Published in final edited form as:

J Am Chem Soc. 2018 November 07; 140(44): 14971–14979. doi:10.1021/jacs.8b09374.

Magnetic Targeting of Nanotheranostics Enhances Cerenkov Radiation-Induced Photodynamic Therapy

Dalong Ni[†], Carolina A. Ferreira[†], Todd E. Barnhart[†], Virginia Quach[†], Bo Yu[†], Dawei Jiang[†], Weijun Wei[†], Huisheng Liu[§], Jonathan W. Engle[†], Ping Hu^{*,‡}, and Weibo Cai^{*,†,#}

[†]Departments of Radiology and Medical Physics, University of Wisconsin–Madison, Wisconsin 53705, United States

[‡]State Key Laboratory of High Performance Ceramics and Superfine Microstructures, Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai 200050, China

[#]University of Wisconsin Carbone Cancer Center, Madison, Wisconsin 53705, United States

[§]Interdisciplinary Innovation Institute of Medicine & Engineering, Beijing Advanced Innovation Center for Big Data-Based Precision Medicine, School of Biological Science and Medical Engineering, Beihang University, Beijing 100191, China

Abstract

The interaction between radionuclides and nanomaterials could generate Cerenkov radiation (CR) for CR-induced photodynamic therapy (PDT) without requirement of external light excitation. However, the relatively weak CR interaction leaves clinicians uncertain about the benefits of this new type of PDT. Therefore, a novel strategy to amplify the therapeutic effect of CR-induced PDT is imminently required to overcome the disadvantages of traditional nanoparticulate PDT such as tissue penetration limitation, external light dependence, and low tumor accumulation of photosensitizers. Herein, magnetic nanoparticles (MNPs) with ⁸⁹Zr radiolabeling and porphyrin molecules (TCPP) surface modification (i.e., ⁸⁹Zr-MNP/TCPP) were synthesized for CR-induced PDT with magnetic targeting tumor delivery. As a novel strategy to break the depth and light dependence of traditional PDT, these ⁸⁹Zr-MNP/TCPP exhibited high tumor accumulation under the presence of an external magnetic field, contributing to excellent tumor photodynamic therapeutic effect together with fluorescence, Cerenkov luminescence (CL), and Cerenkov resonance energy transfer (CRET) multimodal imaging to monitor the therapeutic process. The present study provides a major step forward in photodynamic therapy by developing an advanced phototherapy tool of magnetism-enhanced CR-induced PDT for effective targeting and treatment of tumors.

Graphical Abstract

*Corresponding Authors huping@mail.sic.ac.cn; wcai@uwhealth.org.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.8b09374.

Additional supplementary figures (PDF)

Notes

The authors declare no competing financial interest.

■ INTRODUCTION

With its spatiotemporal selectivity and minimal invasiveness, photodynamic therapy (PDT) has been widely employed in both fundamental research and clinical practice to treat various diseases such as viral infection, macular degeneration, psoriasis, and malignant cancers.^{1–3} However, such light-based intervention suffers from the rapid attenuation of light in tissue and the concomitant tissue penetration limitation, which confines PDT to superficial tissues. To overcome this deficiency, a variety of nanophotosensitizers have been designed to conduct deep PDT by using different excitation sources, including near-infrared (NIR) light,^{4–7} X-ray radiation,^{8,9} and self-luminescence.^{10–12} Among them, Cerenkov radiation (CR)-induced PDT, where Cerenkov luminescence (CL) produced from radionuclides activates nearby photosensitizers to produce detrimental reactive oxygen species (ROS), has been a novel type of PDT without the requirement of external light excitation.^{13,14} This phenomenon is also known as Cerenkov resonance energy transfer (CRET), which has inspired an emerging field that utilizes such interaction for diagnostic imaging and photon-induced therapies.^{15–18} Positron emission tomography (PET) radionuclides such as ¹⁸F, ⁶⁴Cu, or ⁶⁸Ga have been reported to activate TiO₂ nanoparticles (NPs) and photocatalytically generate hydroxyl and superoxide radicals,^{13,19} enabling CR-induced PDT to destruct cancerous cells. However, the relatively weak CR interaction and low tumor accumulation of theranostic agents reduce the therapeutic effect of CR-induced PDT, leaving uncertainty toward the benefits of this CR-induced PDT approach in the clinic.²⁰

Currently, passive tumor accumulation with the enhanced permeability and retention (EPR) effect,²¹ molecular active targeting with specific conjugated ligands on nanoparticle surface,^{22–24} and approaches utilizing physical forces or stimulus (e.g., magnetic field, light, and ultrasound),^{25–28} have been exploited to deliver NPs to tumor regions with high specificity and efficiency. As a general and controllable approach, magnetically guided delivery of therapeutic agents carried by magnetic vectors under an external magnetic field has received significant interests.²⁹ Such magnetic interaction is independent of tumor microenvironment (e.g., specific receptor expression), and has been demonstrated to work well for delivery of drugs/genes,^{25,30–32} photosensitizers,^{33,34} and photothermal agents into tumors.^{35–37} Moreover, magnetic targeted therapy has been successfully applied for patients on early stage clinical trials,^{38–40} opening up new clinical possibilities for the diagnosis and treatment of cancer.

Therefore, rational design and synthesis of a novel strategy to amplify the therapeutic effect of CR-induced PDT to overcome the disadvantage of traditional PDT are of great

significance. In this work, we designed magnetic targeting nanostructures based on high-performance magnetic $(\text{Zn}_{0.4}\text{Mn}_{0.6})\text{Fe}_2\text{O}_4$ nanoparticles (MNPs),^{41,42} with surface conjugating porphyrin molecules of meso-tetrakis(4-carboxyphenyl)porphyrin (TCPP) and chelator-free labeling of ^{89}Zr (^{89}Zr -MNP/TCPP) for magnetism-enhanced CR-induced PDT. The labeled ^{89}Zr on the surface of MNPs could be used for PET imaging to investigate *in vivo* behavior of MNPs and intrinsically excited TCPP for singlet oxygen-mediated destruction of tumor cells (Figure 1a).^{11,43} Our work deliberately demonstrates that ^{89}Zr -MNP/TCPP nanostructures can be used as efficient magnetic vectors to achieve high tumor accumulation under the external magnetic field to greatly enhance the therapeutic effect of CR-induced PDT by overcoming both depth and external light dependence of traditional PDT.

■ RESULTS AND DISCUSSION

The MNPs were synthesized via a previously reported procedure, with Zn^{2+} and Mn^{2+} -doping greatly improving their magnetic characteristics.^{39,40} Transmission electron microscopy (TEM) images indicated that these MNPs were uniform with an average diameter of about 20 nm (Figure 1b), and all expected essential chemical elements (Fe, O, Zn and Mn) were found on the energy dispersive X-ray (EDX) spectrum (Figure S1). The X-ray diffraction pattern (Figure S2) shows that the as-synthesized MNPs have magnetite phase crystals with high-quality crystallinity (JCPDS No. 19-0629). The field-dependent magnetism of MNPs (Figure 1d, Figure S3) showed no hysteresis with high saturation magnetization, rendering these MNPs highly sensitive to the external applied magnetic field (inset in Figure 1d). For further application *in vivo*, the biomimetic DSPE-PEG_{5k} and DSPE-PEG_{5k}-NH₂ were used to transfer the hydrophobic MNPs into an aqueous phase.⁴⁴ These MNP-PEG were stable in water without observable aggregation and TEM image showed that the morphology remained unchanged (Figure 1c) with their dynamic light scattering (DLS) size of ~45 nm (Figure S4).

