Chem Soc Rev

REVIEW ARTICLE

View Article Online
View Journal

Cite this: DOI: 10.1039/d0cs00215a

Emerging combination strategies with phototherapy in cancer nanomedicine

Zhongjian Xie,†[ab] Taojian Fan,†[a] Jusung An,†[c] Wonseok Choi,†[c] Yanhong Duo,†[a] Yanqi Ge,†[a] Bin Zhang,[a] Guohui Nie,[a] Ni Xie,[a] Tingting Zheng,[d] Yun Chen,[d] Han Zhang *[a] and Jong Seung Kim *[c]

Optical techniques using developed laser and optical devices have made a profound impact on modern medicine, with "biomedical optics" becoming an emerging field. Sophisticated technologies have been developed in cancer nanomedicine, such as photothermal therapy and photodynamic therapy, among others. However, single-mode phototherapy cannot completely treat persistent tumors, with the challenges of relapse or metastasis remaining; therefore, combinatorial strategies are being developed. In this review, the role of light in cancer therapy and the challenges of phototherapy are discussed. The development of combinatorial strategies with other therapeutic methods, including chemotherapy, immunotherapy, gene therapy, and radiotherapy, is presented and future directions are further discussed. This review aims to highlight the significance of light in cancer therapy and discuss the combinatorial strategies that show promise in addressing the challenges of phototherapy.

Received 8th March 2020

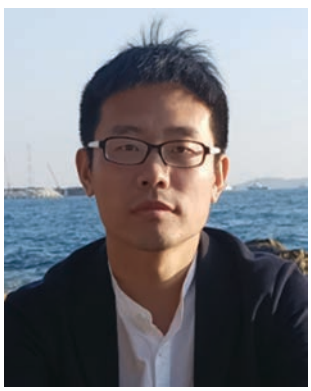
DOI: 10.1039/d0cs00215a

rsc.li/chem-soc-rev

1. Introduction

The use of light in modern medicine was introduced in the 19th century. Based on the advances in our understanding of the physical nature of light and light–matter interactions, medical technologies have been developed in parallel. A representative example of an early achievement in light-induced therapy is the treatment of lupus vulgaris with ultraviolet (UV) light, which was discovered by physician Niels Finsen, who was awarded the Nobel Prize in Physiology or Medicine of 1903 for this discovery. A notable milestone in the 1960s was the treatment of severe hyperbilirubinemia using blue-light phototherapy, which subsequently cured millions of infants with this condition.[1]

[a] Key Laboratory of Optoelectronic Devices and Systems of Ministry of Education and Guangdong Province, College of Physics and Optoelectronic Engineering, and Otolaryngology Department and Biobank of the First Affiliated Hospital, Shenzhen Second People's Hospital, Health Science Center, Shenzhen University, Shenzhen 518060, P. R. China. E-mail: hzhang@szu.edu.cn
[b] Shenzhen International Institute for Biomedical Research, 518116 Shenzhen, Guangdong, China
[c] Department of Chemistry, Korea University, Seoul 02841, Korea. E-mail: jongskim@korea.ac.kr
[d] Shenzhen Key Laboratory for Drug Addiction and Medication Safety, Department of Ultrasound, Peking University Shenzhen Hospital, Shenzhen, 518036, P. R. China
† These authors contributed equally to this work.



Zhongjian Xie

Zhongjian Xie received his PhD from the University of Lyon, France, in 2016. Then, he joined Prof. Han Zhang's lab in Shenzhen University as a post doc. Now, he is a full professor in the Shenzhen International Institute for Biomedical Research (SIIBR). His research interests focus on the application of two-dimensional materials in biological and environmental applications.

Taojian Fan

Taojian Fan was born in Hubei, China, in 1995, and is pursuing his PhD degree under the guidance of Prof. Han Zhang in the Shenzhen Engineering Laboratory of Phosphorene and Optoelectronics, Shenzhen University. His current research interests focus on two-dimensional material based theranostic nanoplatforms for biomedical applications.

Presently, numerous laser-based diagnostic and therapeutic devices are widely used in clinical practice.

Recent efforts in nanomedicine have promoted the rapid development of light-induced theranostics based on versatile nanoagents with rich light-induced functions, including the conversion of near-infrared (NIR) to visible light, photodynamic therapy (PDT), photothermal therapy (PTT),[2] smart drug delivery,[3] and imaging, among others. Phototherapy is a minimally invasive and effective modality that provides a convenient method for ablating tumors using light irradiation in a clinically safe manner.[4,5]

High penetration into biological tissues is a prerequisite for phototherapy. NIR with wavelengths between 650 and 1350 nm has a relatively high tissue penetration capacity and is used to stimulate nanoagents with absorption in this region.[6] In case of photothermal conversion, the light absorbed by nanoagents can be converted to heat through plasmonic heating or non-radiative relaxation. In the photodynamic effect, reactive oxygen species (ROS) are released by intersystem crossing relaxation. PTT kills cells by temperature-dependent necrosis, while PDT uses apoptotic machineries. Earlier, phototherapy was based on the principle of direct ablation of tumor tissue. In recent times, immunogenic cell death has been noted as an interesting phenomenon that accompanies the direct destruction of tumors, wherein cell damage-associated molecular patterns are released, thereby enhancing the immunogenicity in the tumor microenvironment.[7] Despite the rapid progress and distinctive advantage of phototherapy, single-mode phototherapy techniques face several challenges, including limitations toward superficial tumor types, tumor cell targeting, relapse, and metastasis (Fig. 1). Fortunately, combination with chemotherapy, immunotherapy, gene therapy, and radiotherapy can effectively combat these challenges while maximizing the advantage from each therapeutic mode. This review summarizes the significant research progress in combinatorial strategies using phototherapy. First, phototherapeutic agents are introduced systematically. Next, the development of phototherapy is presented and the challenges of single-mode phototherapy

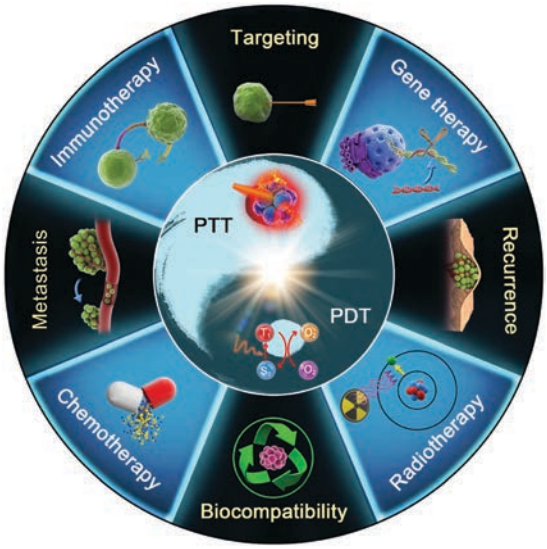


Fig. 1 Combination of different therapies with phototherapy.

Yanhong Duo

Yanhong Duo received her PhD from the Department of Life Sciences at Lanzhou University and the Division of Life and Health Sciences at Tsinghua University in 2017 (joint training). Currently she is a post doc in the Department of Microbiology, Tumor and Cell Biology, Karolinska Institute.

Yanqi Ge

Yanqi Ge was born in Jiangsu Province, China. She received her PhD degree in optical engineering from Shenzhen University, Shenzhen, China, in 2018. She is currently researching on two-dimensional materials, nonlinear optics, optoelectronics technology and mode locked fiber lasers in Shenzhen University.

Han Zhang

Han Zhang is a Full Professor and Director of the Shenzhen Engineering Laboratory of Phosphorene and Optoelectronics, Shenzhen University. He is an expert in low-dimensional optoelectronic devices and applications. He has published over 200 peer-reviewed research and invited review articles in the last 10 years. Related studies have been published in many prestigious journals such as PNAS, Physics Reports, and Nature Communications. All of his publications have received >27 000 citations, with an H-index of 85. He has been awarded as a Highly Cited Researcher by Clarivate Analytics in 2018 and 2019, and an OSA Fellow.

are discussed. Based on these challenges, combinatorial strategies using chemotherapy, immunotherapy, gene therapy, and radiotherapy are highlighted. Finally, we provide insights into the current research challenges and further directions in the use of combinatorial strategies.

2. Phototherapy agents for cancer therapy

Photosensitizers play an important role in phototherapy. Currently, clinically approved photosensitizers are mainly used in PDT. Hematoporphyrin derivatives (HpDs) and porfimer sodium are representative agents in first generation phototherapy agents (PTs). Porfimer sodium exhibits negligible dark toxicity, excellent PDT performance and great water solubility. However, the short excitation wavelength of porfimer sodium limits the PDT effect in deep tissues. In addition, the first generation PTs exhibit long retention times in normal tissue and skin. Therefore, patients need to avoid sunlight for a long time (about 6 weeks) after treatment. The disadvantages of the first generation PTs triggered further research on second generation PTs with near IR (NIR) activation and high production of $^1O_2$. Typically, these PTs are macrocyclic compounds derived from substitutions of the porphyrin moieties or direct modifications of the porphyrin core, or some new non-porphyrinoid photosensitizer molecules. The photosensitive skin window time of second generation PTs was also significantly reduced (<2 weeks). Although some of the early generation photosensitizers have been approved for clinical use, their shortcomings, like the low penetration depths and long retention times of first generation PTs and the low cell/tissue specificity associated with second generation photosensitizers, still hinder their use in clinical therapeutics. Over the past few years, extensive attention has been paid to the investigation of novel nanomaterial-based phototherapy and combined phototherapy. In this part, we mainly discuss novel nanomaterial-based PTs. They can be majorly divided into organic and inorganic agents. In this section, the advantages and disadvantages of these two kinds of agents are compared. For both PTs, surface modifications are required to address the biocompatibility needs and to cope with the complex biological environment. Their surface potential and reactivity can thus be altered, resulting in an enhanced dispersion ability and increased blood circulation times. Furthermore, their tumor site-targeting performance can be enhanced through surface modification to improve their treatment efficacy and avoid their side effects on unaffected organs.

2.1. Organic PTs

Organic PTs primarily include small molecules and semiconducting polymer-based nanoparticles (SPNs). Indocyanine green (ICG) is one of the most popular organic agents approved by the Food and Drug Administration (FDA). PTs are classified into PDT and PTT agents based on their mechanism of action, while organic PTs often share several common advantages and disadvantages. Firstly, organic PTs exhibit biocompatibility as well as, from a clinical viewpoint, long-term stability that results in favorable outcomes in pre-clinical trials. Additionally, their chemical and physical properties can easily be characterized by sophisticated molecular designs; this makes PTs link with tumor-targeting groups, which enables a better therapeutic effect against cancer cells. Moreover, the synthesis of prodrugs with covalent bonds makes organic PTs good combination therapeutic candidates. However, there remain several challenges in the use of organic PTs. Their heavy atom effect, enlarging intersystem crossing (ISC) constants and consequently affording high $^1O_2$ quantum yields, is closely linked to dark toxicity and low fluorescence issues. Because of the high dependence on the Type II PDT process for organic PTs, tumor hypoxia also contributes to one of the main challenges to develop organic PTs. In addition to this, aggregation-caused quenching (ACQ) occurs among most organic PTs under aqueous conditions with reduced fluorescence efficiency and $^1O_2$ quantum yields. This section is designed to cover selected strategies adopted in recent studies that made a significant impact on the limitations of organic PTs, thereby

Left to right, Jusung An, Jong Seung Kim and Wonseok Choi

Prof. Jong Seung Kim received his PhD from the Department of Chemistry and Biochemistry at Texas Tech University in 1993. He has one-year research experience at the University of Houston as a post doc fellow. Currently, he is a full professor in the Department of Chemistry at Korea University in Korea. His research records to date include 420 scientific publications and 80 domestic and international patents. He has been a member of the Korean Academy of Science and Technology since 2014.

Jusung An received his Bachelor's degree from the Department of Chemistry at Hallym University in Korea in 2018. He is currently pursuing his PhD degree under the supervision of Prof. Jong Seung Kim at Korea University. His current research interests are the development of organic-based photosensitizers and diagnosis agents for neurodegenerative disease.

Wonseok Choi received his BS degree from the Department of Chemistry at Chungnam National University in Korea in 2019. He is currently pursuing his master's degree under the supervision of Prof. Jong Seung Kim at Korea University. His research interests are focused on the development of chemical probes and cancer phototherapeutic agents.

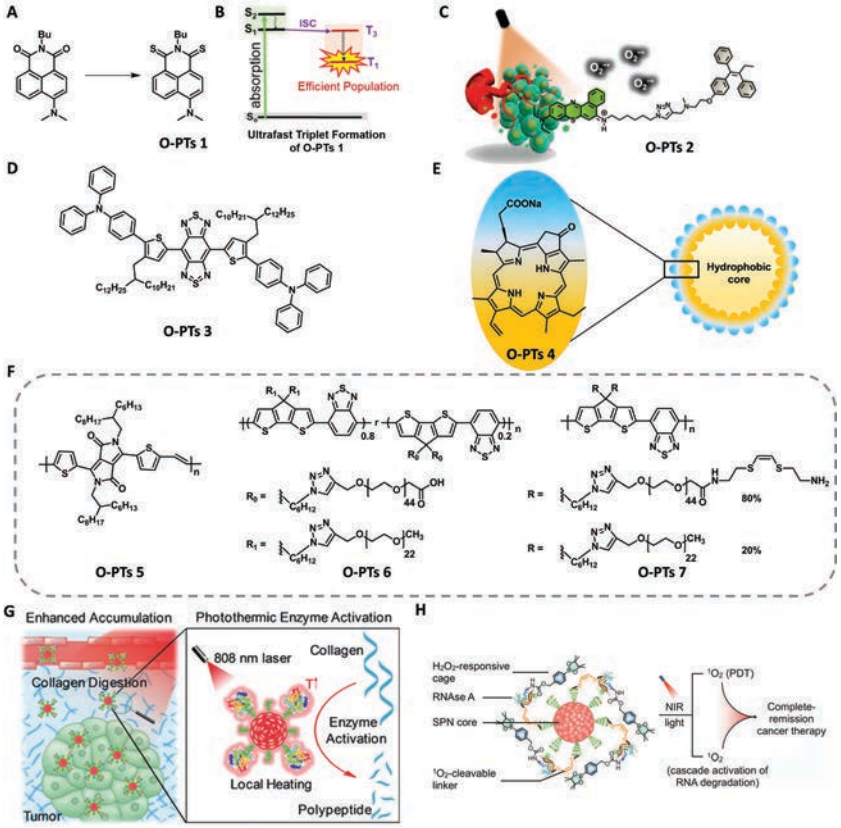


Fig. 2 (A) Molecular structure and formation of **O-PTs 1**. (B) Schematic illustration of the proposed triplet state formation mechanism of control of **O-PTs 1**. Reprinted with permission from ref. 8. Copyright 2019 American Chemical Society. (C) Illustration of the working mechanism of **O-PTs 2**. Reprinted with permission from ref. 9. Copyright 2020 American Chemical Society. (D) Molecular structure of **O-PTs 3**. (E) Nanoemulsion of **O-PTs 4**. (F) Molecular structures of SPNs. (G) Illustration of photothermally triggered enzyme activation of **O-PTs 6** toward collagen digestion. Reprinted with permission from ref. 16. Copyright 2018 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (H) Illustration of the proposed mechanism for the photoactivated synergistic therapeutic action of **O-PTs 7** including PDT and intracellular RNA degradation. Reprinted with permission from ref. 17. Copyright 2019 American Chemical Society.

providing useful alternatives for the role of organic PTs in combination therapy.

2.1.1. Small molecules. Recently, Yoon *et al.* reported a heavy atom free photosensitizer with high ROS generation efficiency.[8] They prepared an atomically-modified naphthalimide derivative, **O-PTs 1** (Fig. 2A), as the PDT reagent. Replacing the two oxygen atoms of naphthalimide with two sulfur atoms induced a large bathochromic shift compared to that of atomically-unmodified naphthalimide. With light irradiation, moreover, the generation of ROS *via* both Type I and Type II processes was subsequently enhanced (Fig. 2B). Consequently, **O-PTs 1** showed little cytotoxicity under dark conditions, whereas it worked well even under extremely hypoxic conditions. To further improve this research, PTs that can be activated *in vivo* should be investigated; however, the abovementioned strategy has the potential to be utilized in further studies.

In another study, Peng and his co-workers reported a similar strategy for PTs based on the Type I mechanism using a Nile Blue analogue.[9] **O-PTs 2** (Fig. 2C) is composed of a sulfur-substituted Nile Blue as an efficient $O_2^{\bullet-}$ generator and FDA approved antiestrogenic drug tamoxifen (TAM) to lower cellular O_2 consumption. Owing to TAM, the reduced endogenous O_2 consumption not only alleviates hypoxic tumor conditions but also meets PDT's high demand for O_2. Besides, its moderate NIR fluorescence emission makes it possible to diagnose tumor tissues accurately in a mouse model. Importantly, this report described a new concept in that overcoming tumor hypoxia resistance, whereas other reports focused on the simultaneous delivery of oxygen.

