [124] synthesized NIR/ROS-responsive black phosphorus quantum dots (BPQD) vesicles (BPNVs) by self-assembly of polyethylene glycol and ROS-sensitive poly (propylene sulfide) (PPS), and encapsulated CpG in those vesicles (Bpnvs CpG). Following accumulation of BPNVs at the tumor site, they were irradiated by NIR laser to produce high levels of ROS that triggered transformation of hydrophobic PPS to hydrophilic
polymer. This in turn dissociated CpG from BPNVs, which then penetrated into the deeper tumor tissues and was captured by the APCs along with tumor antigens, resulting in cytokine secretion and anti-tumor immune response. In addition, the dissociation and release of CpG was controlled by PAI. Thus, BPNVs-CPG achieved effective photodynamic immunotherapy in vivo, which inhibited the proliferation of tumor cells and blocked distant tumor growth and metastasis.

Tumor cells suppress T cell activity through PD-L1 and PD-1 immune checkpoints, which can neutralize PDT-mediated tumor immunotherapy. ICB can reverse the negative regulatory signals between immune cells and tumor cells, and increase the anti-tumor immune response of PDT [125]. Dang et al. [126] designed multifunctional nanoprobes for photodynamic immunotherapy by integrating pH-responsive PDPA micelles, pheophyllin A (PPa) PS and small interference RNA (siRNA) to block PD-1/PD-L1 interaction (Figure 9). The homo fluorescence resonance energy transfer (homo-FRET) between PPa molecules quenched the fluorescence of the micelles. The acidic TME protonated the tertiary amine of PDPA and released PPa, which emitted fluorescence signals for tumor imaging. In addition, laser irradiation of the PS induced ROS formation, and photodynamic tumor ablation released antigens that were presented by the APCs to stimulate adaptive anti-tumor immune response. At the same time, the PD-L1-specific siRNA reversed immunosuppression by silencing the expression of PD-L1 on tumor cells. Compared to PDT alone, the combination of PDT and PD-L1 blockade significantly inhibited tumor growth and distant metastasis in a B16-F10 melanoma model.

Indoleamine 2,3-dioxygenase (IDO) is the rate limiting enzyme of the tryptophan metabolic pathway, and breaks down tryptophan to kynurenine. Increased tryptophan consumption and kynurenine accumulation promotes immune escape of tumor cells [127]. Therefore, IDO is a key target of tumor immunotherapy [128]. Ang et al. [129] showed that inactivating IDO-1 overcame PDT-induced adaptive immune resistance. They combined PEG-PS with NLG919, a prodrug of IDO-1 inhibitors, to synthesize a prodrug vesicle that specifically accumulated at the tumor site. Following cleavage of the PEG corona by matrix metalloproteinase-2 (MMP-2), the PS penetrated into the deep layers of the tumor for FI and PDT. Compared to PDT alone, photodynamic immunotherapy mediated by prodrug vesicles enhanced the immune response against CT26 and 4T1 tumor models and significantly inhibit tumor recurrence, especially for the IDO-overexpressing CT26 tumors. This differential tumor-specific therapeutic potential highlights the importance of IDO-1 expression for efficient photodynamic immunotherapy, and provides novel insights into the development of new multifunctional nanoprobes to overcome adaptive immune resistance.

Figure 8. A. Representative optical images of 4T1 tumor-bearing BALB/c mice at 1, 4, and 24 h after injection of HPPH, GO(HPPH)-PEG, or GO(HPPH)-PEG-HK. Tumors are indicated by arrows. B. Quantitative analysis of HPPH, GO(HPPH)-PEG, and GO(HPPH)-PEG-HK uptake by 4T1 tumors at 1, 4, and 24 h post-injection. C. In vivo optical imaging and BLI of 4T1-fLuc tumor-bearing BALB/c mice 24 h after injection of GO(HPPH)-PEG-HK. D, E. Representative optical images of (D) and quantitative analysis of lung uptake (E) of GO(HPPH)-PEG-HK by 4T1-fLuc tumor-bearing and normal BALB/c mice at 24 h post-injection. *, P <0.05; **, P <0.01. Adapted with permission from [123], copyright 2017 Amer Chemical Soc. GO: graphene oxide; HPPH: 2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-alpha; HK: tumor integrin αvβ6-targeting peptide; PEG: polyethylene glycol.
Image-guided multi-mode therapy combined with immunotherapy

As discussed in previous sections, chemoinmunotherapy, photothermal immunotherapy and photodynamic immunotherapy can achieve synergistic (1+1>2) therapeutic effects compared to any monotherapy. In addition, the combination of three or more modalities can further improve the therapeutic effect on tumors [130] by compensating for the disadvantages of each monotherapy [131,132]. Integrating three or more different therapeutic methods into a single nanoprobe could have a better therapeutic effect than the single or dual-mode treatment [133,134] at lower doses, thereby avoiding the side effects of high drug dosage. Multiple therapeutic and imaging compounds can be assembled into a nanostructure through physical adsorption and chemical forces for multi-mode image-guided multi-mode therapy.