PET imaging, as a highly sensitive, quantitative and noninvasive imaging technique, has been widely used to monitor *in vivo* fate of nanomaterials and promote their clinical translations.⁴⁵ To investigate circulation and biodistribution of MNP-PEG under PET imaging, the radionuclide ^{89}Zr was chelator-free radiolabeled with MNP-PEG since ^{89}Zr is reported to act as a hard Lewis acid and thus prefers to bind with hard Lewis bases such as metal oxides that act as electron donors.^{46,47} The MNP-PEG was incubated with ^{89}Zr for 2 h and thin-layer chromatography (TLC) was used to determine the radiolabeling yields. As shown in Figure 1e, incubation at 37 °C resulted in relatively low labeling yields (about 10%), while relatively high labeling yields were achieved after incubation at 75 °C (about 60%). Notably, ^{89}Zr -MNP-PEG remained intact in PBS within 1 week (Figure S5a) and was found to be highly stable in mouse serum even in an EDTA competitive situation (Figure S5b), further demonstrating the strong binding between ^{89}Zr and the metal oxide surface.

Furthermore, to investigate the long-term fate of MNP-PEG *in vivo*, healthy BALB/c mice were intravenously injected with ^{89}Zr -MNP-PEG and monitored for 16 days. As shown in Figure 2a, the maximum intensity projection images showed dominant heart circulation within 0.5 h postinjection (p.i.), while signals in the liver and spleen were found at 1 h p.i.

with very high signal in the heart, indicating that surface PEGylation endowed MNPs with good circulation. Quantitative region-of-interest (ROI) analysis of PET images showed these ^{89}Zr -MNPs-PEG with a long circulation half-life of 2.46 h (Figure 2b), which is relatively long among inorganic nanoparticles and is desirable for *in vivo* magnetic targeting of the tumor. In addition, dominant liver and spleen uptake were found after 5 h p.i. and remained through the 16 days, which is consistent with previous reports stating that NPs are easily recognized by mononuclear phagocyte systems (e.g., liver/spleen).⁴⁸ The time point of the highest liver uptake was about 3 days p.i. while signals in the spleen increased gradually within 16 days (Figure 2c). It is worth noting that very low bone uptake was found, suggesting good stability of ^{89}Zr -MNP-PEG *in vivo*. *Ex vivo* biodistribution of MNP-PEG in different tissues was performed after final PET scanning, and further confirmed the results from ROI analysis on PET images (Figure S6).

To investigate whether the external magnetic field would greatly enhance tumor-specific uptake of ^{89}Zr -MNP-PEG, PET imaging of bilateral 4T1 tumor-bearing mice was performed at different time points after intravenous injection of ^{89}Zr -MNP-PEG, with a magnet being applied on the right tumor side for 3 h (Figure 2d). To the best of our knowledge, this is the first work reporting the use of PET imaging to evaluate magnetic performance of nanomaterials in detail because PET imaging can provide quantitative information on the whole body under the external magnetic field. As shown in Figure 2e, both magnetic field (MF)-targeted and nontargeted tumors showed time-dependent uptake of ^{89}Zr -MNP-PEG, indicating the passive EPR effect. Importantly, the MF-targeted tumors evidently exhibited higher accumulation of ^{89}Zr -MNP-PEG, especially at the outer side of the tumor which was attached to the magnetic field. This impressive targeting behavior could be attributed to the long circulation half-life of ^{89}Zr -MNP-PEG (Figure 2b), which was attracted to the targeted area through the blood vessel from both normal and tumor tissues under the influence of an external magnetic field. Further ROI analysis showed that the ^{89}Zr -MNP-PEG in MF-targeted tumors reached a large enhancement of $15.2 \pm 4.8\%$ ID/g at 3 h p.i., which was 5 fold higher than that of nontargeted tumors. At 72 h p.i., the MF-targeted tumors still showed very high accumulation of ^{89}Zr -MNP-PEG and *ex vivo* PET imaging of main organs further demonstrated the results found *in vivo* (Figure 2g-f). *Ex vivo* biodistribution of ^{89}Zr -MNP-PEG at 72 h p.i. showed that the magnetically targeted group presented higher tumor accumulation than the nontargeted group. The spleen accumulation was much lower than that of healthy mice (Figure S6), which may be attributed to the fact that the spleen of tumor-bearing mice is usually much larger than that of healthy mice, as well as the fact that the magnetic targeting of the tumor may also affect the distribution of ^{89}Zr -MNP-PEG in main organs (Figure S7). Our results were consistent with previous results and verified the so-called ‘magnetic targeting-enhanced EPR’ effect.³⁷ In addition, our studies provide an approach of radiolabeling magnetic carriers and using PET imaging as a powerful tool for monitoring the whole body while obtaining quantitative information in real time during magnetic targeting process.

Encouraged by excellent magnetic-targeting performance of MNP-PEG on tumors, we furthered conjugated TCPP on the surface of MNP-PEG through conjugation between amino groups on the surface of MNP-PEG and carboxyl groups on TCPP, resulting in ^{89}Zr -MNP/TCPP nanostructures. As shown in Figure 3a, the UV-vis-NIR absorption spectrum of MNP/

TCPP was similar to that of free TCPP and displayed a Soret band around 400 nm and several Q bands from 500 to 650 nm, demonstrating that TCPP had been successfully conjugated on the surface of MNP-PEG. The Fourier transform infrared (FT-IR) spectra also demonstrated the successful conjugation of TCPP on the surface of MNP-PEG (Figure S8), which was further verified by the increase of DLS size and variation of ζ -potential (Figure S4). The MNP/TCPP still kept ferrofluidic behavior under the external magnetic field (Figure 3b), demonstrating that MNPs were efficient magnetic carriers to deliver TCPP into targeted area. In addition, to evaluate the depth penetration ability of the magnetic field in attracting nanostructures, we placed a piece of pork meat of different thickness in between the sample and the magnet. As shown in Figure S9, although it required a longer time with a magnet behind a thicker cut of pork to completely attract the nanostructures, the magnet could still attract MNP/TCPP nanostructures even behind a 15 mm-thick pork tissue.

We have previously demonstrated that TCPP could be intrinsically excited by ^{89}Zr nearby for CRET imaging and generate ROS to destruct tumor cells.¹¹ To evaluate CRET effect and CR-induced PDT in *vitro*, ^{89}Zr -MNP-PEG was surface modified with TCPP to construct ^{89}Zr -MNP/TCPP nanostructures. In consistency with good stability of ^{89}Zr -MNP-PEG, ^{89}Zr -MNP/TCPP exhibited negligible release of free ^{89}Zr within 1 week, as demonstrated by TLC plates in Figure 3c. It is worthy to note that *in vivo* distribution of ^{89}Zr -MNP/TCPP was similar to that of ^{89}Zr -MNP-PEG as confirmed by PET imaging of ^{89}Zr -MNP/TCPP on healthy mice within 16 days (Figure S10), indicating that the *in vivo* behavior of ^{89}Zr -MNP-PEG remained unchanged after surface TCPP modification. Next, we investigated fluorescence imaging (FL), Cerenkov luminescence (CL) and CRET imaging *in vitro* using a conventional small-animal *in vivo* imaging system (IVIS). Among all solution samples, ^{89}Zr -MNP/TCPP showed similar FL imaging to that of free TCPP under 640 nm excitation (Figure 3d) and similar CL imaging to free ^{89}Zr (Figure 3e). Importantly, obvious strong selfillumination of ^{89}Zr -MNP/TCPP was observed due to the CRET effect (Figure 3f). It is found that CL of free ^{89}Zr is more intense at higher frequencies (UV/blue) but will rapidly decrease at a longer wavelength (Figure S11). However, the intensity spectrum of ^{89}Zr -MNP/TCPP was completely different from free ^{89}Zr and exhibited much stronger emission at a long wavelength from 600 to 800 nm, reaching the maximum around 660 nm (Figure S11b). This was similar to free TCPP that was used for PDT by external 660 nm laser.^{11,49} All these results demonstrated that ^{89}Zr -MNP/TCPP could be excited by the UV/blue CL of radiolabeled ^{89}Zr , which then in turn emitted a longer wavelength of light for CRET imaging or CR-induced PDT.