In another report, Tang *et al.*[10] reported an abnormal strategy to overcome ACQ and improved photothermal properties using the small molecule **O-PTs 3** (Fig. 2D) by utilizing the energetic stabilization of the dark twisted intramolecular charge transfer (TICT) state. The 2-decylmyristyl group introduced into the compound interfered with the heavy aggregation in an aqueous solvent. Thus, it provided triphenylamine (TPA) with sufficient space to function as a molecular rotor, leading to the generation of a dark TICT state. While the coplanar structures of normal donor–acceptor–donor (D–A–D) molecules limit their complete heat generation capacity due to the obstruction of the other nonradiative relaxation channels, increased photothermal performances are observed in small molecule **O-PTs 3** due to nonradiative decay *via* the dark TICT state.

Organic PTs could be potentially used in combination therapy by constructing a multifunctional platform. For example, in 2019, Zheng *et al.*[11] designed a porphyrin-based oil-in-water nanoemulsion based on the self-assembly of the pyropheophorbide-a monosodium salt **O-PTs 4** (Fig. 2E). While it could exert a photothermal effect in the intact state, the disrupted mono **O-PTs 4** exhibited a PDT effect. Owing to the oil core of the nanostructure, it can provide a system for drug delivery. The researchers encapsulated paclitaxel (PTX) in the core oil. In an exploratory study to assess its therapeutic effect, mice treated with PTX@nanoemulsion outperformed the control group with respect to tumor growth inhibition. By employing the self-assembling character of **O-PTs 4**, this organic PT could be equipped with phototherapy and drug delivery multimodality as well. Although the combined therapeutic effect was not discussed, it could be a promising strategy in the field of drug delivery systems (DDSs).

2.1.2. Semiconducting polymer-based nanoparticles. With outstanding optical properties, inert photophysical characteristics and biologically compatible composition, SPNs have been employed in various fields of study; their tailorable π-conjugated building blocks are well suited for combination therapy. Since recent literature reviews on SPNs are well organized for application of imaging and single modal phototherapy,[12–14] the purpose of this section is to offer strategies of the ways where recent development and combination therapies have been applied to SPNs.

Biodegradability is one of the important considerations in developing nanomaterials. Pu *et al.*[15] synthesized highly biodegradable SPNs, **O-PTs 5** (Fig. 2F), incorporating oxidizable vinylene bonds into the backbone. With activated myeloperoxidase (MPO) in biological systems, H_2O_2 can be transformed into HOCl, which is a strong oxidant. Thus, the oxidizable vinylene bonds in **O-PTs 5** could be digested. Importantly, the introduced vinylene group does not compensate for optical and phototherapeutic effects. The successful construction of biodegradable SPNs would be highly applicable for combination therapy.

In situ activation of nanoenzymes is highly desirable for cancer therapy. To fully take advantage of SPNs for cancer therapy, **O-PTs 6** (Fig. 2F) with bromelain (Bro) which is a collagen digesting protease was synthesized.[16] **O-PTs 6** could serve as a photothermal therapy agent and showed good photothermal heating efficiency. Importantly, because the enzymatic activity of Bro is optimal at around 45 °C, under NIR laser irradiation, triggered Bro starts to digest tumor extracellular-matrix collagen (Fig. 2G). As a result, **O-PTs 6**-based PTT with synergistic Bro activity was able to noticeably inhibit tumor growth. In a recent study of Pu's group,[17] **O-PTs 7** (Fig. 2F) with the incorporation of an NIR photoactivable proenzyme was synthesized for cancer therapy and inhibition of metastasis. Ribonuclease A (RNase A) that induces cell death is conjugated with SPNs and caged with a hydrogen peroxide (H_2O_2) cleavable group (Fig. 2H). Similarly, upon NIR laser irradiation, $^1O_2$ species generated from **O-PTs 7** not only serve as PDT agents but also initiate a cascade reaction to release free RNase A, thus having an outstanding therapeutic effect on inhibiting tumor growth and metastasis. Although the long-term shelf stability should be of concern, this pro-nanoenzyme strategy allows combination therapy to be practical and highly effective in conquering cancer.

2.2. Inorganic PTs

Many inorganic PTs exhibit strong optical absorption, high photothermal conversion efficiency, and high quantum yields in ROS generation. Inorganic PTs with appropriate size can be passively enriched at tumor sites through the enhanced permeability and retention (EPR) effect. In addition, different inorganic PTs show different unique properties, such as the surface plasmon resonance of noble metals, the upconverted luminescence of upconversion nanoparticles (UCNPs), the adjustable band gap and ultralarge surface area of two-dimensional (2D) materials. There are many factors influencing the phototherapy performance of inorganic PTs, including their size, shape, concentration and surface modification. In this section, we classify inorganic PTs into three categories: zero-dimensional (0D), one-dimensional (1D), and 2D PTs, and the representative materials are illustrated. In addition, all the inorganic PTs and the corresponding information can be found in Table 1.

2.2.1. Zero-dimensional PTs

Noble metal nanoparticles. With large absorption cross section and light stability, noble metal nanoparticles, especially gold nanoparticles, provide considerable advantages in the field of phototherapy. There are a high number of free electrons on the surface of and within the noble metal that can resonate with the incident light and induce "surface plasmon resonance", which distinguishes noble metal nanoparticles from other photosensitizers. Notably, the absorption in surface plasmon resonance is related to various factors, such as the composition, shape, and size of the nanoparticles and the medium surrounding the nanoparticles. As a result, researchers can adjust the optical

Table 1 Inorganic PTs and their corresponding irradiation power densities, phototherapy types, biological models, and irradiation wavelengths

PT	Classification	Irradiation power density (W cm^{-2})	Phototherapy type	Biological model	Irradiation wavelength (nm)	Ref.
Gold nanorods	0D PTs	10	PTT	Oral squamous cell carcinoma	800	18
UCNPs	0D PTs	2.5	PDT	Melanoma	980	19
BPQDs	0D PTs	1.0	PTT	C6 and MCF7 cancer cells	808	20
SWCNTs	1D PTs	0.8	PTT	Metastatic cancer cells in the lymph node	808	21
Graphene	2D PTs	2.0	PTT	Breast cancer	808	23
Mxene	2D PTs	1.0	PTT	Breast cancer	1064	24
BP nanosheets	2D PTs	1.0	PDT	Breast tumors	660	25
MnO_2	2D PTs	1.5	PDT	Breast cancer	980	26

response of noble metal nanoparticles to meet specific biological needs through various approaches. The absorption peak of nanoparticles undergoes a red shift, while the size increases or while aggregation occurs. Notably, gold nanorods exhibit two absorption bands related to longitudinal and lateral vibrations. In addition, the aspect ratio of gold nanorods can control the absorption spectrum and achieve a sensitive response to near-infrared light, which has a relatively greater penetration depth. Huang *et al.*[18] obtained gold nanorods with an aspect ratio of 3.9 by selecting a suitable silver nitrate concentration during the material growth process. The absorption peak of the gold nanorods was located in the NIR region. By conjugating antibodies on their surfaces, gold nanorods bind specifically to cancer cells. It is proved that the energy threshold for killing cancer cells is only half that of normal cells. In addition to gold nanorods, gold nanoshells can also achieve sensitive absorption band regulation. The structure of the gold nanoshells consists of a silica core and a thin gold shell. When the ratio of the thickness of the gold shell to the diameter of the silica core decreases, its absorption band undergoes a significant red shift.

Upconversion nanoparticles. An important factor in the field of phototherapy is the insufficient penetration depth of lasers. Selecting a suitable biological window and increasing the wavelength of the light source is an effective method of increasing the penetration depth. However, a longer wavelength implies that the energy of a single photon is low, which affects the phototherapeutic performance significantly, particularly in PDT. Fortunately, owing to the 4f energy level of the lanthanides in the system, UCNPs exhibit unique anti-Stokes luminescence properties and have great potential in the field of phototherapy. UCNPs can emit short-wavelength light under long-wavelength light excitation, balancing the penetration depth and the therapeutic effect. UCNPs form a dilute guest system, in which lanthanides are dilutely doped. As the luminous center of the system, lanthanides have abundant long-life energy levels, which is the key to the cascading absorption of multiple low-energy photons (long wavelength) and release of high-energy photons (short wavelength). There are several major mechanisms underlying upconversion, including (1) excited-state absorption (ESA); (2) energy transfer upconversion (ETU); (3) cross relaxation (CR); and (4) photon avalanche (PA). Notably, the UCNPs function as a transducer that can convert NIR light to visible light, and active oxygen is generated by other photosensitizers. Idris *et al.*[19] used UCNPs to load two photosensitizers to achieve effective PDT under 980 nm irradiation. After 11 days of treatment, yttrium can only be detected at tumor sites in mice that received intratumoral injections of UCNP-based PTs, indicating their minimal diffusion from the tumor region to other organs.

Quantum dots. Quantum dots contain several types of materials, while some common advantages promote their wide use in the field of phototherapy. Due to their smaller size than the Bohr exciton radius (a few nanometers), quantum dots show obvious quantum confined effects. Researchers can adjust the optical properties of quantum dots by controlling the size for biological needs. Their ultrasmall size also determines not only a fast metabolic rate to avoid long-term toxicity, but also an efficient production of ROS from their fully exposed surface. In addition, defects are easily introduced during the preparation process; they work as effective nonradiative recombination centers and enhance their photothermal performance. Herein, we consider black phosphorus quantum dots (BPQDs) as an example, which can be used as a novel photothermal agent with excellent biodegradability. Sun *et al.*[20] designed BPQDs by liquid phase exfoliation for PTT of cancer. The BPQDs exhibit great light stability, a large extinction coefficient, and photothermal conversion efficiency. Cell experiments revealed that the heat generated by low-concentration BPQDs (50 ppm) is sufficient to almost completely kill tumor cells under irradiation with an 808 nm laser. Additionally, high-concentration BPQDs still exhibit negligible toxicity without irradiation, indicating their good biocompatibility. In addition to its attractive photothermal properties, the unique biodegradability of black phosphorus (BP) distinguishes it from ordinary photosensitizers. Under physiological conditions, BP can undergo oxidative degradation into non-toxic phosphate and phosphonate, which are abundant in the human body.

2.2.2. One-dimensional PTs. Carbon nanotubes are single or multiple coaxial tubes composed of sp^2 carbon atoms. They are divided into single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs), based on the number of coaxial tube wall layers. Each layer of carbon nanotubes can be considered as rolled graphene. Carbon nanotubes can effectively absorb light energy in the biological transparent window (750–1000 nm) and generate heat through non-radiative relaxation. Owing to their aromatic ring and delocalized π electrons, carbon nanotubes can also effectively load drugs. After modification, the carbon nanotubes display suitable blood circulation times and considerable biocompatibility. Unlike ordinary PTs, carbon nanotubes exert therapeutic effects on tumor metastasis. Liang *et al.*[21] used SWCNT-based PTT to kill metastatic cancer cells in sentinel lymph nodes, which are important target organs for cancer metastasis (Fig. 3B). Enrichment of SWCNTs in sentinel lymph nodes improved 20 minutes after injection into the primary tumor, possibly due to lymphatic drainage. 90 minutes after injection, the enrichment of SWCNTs in the sentinel lymph nodes peaked, and the signal from the lymph nodes was 4–5 times stronger than that from the surrounding muscles. The researchers then removed the lymph nodes of the mice for further analysis, and the results confirmed the positive enrichment effect exerted by SWCNTs in the lymph nodes. To verify the inhibition of tumor metastasis by SWCNT-based PTT, the researchers injected SWCNTs intratumorally and irradiated the tumors and sentinel lymph nodes. Compared to the control group, in which the sentinel lymph nodes were unexposed to light, the experimental group displayed a significantly longer survival time.

2.2.3. Two-dimensional PTs. With the unique quantum confined effect, 2D materials have a wide range of applications in the fields of optics, devices, and biomedicine.[22] In particular in the field of phototherapy, 2D materials exhibit several attractive properties. The band gap of 2D materials can be

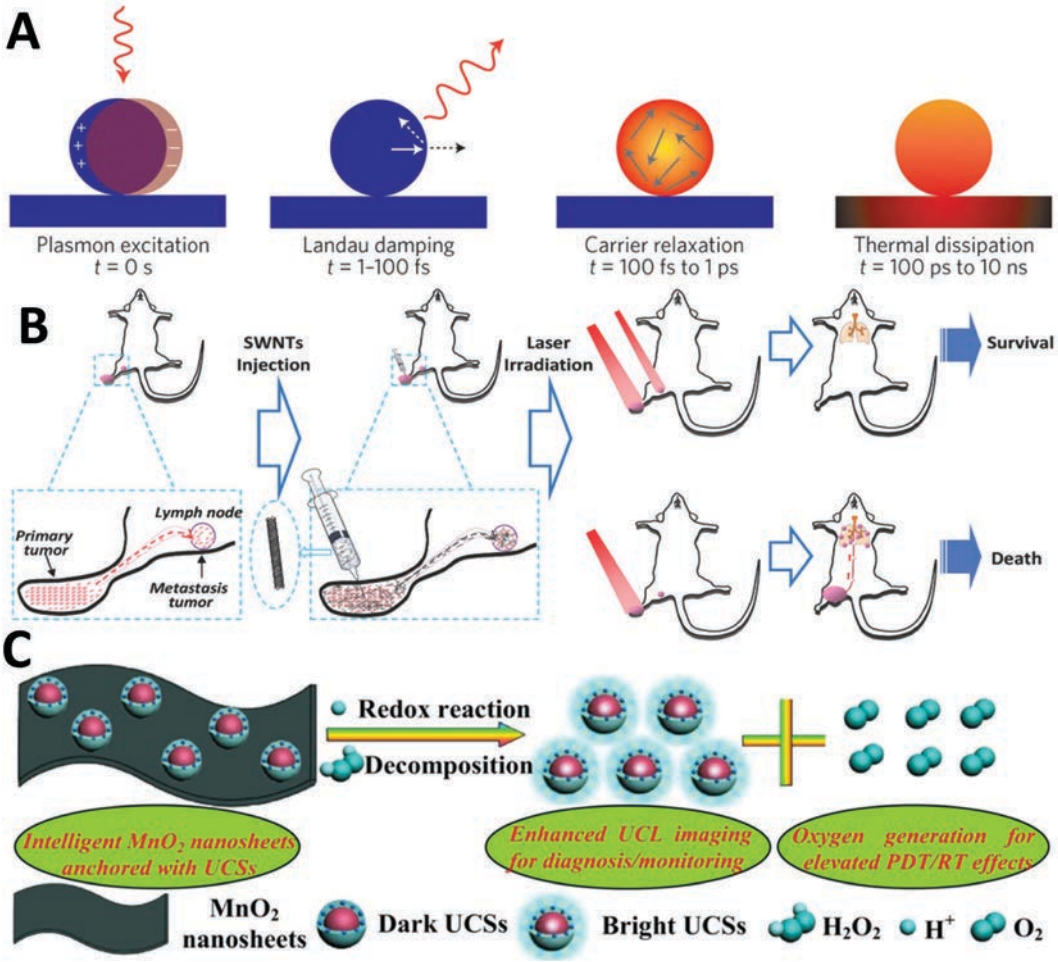


Fig. 3 (A) Schematic representation of the degradation process of BPQD-based PTs in a physiological environment. Reprinted with permission from ref. 20, Copyright 2015 Nature Publishing Group. (B) Scheme showing the anti-tumor metastasis of SWCNTs. Mice with tumor cells that had spread to sentinel lymph nodes were selected for experiments. After both primary tumors and lymph nodes undergo SWCNT-based PTT, most mice can survive for more than 60 days. Mice with only the primary tumor but not the sentinel lymph node ablated by photothermal therapy or cut by surgery gradually died from tumor metastasis. Reprinted with permission from ref. 21, Copyright 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (C) Schematic illustration of the decomposition of MnO_2 nanosheets arising from the redox reaction between UCSMs and acidic H_2O_2. Reprinted with permission from ref. 26, Copyright 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

adjusted by the number of layers, making them sensitive to broadband light. Under irradiation, non-radiative recombination of photoexcited electrons and holes occurs in 2D materials, which induces the generation of phonons and heat. In this process, deep-level defects play an important role, leading to capture of electrons and holes at the center of non-radiative recombination. It is easy to induce defects on 2D material surfaces, which can be used as a phonon scattering mechanism and improve the light-to-heat conversion ability. Graphene, which has delocalized π-electrons that are advantageous for binding various aromatic drug molecules through π–π stacking, is the first 2D material used for phototherapy. Yang *et al.*[23] prepared PEG-modified graphene oxide (GO) with a radial size of 10–50 nm and a thickness of 1–2 layers for application in PTT. The temperature of the GO solution increased by more than 30 °C under 808 nm laser (2 W cm^{-2}) irradiation, while the control group showed negligible temperature rise. The blood circulation half-life of GO is 1.5 hours. Fluorescence imaging indicated the gradual accumulation of graphene-based photothermal agents in the tumor site over time after injection into mice; due to their appropriate shape and size, their enrichment efficiency is higher than that of carbon tubes. After photothermal treatment, the tumors subsided and the experimental mice survived for over 40 days, while the average life expectancy of the control group was only 16 days. No significant weight loss or organ damage was observed in the animals after the treatment, indicating the biocompatibility of graphene-based PTs. In most PTTs, the selected laser wavelength is 808 nm, which is scattered by the tissue and therefore has a limited penetration depth. In order to increase the depth of treatment, Lin *et al.*[24] developed ultrathin Mxene (Nb_2C) nanosheets with a radial size of approximately 150 nm and a thickness of 0.3–0.8 nm for use as photosensitizers in the second NIR light biowindows, which have a higher allowable exposure as well as deeper penetration. Owing to the high extinction coefficient and the high photothermal conversion efficiency, the temperature of the Nb_2C

solution increased above 60 °C under irradiation with a 1064 nm laser. In addition, Nb_2C exhibited much higher photostability than gold nanorods and ICG. Additionally, *in vitro* experiments indicate that Nb_2C can effectively be degraded in the presence of human myeloperoxidase and H_2O_2, indicating its good biodegradability. Using chicken breast as a model, better penetration of the 1064 nm laser light was observed. After administration of Nb_2C in the tail vein, 4 mm deep tumor tissues were ablated significantly under light.