PTT and PDT are noninvasive and highly selective [135], and rely on photoactivation to convert light to local hyperthermia [136] or ROS [120] respectively and trigger tumor cell apoptosis. Several nanoprobes have been developed for combination PDT/PTT in recent years [137], especially the carbon-based nanoprobes that have the advantages of simple manufacture, strong NIR absorption and high photothermal conversion efficiency [138]. Hong et al. [139] designed biodegradable carbon-silica multi-functional nanoprobes (CSN) with immunoadjuvant and PAI abilities for image-guided PDT/PTT. The nanoprobes effectively inhibited the growth of 4T1 and patient-derived xenograft (PDX) tumor models by 93.2% and 92.5% respectively. In addition, CSN was degraded into particles smaller than 5.5 nm upon NIR irradiation, and were excreted via the urine given the kidney filtration threshold (KFT) of ~5.5 nm, thus avoiding the long-term toxicity of carbon-based nanoprobes.

Chun et al. [140] conjugated graphene quantum dots (GQDs) with Ce6 and coated them with polydopamine to form a functional photosensitive GQD complex (GCpD), which was then integrated with Cpg and Gd³⁺/Cy3 MRI/FI contrast agent (PC@GCpD(Gd)) for cancer photoinmunotherapy and dual-mode imaging. The probes effectively killed tumor cells through GCpD-mediated PTT and PDT. In addition, CpG activated the innate immunity-related Toll-like receptor 9 (TLR9), resulting in continuous secretion of pro-inflammatory cytokines, DC maturation, and T lymphocyte activation and infiltration. PC@GCPD(Gd) had a strong inhibitory effect on the EMT6 mouse breast tumors under laser irradiation, indicating synergistic photothermal, photodynamic and immunotherapy. Furthermore, dual-mode MRI/FI can track the in vivo biodistribution of PC@GCPD(Gd). The MRI signals in the tumor were strongest at 6 h after administration and persisted after 48 h, indicating that PC@GCPD(Gd) can rapidly accumulate in the tumor tissues and remain in situ for a longer time, thereby maximizing the therapeutic effects. The MRI findings were also confirmed by FI of the tumors and other organs (Figure 10). PC@GCPD(Gd) also migrated to the draining lymph nodes, which is the primary site of DC maturation and activation of antigen-specific T cells for immunotherapy.
Although chemotherapy is the mainstay of cancer treatment, it is fraught with disadvantages such as non-specific action, low bioavailability, inability to overcome tumor heterogeneity and development of multidrug resistance (MDR), resulting in serious systemic side effects and tumor recurrence [141]. The integration of non-invasive photodynamic [142], photothermal [143] and photodynamic photothermal [144] with chemotherapy in a single nanosystem can greatly improve the latter’s efficacy while minimizing adverse side effects. In addition, chemotherapy, PTT and PDT can induce ICD to stimulate the immune system, which when combined with ICB can target the metastatic cancer cells and generate immune memory.

Camptothecin can effectively kill tumor cells in the S phase and G2 phase, but its high toxicity limits clinical applications. Sun et al. [144] synthesized multifunctional nanoprobes by coupling CD44-targeted hyaluronic acid and camptothecin to encapsulate polypyrrole, and attached the NIR fluorescent dye IRDye800CW on the surface. The multifunctional nanoprobes (Figure 11) combined the chemotherapy function of camptothecin, the photoacoustic and photothermal functions of polypyrrole, and NIR FI capacity of IRDye800CW. Breast tumor-bearing mice were injected with P@CH and laser irradiated, followed by five doses of anti-PD-L1 antibodies every three days. The nanoparticles not only mitigated the toxicity of camptothecin in normal tissues but also produced anti-tumor immune response via PTT or chemotherapy-mediated cell death [145], eventually clearing the primary tumor cells and preventing tumor recurrence and metastasis in combination with immunotherapy [146,147].

Paclitaxel (PTX) is a commonly used chemotherapeutic drug [148,149] with good anticancer activity but low water solubility, which limits its applications. Therefore, a PTX loading system that can reduce its side effects and the risk of drug resistance would be of clinical significance. Xian et al. [133] encapsulated PTX, PS (IR820) and the TLR7
agonist imiquimod (R837) in a thermosensitive liposome (TSL) (Figure 12). NIR irradiation of PTX-R837-IR820@TSL at 808 nm led to the conversion of light energy into thermal energy and singlet oxygen by IR820, thus triggering PTT and PDT. Hyperthermia not only killed the tumor cells directly but also ruptured the TSLs to release the encapsulated PTX for a chemotherapeutic effect. Furthermore, R837 also acted as an immune adjuvant that augmented the immune response to the antigens released from the ablated tumors by triggering DC maturation and secretion of cytokines [59]. Thus, PTX-R837-IR820@TSL can deliver chemotherapy drugs to target sites and enhance their accumulation to maximize the therapeutic effect. Taken together, the combination of PTT/PDT with chemotherapy and immunotherapy can synergistically inhibit tumor growth and metastasis, and achieve a stronger therapeutic effect.