Furthermore, the photodynamic property of ^{89}Zr -MNP/TCPP was evaluated using a singlet oxygen sensor green (SOSG) probe to monitor the release of singlet oxygen ($^1\text{O}_2$). As shown in Figure 4a, significant time-dependent $^1\text{O}_2$ generation was observed for solutions containing ^{89}Zr -MNP/TCPP, while significantly lower increase in SOSG fluorescence was found in pure free ^{89}Zr samples. The $^1\text{O}_2$ generation also depended on the radioactivity and increased continuously within 24 h. The $^1\text{O}_2$ generation by continuous CR irradiation was found to be similar to that of TCPP-containing metal-organic framework under traditional PDT treatment^{50,51} It was reasonably expected that such a process could last longer due to the long half-life of ^{89}Zr (78.4 h). These encouraging findings motivated us to evaluate CR-induced PDT on 4T1 cancer cells. The *in vitro* toxicity of cells was tested by a standard

MTT assay after incubation of MNP-PEG and MNP/TCPP at varying concentrations with 4T1 cells for 24 and 48 h. Both nanostructures showed negligible toxicity even at a high concentration (Figure S12). Subsequently, ^{89}Zr -MNP/TCPP at varying radioactivity (10, 20, 40 and 80 μCi) was incubated with 4T1 cells for 24 and 48 h. It was found that the relative viabilities of the 4T1 cells decreased remarkably at higher radioactivity levels, indicating the therapeutic effect of CR-induced PDT. To further reveal such process, confocal microscopic imaging of 4T1 cells after incubation with ^{89}Zr -MNP/TCPP or MNP/TCPP for 24 h was performed. The fluorescence in both groups could be observed around the nucleus of the 4T1 cells, which indicated that ^{89}Zr -MNP/TCPP or MNP/TCPP had been uptaken by cells into the cytoplasm. Importantly, a large amount of ROS generation in cells treated with ^{89}Zr -MNP/TCPP was observed, but negligible ROS was found in cells treated with MNP/TCPP (Figure 4c). These encouraging results clearly demonstrated that ^{89}Zr -MNP/TCPP could be used as an effective nanoplatform for CR-induced PDT.

A main concern with CR-induced PDT is its low efficacy.²⁰ The therapeutic effect of CR-induced PDT greatly depends on the intensity of CR. For porphyrin chelators, ^{64}Cu has been widely used for radiolabeling due to their natural capture capability of Cu^{2+} ions.⁵² Due to weak CR productivity of ^{64}Cu and the very low injected dose for imaging,¹⁴ few of these porphyrin-based probes have noticed CR-PDT induced toxicity. In contrast, ^{89}Zr decays to release a positron with an average energy of 902 keV and provides 2.29 photons per decay in aqueous solution.¹⁴ The designed ^{89}Zr -MNP/TCPP has exhibited efficient ROS production, which could be attributed to the close distance between radionuclides and photosensitizers confined on the same nanostructures that greatly improved CR-induced PDT effect.¹¹ Furthermore, magnetic targeting delivery under magnetic field significantly enhances accumulation of ^{89}Zr -MNP/TCPP into tumor areas to amplify the therapeutic effect of CR-induced PDT. However, it is worthy to note that the interactions involving radionuclides and nanomaterials are complex,⁵³ and much attention should be paid to investigate all the potentially involved ionizing radiation processes in depth for ^{89}Zr -MNP/TCPP in future studies.

Taking advantage of such high-performance magnetic and CR-induced PDT effects of the ^{89}Zr -MNP/TCPP nanoplatform, we investigated magnetic-targeted CR-induced PDT using bilateral 4T1 tumor-bearing mice with a magnet being applied on the right tumor side for 3 h after intravenous injection of ^{89}Zr -MNP/TCPP. The mice that received PBS or MNP/TCPP injections were used as control groups. FL, CL and CRET imaging were performed to monitor therapeutic effect over 2 weeks. As shown in Figure 5a, FL imaging (Ex: 640 nm, Em: 740 nm) displayed a very high uptake of ^{89}Zr -MNP/TCPP on the right tumor side with MF. Interestingly, 4T1 tumors typically grew fast within 2 weeks, but showed visible growth retardation in MF-targeting group during the study period, as clearly seen from FL images. The CL and CRET imaging further verified the MF-enhanced tumor accumulation observed from FL imaging results. However, only FL signal was found for MNP/TCPP-treated mice under the same condition (Figure S14). Both CL and CRET signal could be observed for at least 4 days, indicating the persistent CR-induced PDT to gradually generate $^1\text{O}_2$ for the destruction of tumor cells. The CL and CRET signal without requiring external light can also be used for luminescence imaging-guided surgery *in vivo* in the future. Good overlap of

signals from the FL, CL and CRET modalities at all time points further validated the robust nature of these nanostructures.

Tumor volume, photographs of mice and body weight of mice were monitored regularly. Remarkably, for the MF-treated group, tumor growth of mice that received ^{89}Zr -MNP/TCPP treatment was significantly inhibited, whereas the MNP/TCPP-treated group demonstrated rapid tumor growth (Figure 5b-d). In contrast, non-MF targeted group showed lower tumor growth inhibition rates. Hematoxylin and eosin (H&E) staining of tumor tissues from various groups clearly demarcated the difference in tissue morphology, while the widespread tumor tissue loss and undergoing apoptosis in tumor cells were observed for ^{89}Zr -MNP/TCPP and MF-treated mice (Figure 5c). Control groups (non-MF or nonradioactivity) showed minimal damage to tumor tissues with pleomorphic nuclei and thriving tumor cells, suggesting that both ^{89}Zr and MF were essential for effective tumor cell killing with CR-induced PDT. No abnormal behavior or significant weight loss was observed in any group (Figure S15), indicating minimal side effects of treatment. Furthermore, the CR-induced PDT was also evaluated on large-sized tumors (double the size of previously used tumors) using the ^{89}Zr -MNP/TCPP nanostructures, which also exhibited high antitumor effect with suppressed tumor growth in the MF-targeted group (Figure S16). To investigate whether ^{89}Zr -MNP/TCPP caused long-term detrimental effects, H&E staining of different organs (heart, liver, spleen, lung, and kidney) of healthy mice that received an intravenous injection of ^{89}Zr -MNP/TCPP were monitored for two months. No noticeable organ damage or inflammatory lesions over 60 days was found except in the liver. The liver was damaged at 7 days p.i. but self-recovered within two months (Figure S17), which was reasonable since most of the nanostructures accumulated in the liver and spleen (Figure S10). Future studies should be focused on developing tumor microenvironment responsive nanoplatform for CR-induced PDT. For example, the surface TCPP center can be filled with Cu^{2+} ions so that the fluorescence will be quenched, and only be activated by H_2S in the tumors,⁵⁴⁻⁵⁶ which will undoubtedly allow for controllable CR-induced PDT *in vivo*.