In addition to PTT, 2D materials also have important applications in PDT. Wang *et al.*[25] created highly biocompatible BP nanosheets with a radial dimension of more than 200 nm and a thickness of approximately 2 nm by liquid phase exfoliation in water. Under light irradiation, the BP nanosheets could effectively generate singlet oxygen with a quantum yield of 0.91. Owing to their ultrathin properties, the BP nanosheets contain sufficient active sites and have a low electron hole recombination rate, thereby achieving a remarkably high production efficiency of singlet oxygen. With an ultra-low concentration of 0.2 μg mL^{-1}, the BP-based photosensitizer could achieve an effective photodynamic killing effect. In addition, the results obtained from ICP-AES confirmed that the BP nanosheets underwent almost complete photo-controlled degradation into P_xO_y substances dominated by phosphite ions, indicating their good biodegradability. Although ROS can effectively kill tumor cells, insufficient oxygen supply inside solid tumors limits clinical applications of photodynamics. In addition to the hypoxic environment of tumors, the insufficient penetration depth of a 660 nm laser, which is commonly used in PDT, poses a challenge. To solve these two issues, Fan *et al.*[26] combined MnO_2 nanosheets and upconversion nanoparticles to facilitate NIR-triggered PTT under hypoxic conditions (Fig. 3C). In this system, upconversion nanoparticles convert NIR light into short-wavelength light that can be used for PTT. MnO_2 nanosheets exert dual effects: (1) H_2O_2 consumption, which is abundant in tumor sites, to produce oxygen to enhance the PTT effect; and (2) quenching of the emitted light of upconversion nanoparticles in normal tissues to protect normal tissues from ROS-mediated damage, and activation of photodynamic effects in response to pH changes at tumor sites.

3. Single-mode phototherapy

3.1. PTT

The mechanism underlying PTT is complicated. Light-induced heat can significantly damage the integrity of a cell membrane, and a large influx of Ca^{2+} ions induces chemical damage. When the temperature of light-induced tissues exceeds 39 °C, some proteins tend to aggregate and denature. When the temperature increases further, the cells temporarily become inactivated. In this process, cells reduce heat-induced damage by producing heat shock proteins and through other approaches. When the temperature increases to 43–45 °C, the biochemical reactions in the cells are significantly accelerated, thereby producing ROS. When an extremely high temperature is applied (for example, the light source is an ultra-short pulse laser), the photosensitizer undergoes local overheating in a short time and evaporates the surrounding liquid and generates air bubbles. The bubbles collapse shortly, immediately lysing the cancer cells through a mechanical process. Notably, the position of the photosensitizer exerts a greater effect on the photothermal killing effect. When the photosensitizer is in the extracellular solution, the photothermal killing effect observed is the most severe. Interestingly, the photosensitizer attached to the membrane plays a greater role than the photosensitizer present within the cell, possibly for multiple reasons: (1) the destruction of the cell membrane by heat directly induces cell death; (2) a higher concentration can be achieved by attachment to the cell membrane than by entering the cell; and (3) the thermal conductivity of the cell membrane is relatively low, which ensures the higher local temperature of the cell membrane.

3.2. PDT

In PDT, photosensitizers absorb photons and excite electrons into high-energy orbitals, known as the excited singlet state. The life time is too short to allow energy exchange or electron transfer with the surrounding biomolecules. Singlet photosensitizers can alter the spin state of the excited electrons by intersystem crossing and form a triplet state with a drastically extended life time. Triplet photosensitizers can transfer their energy to molecular oxygen (O_2) to produce singlet oxygen ($^1O_2$), which is known as the Type II process. In addition, Type I PDT procedures with more complex mechanisms are also used for cancer treatment. In the Type II process, photosensitizers usually undergo light-induced electron transfer with organic molecules in a physiological environment to produce various ROS, including superoxide anion radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($HO^{\bullet}$). There are three major cell death pathways in PDT, namely apoptotic, necrotic, and autophagy-associated cell death pathways, among which apoptosis is most common. During apoptosis, PDT can affect mitochondrial outer membrane permeabilization and release caspase activators, including cytochrome *c*, Smac/DIABLO, and apoptosis-inducing factor. The lysosomes targeted by PDT undergo membrane rupture and cathepsin leakage. Moreover, other apoptotic pathways such as calpain also occur after PDT. Notably, cancer cells possess some cytoprotective mechanisms to protect themselves from PDT toxicity. First, there are antioxidant molecules within tumor cells, including glutathione, vitamin C, vitamin E, and some amino acids. Extensive research has been conducted to improve PDT effects through antioxidant consumption. Cancer cells possess enzymes that promote ROS metabolism, which can also increase the resistance to PDT in cancer cells. Finally, the cell itself can regulate the PDT-induced apoptotic process and repair the ROS-induced damage.

3.3. Advantages and disadvantages of phototherapy

Compared to traditional therapies, phototherapy offers several advantages. First, phototherapy can precisely control the area, time, and efficacy of treatment by changing the irradiation site, duration, and power. As a result, it is easy to adjust the

treatment strategy in a timely manner according to the clinical needs. The limited irradiation area ensures fewer side effects and more concentrated energy, which guarantees a significant treatment effect. After phototherapy, tumor-bearing mice display significantly longer survival and suppressed tumor size. However, despite these advantages, the clinical application of single-mode phototherapy is limited. Although it has exhibited good therapeutic effects in solid tumors in animal models, phototherapy cannot address the issues of tumor relapse, which claims thousands of lives. In addition, the heat generated during PTT will inevitably damage the tissues surrounding the tumor. Finally, the scope of phototherapy is limited by the penetration depth of lasers, which can only penetrate small, shallow tumors. As a result, the clinical performance of phototherapy is not as commendable as in animal experiments.

4. Combined phototherapy

To address the intractability of cancer, single-mode therapy is insufficient for clinical use. Phototherapy combined with other therapeutic methods provides a better alternative for tumor therapy.

4.1. Combination with chemotherapy

Since the early 1900s, researchers have worked on developing anticancer drugs using small molecules, and until now, chemotherapy has been the most commonly used modality in both the oncological research and clinical fields.[27] However, chemotherapy still faces challenges, such as several inherent side effects due to the absence of tumor selectivity, and incomplete treatment that may result in relapse due to multidrug resistance. To overcome these difficulties, chemotherapy in combination with phototherapy has been highlighted significantly in a considerably recent study. Nanosystems offer a clue to the solution to the drawbacks of chemotherapy while introducing a more advanced therapeutic effect.[28–31]

4.1.1. Enhanced chemotherapy through combination with phototherapy. So far, while the combination of the two strategies has shown a notable therapeutic outcome, above all, considering the benefits of the combination, it is important to prove an obvious synergy, not a merely mixed therapy. The key to this combination for enhanced chemotherapy is that nanocomposites simultaneously play the role of PTs and nanocarriers in selectively delivering small molecule anticancer drugs to tumors. Therefore, in order to ensure strong and reliable therapeutic effects due to this strategy, a safe and efficient delivery system is pertinent.

By using loadable PTs as nanocarriers for chemotherapeutic drugs including doxorubicin (DOX) and camptothecin (CPT), an effective and safe delivery system has been realized. Selective delivery systems of the desired material targeting a particular tissue can be classified into broad categories such as 'active targeting' and 'passive targeting' delivery systems (Fig. 4).[32] First, as a passive delivery strategy, the EPR effect offers a promising approach to improve therapeutic efficiency and reduce harmful

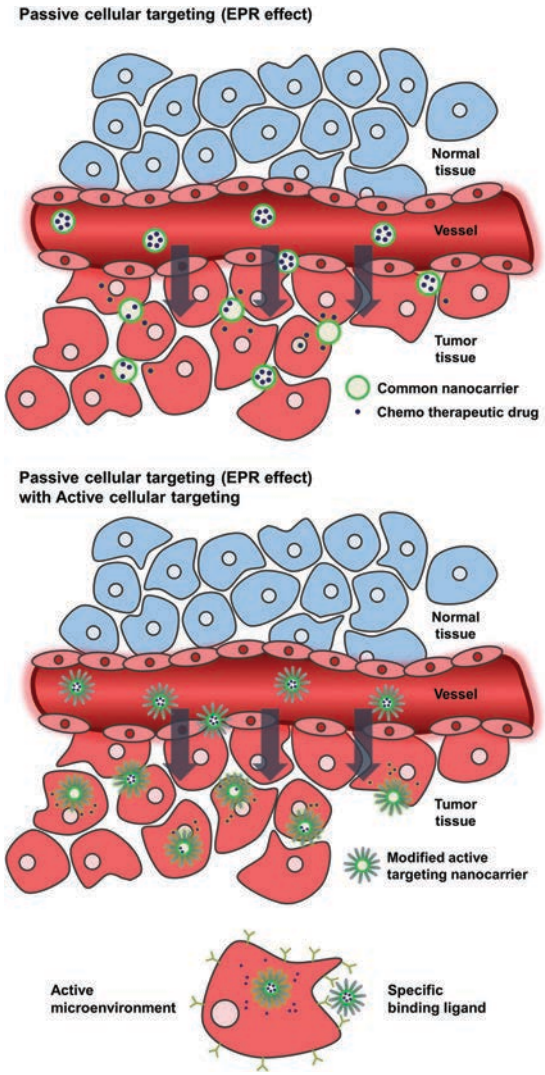


Fig. 4 Schematic illustrating the passive cellular targeting (EPR effect) and the EPR effect with active cellular targeting in tumor tissue compared with that in normal tissue.

side effects experienced during cancer treatment. It is rationalized to describe a condition in which the small material accumulates in tumor tissues while avoiding the normal tissue. To date, it has been considered controversial whether the corresponding nanoparticles accumulate in tumors through this pathway or not. The most logical basis for an argument on this phenomenon is that in cancer angiogenesis, along with the rapid growth of tumor cells, the growth of the adjacent vascular apparatus is also stimulated by the overexpressed vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), sphingosine-1-phosphate (S1P) signal, and prostaglandin E2 (PGE_2). Therefore, given the abnormal vascular architecture, approximately 10 to 100 nm particles can easily pass through the cell boundaries. Therefore, the correspondingly sized PTs are readily delivered to target tissues. In recent studies, various effective bioapplicable phototherapeutic nanomaterials, including inorganic-based nanoparticles (gold nanostructures), 2D nanosheets (Mxenes, BP, and GO), organic-based semiconductors, and

cell-based biocompatible nanoparticles (liposomes), have been reported for use in combination with chemotherapy. Secondly, for a more sophisticated approach, advanced studies have incorporated active cellular targeting strategies to design a more enhanced delivery system. With passive cellular targeting reinforced by active cellular targeting on nanoparticles, its ability to deliver can target more specific sites *in vivo*. The method involves nanocarriers that bind to a specific binding position on the target site, such as a receptor, an enzyme, or a cell–ligand, and remain active only in certain microenvironments based on the difference in pH, tumor hypoxia, or glutathione levels. In particular, this strategy is more selective to the tumor and uses the properties of the tumor and not of the normal cell, or it uses a carrier activated only by additional external stimuli, thereby effectively increasing the potential of chemotherapy. Herein, we introduce several examples of recent advances in the combination of phototherapy and chemotherapy through the use of effective DDSs with nanosystems.

Efforts have been directed in recent years towards overcoming these limitations and developing effective synergistic therapeutic methods having greater compatibility than when used individually. Zhang *et al.*[28] designed porphyrin-based metal–organic framework-coated gold nanorods (**AuNR@MOFs@CPT**) as a versatile nanoplatform for combined phototherapy and chemotherapy of tumors (Fig. 5A). Metal–organic frameworks (MOFs), based on a promising strategy developed using porous materials, have received considerable attention as DDSs. Due to their modifiable inorganic building blocks and organic linkers, MOFs integrate photosensitizers into periodic arrays and have large pore sizes and high surface areas for drug encapsulation. By using MOFs, the loading efficiency, which is a limiting factor in traditional combination therapy, was increased by more effective binding than that achieved when a single nanoparticle was used. Hence, the effective delivery of CPT, a type of anticancer drug, increased the efficacy of chemotherapy in addition to the use of PDT/PTT of nanorods (Fig. 5A1). Owing to these properties, **AuNR@MOFs@CPT** exerts a drastically enhanced therapeutic effect on tumors. According to *in vitro* and *in vivo* studies, **AuNR@MOFs@CPT** displayed excellent tumor accumulation (Fig. 5A2). Furthermore, a combination of chemotherapy and PDT using **AuNR@MOFs@CPT** under irradiation with an NIR (808 nm) laser remarkably suppressed cancer growth (Fig. 5A3 and A4).

2D nanomaterials were also studied for their excellent potential in combination therapy. The nanomaterials exhibited a significant loading efficiency, besides exerting effective phototherapy effects. Wu *et al.*[29] developed a layer-by-layer engineered nanocomposite for a synergistic effect by combining chemotherapy with PTT (Fig. 5B1). The drug-loading capacity of the **pRGO@MS(DOX)–HA** nanocomposite was significantly enhanced by completely covering mesoporous silica (MS). Besides, the surfaces were modified with hyaluronic acid (HA) to further reinforce the targeting ability toward specific cancers. Using the photothermal effect of GO, **pRGO@MS(DOX)–HA** efficiently accumulated in the tumor by the EPR and displayed a synergistically improved therapeutic effect (Fig. 5B2). The therapeutic

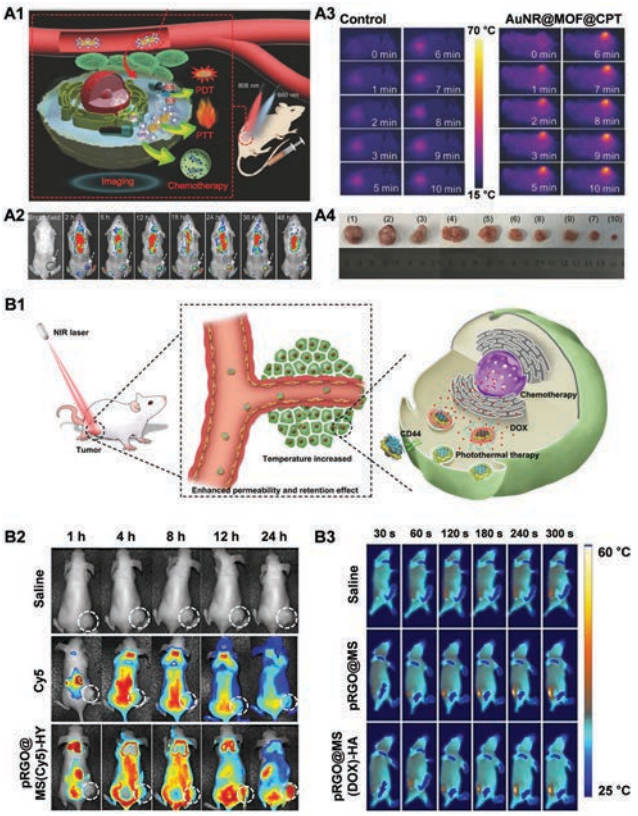


Fig. 5 (A1) Schematic of **AuNR@MOFs@CPT** as a multifunctional theranostic system. (A2) Fluorescence *in vivo* images of **AuNR@MOFs@CPT** and (A3) *in vivo* thermal images of mice after intravenous injection. (A4) Representation of cancer growth inhibition over time. Reprinted with permission from ref. 28. Copyright 2018 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (B) *In vivo* synergistic therapeutic effect of the **pRGO@MS(DOX)–HA** nanocomposite. (B1) Schematic of **pRGO@MS(DOX)–HA** as a multifunctional therapeutic system. (B2) Time-dependent *in vivo* fluorescence images (tumors indicated by the white circles) of the mice after administration of saline, Cy5, or pRGO@MS(Cy5)–HA. (B3) IR thermal images upon applying NIR (808 nm) laser irradiation to HeLa tumor-bearing mice at regular intervals. Reprinted with permission from ref. 29. Copyright 2017 ACS Publications.

effect of **pRGO@MS(DOX)–HA** was demonstrated by the *in vivo* experiment on HeLa tumor-bearing mice. Monitoring studies through an IR camera revealed that the nanocomposite accumulating in cancer cells increased the local temperature significantly (above 50 °C) during a 5 minute NIR irradiation period (Fig. 5B3), which is sufficient to treat cancer through PTT.

Unfortunately, despite profound research findings, current DDSs based on nanosystems still have potential limitations such as: (1) the inadequate potential of general nanocarriers leads to difficulties in achieving the required pharmaceutical efficacy of drugs in a living organism. Besides, it is difficult to accurately predict the dose of the drug being delivered; hence, rather inappropriate concentrations may be administered. (2) Nanoparticles of the particular size that display the EPR effect are prone to be eliminated by phagocytes in the metabolic system. As a result, the encapsulated drugs are rapidly released before reaching the pharmacological target point, also known

as 'burst release.' These results cause adverse therapeutic effects and significant side effects due to chemotherapeutic drugs.