Figure 11. Schematic illustration of the formation and functions of multifunctional nanoprobes. Adapted with permission from [144], copyright 2019 Elsevier Sci Ltd. CPT: camptothecin; HA: hyaluronic acid; Hyal: hyaluronidase; NIR: the near infrared; FI: fluorescence imaging; PTT: photothermal therapy; PAI: photoacoustic imaging; a-PD-L1: atezolizumab.

Figure 12. Schematic illustration of the synthesis of PTX-R837-IR820@TSL complexes and the mechanism of combining NIR-mediated PDT/PTT with chemotherapy for cancer therapy. Adapted with permission from [133], copyright 2019 Wiley-V C H Verlag Gmbh. DPPG: 1,2-dipalmitoyl-sn-glycerol-3-phospho-(1′-rac-glycerol); DSPE-PEG 2000: 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N [methoxy (polyethylene glycol)]-2000; DPPC: 1,2-dioleoyl-sn-glycerol-3-phosphocholine; IR820: a photosensitizer; PTX: paclitaxel; R837: imiquimod; TSL: thermosensitive liposomal.
Conclusion and outlook

Cancer immunotherapy has the advantages of strong specificity, excellent durability and high safety, and is often called the “green therapy” for cancer. However, the current immunotherapeutic strategies are still limited by tumor heterogeneity, complexity of the TME, off-target effects and low immunogenicity. It is clinically challenging to “awaken” the immune response in such a complex system. Multifunctional nanoprobes have heralded a new era of cancer immunotherapy that can overcome existing limitations.

Multifunctional nanoprobes can effectively and continuously deliver tumor antigens and immune adjuvants to DCs and other APCs, thereby increasing antigen presentation and resulting in a sustained immune response. Furthermore, incorporation of contrast agents in multifunctional nanoprobes can achieve image-guided precise cancer immunotherapy. Multi-mode imaging combines the advantages of two or more imaging methods, for example high spatial resolution and high sensitivity, which can significantly improve cancer diagnosis. In addition, molecular imaging can visualize the interactions between immune cells and tumor cells in the TME, such as tumor T cell infiltration, cancer cell killing and migration of myeloid cells, and shed light on the mechanisms underlying immunotherapy.

The efficacy of cancer immunotherapy can be enhanced when combined with other therapies that further strengthen host immunity and overcome the immunosuppressive TME. For example, chemotherapy, PDT and PTT trigger the release of tumor antigens from the dying cells, which elicits a systemic anti-tumor immune response. When combined with ICB, the immune cells can be activated to attack metastatic cancer cells and generate long-term immune memory to inhibit tumor recurrence. Multifunctional nanoprobes that combine different monotherapies with immunotherapy, as well as multiple imaging modalities, can significantly improve the therapeutic effect against residual tumor cells.

Despite the encouraging results with pre-clinical models, there are several hurdles in the clinical applications of multifunctional nanoprobes. It is crucial to optimize the dose of drugs loaded into nanoprobes in order to minimize toxicity and maximize the synergistic effects. Although nanoprobes can accumulate in tumor tissues through enhanced permeability and retention effect (EPR), they are easily intercepted by the mononuclear phagocytic system (MPS) before reaching the tumor site, which not only decreases the effective dosage but also leads to off-target effects. While prolonging blood nanoprobes circulation can increase their accumulation at the tumor site, it can also increase their exposure to the immune system, generating a more complex immune response. The TME is physiologically complex, and differs from normal tissues in terms of uneven blood flow, hypoxia and acidic pH value among others. Therefore, nanoprobes responsive to hypoxia, acidity, redox, and tumor-specific enzymes, nucleic acids etc. can release their cargo in the TME in a controlled manner, with minimal effects on healthy tissues. Nevertheless, it is challenging to cross the immune defense barrier and achieve accurate and efficient drug delivery. One major concern is that animal models and cell lines cannot simulate the complexity and heterogeneity of human tumors and the TME, which is responsible for high disparity between patients in their response to immunotherapies. In addition, subcutaneous xenografts cannot replicate the immune status of tumors in situ. Finally, some immunotherapies may have significant side effects in humans that animal models cannot predict.

While multifunctional nanoprobes are a step towards safe and effective immunotherapy, and may even replace the current therapeutic strategies, a deeper understanding of the complex interactions between cancer cells and the immune system is needed to increase therapeutic efficacy.

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Competing Interests

The authors have declared that no competing interest exists.

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