■ CONCLUSION

In summary, we presented here a novel strategy for enhanced delivery of photosensitizers and radioisotopes into tumor area based on magnetic vectors to realize efficient CR-induced PDT under the external magnetic field. Both circulation, long-term fate *in vivo*, and magnetic targeting performance of MNPs have been investigated in detail with ^{89}Zr radiolabeling by PET imaging. By overcoming the depth and light dependency of traditional PDT, our findings revealed that ^{89}Zr -MNP/TCPP nanostructures exhibited efficient singlet oxygen generation for tumor PDT. With high MF-targeted uptake by the tumor, these magnetic nanostructures displayed excellent FL, CL and CRET multimodal imaging, which could be used to monitor the therapeutic effects. Magnetic targeted CR-induced PDT has been demonstrated on bilateral 4T1 tumor-bearing mice, with rapid and significant inhibition of tumor growth *in vivo*. Overall, the strategy described herein is versatile and is not only confined to cancer, holding great promise as an advanced phototherapy tool for treating a variety of lesions in clinics.

■ EXPERIMENTAL SECTION

Materials.

ZnCl₂, MnCl₂, [Fe(acac)₃], oleic acid, oleylamine, octyl ether, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich. Tetrakis(4-carboxyphenyl)porphyrin (TCPP) was obtained from TCI America (Portland, OR). DSPE-PEG_{5K} and DSPE-PEG_{5K}-NH₂ were purchased from Creative PEGworks. All reagents were used without any purification.

Material Characterization.

Transmission electron microscopy images and energy-dispersive X-ray analysis were conducted on a JEOL 200CX microscope with an accelerating voltage of 200 kV. Powder X-ray diffraction pattern was obtained on a Rigaku D/MAX-2250 V diffractometer with graphite-monochromatized Cu K α radiation. Dynamic light scattering (DLS) measurements were performed on a Nano-Zetesizer (Malvern Instruments Ltd.). XPS measurement was conducted on a Thermo Scientific K-alpha XPS. UV-vis spectra were recorded on an Agilent Cary 60 spectrophotometer. Figure 1a were reproduced and modified under the Creative Commons Attribution License 3.0 from Servier Medical Art (<https://creativecommons.org/licenses/by/3.0/>). H&E staining was observed on an inverted optical microscope (Nikon, Eclipse Ti-U, Japan).

Synthesis of Magnetic Nanoparticles (MNPs).

To obtain MNPs with enhanced magnetic performance, Zn and Mn ions were usually codoped into iron oxide nanoparticles. The (Zn_{0.4}Mn_{0.6})Fe₂O₄ MNPs were synthesized according to the reported procedures as follows:⁴¹ ZnCl₂ (0.03 g), MnCl₂ (0.04 g) and [Fe(acac)₃] (0.353 g) were dissolved in a 50 mL three-neck round-bottomed flask containing surfactants (oleic acid and oleylamine) in octyl ether. The reaction mixture was heated at 300 °C for 1 h, which were then cooled to room temperature. The resulting black powder was precipitated by adding ethanol, collected by centrifugation, and finally dispersed in chloroform for further research.

Surface PEGylation of MNPs.

For hydrophilic transferring and improving the biocompatibility, commercially available DSPE-PEG_{5K}-NH₂ and DSPE-PEG_{5K} was used for surface modification of hydrophobic MNPs following a slightly modified procedure.^{44,57–59} Typically, 5 mL of MNPs solution in chloroform (~3 mg/mL) was added into one-neck round-bottom flask containing 4.5 mL DSPE-PEG_{5K} solution in chloroform (20 mg/mL) and 0.5 mL DSPE-PEG_{5K}-NH₂ solution in chloroform (20 mg/mL) and then stirred for 5 min. The mixture was incubated under the vacuum in a rotary evaporator for 2 h at 60 °C to evaporate the solvent. Subsequently, 5 mL of water was added into the flask and then sonicated for 15 min. The resulting MNPs-PEG were collected by centrifugation at 15000 rpm for 10 min to remove the free DSPE-PEG_{5K}-NH₂ and DSPE-PEG_{5K}. The final MNP-PEG were redispersed in water or PBS for further application.

Chelator-Free Radiolabeling of MNP-PEG.

To evaluate *in vivo* circulation behavior and biodistribution of MNP-PEG, ^{89}Zr was used to radiolabel the MNP-PEG for PET imaging by a chelator-free method.^{47,60} Such chelator-free labeling strategy without introducing chelator could be more accurate to monitor *in vivo* behavior of MNPs. Briefly, for ^{89}Zr -labeling, 100 μL of MNP-PEG dispersed in HEPES buffer was directly mixed with 1 mCi (or 37 MBq) of ^{89}Zr -oxalate. The final pH value was adjusted to 7–8 with 1 M Na_2CO_3 . After shaking for 2 h at 75 °C, ^{89}Zr -MNP-PEG was collected by centrifugation and finally dispersed in PBS. ^{89}Zr labeling yield was monitored and quantified by using thin layer chromatography (TLC) with subsequent autoradiography.

In vivo PET Imaging of ^{89}Zr -MNP-PEG.

All animal studies were performed under a protocol approved by the University of Wisconsin Institutional Animal Care and Use Committee. The BALB/c mice were anesthetized and intravenously injected with 150 μL (~200 μCi or 7.4 MBq) of ^{89}Zr -MNP-PEG in PBS (n = 4). Serial PET scans were performed at various time points postinjection (p.i.) from 0.5 h to 16 days. ROI analysis of each PET scan was conducted to calculate the percentage of injected dose per gram of tissue (%ID/g) in mouse organs, using vendor software (Inveon Research Workplace [IRW]) on decay-corrected whole-body images.

After the last PET imaging at 16 days p.i., all major organs and tissues were collected and wet-weighted. The radioactivity of each tissue and organ was measured using a gamma counter and calculated as %ID/g.

Tumor Models.

4T1 tumor-bearing BALB/c mice were established and used for *in vivo* tumor magnetic target PET imaging: 4T1 cells (10^6 in 50 μL PBS) were subcutaneously injected into the bilateral rear legs, respectively. Once the tumors reached the required volume, the tumor-bearing mice were used for imaging or therapy.

In Vivo Tumor Magnetic Targeted PET Imaging of ^{89}Zr -MNP-PEG.

Bilateral 4T1 tumor-bearing BALB/c mice with tumor volume about 150 mm^3 were used for *in vivo* tumor magnetic target PET imaging. About 100 μL (~100 μCi or 3.7 MBq) of ^{89}Zr -MNP-PEG in PBS was intravenously injected into 4T1 tumor-bearing mice (n = 4). Afterward, the tumors on the right were attached to a strong magnet (Neodymium rare earth permanent magnet with grade N42 magnetic energy, diameter around 10 mm) for 3 h while the left was not. PET scans at various time points p.i. were conducted by using a microPET/CT Inveon small animal scanner (Siemens Medical Solutions USA, Inc.). ROI analysis of both side tumors on each PET images was performed to calculate the in tumors (percentage of injected dose per gram of tissue, %ID/g) values, using vendor software (Inveon Research Workplace [IRW]) on decay-corrected whole-body images.

Biodistribution studies were performed to confirm the quantitative uptake obtained through PET imaging. After the last *in vivo* PET imaging at 3 days p.i., all major tissues and organs were collected and wet-weighted. The major organs (heart, liver, spleen, lung and kidneys)

and tumors on both sides were scanned by PET. Finally, the radioactivity in each tissue and organ was measured using a gamma counter and calculated as %ID/g.

Conjugating MNPs with TCPP and Radiolabeling.