4.1.2. Enhanced phototherapy through combination with chemotherapy. With regard to the complementary synergistic effects of phototherapy and chemotherapy, it is not possible to simply say that one strategy unilaterally subsidizes one strategy. However, some of the findings were described as explicitly addressing complete cancer suppression through combination with chemotherapy, which was not met by phototherapy alone. Pu *et al.*[30] designed **DSPN$_5$**, an amphiphilic semiconducting polymer, as a multifunctional nanocarrier (Fig. 6A). The nanomaterials in this organic structure base selectively delivered drugs and photosensitizers to tumors through the EPR effect (Fig. 6A1). Concurrently, **DSPN$_5$** based on NIR fluorescence/photoacoustic (PA) imaging represents an *in vitro* and *in vivo* DDS. Poly(cyclopentadithiophene-*alt*-benzothiadiazole) (PEG-PCB), a π-conjugated framework of these dual-component nanotheranostic systems, has been used to form a homogeneous type of nanoparticle by self-assembly with DOX in aqueous solution. Therefore, PEG-PCB facilitated diagnosis based on NIR fluorescence and PA imaging and also led to effective combination therapy with chemotherapeutic drugs. Owing to this property, **DSPN$_5$** displayed remarkable results in combination therapy with PTT and chemotherapy. PEG-PCB and **DSPN$_5$** induced significant increases in local temperature by NIR (808 nm) laser irradiation in 4T1 tumor-bearing mice compared to that induced by saline (Fig. 6A2). In particular, **DSPN$_5$** demonstrated a PTT effect that is functional for inhibiting tumor growth with chemotherapy as well (Fig. 6A3). PEG-PCB and **DSPN$_5$**, moreover, induced significant increases in local temperature by NIR (808 nm) laser irradiation in 4T1 tumor-bearing mice compared to that induced by saline (Fig. 6A2). In particular, **DSPN$_5$** demonstrated a PTT effect that is functional for inhibiting tumor growth with chemotherapy as well (Fig. 6A3). Employing PA imaging as well, SPNs could be a powerful platform as a theranostic agent in that it extends beyond the optical diffusion limit.

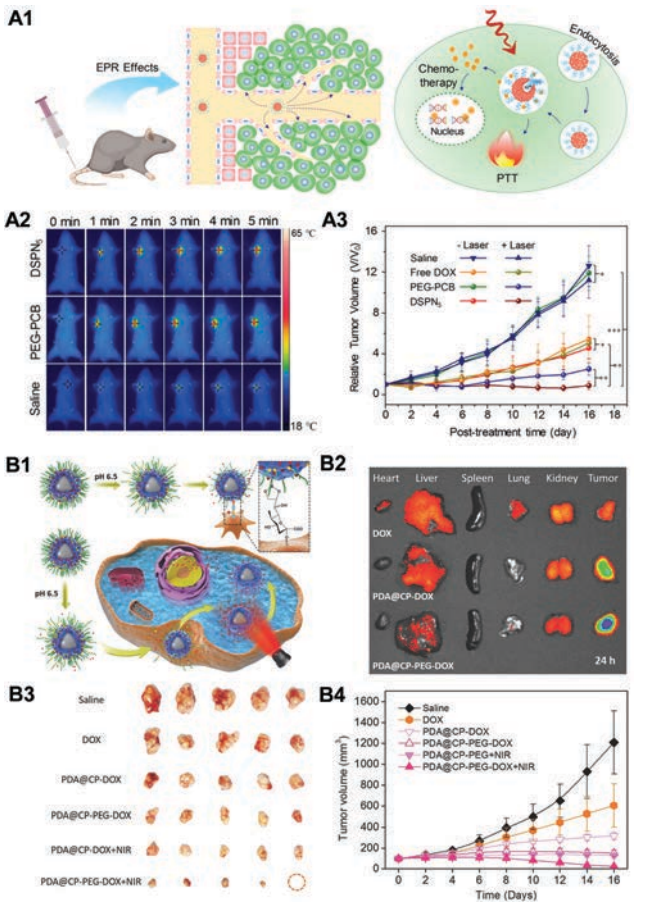


Fig. 6 (A) *In vivo* therapeutic effect of **DSPN$_5$** SPNs. (A1) Schematic of **DSPN$_5$** SPNs and working and DDS mechanisms through EPR as nanocarriers. Results obtained from *in vivo* combination therapy were displayed. (A2) IR thermal images upon applying NIR (808 nm) laser irradiation to 4T1 tumor-bearing mice 8 hours post-injection of **DSPN$_5$** and (A3) the tumor growth was significantly inhibited by the synergistic combination therapy using **DSPN$_5$**. Reprinted with permission from ref. 30. Copyright 2017 Elsevier. (B1) Schematic illustration of **PDA@CP-PEG**, PBA-based dynamic PEGylation multifunctional nanoparticles for a synergetic targeting mechanism and combination cancer therapy. (B2) DOX fluorescence images of major organs and tumors *ex vivo*. (B3) Changes in morphologies and (B4) decreases in tumor volumes as *in vivo* antitumor effects. Reprinted with permission from ref. 31. Copyright 2018 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

To introduce more suitable and dramatic DDSs for backing phototherapy, an active cellular targeting strategy is more selective to cancer cells over healthy cells. Independent passive targeting can be complemented more. Recently, Pan *et al.*[31] improved DDSs through an active cellular targeting strategy combined with the EPR effect that is only active in a specific tumor microenvironment (Fig. 6B). Owing to this property, selective delivery to the tumor, and not to normal cells, can be ensured with greater precision. **PDA@CP-PEG**, poly(dopamine) (PDA) based nanoparticles, effectively demonstrated active cellular targeting under a mildly acidic tumor microenvironment (~pH 6.5) by modifying the surface using the phenylboronic acid (PBA) group (Fig. 6B1). PBA can form a bond with sialic acid (SA), which is usually overexpressed in metastatic cancer cells. Therefore, SA can be used as an appropriate target ligand for PBA. These potential nanoparticles exhibited high cancer selectivity in *ex vivo* fluorescence imaging for DOX (Fig. 6B2). These multifunctional therapeutic nanoparticles also displayed a remarkable *in vivo* antitumor effect in MCF-7 tumor-bearing mice. Changes in tumor volume as time elapsed demonstrated the clinical significance of **PDA@CP-PEG** (Fig. 6B3 and B4).

In summary, the mutually complementary combination of phototherapy and chemotherapy considerably suppresses the proliferative capacity and completely eradicates the tumor relapse potential. Therefore, the combination of other chemotherapeutic drugs with phototherapy has considerable potential to improve the clinical outcome by creating a selective DDS and blocking tumor growth. Based on these advantages, nanocomposites can address the diverse limitations of chemotherapy; hence, this strategy warrants in-depth research. However, even though advances in molecular pharmacology combined with the use of functional materials have yielded multiple effective and promising therapeutic results, some limitations remain. As mentioned earlier, insufficient loading efficiency, uncertain

doses of combination therapy agents, and a lack of biocompatibility with macrophages in the bloodstream have resulted in a lack of sufficiently positive outcomes at the clinical stage. Therefore, a wider variety of combinations needs to be focused on; some combinations have been discussed below.

4.2 Combination with immunotherapy

The clinical success of immune checkpoint blockers has been a major milestone in the use of immunotherapy against cancer, and this was further highlighted by the 2018 Nobel Prize in Physiology or Medicine, which was awarded to James Allison and Tasuku Honjo. The rationale behind cancer immunotherapy is to train antitumor immune cells in the tumor microenvironment and employ immune cells in lymphoid tissues to target tumor cells and destroy them. When the systemic immune surveillance is mobilized, both local and metastatic tumor cells can be killed. Furthermore, long-lasting immune memory may be established to defend against tumor recurrence.

The combination of phototherapy and immunotherapy has achieved synergistic therapeutic efficacy. The effect of phototherapy is limited to superficial tumors and tumor relapse is common after treatment. Immunotherapy can trigger systemic antitumor immunity and target tumor cells to prevent tumor recurrence after phototherapy. In contrast, phototherapy can be employed to convert cold tumors into immunogenic, hot tumors (Fig. 7).[33] This part of the review discusses the recent reports on synergistic phototherapy and immunotherapy used in tumor treatment. Several phototherapeutic agents have been employed in combination with immuno-adjuvants or immune checkpoint blockers.

4.2.1. Phototherapy enhancement by immunotherapy

PTT enhancement by immunotherapy. Nanomaterial-based PTT can activate antitumor immune response through the generation of tumor antigens or immune-related molecules from dead tumor cells. However, distal or metastatic tumors cannot be ablated by single-mode PTT due to the weak immune activation induced by PTT. Moreover, PTT-induced immune responses can be easily inhibited if the tumor temperature is above 45 °C, possibly owing to the heat-induced damage to the vasculature, chemokines, and cytokines, and the temperature-induced stress in stromal and tumor cells. However, high temperatures are necessary in PTT for rapid tumor ablation; hence, only limited immune activation can be achieved. Moreover, relapse of large tumors is fairly common owing to the presence of residual tumor cells; therefore, single-mode PTT cannot be competent. An effective, nontoxic, tumor-specific immunotherapy effectively addresses these issues.

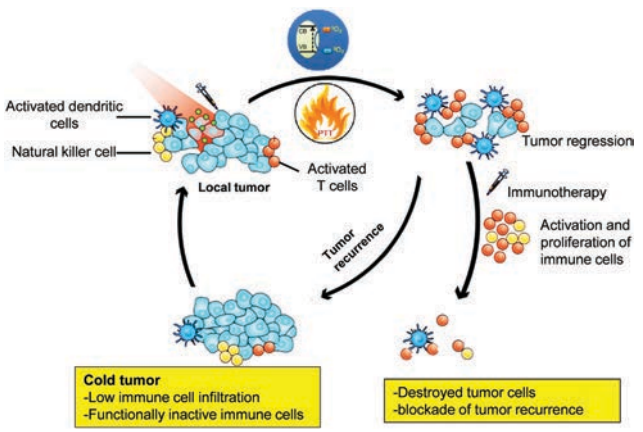


Fig. 7 Combination of phototherapy and immunotherapy.

PTT-induced immunosuppression in the tumor microenvironment can be reversed through combination with immune checkpoint blockers. The cytotoxic T-lymphocyte-associated protein 4 (CTLA4) checkpoint, expressed in T_{reg} cells, is frequently used. Immunity can be restored by blocking this checkpoint with an anti-CTLA4 antibody in antitumor immunotherapy.[34] Different materials have been employed for combination with CTLA4 checkpoint blockers, such as combination with carbon nanotubes (SWCNTs) (Fig. 8A1)[35] and ICG,[36] among others. It was observed that photothermally-induced tumor ablation could release tumor-associated antigens, which then serve as an immunological adjuvants to facilitate significant maturation of dendritic cells and release of antitumor cytokines.[35] Further exposure to CTLA-4 blockers after photothermal destruction of the major tumors would promote infiltration of effective T cells on one hand, while on the other hand the T_{reg} cells would be significantly abrogated in distant tumors. Compared with the conventional surgical removal of primary tumors, synergetic therapies combining PTT and anti-CTLA4 blockers significantly reduced metastasis (Fig. 8A2 and A4). A similar result was achieved through the combination of CTLA4 blockers and ICG,[36] which imparted the ability to attack residual tumor cells, consequently inhibiting metastasis. Furthermore, a persistent immunological memory effect can be obtained by such strategies, which can offer a protective mechanism against tumor relapse post removal of the primary tumors (Fig. 8B1 and B2).

In cancer immunotherapy, immunoadjuvants can stimulate specific antitumor immunity by promoting the uptake and presentation of antigens *via* specific antigen-presenting cells. Guo *et al.* designed an NIR light-triggered transformative nanoplatform that combined PTT with immunoadjuvant-based immunotherapy.[37] The nanoplatform was built by assembling chitosan-coated hollow CuS and cytosineguanine (CpG) motif-based immunoadjuvants. It was a photothermally-triggered smart system that facilitated the production of separated Chi-CpG and CuS nanoparticles (Fig. 9A1), which could enhance the tumor retention time and increase the CpG uptake by plasmacytoid dendritic cells. The tumor cells killed by PTT could release tumor antigens into the tumor environment, and the host antitumor immunity could be strengthened further by the immunoadjuvants (Fig. 9A2). Apart from CuS, gold nanomaterials represent an alternative category of popular PTs that can be combined with immunoadjuvants.[38,39] The popularity of gold nanomaterials can be ascribed to their excellent biocompatibility, simple synthesis, and easy functionalization by different biological molecular pathways *via* simple chemical procedures.[38] Yata *et al.* designed an immunostimulatory DNA hydrogel composed of gold nanoparticles and DNA fragments

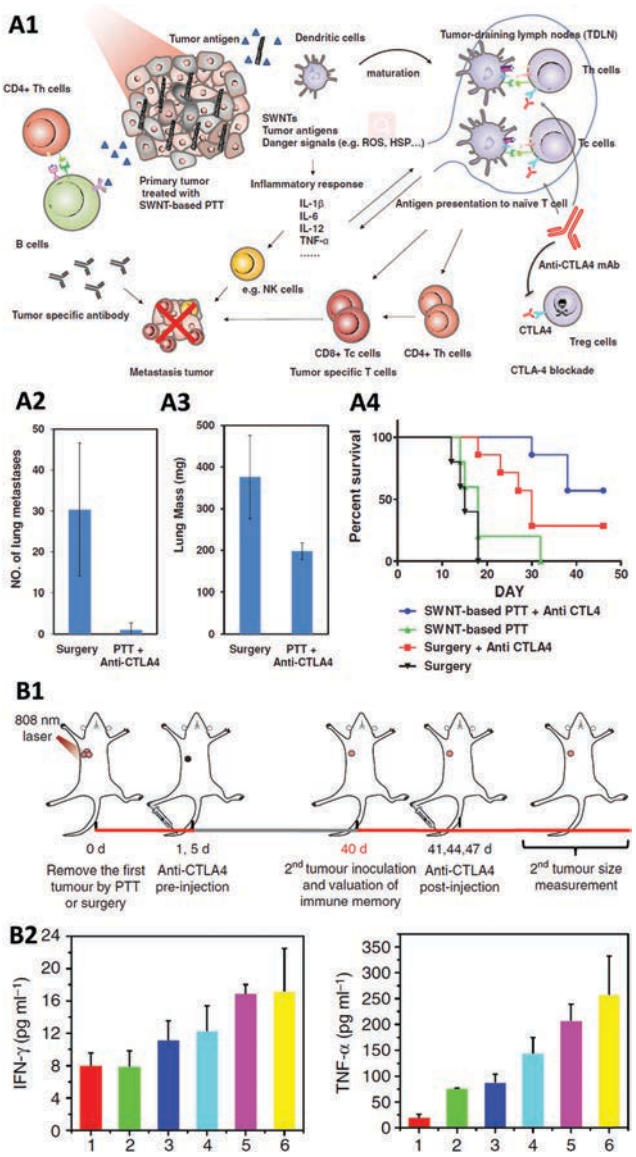


Fig. 8 (A1) The antitumor immune response mechanism stimulated by the combination of SWCNT-based PTT and anti-CTLA-4 antibody. (A2) Quantification of lung metastasis nodules and (A3) lung masses after different treatments. (A4) The survival rates of mice post various treatments for 50 days. Reprinted with permission from ref. 35. Copyright 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (B) Long-term immunologic memory effects. (B1) Schematic illustration of the therapeutic procedure combining ICG-based PTT and CTLA-4 antibody to prevent tumor relapse. (B2) Cytokine levels in mouse sera after re-challenge with secondary tumors. Reprinted with permission from ref. 36. Copyright 2016 Nature Publishing Group.

with CpG sequences.[39] Laser irradiation would induce the release of DNA from the hydrogel, which would effectively drive immune cells to generate proinflammatory cytokines (Fig. 9B1). Moreover, laser-induced hyperthermia promoted the production of heat shock proteins from mRNA expression in tumor tissue, enhanced tumor-related antigen-specific IgG levels, and produced tumor-related antigen-specific interferon-γ (Fig. 9B2). As a result, the tumor growth was significantly retarded and the survival time of the tumor-bearing mice increased.

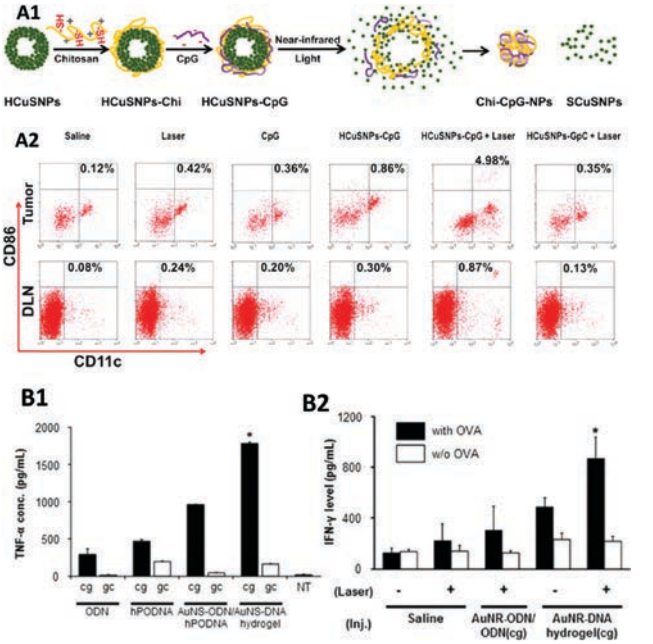


Fig. 9 (A1) Assembly of a CuS–CpG nanoplatform and its photothermally-triggered decomposition. (A2) Stimulated myeloid dendritic cells in draining lymph nodes or tumors detected by flow cytometry. Reprinted with permission from ref. 37. Copyright 2014 American Chemical Society. (B1) The release of cytokines from immune cells in different treatments. (B2) IFN-γ production from splenocytes. Reprinted with permission from ref. 39. Copyright 2017 Elsevier.

PDT enhancement by immunotherapy. In the past decade, PDT has received significant attention in various platforms, ranging from preclinical studies to clinical practices, owing to its minimally invasive strategy. However, its overall therapeutic efficiency needs to be improved further. Firstly, non-irradiated tumors cannot be removed because of the limitations imposed by conditions of local light treatment in PDT; hence, tumor metastases cannot be controlled. Secondly, the antitumor immune response induced by PDT is usually too weak to affect the immunosuppressed tumor.[40] Thirdly, PDT would inevitably damage the surrounding healthy cells and release the self-antigen, which induces immune suppression or tolerance. Therefore, the multiple possibilities offered by immunotherapy can be used to cope with tumor metastases and the immunosuppressive effects of mono-PDT.

Smart drug delivery systems play a critical role in combining PDT and immunotherapy. Dai *et al.* reported a synergistic PDT and immunotherapeutic method based on a smart micellar nanocomplex responsive to the acidic tumor environment, composed of a mitochondrion-targeting photosensitizer and programmed death-ligand 1 (PD-L1)-blockade siRNA (Fig. 10A1).[41] Compared with the high tumor relapse rate of the single irradiation group (75%) and the single PD-L1 group (87.5%), the laser irradiated smart nanocomplex group exhibited a tumor relapse rate of only 25% (Fig. 10A2). This phenomenon can be attributed to the significantly higher proportion of induced effector memory T cells (T_{EM}) in the nanocomplex from the laser irradiation group and the lower proportion of central memory T cells (T_{CM})

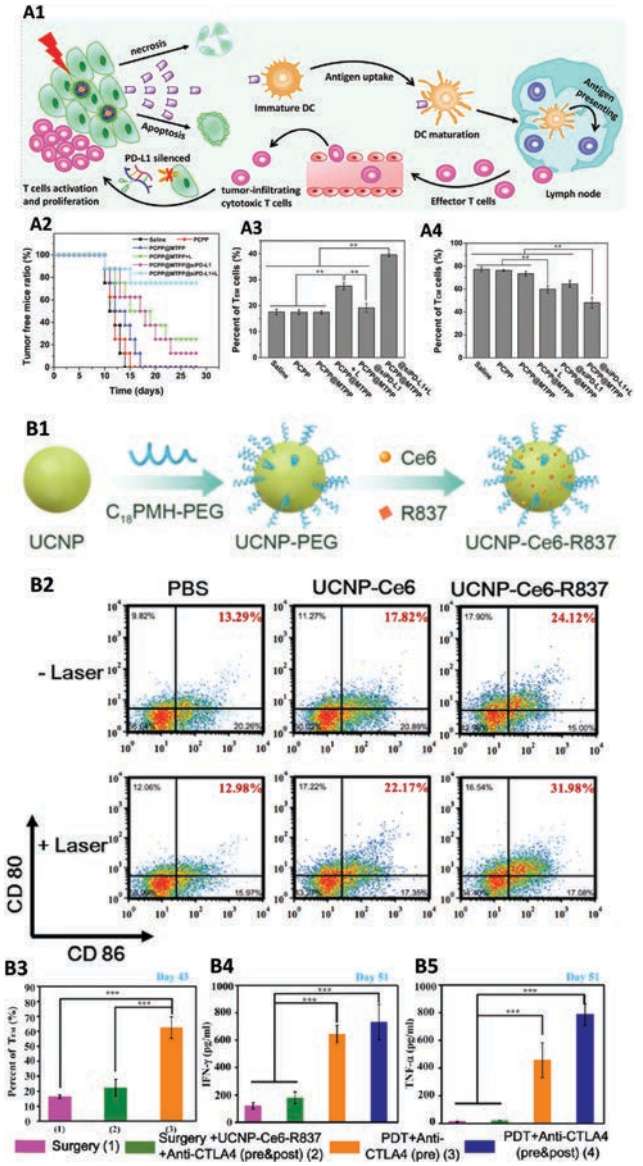


Fig. 10 (A1) Schematic of a pH-responsive micellar nanocomplex for tumor photodynamic immunotherapy. (A2) Tumor recurrence ratios after various treatments. The major tumors were removed, and the mice were retreated with tumor cells. (A3 and A4) The percentages of T_{CM} and T_{EM} cells in spleen cell populations on day 8 when the mice were retreated with secondary tumor cells. Reprinted with permission from ref. 41. Copyright 2018 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (B1) Schematic fabrication procedure of UCNP–Ce6–R837. (B2) Dendritic cell maturation induced by PDT and immune adjuvants. (B3) Proportion of T_{EM} in the spleen. (B4 and B5) Cytokine levels in sera 7 days post re-challenge with secondary tumor cells. Reprinted with permission from ref. 42. Copyright 2017 American Chemical Society.

compared to those in the other treatment groups (Fig. 10A3 and A4). The results indicated that the immune memory response can be successfully stimulated through the combination group with long-lasting protective effects.

To cope with the limited penetration depth of irradiation light in PDT, upconversion nanoparticles (UCNPs) were employed in the design of photodynamic nanoagents by Xu *et al.* Chlorin e6 (Ce6) was chosen as the PDT agent, and a receptor of imiquimod (R837) was used as the immune adjuvant (Fig. 10B1).[42] The prepared UCNP–Ce6–R837 nanoplatform could achieve photodynamic ablation of deep-seated tumors and attract numerous tumor-related antigens, which induced systemic antitumor immune responses with the aid of R837 (Fig. 10B2). Further combination with the anti-CTLA-4 antibody could prevent the growth of the distant tumors remaining after PDT by inducing a long-term immune memory effect (Fig. 10B3–B5).

Typical immunoregulatory enzymes, such as indoleamine 2,3-dioxygenase (IDO), which can catalyze the oxidative catabolism of tryptophan and are highly expressed in tumors, can induce the apoptosis of and alleviate the expansion of T cells.[43] Synergistic PDT and IDO blockade stimulates systemic antitumor immunity and the expansion of local and particular metastatic tumors can be inhibited.[44,45] For example, Lu *et al.* investigated a synergetic approach that combines PDT and an IDO inhibitor (IDOi). A novel nanoscale MOF (nMOF), TBC-Hf, was selected as the PDT agent (Fig. 11A1).[44] Exposure of IDOi@TBC-Hf to light increased the proportions of B cells and tumor-infiltrating neutrophils (Fig. 11A2 and A3). The levels of tumor-infiltrating leukocytes after PDT and IDOi treatment were further investigated. There was an apparent augmentation in the proportion of infiltrating $CD8^+$ T cells in the distal tumor in response to IDOi@TBC-Hf exposure with laser irradiation (Fig. 11A4), indicating the stimulation of antitumor immune response. Song *et al.* reported a smart caspase-responsive chimeric peptide, PpIX-1MT, composed of a photosensitizer (PpIX) and an IDO inhibitor (1MT) attached by a caspase-responsive peptide sequence that imparts a cascaded synergistic therapeutic property (Fig. 11B1).[45] Upon light irradiation, ROS

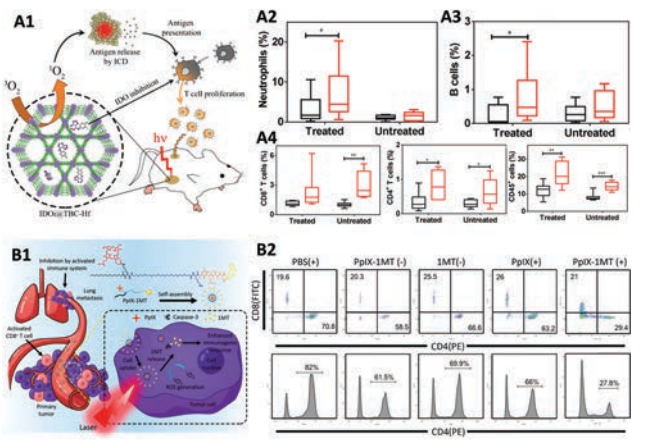


Fig. 11 (A1) Schematic illustration of IDOi@TBC-Hf-based synergetic PDT and immunotherapy. (A2) The proportions of tumor-infiltrating neutrophils and (A3) B cells relative to the total number of tumor cells. (A4) The proportions of tumor-infiltrating $CD8^+$ T cells, $CD4^+$ T cells, and $CD45^+$ cells relative to the total number of tumor cells. Reprinted with permission from ref. 44. Copyright 2016 American Chemical Society. (B1) Cascaded immune response activation based on PpIX-1MT. (B2) Evaluation of the immune response through flow cytometry results and proportion of $CD3^+CD4^+$ T cells in mouse blood. Reprinted with permission from ref. 45. Copyright 2018 American Chemical Society.

could be produced by the PpIX–1MT nanoparticles, which induced tumor cell apoptosis, besides enhancing the expression of caspase-3. The 1MT was subsequently released in response to the cleavage effect of caspase-3 and activated $CD8^+$ T cells to strengthen the immune system (Fig. 11B2). Finally, the sequential synergistic therapy could prevent the growth of primary tumors and remove the metastasis.

4.2.2. Immunotherapy enhancement by phototherapy

Immunotherapy enhancement by PTT. There are several challenges to be resolved in single-mode immunotherapy, such as low immune response rates and dose-limited toxicities. Long-lasting clinical responses can be achieved in patients with immunogenic tumors (hot tumors) characterized by numerous tumor-infiltrating T cells or a high expression of PD-L1, both of which can effectively respond to immune checkpoint blockers. However, cold tumors, or non-immunogenic tumors, induce limited immune response. Moreover, the systemic administration of checkpoint blockers can induce off-target adverse effects by activating self-antigen-reactive T cells. Therefore, for the effective clinical application of tumor immunotherapy, certain strategies should be devised that expand the scope of therapy by converting cold tumors to hot tumors and reducing off-target induced adverse effects. The introduction of phototherapy enables activation of antitumor immune response and improves tumor sensitivity to immunotherapy in a safe and effective manner. For example, Ye *et al.* reported a photothermally-mediated tumor immunotherapy approach based on a transdermal microneedle patch (Fig. 12A).[46] Under NIR light irradiation, melanin in the patch produces heat that can facilitate the uptake of tumor-antigen by dendritic cells and enhance antitumor immune response. This strategy also ensures effective treatment in some persistent forms of cancer, such as a basal-like breast tumor. Liang *et al.* designed a biomimetic two-dimensional BPQD nanosystem that can induce the apoptosis of breast tumor cells *in situ* upon NIR irradiation and further drive the immune response to attack the residual and metastatic tumor cells post PTT.[47] Biomimetic modification was achieved by coating erythrocyte membranes (RMs) on BPQDs, leading to the formulation of BPQD-RM nanovesicles (BPQD-RMNVs). Longer blood circulation times and excellent tumor accumulation effects could thus be achieved. PTT-induced apoptosis and necrosis of tumor cells employed dendritic cells to attack tumor antigens. The PD-1 antibody (aPD-1) was used synergistically to retard the exhaustion of $CD8^+$ T cells (Fig. 12B1). As a result, BPQD-RMNV-based PTT with synergistic aPD-1 treatment markedly inhibited the occurrence and growth of metastatic tumors (Fig. 12B2).

Immunotherapy enhancement by PDT. Tumor cell necrosis by PDT can promote the presentation of tumor-derived antigens to T cells and activate systemic immune response. He *et al.*[48] and Duan *et al.*[49] reported the use of immunogenic nanoparticle-based PDT to enhance PD-L1 antibody-mediated tumor immunotherapy (Fig. 13A and B). Core–shell nanoplatforms (NCP@pyrolipid) based on nanoscale coordination polymers (NCP) were designed with the chemotherapy drug oxaliplatin present in the core and the photosensitizer pyrolipid in the shell for synergistic PDT

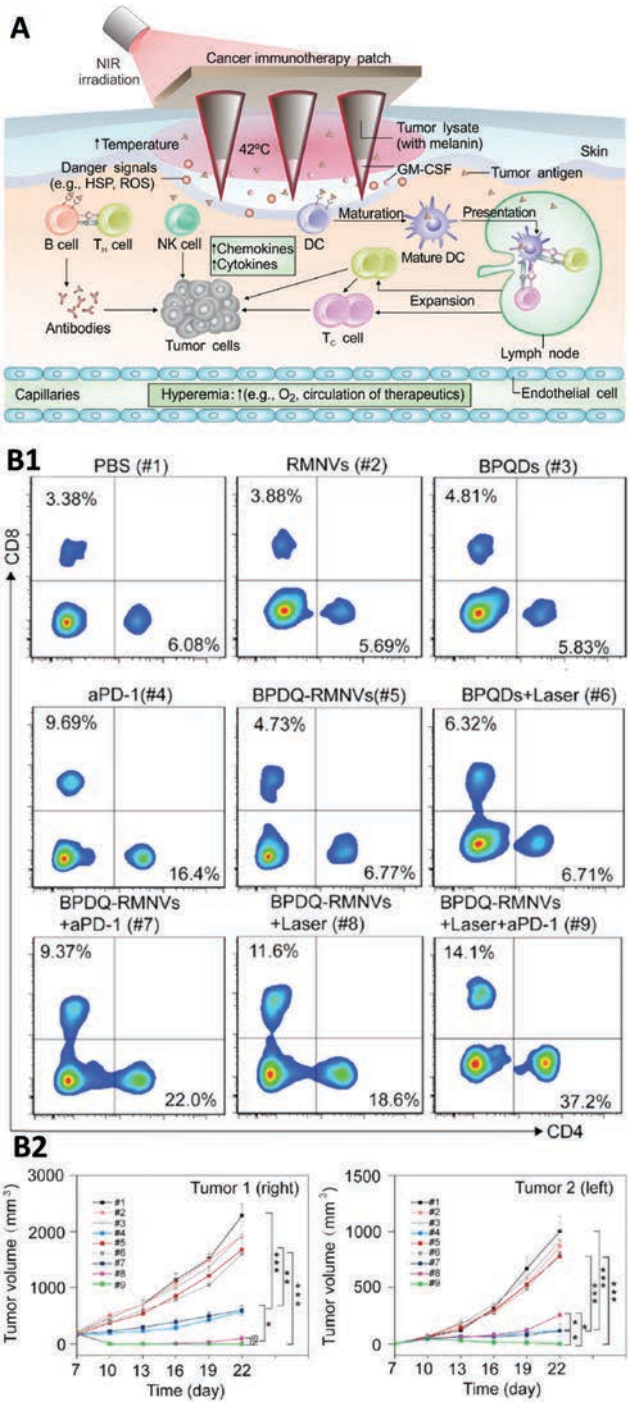


Fig. 12 (A) Schematic representation of photothermally-mediated tumor immunotherapy through a transdermal microneedle-based vaccine patch. Reprinted with permission from ref. 46. Copyright 2017 American Association for the Advancement of Science. (B1) Flow cytometry results of T cells in tumor tissue for the different BPQD-RMNV-based therapy groups. (B2) The evolution of the average tumor volumes of the NIR irradiated tumor (right) and non-NIR-treated tumor (left). #1: PBS; #2: BPQDs; #3: RMNVs; #4: aPD-1; #5: BPQD-RMNVs; #6: BPQDs + laser; #7: BPQD-RMNVs + aPD-1; #8: BPQD-RMNVs + laser; and #9: BPQD-RMNVs + laser + aPD-1. Reprinted with permission from ref. 47. Copyright 2019 Elsevier.