For the TCPP conjugation, the amine group on the surface of MNP-PEG was covalently conjugated with the carboxyl group of TCPP by using cross-linking reagents EDC and NHS. Adequate amounts of EDC (125 μmol) and NHS (210 μmol) were added to the water, followed by adding TCPP (~ 2 μmol , dispersed in DMSO), and sonicated at room temperature for 10 min. Afterward, 15 mg MNPs-PEG was added and adjusted the pH to 8.0 using 0.1 M Na_2CO_3 . The mixture was incubated at room temperature with slow stirring for 2 h. Then, excess EDC, NHS and TCPP were removed by repeatedly washing nanoparticles with deionized water at least 2 times (15000 r/min, 20 min). The final MNP/TCPP was dispersed in water or PBS for future application. For ^{89}Zr -MNP/TCPP radiolabeling, ^{89}Zr -MNP-PEG were used for TCPP conjugation and followed by the above-mentioned process. The stability was monitored and quantified by using TLC with subsequent autoradiography.

Singlet Oxygen Detection.

In brief, 100 μL of ^{89}Zr -MNP/TCPP with a different activity (25, 50 and 100 μCi) was added into a black 96-well cell culture plate ($n = 4$). 100 μL of free ^{89}Zr (100 μCi) was used as the control. Then, 5 μL of SOSG (0.5 μM , Molecular Probes, USA) was added to each well. The plate was read to measure the fluorescence intensity of SOSG of each well at a different time within 24 h with an excitation wavelength of 494 nm.

Cell Culture and Cytotoxicity Assessment.

Murine breast cancer 4T1 cells were cultured at 37 $^{\circ}\text{C}$ and with 5% CO_2 in Roswell Park Memorial Institute medium (RPMI) 1640 supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were seeded into 96-well cell culture plates at 10^4 /well and then incubated for 24 h at 37 $^{\circ}\text{C}$ under 5% CO_2 . Culture media solutions of MNP-PEG or MNP/TCPP with different concentrations (0, 8, 16, 32, 62.5, 125, 250 and 500 μg per mL) were added to the wells. The cells were then incubated for 24 or 48 h at 37 $^{\circ}\text{C}$ under 5% CO_2 , and the cell viability was detected by MTT assay.

In Vitro Cerenkov Radiation-Induced Photodynamic Therapy.

4T1 cells were seeded into a 96-well culture plate at a density of 10^4 /well and then incubated at 37 $^{\circ}\text{C}$ under 5% CO_2 for 24 h prior to treatment ($n=4$). The ^{89}Zr -MNP/TCPP were dispersed in RPMI 1640 media with a different activity (10, 20, 40 and 80 μCi) were then added into the wells. Cells without any treatment were used as control group. After incubation for 24 or 48 h in the dark, the cell viability was measured using a typical MTT assay.

For ROS staining in live cells, a final concentration of 5 μM of fluorogenic probes (CellROX Oxidative Stress Reagents) and 5 μM of Hoechst 33324 (Invitrogen) was added to the cells and incubated for 0.5 h at 37 $^{\circ}\text{C}$ in the dark, after the aforementioned treatment of cells with ^{89}Zr -MNP/TCPP (100 μCi) after 24 h. After washing with PBS, phenol red-free DMEM

medium was added and the fluorescence images were obtained with a Nikon A1RS confocal microscope.

***In Vivo* PET Imaging of ^{89}Zr -MNP/TCPP.**

The BALB/c mice were anesthetized and intravenously injected with 150 μL ($\sim 200 \mu\text{Ci}$ or 7.4 MBq) of ^{89}Zr -MNP/TCPP in PBS ($n = 4$). Serial PET scans were performed at various time points p.i. from 1 day to 16 days. ROI analysis of each PET scan was conducted to calculate the percentage of injected dose per gram of tissue (%ID/g) in mouse organs, using vendor software (Inveon Research Workplace [IRW]) on decay-corrected whole-body images.

***In Vivo* Tumor Magnetic Targeted Imaging and Therapy of ^{89}Zr -MNP/TCPP.**

For therapeutic studies, about 200 μL of ^{89}Zr -MNP/TCPP (14.8 MBq, $\sim 3.5 \text{ mg/mL}$ of MNP/TCPP) was intravenously injected into the tail vein of bilateral 4T1 tumor-bearing mice with tumor volume about 50–70 mm^3 ($n = 4$). As a comparison, the same dose of MNP/TCPP (200 μL , $\sim 3.5 \text{ mg/mL}$) was also intravenously injected into bilateral 4T1 tumor-bearing mice ($n = 4$). Afterward, the tumors on the right side were attached to a strong magnet for 3 h while the left was not. For CR-induced therapy using large-sized tumor, the mice with bilateral 4T1 tumor of about 150–200 mm^3 volume were used. Serial fluorescence imaging (Ex, 640 nm; Em, 720 nm), Cerenkov luminescence (CL) imaging (Ex, close; Em, open), and Cerenkov radiation energy transfer (CRET) imaging (Ex, close; Em, 740 nm) were carried out at 3 h, 1 day, 4 days, 8 days and 14 days p.i. At the end of the last scans at 14 day p.i., the mice were euthanized and tumor tissues were harvested for hematoxylin-eosin (H&E) staining to compare the therapeutic efficacy of different treatment groups. The tumor volumes after treatments were recorded every other day for 2 weeks using caliper measurements. Relative tumor volume (V/V_0 , where V_0 represents the initial tumor volume, i.e., day 0), body weight and tumor appearance were monitored.

Toxicity Analysis *in Vivo*.

Most of ^{89}Zr -MNP/TCPP was accumulated in reticuloendothelial system (RES) organs (e.g., liver and spleen). The *in vivo* biocompatibility of ^{89}Zr -MNP/TCPP was evaluated using standard H&E staining. BALB/c mice were euthanized at 2, 7, 16, 30 days, and 60 days post intravenous injection of $\sim 200 \mu\text{L}$ ^{89}Zr -MNP/TCPP (14.8 MBq, $\sim 3.5 \text{ mg/mL}$). Tissues were H&E-stained to monitor the histological changes in the heart, liver, spleen, lung and kidney of mice. The histological sections were observed under an inverted optical microscope (Nikon, Eclipse Ti-U, Japan).

Statistical Analysis.

Statistical comparisons were performed by using a Student's two-tailed t test. Quantitative data were expressed as mean $\pm$ s.d, *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was supported, in part, by the University of Wisconsin–Madison, the National Institutes of Health (P30CA014520), the National Natural Science Foundation of China (Grant No. 51702349), and Shanghai Yangfan Program (16YF1412800).