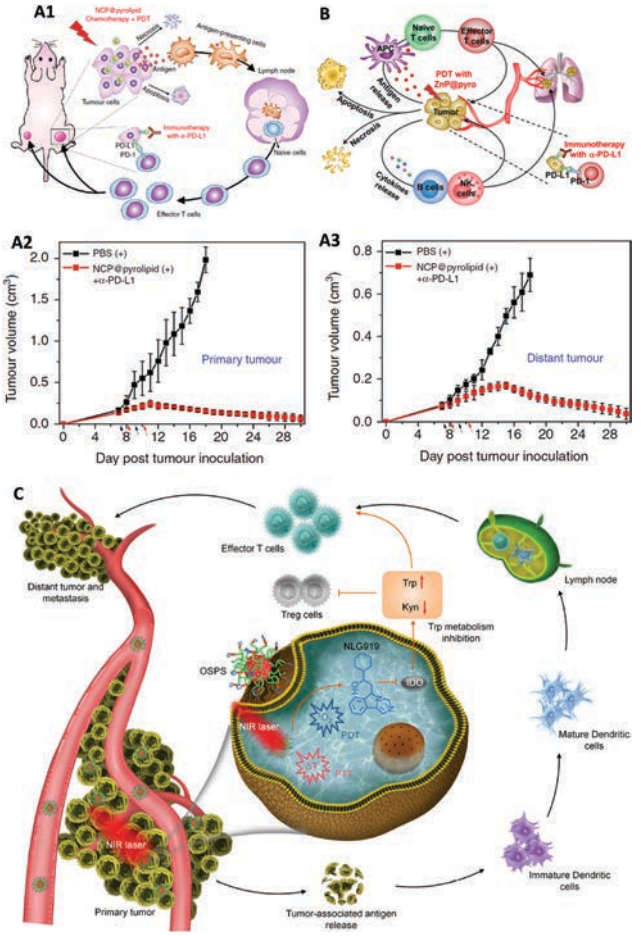


Fig. 13 (A1) The potentiation effect of PDT on a PD-L1 inhibitor to stimulate systemic antitumor immune response. Reprinted with permission from ref. 48. Copyright 2016 Nature Publishing Group. (B) Immunogenic ZnP@pyrolipid-based PDT enhances the response rate of tumors to PD-L1 blockade to prevent the occurrence of metastatic tumors. Reprinted with permission from ref. 49. Copyright 2016 American Chemical Society. (A2 and A3) Tumor evolution of the primary and distant tumors based on the synergetic therapy. (C) Schematic of OSPS-mediated photo-induced immunotherapy. Reprinted with permission from ref. 50. Copyright 2019 John Wiley & Sons, Inc.

and chemotherapy. The combination of oxaliplatin-induced chemotherapy and pyrolipid-based PDT can create an immunogenic tumor environment, which helps execute the crucial functions of a PD-L1 checkpoint inhibitor. Finally, NIR light-irradiated primary tumors as well as non-irradiated distant tumors were ablated by the enhanced systemic antitumor immunity (Fig. 13A2 and A3).

More interestingly, PTT and PDT can be employed together for immunotherapy enhancement. Li *et al.* investigated the NIR-induced synergetic therapeutic efficacy by using an organic semiconducting pronanostimulant (OSPS), which is composed of an immunostimulant and semiconducting polymer nanoparticles connected by a $^1O_2$ cleavable linker (Fig. 13C).[50] When exposed to NIR light, the smart nanosystem produces heat and $^1O_2$ simultaneously, which not only works for direct phototherapy but also generates tumor-associated antigens. Furthermore, the $^1O_2$ cleaves the linker and releases the immunostimulant from the OSPS to trigger the immune response. This study offers a smart molecule design strategy for photo-induced synergistic immune systems.

4.3. Combination with gene therapy

Nanomaterial-based treatments exhibit extraordinary application potential against cancer by driving therapeutic agents to target tumors.[51] Owing to the EPR effect of nanoparticles, nanomaterials can selectively accumulate in solid tumors, which can reduce the toxic side effects of encapsulated agents and enhance their bioavailability. There are other noteworthy features of nanomaterials with respect to tumor treatment, such as the delivery of genetic agents for gene therapy. Gene therapy is a promising tool for improving the therapeutic effects of current antitumor treatment methods. Various strategies, such as gene-regulated enzyme prodrug therapy, application of replication-competent and oncolytic viral vectors, hypoxia-induced gene expression, and sensitization of an antitumor immune attack, can be used to target tumors. Photo-response nanoplatforms loaded with gene editing molecules (shRNA, siRNA, microRNA, cas9, and cas13a, among others) can be employed for efficient gene delivery and combination therapy based on the synergistic anticancer effects of nanomaterials and phototherapy (PDT and PTT) (Fig. 14).[52] Several advances in the synergistic anticancer effects of gene therapy and phototherapy using different nanomaterials have been reported recently.[53]

4.3.1. Phototherapy enhancement by gene therapy. Currently, the combination of phototherapy with gene-based delivery therapy constitutes a major trend in clinical practice and research.[54] The combination of phototherapy and gene therapy can enhance the antitumor effect. Phototherapy can inhibit the growth of tumors first, following which gene therapy can treat cancer by an internal mechanism. While PTT kills tumor cells rapidly, it can induce tolerance after prolonged treatment. A gold nanorod (GNR)–siRNA nanoplex preparation can release genes (siRNA) on the surfaces of the gold nanorods

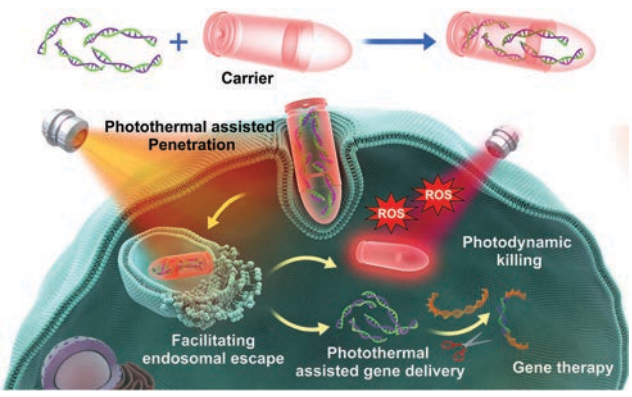


Fig. 14 Graphical illustration of the combination of gene therapy and phototherapy. The photothermal temperature can increase the penetration of the cell membrane and facilitate gene delivery. Photodynamic-induced ROS generation can cooperatively kill cancer cells with gene therapy.

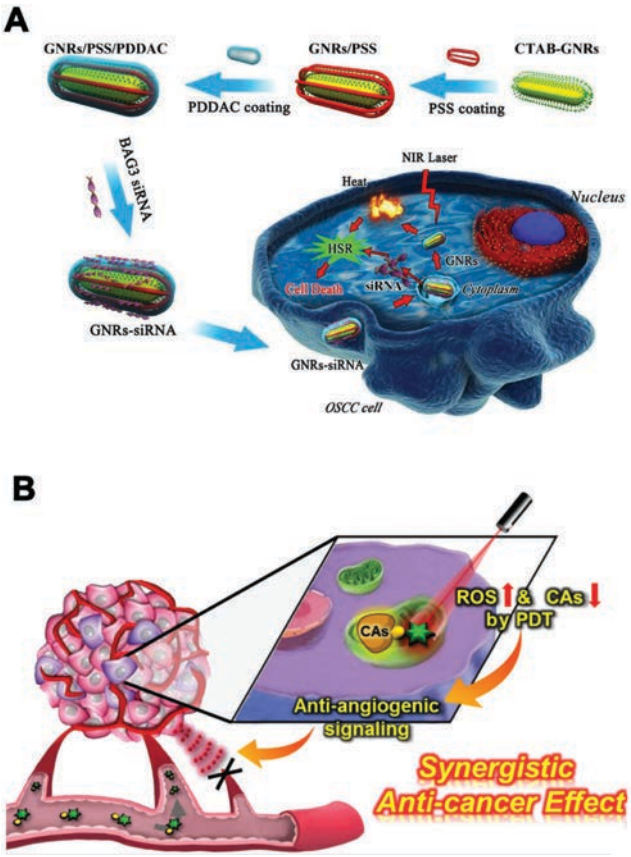


Fig. 15 Phototherapy enhancement by gene therapy. (A) Schematic diagram of GNR-siRNA design in an improved PTT platform: it can downregulate the heat-resistant gene in cancer cells, improving the sensitivity of tumor cells to phototherapy. Reprinted with permission from ref. 55. Copyright 2016 Elsevier Ltd. (B) The schematic diagram of the synergistic antitumor effect of gene therapy and PDT: gene therapy regulates the expression of hypoxia-related genes to realize hypoxia PDT. Reprinted with permission from ref. 56. Copyright 2017 American Chemical Society.

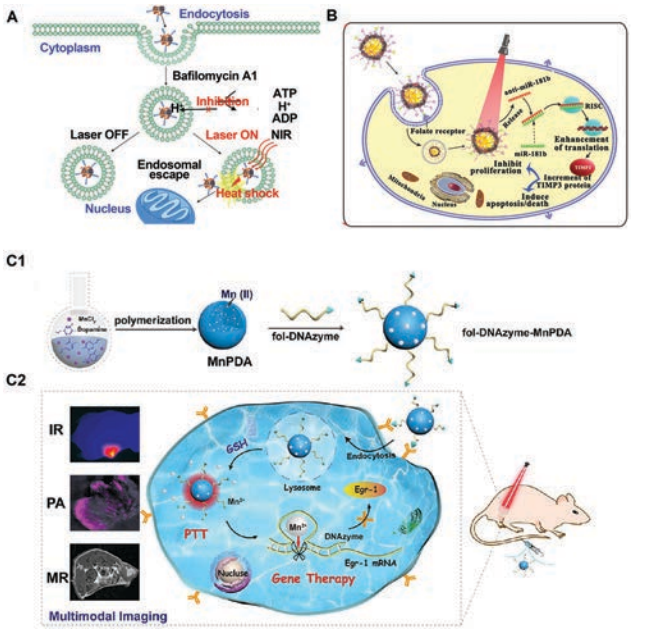


Fig. 16 Gene therapy enhancement by phototherapy. (A) Schematic diagram of the inhibition mechanism underlying the proton sponge effect. (B) Near infrared laser induced gene photothermal therapy of nanocomposites. Reprinted with permission from ref. 62. Copyright 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (C1 and C2) The schematics suggest that the fol-DNAzyme-MnPDA nanoplatform is a multipurpose carrier for multimodal imaging guided therapy. Reprinted with permission from ref. 63. Copyright 2018 American Chemical Society.

under NIR light irradiation by gene silencing to resolve the heat problems in phototherapy technology. It can downregulate the expression of heat-resistant genes in cancer cells, enhance the sensitivity of tumor cells to phototherapy, and utilize the photothermal effect of the preparation to rapidly heat the tumor area, so as to effectively kill the tumor cells (Fig. 15A).[55] Combined with PDT, gene therapy can regulate the expression of hypoxia-inducible factor-1α (HIF-1α) and other hypoxia-related genes (such as VEGF) to realize hypoxia PDT (Fig. 15B).[56] The combination of gene therapy and phototherapy has broad application prospects owing to its safety, low toxicity, high efficiency, and controllability.

4.3.2. Gene therapy enhancement by phototherapy. Cancer-related genes are key regulatory factors in cancer oncogenesis, development, and dissemination. Several studies suggest that the escalation of low-expressed carcinostatic genes and inhibition of over-expressed oncogenic genes can be an effective strategy for anticancer therapy.[57] Additional evidence indicates the potential of gene-based therapies. However, there are disadvantages such as the poor *in vivo* stability, endosomal degradation, low delivery efficiency, poor cytosolic release of the nanoparticles, and the non-specificity of treatment.[58] To address these issues, photothermally-based technologies, such as photochemical internalization to escape the endoplasmic body of the transferred nanoparticles, and photothermally regulated gene expression to negate non-specific effects, can be adopted (Fig. 16A).[59,60] In addition, low power photothermal irradiation can increase the penetration through cell membranes and facilitate gene delivery, since mild photothermal heating enhances the permeability of the cell membrane without any obvious harm to cells.[61] The results indicated that nanocarriers as photoresponsive gene carriers could remarkably improve the efficiency of gene transfection induced by NIR lasers. Moreover, nanocarriers can transfer microRNA into cells under exposure to NIR light, resulting in the downregulation of target genes under laser irradiation. This indicates that the photo-controlled gene transfer is mediated by the intracellular trafficking of photothermally enhanced nanocarriers (Fig. 16B).[62] Concurrently, PDT can induce apoptosis through ROS, while PTT can increase the local temperature of the tumor site, and also effectively ablate the tumor. Recently, many researchers have reported that the photothermal effect produced by nanophotothermal materials can kill tumor cells directly and inhibit tumor metastasis as well. In addition, photothermal nanomaterials can also play a contrasting role through surface modification and other methods, or act synergistically with gene therapy to produce a multi-functional diagnostic and treatment reagent for effective

antitumor therapy (Fig. 16C1 and C2). Therefore, the combination of gene therapy and phototherapy constitutes a cancer therapy method with greater safety and effectiveness, providing a novel idea for cancer treatment with good application prospects.[63]

Moreover, a considerable number of antitumor drug delivery systems have been widely used in cancer treatment; however, due to the complicatedness of the molecules and the multidrug resistance of tumors, single oncology therapy remains unsatisfactory. Therefore, the combination of various therapies to treat cancer is gaining ground. Zhao *et al.* developed a multifunctional nanotherapeutic platform that can be employed for effective cancer treatment. The results reveal that this new nanotherapeutic platform can provide a novel method for cancer treatment by using image-guided targeting and the ingenious comprehensive chemotherapy, gene therapy, and phototherapy (Fig. 17A1 and A2).[64]

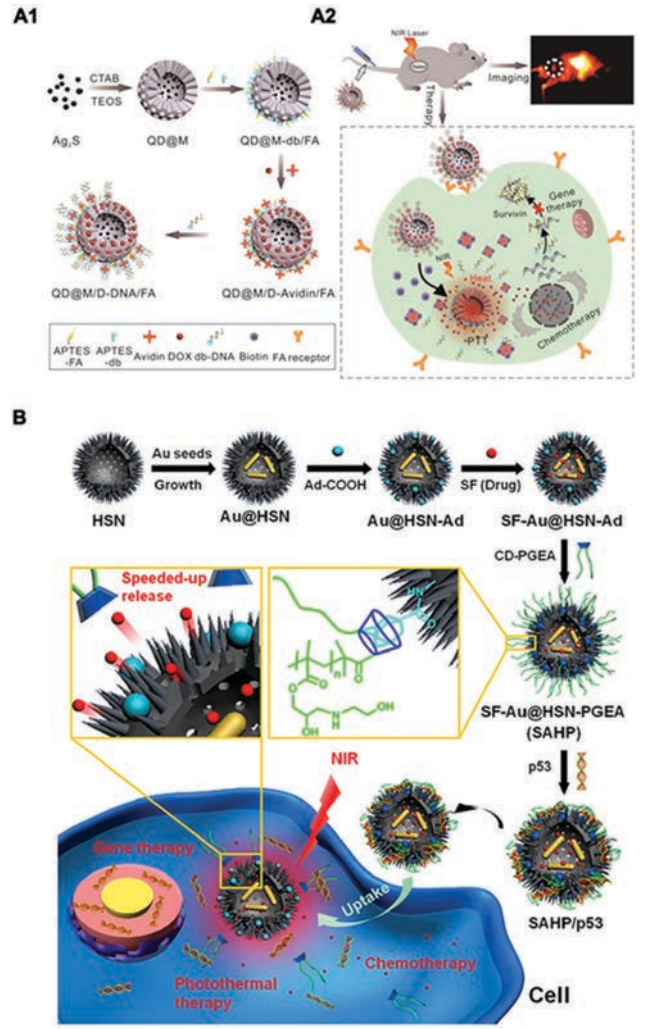


Fig. 17 (A1) Schematic illustration of the route of QD@M/D-DNA/FA synthesis. (A2) Tumor-targeted fluorescence imaging and combined chemotherapy, photothermal therapy, and gene therapy. Reprinted with permission from ref. 64. Copyright 2018 Ivyspring International Publisher. (B) Schematic diagram of the SAHP preparation and its reactive drug/gene co-delivery process. Reprinted with permission from ref. 65. Copyright 2018 American Chemical Society.

Furthermore, Xu *et al.* reported a three peak complementary tumor treatment system consisting of an Au-NR core and a polycation MS shell known as a rattle-structured rough nanocapsule (Au@HSN–PGEA, AHPs). The effects of the rough surface properties of AHPs on cell uptake and gene transfer were studied. The practical possibilities of using photothermal AU-NR nuclei to trigger sorafenib release under NIR light were tested. Notably, the synergistic effect of enhanced gene therapy and chemotherapy based on PTT was implemented.[65] This research expands the biomedical application of rough nanoparticles and provides a flexible strategy to design a multipotent platform for complementary gene/photothermal/chemotherapy (Fig. 17B).

All of the above examples include the combination of phototherapy and gene therapy, along with other different treatment methods. The combination of different therapies for treating cancer signifies an inevitable trend in cancer treatment development.

4.4. Combination with radiotherapy

High-energy ionizing radiation, including X-rays or γ-rays, can be used to ionize water and cellular components to produce ROS, which can damage DNA and induce apoptosis. Radiotherapy is one of the most widely employed treatment methods in clinical tumor therapy. Single-mode phototherapy is disadvantageous for treatment of deep-seated tumors due to the superficial penetration of light. High energy X-rays used in radiotherapy exert an in-depth tumor penetrative effect on tumor tissue that can kill deep-located tumor cells. In contrast, photothermally-induced high temperature can enhance blood flow and promote ROS generation, allowing the reduction of the harmful X-ray dose. The combination of radiotherapy and phototherapy has potential for cancer treatment (Fig. 18). The therapeutic efficacy can be improved and the negative effects on healthy surrounding tissues can be minimized.

4.4.1. Radiotherapy enhancement by photothermal effects. Radiotherapy exerts limited inhibitory effects on hypoxic solid tumors. On one hand, the hypoxic tumor environment can alleviate oxidation-induced destruction; on the other hand, reductive chemical groups, such as –SH, facilitate tumor cell survival through the postirradiation stages using DNA repair mechanisms. Consequently, hypoxic tumors are resistant to radiotherapy.[66] Additional external stimulus-based therapeutic modes may help improve the radiosensitivity of hypoxic tumors. Various nanoplatforms have been shown to increase the therapeutic efficacy and minimize the side effects of radiotherapy.[67,68] Recently, NIR light has been used for phototherapy-enhanced radiotherapy.