REFERENCES

- (1). Vankayala R; Hwang KC Near-Infrared-Light-Activatable Nanomaterial-Mediated Phototheranostic Nanomedicines: An Emerging Paradigm for Cancer Treatment. *Adv. Mater.* 2018, 30, 1706320.
- (2). Fan W; Huang P; Chen X Overcoming the Achilles' Heel of Photodynamic Therapy. *Chem. Soc. Rev.* 2016, 45, 6488–6519. [PubMed: 27722560]
- (3). Lucky SS; Soo KC; Zhang Y Nanoparticles in Photodynamic Therapy. *Chem. Rev.* 2015, 115, 1990–2042. [PubMed: 25602130]
- (4). Liu YY; Liu Y; Bu WB; Cheng C; Zuo CJ; Xiao QF; Sun Y; Ni DL; Zhang C; Liu JA; Shi JL Hypoxia Induced by Upconversion-Based Photodynamic Therapy: Towards Highly Effective Synergistic Bioreductive Therapy in Tumors. *Angew. Chem., Int. Ed.* 2015, 54, 8105–8109.
- (5). Ai X; Ho CJH; Aw J; Attia ABE; Mu J; Wang Y; Wang X; Wang Y; Liu X; Chen H; Gao M; Chen X; Yeow EKL; Liu G; Olivo M; Xing B In Vivo Covalent Cross-Linking of Photon-Converted Rare-Earth Nanostructures for Tumour Localization and Theranostics. *Nat. Commun.* 2016, 7, 10432. [PubMed: 26786559]
- (6). Huang L; Li Z; Zhao Y; Zhang Y; Wu S; Zhao J; Han G Ultralow-Power near Infrared Lamp Light Operable Targeted Organic Nanoparticle Photodynamic Therapy. *J. Am. Chem. Soc.* 2016, 138, 14586–14591. [PubMed: 27786443]
- (7). Zhu H; Li J; Qi X; Chen P; Pu K Oxygenic Hybrid Semiconducting Nanoparticles for Enhanced Photodynamic Therapy. *Nano Lett.* 2018, 18, 586–594. [PubMed: 29220576]
- (8). Zhang C; Zhao K; Bu W; Ni D; Liu Y; Feng J; Shi J Marriage of Scintillator and Semiconductor for Synchronous Radiotherapy and Deep Photodynamic Therapy with Diminished Oxygen Dependence. *Angew. Chem. Int. Ed.* 2015, 54, 1770–1774.
- (9). Kamkaew A; Chen F; Zhan Y; Majewski RL; Cai W Scintillating Nanoparticles as Energy Mediators for Enhanced Photodynamic Therapy. *ACS Nano* 2016, 10, 3918–3935. [PubMed: 27043181]
- (10). Kamkaew A; Cheng L; Goel S; Valdovinos HF; Barnhart TE; Liu Z; Cai W Cerenkov Radiation Induced Photodynamic Therapy Using Chlorin e6-Loaded Hollow Mesoporous Silica Nanoparticles. *ACS Appl. Mater. Interfaces* 2016, 8, 26630–26637. [PubMed: 27657487]
- (11). Goel S; Ferreira CA; Chen F; Ellison PA; Siamof CM; Barnhart TE; Cai W Activatable Hybrid Nanotheranostics for Tetramodal Imaging and Synergistic Photothermal/Photodynamic Therapy. *Adv. Mater.* 2018, 30, 1704367.
- (12). Sun X; Huang X; Guo J; Zhu W; Ding Y; Niu G; Wang A; Kiesewetter DO; Wang ZL; Sun S; Chen X Self-Illuminating ^{64}Cu -Doped CdSe/Zns Nanocrystals for in Vivo Tumor Imaging. *J. Am. Chem. Soc.* 2014, 136, 1706–1709. [PubMed: 24401138]
- (13). Kotagiri N; Sudlow GP; Akers WJ; Achilefu S Breaking the Depth Dependency of Phototherapy with Cerenkov Radiation and Low-Radiance-Responsive Nanophotosensitizers. *Nat. Nanotechnol.* 2015, 10, 370–379. [PubMed: 25751304]
- (14). Shaffer TM; Pratt EC; Grimm J Utilizing the Power of Cerenkov Light with Nanotechnology. *Nat. Nanotechnol.* 2017, 12, 106–117. [PubMed: 28167827]
- (15). Kotagiri N; Cooper ML; Rettig M; Egbulefu C; Prior J; Cui G; Karmakar P; Zhou M; Yang X; Sudlow G; Marsala L; Chanswangphuwana C; Lu L; Habimana-Griffin L; Shokeen M; Xu X; Weilbaecher K; Tomasson M; Lanza G; DiPersio JF; Achilefu S Radionuclides Transform Chemotherapeutics into Phototherapeutics for Precise Treatment of Disseminated Cancer. *Nat. Commun.* 2018, 9, 275. [PubMed: 29348537]
- (16). Thorek DL; Ogirala A; Beattie BJ; Grimm J Quantitative Imaging of Disease Signatures through Radioactive Decay Signal Conversion. *Nat. Med.* 2013, 19, 1345–1350. [PubMed: 24013701]

- (17). Kotagiri N; Niedzwiedzki DM; Ohara K; Achilefu S Activatable Probes Based on Distance-Dependent Luminescence Associated with Cerenkov Radiation. *Angew. Chem., Int. Ed.* 2013, 52, 7756–7760.
- (18). Zhou C; Hao G; Thomas P; Liu J; Yu M; Sun S; Oz OK; Sun X; Zheng J Near-Infrared Emitting Radioactive Gold Nanoparticles with Molecular Pharmacokinetics. *Angew. Chem., Int. Ed.* 2012, 51, 10118–10122.
- (19). Duan D; Liu H; Xu Y; Han Y; Xu M; Zhang Z; Liu Z Activating TiO₂ Nanoparticles: Gallium-68 Serves as a High-Yield Photon Emitter for Cerenkov-Induced Photodynamic Therapy. *ACS Appl. Mater. Interfaces* 2018, 10, 5278–5286. [PubMed: 29368518]
- (20). Pratz G; Kapp DS Is Cerenkov Luminescence Bright Enough for Photodynamic Therapy? *Nat. Nanotechnol.* 2018, 13, 354.
- (21). Iyer AK; Khaled G; Fang J; Maeda H Exploiting the Enhanced Permeability and Retention Effect for Tumor Targeting. *Drug Discovery Today* 2006, 11, 812–818. [PubMed: 16935749]
- (22). Weissleder R; Mahmood U Molecular Imaging. *Radiology* 2001, 219, 316–333. [PubMed: 11323453]
- (23). Bjornmalm M; Thurecht KJ; Michael M; Scott AM; Caruso F Bridging Bio-Nano Science and Cancer Nanomedicine. *ACS Nano* 2017, 11, 9594–9613. [PubMed: 28926225]
- (24). Wei W; Ni D; Ehlerding EB; Luo Q-Y; Cai W Pet Imaging of Receptor Tyrosine Kinases in Cancer. *Mol. Cancer Ther.* 2018, 17, 1625–1636. [PubMed: 30068751]
- (25). Mikhaylov G; Mikac U; Magaeva AA; Itin VI; Naiden EP; Psakhye I; Babes L; Reinheckel T; Peters C; Zeiser R; Bogoyo M; Turk V; Psakhye SG; Turk B; Vasiljeva O Ferri-Liposomes as an MRI-Visible Drug-Delivery System for Targeting Tumours and Their Microenvironment. *Nat. Nanotechnol.* 2011, 6, 594–602. [PubMed: 21822252]
- (26). Yan H; Teh C; Sreejith S; Zhu L; Kwok A; Fang W; Ma X; Nguyen KT; Korzh V; Zhao Y Functional Mesoporous Silica Nanoparticles for Photothermal-Controlled Drug Delivery in Vivo. *Angew. Chem., Int. Ed.* 2012, 51, 8373–8377.
- (27). Kinoshita M; McDannold N; Jolesz FA; Hynynen K Noninvasive Localized Delivery of Herceptin to the Mouse Brain by MRI-Guided Focused Ultrasound-Induced Blood-Brain Barrier Disruption. *Proc. Natl. Acad. Sci. U. S. A.* 2006, 103, 11719–11723. [PubMed: 16868082]
- (28). von Maltzahn G; Park JH; Lin KY; Singh N; Schwoppe C; Mesters R; Berdel WE; Ruoslahti E; Sailor MJ; Bhatia SN Nanoparticles That Communicate in Vivo to Amplify Tumour Targeting. *Nat. Mater.* 2011, 10, 545–552. [PubMed: 21685903]
- (29). Huang J; Li Y; Orza A; Lu Q; Guo P; Wang L; Yang L; Mao H Magnetic Nanoparticle Facilitated Drug Delivery for Cancer Therapy with Targeted and Image-Guided Approaches. *Adv. Funct. Mater.* 2016, 26, 3818–3836. [PubMed: 27790080]
- (30). Namiki Y; Namiki T; Yoshida H; Ishii Y; Tsubota A; Koido S; Nariai K; Mitsunaga M; Yanagisawa S; Kashiwagi H; Mabashi Y; Yumoto Y; Hoshina S; Fujise K; Tada N A Novel Magnetic Crystal-Lipid Nanostructure for Magnetically Guided in Vivo Gene Delivery. *Nat. Nanotechnol.* 2009, 4, 598–606. [PubMed: 19734934]
- (31). Yang K; Liu Y; Zhang Q; Kong C; Yi C; Zhou Z; Wang Z; Zhang G; Zhang Y; Khashab NM; Chen X; Liu Y; Nie Z Cooperative Assembly of Magneto-Nanovesicles with Tunable Wall Thickness and Permeability for MRI-Guided Drug Delivery. *J. Am. Chem. Soc.* 2018, 140, 4666–4677. [PubMed: 29543442]
- (32). Zhang F; Braun GB; Pallaoro A; Zhang Y; Shi Y; Cui D; Moskovits M; Zhao D; Stucky GD Mesoporous Multifunctional Upconversion Luminescent and Magnetic “Nanorattle” Materials for Targeted Chemotherapy. *Nano Lett.* 2012, 12, 61–67. [PubMed: 22133237]
- (33). Xuan M; Shao J; Zhao J; Li Q; Dai L; Li J Magnetic Mesoporous Silica Nanoparticles Cloaked by Red Blood Cell Membranes: Applications in Cancer Therapy. *Angew. Chem., Int. Ed.* 2018, 57, 6049–6053.
- (34). Huang P; Li Z; Lin J; Yang D; Gao G; Xu C; Bao L; Zhang C; Wang K; Song H Photosensitizer-Conjugated Magnetic Nanoparticles for in Vivo Simultaneous Magnetofluorescent Imaging and Targeting Therapy. *Biomaterials* 2011, 32, 3447–3458. [PubMed: 21303717]

- (35). Yu J; Ju Y; Zhao L; Chu X; Yang W; Tian Y; Sheng F; Lin J; Liu F; Dong Y; Hou Y Multistimuli-Regulated Photo-chemothermal Cancer Therapy Remotely Controlled via Fe₅C₂ Nanoparticles. *ACS Nano* 2016, 10, 159–169. [PubMed: 26602632]
- (36). Zhou Z; Sun Y; Shen J; Wei J; Yu C; Kong B; Liu W; Yang H; Yang S; Wang W Iron/Iron Oxide Core/Shell Nanoparticles for Magnetic Targeting MRI and Near-Infrared Photothermal Therapy. *Biomaterials* 2014, 35, 7470–7478. [PubMed: 24881997]
- (37). Li Z; Yin S; Cheng L; Yang K; Li Y; Liu Z Magnetic Targeting Enhanced Theranostic Strategy Based on Multimodal Imaging for Selective Ablation of Cancer. *Adv. Funct. Mater.* 2014, 24, 2312–2321.
- (38). Wilson MW; Kerlan RK, Jr.; Fidelman NA; Venook AP; LaBerge JM; Koda J; Gordon RL Hepatocellular Carcinoma: Regional Therapy with a Magnetic Targeted Carrier Bound to Doxorubicin in a Dual MR Imaging/Conventional Angiography Suite-Initial Experience with Four Patients. *Radiology* 2004, 230, 287–293. [PubMed: 14695402]
- (39). Lübke AS; Bergemann C; Riess H; Schriever F; Reichardt P; Possinger K; Matthias M; Dörken B; Herrmann F; Gürtler R Clinical Experiences with Magnetic Drug Targeting: A Phase I Study with 4'-Epidoxorubicin in 14 Patients with Advanced Solid Tumors. *Cancer Res.* 1996, 56, 4686–4693. [PubMed: 8840985]
- (40). Lubbe AS; Alexiou C; Bergemann C Clinical Applications of Magnetic Drug Targeting. *J. Surg. Res.* 2001, 95, 200–206. [PubMed: 11162046]
- (41). Jang JT; Nah H; Lee JH; Moon SH; Kim MG; Cheon J Critical Enhancements of MRI Contrast and Hyperthermic Effects by Dopant-Controlled Magnetic Nanoparticles. *Angew. Chem., Int. Ed.* 2009, 48, 1234–1238.
- (42). Lee JH; Huh YM; Jun YW; Seo JW; Jang JT; Song HT; Kim S; Cho EJ; Yoon HG; Suh JS; Cheon J Artificially Engineered Magnetic Nanoparticles for Ultra-Sensitive Molecular Imaging. *Nat. Med.* 2007, 13, 95–99. [PubMed: 17187073]
- (43). Ni D; Jiang D; Ehlerding EB; Huang P; Cai W Radiolabeling Silica-Based Nanoparticles via Coordination Chemistry: Basic Principles, Strategies, and Applications. *Acc. Chem. Res.* 2018, 51, 778–788. [PubMed: 29489335]
- (44). Li LL; Zhang R; Yin L; Zheng K; Qin W; Selvin PR; Lu Y Biomimetic Surface Engineering of Lanthanide-Doped Upconversion Nanoparticles as Versatile Bioprobes. *Angew. Chem., Int. Ed.* 2012, 51, 6121–6125.
- (45). Ni D; Ehlerding EB; Cai W Multimodality Imaging Agents with Pet as the Fundamental Pillar. *Angew. Chem., Int. Ed.* 2018, DOI: 10.1002/anie.201806853.
- (46). Ni D; Jiang D; Valdovinos HF; Ehlerding EB; Yu B; Barnhart TE; Huang P; Cai W Bioresponsive Polyoxometalate Cluster for Redox-Activated Photoacoustic Imaging-Guided Photo-thermal Cancer Therapy. *Nano Lett.* 2017, 17, 3282–3289. [PubMed: 28418679]
- (47). Cheng L; Shen S; Jiang D; Jin Q; Ellison PA; Ehlerding EB; Goel S; Song G; Huang P; Barnhart TE; Liu Z; Cai W Chelator-Free Labeling of Metal Oxide Nanostructures with Zirconium-89 for Positron Emission Tomography Imaging. *ACS Nano* 2017, 11, 12193–12201. [PubMed: 29178789]
- (48). Tsoi KM; MacParland SA; Ma XZ; Spetzler VN; Echeverri J; Ouyang B; Fadel SM; Sykes EA; Goldaracena N; Kathis JM; Conneely JB; Alman BA; Selzner M; Ostrowski MA; Adeyi OA; Zilman A; McGilvray ID; Chan WC Mechanism of Hard-Nanomaterial Clearance by the Liver. *Nat. Mater.* 2016, 15, 1212–1221. [PubMed: 27525571]
- (49). Cheng L; Jiang D; Kamkaew A; Valdovinos HF; Im HJ; Feng L; England CG; Goel S; Barnhart TE; Liu Z; Cai W Renal-Clearable Pegylated Porphyrin Nanoparticles for Image-Guided Photodynamic Cancer Therapy. *Adv. Funct. Mater.* 2017, 27, 1702928. [PubMed: 29151826]
- (50). Li B; Wang X; Chen L; Zhou Y; Dang W; Chang J; Wu C Ultrathin Cu-TCP MOF Nanosheets: A New Theragnostic Nanoplatform with Magnetic Resonance/Near-Infrared Thermal Imaging for Synergistic Phototherapy of Cancers. *Theranostics* 2018, 8, 4086–4096. [PubMed: 30128038]
- (51). Park J; Jiang Q; Feng D; Mao L; Zhou HC Size-Controlled Synthesis of Porphyrinic Metal-Organic Framework and Functionalization for Targeted Photodynamic Therapy. *J. Am. Chem. Soc.* 2016, 138, 3518–3525. [PubMed: 26894555]