PTT is not limited to the hypoxic tumor environment and can kill tumor cells that are resistant to radiotherapy. Moreover, the oxygenation status in the tumor can be improved by the enhanced intratumoral blood flow induced by the photothermal effect, endowing tumor cells with high sensitivity to radiotherapy. For example, Song *et al.* used hollow Bi_2Se_3 nanoparticles for photothermally-enhanced radiotherapy.[69] The hollow Bi_2Se_3 nanoparticles were used to load perfluorohexane (PFC), which

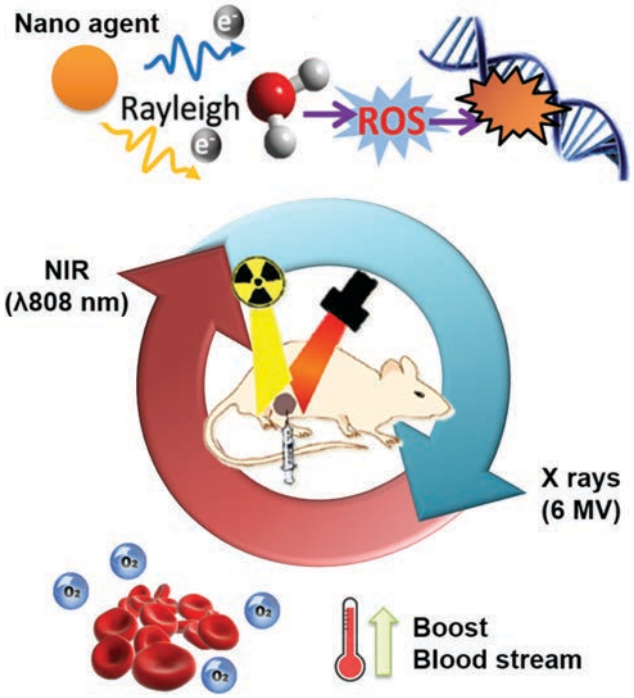


Fig. 18 Combination of phototherapy and radiotherapy. Enhanced photothermal temperature can boost blood flow in a tumor to increase the oxygen concentration, thus overcoming hypoxia-induced radioresistance.

further served as an oxygen reservoir, resulting in the formation of **PEG-Bi2Se3@PFC@O_2**. When this oxygen-loaded nanoplatform was exposed to NIR light, oxygen was released spontaneously (Fig. 19A). The increase in the tumor oxygenation status was confirmed as well (Fig. 19B). The lowest hypoxic signals were observed in the PEG–Bi_2Se_3@PFC@O_2 + NIR treatment group,

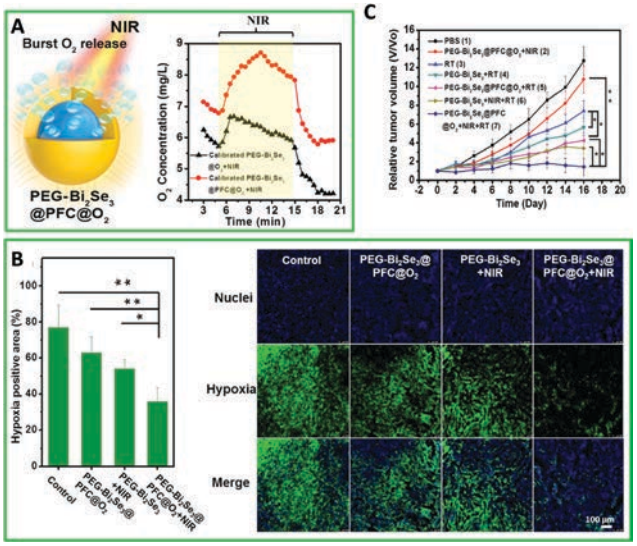


Fig. 19 (A) The release of oxygen from PEG–Bi_2Se_3@PFC@O_2 upon NIR light irradiation. (B) Enhanced oxygenation level and immunofluorescence staining of tumor slices through the hypoxia-probe. (C) Relative tumor volume evolution for different mice groups. Reprinted with permission from ref. 69. Copyright 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

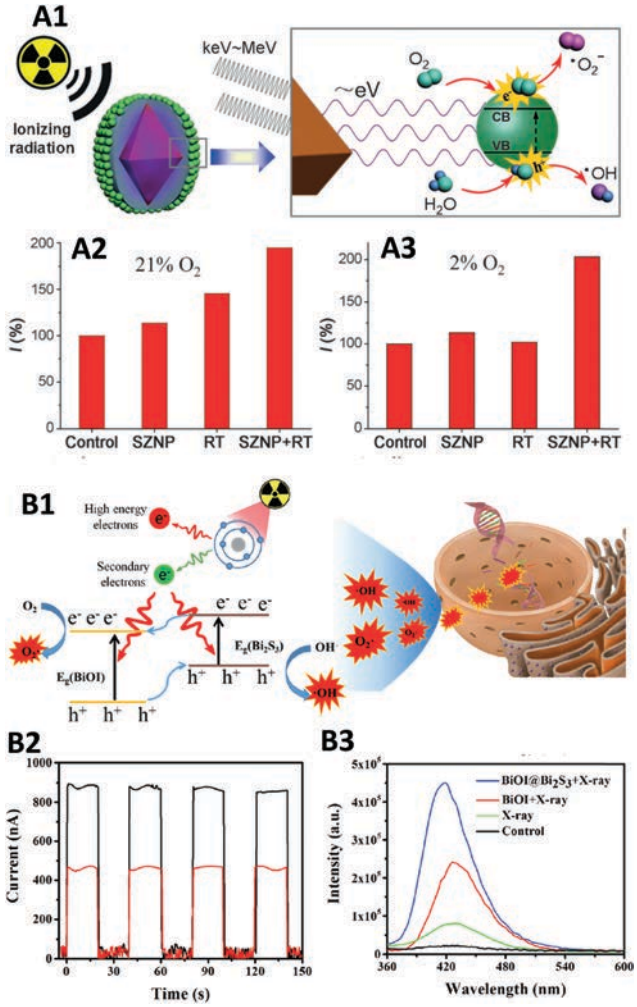


Fig. 20 (A1) The mechanism underlying ionizing radiation-enhanced PDT. (A2 and A3) Relative intensities of ROS-induced fluorescence after incubation of the cells with a photosensitizer in (A2) normoxic (21% O_2) and (A3) hypoxic (2% O_2) tumor cells. Reprinted with permission from ref. 70. Copyright 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (B1) The schematic mechanism of X-ray induced PDT. (B2) X-ray-stimulated photocurrent for BiOI (red) and BiOI@Bi_2S_3 (black) in the on/off irradiation cycle. (B3) Fluorescence spectra evaluating ROS production for different treatments. Reprinted with permission from ref. 71. Copyright 2017 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

indicating the efficient photothermally-induced oxygen supply from this nanoplatform. Therefore, an effective therapeutic outcome can be achieved while overcoming tumor hypoxia-resistant radiotherapy (Fig. 19C).

4.4.2. PDT enhancement by radiotherapy. The clinical use of PDT is faced with issues such as its dependence on oxygen levels and its limited penetration. In contrast, the ionizing radiation of X-rays is highly penetrative and cannot ensure effective energy accumulation in tumor sites, leading to therapeutic ineffectiveness. Given the complementarity of X-rays and light, Zhang *et al.* reported synergistic radiotherapy and X-ray induced deep-seated PDT (Fig. 20A1).[70] This strategy exhibits a limited dependence on intratumor oxygen levels by integrating a scintillator and a photosensitizer. The scintillator can down-convert X-ray radiation to match the energy gap of the photosensitizer for generation of ROS

Table 2 Combination strategies with phototherapy

Combination way	Phototherapy mode	Phototherapy agent	Combined agent	Ref.
Chemotherapy	PTT	Gold nanorods	Camptothecin (CPT)	28
		Graphene oxide	Doxorubicin (DOX)	29
		Semiconducting polymer	Doxorubicin (DOX)	30
		Polydopamine (PDA) nanoparticles	Doxorubicin (DOX)	31
Immunotherapy	PTT	Carbon nanotubes	CTLA-4	35
		ICG	CTLA-4	36
		CuS	CpG	37
		Gold nanoparticles	CpG	39
		Melanin	Tumor-antigen	46
		BPQDs	aPD-1	47
	PDT	MTPP[a]	PD-L1	41
		Chlorin e6	R837	42
		TBC-Hf[b]	IDO inhibitor	44
		PpIX	IDO inhibitor	45
		Pyrolipid	PD-L1	48
		ZnP@pyrolipid	PD-L1	49
Gene therapy	PTT	Gold nanorods	siRNA	34
		Gold nanocages (AuNCs)	Anti-miR-181b	54
		Branched polyethylenimine and reduced graphene oxide (PEG–BPEI–rGO)	Plasmid DNA (pDNA)	53
		Polydopamine Mn^{2+} nanoparticles (MnPDA)	DNAzyme (Egr-1)	42
		Folic acid-modified silver sulfide@mesoporous silica core–shell nanoparticles (Ag_2S QDs)	Db-Modified survivin antisense oligonucleotide (db-DNA)	43
		Gold nanorods (Au NRs)	AHP/pDNA	44
	PDT	Acetazolamide (AZ)-conjugated BODIPY photosensitizer (AZ-BPS)	Carbonic anhydrase IX (CAIX)	35
Radiotherapy	PTT	Bi_2Se_3	Bi_2Se_3	70
		Bi_2S_3	BiOI	62
	PDT	ZnO	Ce^{III}-Doped $LiYF_4$	71

[a] (5-(3-Hydroxy-*p*-(4-trimethylammonium)butoxyphenyl)-10,15,20-triphenylporphyrin chlorine), abbreviated as MTPP. [b] Chlorin-based nanoscale metal–organic framework (nMOF), abbreviated as TBC-Hf.

from water molecules, resulting in efficient X-ray radiation-induced Type I PDT. Therefore, the combination with radiotherapy significantly enhances therapeutic efficacy without being limited by the tumor oxygen levels (Fig. 20A2 and A3). Guo *et al.* were the first to use bismuth oxyiodide (BiOI),[71] an effective therapeutic agent that can act as a radiosensitizer owing to the high-*Z* elements of I and Bi atoms and can also serve as an X-ray-induced photosensitizer owing to the generation of electron–hole pairs upon X-ray irradiation for ROS production (Fig. 20B1). Moreover, the Bi_2S_3 coating on BiOI enhances the generation efficiency of electron–hole pairs to alleviate their recombination, observed in terms of higher photocurrent (Fig. 20B2). Therefore, a high yield of ROS can be achieved regardless of the oxygen levels (Fig. 20B3). In addition, the Bi_2S_3 is an efficient PT. Consequently, the elaborately designed heterojunction can overcome the oxygen shortage problem in PDT by employing X-rays and a multi-therapy nanoplatform can be created. Finally, all the combination strategies can be found in Table 2, which shows the rich kinds of combination ways and promising combination strategies for cancer nanomedicine.

5. Conclusions and perspectives

Light-induced phototherapy is an environment-friendly mode of therapy owing to mild irradiation compared with other types of high-energy ionizing radiation. In particular, metronomic photodynamic therapy (mPDT), which uses extremely low-power light irradiation, leads to the negligible risk of normal cells for thermal damage, and thus is widely used to tackle a variety of cancers.[72] In this review, different PTs were introduced and the challenges in single-mode phototherapy were presented. Tissue penetration can be the main challenge for NIR-I (650–950 nm) phototherapy. The second NIR window (NIR-II, 1000–1700 nm) provides a solution for deep-tissue therapy and diagnosis. Jiang *et al.* first reported the dual-peak absorption of an organic photothermal agent in both NIR windows and its utilization for PTT.[73] They also reported photoacoustic imaging in the NIR-II region based on semiconducting polymer nanoparticles.[74] NIR-II is also appropriate for fluorescence imaging. Huang *et al.* used an NIR-II fluorescent molecular semiconductor for real-time monitoring of kidney dysfunction in mice.[75] Based on these investigations, Lyu *et al.* summarized the recent development of NIR-II based photoacoustic imaging and PTT, and provided further potential.[76] Moreover, light sources, including how to localize and deliver light to tumor sites, should be considered to improve the therapeutic efficacy of PDT and PTT. For example, "metronomic PDT" can be realized for a long-term yet low power treatment by implanting optoelectronics with negligible thermal damage.[77–79] Mono-phototherapy is usually ineffective in ablating tumors

completely without relapse, especially deep-seated tumors limited by the weak penetration of light and hypoxic tumors limited by oxygen-dependent PDT. To address these issues, we have discussed different combination strategies using chemotherapy, immunotherapy, radiotherapy, and gene therapy in this review. Meanwhile, the drawbacks of other single therapy modes can also be overcome by using phototherapy, achieving a complementary multitherapeutic efficacy.

5.1 Phototherapy + chemotherapy

A nanocomposite which can function as a carrier, as well as a photosensitizer, has been able to overcome the drawbacks of chemotherapy. Using a nanosystem that can load small molecules exerting anticancer effects, it exhibits chemotherapeutic effects on tumors through passive or active delivery systems, leading to remission of cancer and improvement of the clinical aspects of the treatment.

The local therapeutic effect resulting from laser irradiation in the presence of a photosensitizer could lead to significant tumor elimination. However, the possibility of a residual tumor being present cannot be excluded and poses a limitation. The mutually complementary combination of phototherapy and chemotherapy significantly suppresses the capacity for proliferation and sustenance while destroying the potential for recurrence. Therefore, the combination of other chemotherapeutic drugs with phototherapy has the potential to improve the clinical outcome compared to that achieved by selective DDSs and tumor growth blockade.

However, even though advances in molecular pharmacology combined with functional materials have yielded multiple effective or promising results, and displayed therapeutic potential, there remain certain limitations: insufficient loading efficiency, uncertain doses of combination therapy agents, lack of biocompatibility against macrophages in the bloodstream.

5.2 Phototherapy + immunotherapy

Although cancer immunotherapy has been widely successful, the low immune response rate is the major limitation in its clinical use. By combining immunotherapy with phototherapy, the antitumor immune response can be amplified and the tumor becomes sensitive to immunotherapy in an effective and safe manner. In contrast, the antitumor immunity triggered by PDT and PTT is too weak to treat distal tumors or metastases. In addition, PDT always induces immune suppression or tolerance by releasing excess self-antigens from damaged normal cells. Through combination with immunotherapy, the immunosuppressive and tumor metastatic effects of mono-phototherapy can be overcome.

For an effective combination, the temperature must be considered carefully and the criteria for the application of a tumor model should be chosen. Immune responses induced by PTT can be inhibited when the temperature of the tumor is above 45 °C. Moreover, the appropriate stage of the tumor model should be chosen for the combined use of phototherapy and immunotherapy. Small tumors can be completely ablated through single-mode PTT without inducing an immune response. For large tumors, a high temperature is necessary for rapid ablation of the primary tumor; therefore, limited immune activation can be achieved. Following this, it is prone to tumor relapse caused by the remaining tumor cells. Such circumstances warrant the combination of phototherapy and immunotherapy.

5.3 Phototherapy + gene therapy

Gene therapy is expected to effectively remove tumors without harming normal human tissues, so as to improve the efficacy of tumor therapy and reduce off-target side effects. It can resist, inhibit, and kill cancer cells by stimulating suitable immune responses. Gene therapy differs from traditional therapeutic methods, and primarily utilizes the natural anticancer ability of the human body, restores the balance of the internal environment, and is the latest treatment method based on autoimmune anticancer. However, owing to its disadvantages such as the low controllability of *in vivo* gene expression, the low efficiency of gene transfer, and the instability of gene carriers, the treatment is slow and time-consuming. Combined with phototherapy, it can first inhibit the growth of tumors, following which gene therapy treats tumors based on an internal mechanism. Conversely, PDT and PTT can cause tumor recurrence and metastasis, which requires gene therapy for long-term treatment. In addition, gene-directed enzyme therapy requires a specific temperature (phototherapy) for activation. In further studies, the aspects of combined gene therapy and phototherapy, such as the instability of gene carriers *in vivo*, or the low concentration of PTs in tumors, need to be improved.

5.4 Phototherapy + radiotherapy

The efficiency of the treatment of hypoxic tumors using radiotherapy is considerably low. This limitation does not exist for PTT, by which hypoxic tumors can be ablated efficiently. In addition, the hyperthermia-induced could boost intratumoral blood flow and relieve the hypoxic state, rendering the hypoxic tumor more sensitive to radiotherapy. Contrastingly, radiotherapy can act on deep-seated tumors owing to its high-energy electromagnetic waves, which can compensate for the superficial limitation of phototherapy. Therefore, phototherapy combined with radiotherapy is required for enhanced synergistic therapeutic efficacy, since it could completely utilize the advantages of each mode and avoid the respective drawbacks.

Several challenges in combined phototherapy and radiotherapy remain unaddressed. Firstly, the high energy X-rays in the tumor therapy process would damage the surrounding normal cells. This is the primary drawback, compared to the mild radiation used in phototherapy. A radiosensitizer with strong X-ray absorption and transformation abilities is required to improve the local and precise treatment efficacy at a relatively safe dose. Moreover, the bioimaging system needs to be configured to realize a precisely controlled therapeutic mode. Therefore, designing a nanoagent for imaging-guided low-dose radiotherapy is highly necessary. Secondly, the difference in energy required for the activation of radiotherapy and phototherapy remains an issue. The energy of X-ray radiation is much

higher than the singlet–triplet energy gap (1–3 eV) in photosensitizers and it cannot initiate the photo-induced energy conversion (photothermal and photodynamic). Novel nanosystems need to be designed to transform between the two different energy stimuli. X-ray induced luminescence may be a suitable choice.