- (52). Liu TW; MacDonald TD; Shi J; Wilson BC; Zheng G Intrinsically Copper-64-Labeled Organic Nanoparticles as Radiotracers. *Angew. Chem., Int. Ed.* 2012, 51, 13128–13131.
- (53). Pratt EC; Shaffer TM; Zhang Q; Drain CM; Grimm J Nanoparticles as Multimodal Photon Transducers of Ionizing Radiation. *Nat. Nanotechnol* 2018, 13, 418–426. [PubMed: 29581551]
- (54). Ma Y; Li X; Li A; Yang P; Zhang C; Tang B H₂S-Activable Mof Nanoparticle Photosensitizer for Effective Photodynamic Therapy against Cancer with Controllable Singlet-Oxygen Release. *Angew. Chem., Int. Ed.* 2017, 56, 13752–13756.
- (55). Sarkar S; Ha YS; Soni N; An GI; Lee W; Kim MH; Huynh PT; Ahn H; Bhatt N; Lee YJ; Kim JY; Park KM; Ishii I; Kang SG; Yoo J Immobilization of the Gas Signaling Molecule H₂S by Radioisotopes: Detection, Quantification, and in Vivo Imaging. *Angew. Chem., Int. Ed.* 2016, 55, 9365–9370.
- (56). Xu G; Yan Q; Lv X; Zhu Y; Xin K; Shi B; Wang R; Chen J; Gao W; Shi P; Fan C; Zhao C; Tian H Imaging of Colorectal Cancers Using Activatable Nanoprobes with Second Near-Infrared Window Emission. *Angew. Chem., Int. Ed.* 2018, 57, 3626–3630.
- (57). Xing H; Zheng X; Ren Q; Bu W; Ge W; Xiao Q; Zhang S; Wei C; Qu H; Wang Z; Hua Y; Zhou L; Peng W; Zhao K; Shi J Computed Tomography Imaging-Guided Radiotherapy by Targeting Upconversion Nanocubes with Significant Imaging and Radio sensitization Enhancements. *Sci. Rep.* 2013, 3, 1751. [PubMed: 23624542]
- (58). Ni D; Zhang J; Bu W; Xing H; Han F; Xiao Q; Yao Z; Chen F; He Q; Liu J; Zhang S; Fan W; Zhou L; Peng W; Shi J Dual-Targeting Upconversion Nanoprobes across the Blood-Brain Barrier for Magnetic Resonance/Fluorescence Imaging of Intracranial Glioblastoma. *ACS Nano* 2014, 8, 1231–1242. [PubMed: 24397730]
- (59). Ni D; Bu W; Zhang S; Zheng X; Li M; Xing H; Xiao Q; Liu Y; Hua Y; Zhou L; Peng W; Zhao K; Shi J Single Ho³⁺-Doped Upconversion Nanoparticles for High-Performance 2-Weighted Brain Tumor Diagnosis and MR/UCL/CT Multimodal Imaging. *Adv. Funct. Mater.* 2014, 24, 6613–6620.
- (60). Boros E; Bowen AM; Josephson L; Vasdev N; Holland JP Chelate-Free Metal Ion Binding and Heat-Induced Radiolabeling of Iron Oxide Nanoparticles. *Chem. Sci.* 2015, 6, 225–236. [PubMed: 28553472]

Figure 1.

(a) Schematic illustration of magnetism-enhanced Cerenkov radiation (CR)-induced PDT. TEM images of MNPs (b) before and (c) after transferring into the aqueous solution. Scale bar: 20 nm. (d) Field-dependent magnetization hysteresis loop of the MNPs at 300 K. Inset: photograph showing the ferrofluidic behavior of MNP-PEG in PBS. (e) Time-dependent ^{89}Zr -labeling yields of MNP-PEG at 37 and 75 °C ($n = 3$, mean $\pm$ s.d.). Inset: autoradiographic image of thin layer chromatography (TLC) plates of ^{89}Zr -MNP-PEG. Free ^{89}Zr was used as a control.

Figure 2.

(a) Representative MIP PET images of healthy BALB/c mice at a various time point after intravenous injection of ^{89}Zr -MNP-PEG. (b) Time-activity curves of ^{89}Zr -MNP-PEG in the blood ($n = 4$, mean $\pm$ s.d.). H, heart; L, liver; S, spleen. (c) Quantification of ^{89}Zr -MNP-PEG uptake in the blood, liver, spleen, kidney and muscle at various time point p.i. ($n = 4$, mean $\pm$ s.d.). (d) Schematic illustration of bilateral 4T1 tumor-bearing mice with external magnetic field attaching onto the right tumor side for 3 h. (e) Representative coronal PET imaging slices of bilateral 4T1 tumorbearing mice after intravenous injection of ^{89}Zr -MNP-PEG at different time points. (f) Quantification uptake of ^{89}Zr -MNP-PEG in the tumor from both sides at various time points p.i. ($n = 4$, mean $\pm$ s.d.). MF: magnetic field. (g) Photograph and (h) PET imaging of main organs of bilateral 4T1 tumor-bearing mice at 72 h p.i. of ^{89}Zr -MNP-PEG.

Figure 3.

(a) Adsorption spectra of MNP/TCPP (black), MNP-PEG (blue), and free TCPP (red). (b) Photograph showing the ferrofluidic behavior of MNP/TCPP in PBS. (c) Time-dependent stability of ^{89}Zr -MNP/TCPP at room temperature ($n = 3$, mean $\pm$ s.d.). Inset: autoradiographic image of thin layer chromatography (TLC) plates of ^{89}Zr -MNP/TCPP. Free ^{89}Zr was used as a control. Representative (d) FL, (e) CL and (f) CRET image of different solution samples. FL, fluorescence; CL, Cerenkov luminescence; CRET, Cerenkov resonance energy transfer.

Figure 4.

(a) Generation of singlet oxygen by measuring the fluorescence intensity changes of SOSG from different samples. ($n = 4$, mean $\pm$ s.d.). (b) Relative viabilities of 4T1 cells after incubating with $^{89}\text{Zr-MNP/TCPP}$ for CR-induced PDT at varied concentrations of radioactivity ($n = 4$, mean $\pm$ s.d., $**P < 0.01$, $***P < 0.001$). (c) Representative confocal images of 4T1 cells after incubation with $^{89}\text{Zr-MNP/TCPP}$ or MNP/TCPP for 24 h. Scale bar: 50 μm .

Figure 5.

(a) Magnetic-targeted FL, CL and CRET imaging of bilateral 4T1 tumor-bearing mice after intravenous injection of ^{89}Zr -MNP/TCPP at different time points. The external magnetic field was attached to the right tumor side after injection for 3 h. Red arrows indicate nonmagnetic targeting tumors while green arrows point out magnetic targeting tumors. FL, fluorescence; CL, Cerenkov luminescence; CRET, Cerenkov resonance energy transfer. (b) Representative photographs of mice and dissected tumors at 14 days post-treatments. Scale bar: 5 mm. Red arrows indicate nonmagnetic targeting tumors while green arrows point out magnetic targeting tumors. (c) H&E staining of tumor sections after various treatments. Scale bar: 100 μm . (d) Tumor growth profiles of 4T1 tumors after each treatment (n = 4, mean $\pm$ s.d., *** P < 0.001).