Therefore, considering the drawbacks of single-mode phototherapy and the limitations of other therapeutic modes, combination strategies represent a promising treatment method suitable for clinical use. The development of nanotechnology will further improve the relationship between the different therapeutic modes and help develop efficient combinations that have the potential to treat cancer.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by the State Key Research Development Program of China (Grant No. 2019YFB2203503), the National Natural Science Fund (Grant No. 61875138, 61435010, 81871358, U1801254 and 61961136001), and the Science and Technology Innovation Commission of Shenzhen (KQTD20150324162703 85, JCYJ20180307164612205, and GJHZ20180928160209731). This work was also supported by a CRI project (No. 2018R1A3B1052702, JSK) of the National Research Foundation of Korea. The Natural Science Foundation of Guangdong Province (Grant No. 2020A151501612) is grateful acknowledged. Guangdong Basic and Applied Basic Research Foundation (2019B1515120043), the Science and Technology Project of Shenzhen (JCYJ20180508152903208), Longhua District Science and Innovation Commission Project Grants of Shenzhen (JCYJ201904) are grateful acknowledged. The authors also acknowledge the support from the Instrumental Analysis Center of Shenzhen University (Xili Campus).

Notes and references

1 M. J. Maisels and A. F. McDonagh, *N. Engl. J. Med.*, 2008, **358**, 920–928.
2 C. Xing, S. Chen, M. Qiu, X. Liang, Q. Liu, Q. Zou, Z. Li, Z. Xie, D. Wang, B. Dong, L. Liu, D. Fan and H. Zhang, *Adv. Healthcare Mater.*, 2018, **7**, 1701510.
3 M. Qiu, D. Wang, W. Liang, L. Liu, Y. Zhang, X. Chen, D. K. Sang, C. Xing, Z. Li and B. Dong, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**, 501–506.
4 W. Tao, N. Kong, X. Ji, Y. Zhang, A. Sharma, J. Ouyang, B. Qi, J. Wang, N. Xie, C. Kang, H. Zhang, O. C. Farokhzad and J. S. Kim, *Chem. Soc. Rev.*, 2019, **48**, 2891–2912.
5 M. Qiu, W. X. Ren, T. Jeong, M. Won, G. Y. Park, D. K. Sang, L.-P. Liu, H. Zhang and J. S. Kim, *Chem. Soc. Rev.*, 2018, **47**, 5588–5601.
6 A. M. Smith, M. C. Mancini and S. Nie, *Nat. Nanotechnol.*, 2009, **4**, 710–711.
7 L. Galluzzi, A. Buqué, O. Kepp, L. Zitvogel and G. Kroemer, *Nat. Rev. Immunol.*, 2017, **17**, 97–111.
8 V.-N. Nguyen, S. Qi, S. Kim, N. Kwon, G. Kim, Y. Yim, S. Park and J. Yoon, *J. Am. Chem. Soc.*, 2019, **141**, 16243–16248.
9 M. Li, Y. Shao, J. H. Kim, Z. Pu, X. Zhao, H. Huang, T. Xiong, Y. Kang, G. Li, K. Shao, J. Fan, J. W. Foley, J. S. Kim and X. Peng, *J. Am. Chem. Soc.*, 2020, **142**, 5380–5388.
10 S. Liu, X. Zhou, H. Zhang, H. Ou, J. W. Y. Lam, Y. Liu, L. Shi, D. Ding and B. Z. Tang, *J. Am. Chem. Soc.*, 2019, **141**, 5359–5368.
11 W. Hou, J. W. H. Lou, J. Bu, E. Chang, L. Ding, M. Valic, H. H. Jeon, D. M. Charron, C. Coolens, D. Cui, J. Chen and G. Zheng, *Angew. Chem., Int. Ed.*, 2019, **58**, 14974–14978.
12 J. Li and K. Pu, *Chem. Soc. Rev.*, 2019, **48**, 38–71.
13 K. K. Ng and G. Zheng, *Chem. Rev.*, 2015, **115**, 11012–11042.
14 J. Li, F. Cheng, H. Huang, L. Li and J. J. Zhu, *Chem. Soc. Rev.*, 2015, **44**, 7855–7880.
15 Y. Lyu, J. Zeng, Y. Jiang, X. Zhen, T. Wang, S. Qiu, X. Lou, M. Gao and K. Pu, *ACS Nano*, 2018, **12**, 1801–1810.
16 J. Li, C. Xie, J. Huang, Y. Jiang, Q. Miao and K. Pu, *Angew. Chem., Int. Ed.*, 2018, **57**, 3995–3998.
17 J. Li, J. Huang, Y. Lyu, J. Huang, Y. Jiang, C. Xie and K. Pu, *J. Am. Chem. Soc.*, 2019, **141**, 4073–4079.
18 X. Huang, I. H. El-Sayed, W. Qian and M. A. El-Sayed, *J. Am. Chem. Soc.*, 2006, **128**, 2115–2120.
19 N. M. Idris, M. K. Gnanasammandhan, J. Zhang, P. C. Ho, R. Mahendran and Y. Zhang, *Nat. Med.*, 2012, **18**, 1580–1585.
20 Z. Sun, H. Xie, S. Tang, X. F. Yu, Z. Guo, J. Shao, H. Zhang, H. Huang, H. Wang and P. K. Chu, *Angew. Chem., Int. Ed.*, 2015, **54**, 11526–11530.
21 C. Liang, S. Diao, C. Wang, H. Gong, T. Liu, G. Hong, X. Shi, H. Dai and Z. Liu, *Adv. Mater.*, 2014, **26**, 5646–5652.
22 Z. Xie, C. Xing, W. Huang, T. Fan, Z. Li, J. Zhao, Y. Xiang, Z. Guo, J. Li and Z. Yang, *Adv. Funct. Mater.*, 2018, **28**, 1705833.
23 K. Yang, S. Zhang, G. Zhang, X. Sun, S. T. Lee and Z. Liu, *Nano Lett.*, 2010, **10**, 3318–3323.
24 H. Lin, S. Gao, C. Dai, Y. Chen and J. Shi, *J. Am. Chem. Soc.*, 2017, **139**, 16235–16247.
25 H. Wang, X. Yang, W. Shao, S. Chen, J. Xie, X. Zhang, J. Wang and Y. Xie, *J. Am. Chem. Soc.*, 2015, **137**, 11376–11382.
26 W. Fan, W. Bu, B. Shen, Q. He, Z. Cui, Y. Liu, X. Zheng, K. Zhao and J. Shi, *Adv. Mater.*, 2015, **27**, 4155–4161.
27 V. T. DeVita and E. Chu, *Cancer Res.*, 2008, **68**, 8643–8653.
28 J.-Y. Zeng, M.-K. Zhang, M.-Y. Peng, D. Gong and X.-Z. Zhang, *Adv. Funct. Mater.*, 2018, **28**, 1705451.
29 L. Shao, R. Zhang, J. Lu, C. Zhao, X. Deng and Y. Wu, *ACS Appl. Mater. Interfaces*, 2017, **9**, 1226–1236.
30 Y. Jiang, D. Cui, Y. Fang, X. Zhen, P. K. Upputuri, M. Pramanik, D. Ding and K. Pu, *Biomaterials*, 2017, **145**, 168–177.
31 S. Liu, J. Pan, J. Liu, Y. Ma, F. Qiu, L. Mei, X. Zeng and G. Pan, *Small*, 2018, **14**, 1703968.
32 M. F. Attia, N. Anton, J. Wallyn, Z. Omran and T. F. Vandamme, *J. Pharm. Pharmacol.*, 2019, **71**, 1185–1198.

33 J. Nam, S. Son, K. S. Park, W. Zou, L. D. Shea and J. J. Moon, *Nat. Rev. Mater.*, 2019, **4**, 398–414.
34 F. S. Hodi, S. J. O'Day, D. F. McDermott, R. W. Weber, J. A. Sosman, J. B. Haanen, R. Gonzalez, C. Robert, D. Schadendorf, J. C. Hassel, W. Akerley, A. J. M. van den Eertwegh, J. Lutzky, P. Lorigan, J. M. Vaubel, G. P. Linette, D. Hogg, C. H. Ottensmeier, C. Lebbé, C. Peschel, I. Quirt, J. I. Clark, J. D. Wolchok, J. S. Weber, J. Tian, M. J. Yellin, G. M. Nichol, A. Hoos and W. J. Urba, *N. Engl. J. Med.*, 2010, **363**, 711–723.
35 C. Wang, L. Xu, C. Liang, J. Xiang, R. Peng and Z. Liu, *Adv. Mater.*, 2014, **26**, 8154–8162.
36 Q. Chen, L. Xu, C. Liang, C. Wang, R. Peng and Z. Liu, *Nat. Commun.*, 2016, **7**, 13193–13200.
37 L. Guo, D. D. Yan, D. Yang, Y. Li, X. Wang, O. Zalewski, B. Yan and W. Lu, *ACS Nano*, 2014, **8**, 5670–5681.
38 I.-H. Lee, H.-K. Kwon, S. An, D. Kim, S. Kim, M. K. Yu, J.-H. Lee, T.-S. Lee, S.-H. Im and S. Jon, *Angew. Chem., Int. Ed.*, 2012, **51**, 8800–8805.
39 T. Yata, Y. Takahashi, M. Tan, H. Nakatsuji, S. Ohtsuki, T. Murakami, H. Imahori, Y. Umeki, T. Shiomi, Y. Takakura and M. Nishikawa, *Biomaterials*, 2017, **146**, 136–145.
40 A. P. Castano, P. Mroz, M. X. Wu and M. R. Hamblin, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 5495–5500.
41 L. Dai, K. Li, M. Li, X. Zhao, Z. Luo, L. Lu, Y. Luo and K. Cai, *Adv. Funct. Mater.*, 2018, **28**, 1707249.
42 J. Xu, L. Xu, C. Wang, R. Yang, Q. Zhuang, X. Han, Z. Dong, W. Zhu, R. Peng and Z. Liu, *ACS Nano*, 2017, **11**, 4463–4474.
43 W. Zou, *Nat. Rev. Cancer*, 2005, **5**, 263–274.
44 K. Lu, C. He, N. Guo, C. Chan, K. Ni, R. R. Weichselbaum and W. Lin, *J. Am. Chem. Soc.*, 2016, **138**, 12502–12510.
45 W. Song, J. Kuang, C.-X. Li, M. Zhang, D. Zheng, X. Zeng, C. Liu and X.-Z. Zhang, *ACS Nano*, 2018, **12**, 1978–1989.
46 Y. Ye, C. Wang, X. Zhang, Q. Hu, Y. Zhang, Q. Liu, D. Wen, J. Milligan, A. Bellotti, L. Huang, G. Dotti and Z. Gu, *Sci. Immunol.*, 2017, **2**, eaan5692.
47 X. Liang, X. Ye, C. Wang, C. Xing, Q. Miao, Z. Xie, X. Chen, X. Zhang, H. Zhang and L. Mei, *J. Controlled Release*, 2019, **296**, 150–161.
48 C. He, X. Duan, N. Guo, C. Chan, C. Poon, R. R. Weichselbaum and W. Lin, *Nat. Commun.*, 2016, **7**, 12499.
49 X. Duan, C. Chan, N. Guo, W. Han, R. R. Weichselbaum and W. Lin, *J. Am. Chem. Soc.*, 2016, **138**, 16686–16695.
50 J. Li, D. Cui, J. Huang, S. He, Z. Yang, Y. Zhang, Y. Luo and K. Pu, *Angew. Chem., Int. Ed.*, 2019, **58**, 12680–12687.
51 *Global Cancer Facts & Figures*, American Cancer Society, Atlanta, 3rd edn, 2015.
52 S. M. Ayuk and H. Abrahamse, *Cells*, 2019, **8**, 431.
53 Z. Yang, Z. Sun, Y. Ren, X. Chen, W. Zhang, X. Zhu, Z. Mao, J. Shen and S. Nie, *Mol. Med. Rep.*, 2019, **20**, 5–15.
54 Y. Chang, Y. Feng, Y. Cheng, R. Zheng, X. Wu, H. Jian, D. Zhang, Z. Tang, Z. Wang, J. Hao and H. Zhang, *Adv. Sci.*, 2019, **6**, 1900158.
55 B. K. Wang, X. F. Yu, J. H. Wang, Z. Bin Li, P. H. Li, H. Wang, L. Song, P. K. Chu and C. Li, *Biomaterials*, 2016, **78**, 27–39.
56 H. S. Jung, J. Han, H. Shi, S. Koo, H. Singh, H.-J. Kim, J. L. Sessler, J. Y. Lee, J.-H. Kim and J. S. Kim, *J. Am. Chem. Soc.*, 2017, **139**, 7595–7602.
57 R. J. C. Bose, S. Uday Kumar, Y. Zeng, R. Afjei, E. Robinson, K. Lau, A. Bermudez, F. Habte, S. J. Pitteri, R. Sinclair, J. K. Willmann, T. F. Massoud, S. S. Gambhir and R. Paulmurugan, *ACS Nano*, 2018, **12**, 10817–10832.
58 R. Robinson, *PLoS Biol.*, 2004, **2**, e28.
59 Y. Zhang, Z. Wang and R. A. Gemeinhart, *J. Controlled Release*, 2013, **172**, 962–974.
60 M. K. G. Jayakumar, A. Bansal, K. Huang, R. Yao, B. N. Li and Y. Zhang, *ACS Nano*, 2014, **8**, 4848–4858.
61 L. Feng, X. Yang, X. Shi, X. Tan, R. Peng, J. Wang and Z. Liu, *Small*, 2013, **9**, 1989–1997.
62 S. Huang, S. Duan, J. Wang, S. Bao, X. Qiu, C. Li, Y. Liu, L. Yan, Z. Zhang and Y. Hu, *Adv. Funct. Mater.*, 2016, **26**, 2532–2544.
63 J. Feng, Z. Xu, F. Liu, Y. Zhao, W. Yu, M. Pan, F. Wang and X. Liu, *ACS Nano*, 2018, **12**, 12888–12901.
64 C. Li, X.-Q. Yang, M.-Z. Zhang, Y.-Y. Song, K. Cheng, J. An, X.-S. Zhang, Y. Xuan, B. Liu and Y.-D. Zhao, *Theranostics*, 2018, **8**, 5662–5675.
65 X. Chen, Q. Zhang, J. Li, M. Yang, N. Zhao and F.-J. Xu, *ACS Nano*, 2018, **12**, 5646–5656.
66 H. E. Barker, J. T. E. Paget, A. A. Khan and K. J. Harrington, *Nat. Rev. Cancer*, 2015, **15**, 409–425.
67 Y. Duo, Y. Huang, W. Liang, R. Yuan, Y. Li, T. Chen and H. Zhang, *Adv. Funct. Mater.*, 2020, **30**, 1906010.
68 Q. Huang, S. Zhang, H. Zhang, Y. Han, H. Liu, F. Ren, Q. Sun, Z. Li and M. Gao, *ACS Nano*, 2019, **13**, 1342–1353.
69 G. Song, C. Liang, X. Yi, Q. Zhao, L. Cheng, K. Yang and Z. Liu, *Adv. Mater.*, 2016, **28**, 2716–2723.
70 C. Zhang, K. Zhao, W. Bu, D. Ni, Y. Liu, J. Feng and J. Shi, *Angew. Chem., Int. Ed.*, 2015, **54**, 1770–1774.
71 Z. Guo, S. Zhu, Y. Yong, X. Zhang, X. Dong, J. Du, J. Xie, Q. Wang, Z. Gu and Y. Zhao, *Adv. Mater.*, 2017, **29**, 1704136.
72 Y. Lee and D.-H. Kim, *Nat. Biomed. Eng.*, 2019, **3**, 5–6.
73 Y. Jiang, J. Li, X. Zhen, C. Xie and K. Pu, *Adv. Mater.*, 2018, **30**, 1705980.
74 Y. Jiang, P. K. Upputuri, C. Xie, Z. Zeng, A. Sharma, X. Zhen, J. Li, J. Huang, M. Pramanik and K. Pu, *Adv. Mater.*, 2019, **31**, 1808166.
75 J. Huang, C. Xie, X. Zhang, Y. Jiang, J. Li, Q. Fan and K. Pu, *Angew. Chem., Int. Ed.*, 2019, **58**, 15120–15127.
76 Y. Lyu, J. Li and K. Pu, *Small Methods*, 2019, **3**, 1900553.
77 A. Bansal, F. Yang, T. Xi, Y. Zhang and J. S. Ho, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**, 1469–1474.
78 S. I. Park, D. S. Brenner, G. Shin, C. D. Morgan, B. A. Copits, H. U. Chung, M. Y. Pullen, K. N. Noh, S. Davidson, S. J. Oh, J. Yoon, K.-I. Jang, V. K. Samineni, M. Norman, J. G. Grajales-Reyes, S. K. Vogt, S. S. Sundaram, K. M. Wilson, J. S. Ha, R. Xu, T. Pan, T.-I. Kim, Y. Huang, M. C. Montana, J. P. Golden, M. R. Bruchas, R. W. Gereau and J. A. Rogers, *Nat. Biotechnol.*, 2015, **33**, 1280–1286.
79 J. Kim, R. Ghaffari and D.-H. Kim, *Nat. Biomed. Eng.*, 2017, **1**, 0049.