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Review

Enhancing Combined Immunotherapy and Radiotherapy through Nanomedicine

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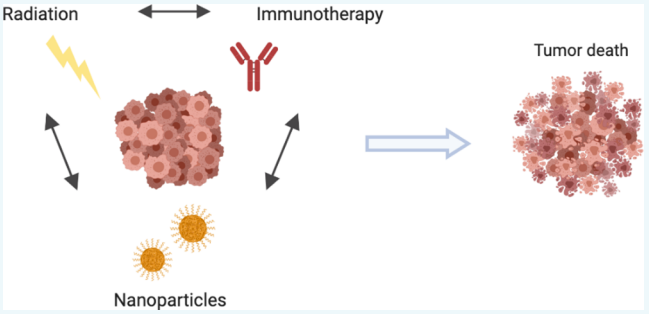
ABSTRACT: Radiotherapy and immunotherapy are two key treatments for cancer. There is growing evidence that they are also synergistic, and combination treatments are being studied extensively in the clinical setting. In addition, there is emerging evidence that nanotechnology-enabled therapeutics can potentiate both radiotherapy and immunotherapy, in turn improving both treatments. This is an exciting new area of interdisciplinary science and has significant potential for major clinical impact. Some of the approaches in this area have already reached the clinical stage. In this review, we will discuss recent advances in the interface between radiotherapy, immunotherapy, and nanomedicine. We plan to review the many approaches to combine these three fields for cancer treatment.



INTRODUCTION

Radiotherapy is a key cancer treatment modality, and more than 50% of all cancer patients will receive radiotherapy during their treatment course. Importantly, the concurrent administration of chemotherapy and radiotherapy, also called chemoradiotherapy (CRT), is a critical treatment paradigm in the curative management of many solid tumors, including brain, head and neck, esophageal, gastric, pancreatic, small cell and non-small cell lung, rectal, bladder, anal, vulvar, and cervical cancers.[1−4] Despite the success of radiotherapy, it is not without limitations. Radiotherapy cannot always eradicate the primary tumor, especially in diseases such as pancreatic cancer. Radiotherapy can also lead to significant toxicity.[5,6] Thus, there has been strong interest in strategies to improve radiotherapy for cancer.

Cancer immunotherapy, the utilization of the patients' own immune system to treat cancer, has emerged as a powerful new strategy in cancer treatment.[7] The development of antibodies that can block negative immune regulatory pathways, such as the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and the programmed cell death protein 1 (PD-1), have resulted in clinical improvements in cancer patients that have not been seen previously.[8−13] A clinical approach of substantial interest to improving immunotherapy has been to combine radiotherapy with the use of immunotherapeutic agents.[14] Radiotherapy has been shown to enhance immunotherapy clinically.[15] Preclinical data have also shown that immune checkpoint inhibitors improve CRT.[16] Recently, investigators have shown that adjuvant immunotherapy with durvalumab (anti-programmed death-ligand 1 (αPD-L1)) following CRT significantly increased the progression-free survival in stage III non-small cell lung cancer patients (PACIFIC study). At 18 months post treatment, the progression free survival for patients that received both radiotherapy and αPD-L1 was 44% vs 27% for those that received radiotherapy alone.[17] Currently, there are many trials examining the use of immune checkpoint blockade agents with radiotherapy in the curative management of cancers.

Another strategy to improve both radiotherapy and immunotherapy is through the use of nanotherapeutics. There is growing evidence that nanoparticles (NPs) can improve both treatments by increasing delivery of drugs to tumors,[18] enhancing antigen presentation to antigen presenting cells (APCs),[19−21] and improving immunotherapeutic agents' effects.[22−24] The synergistic actions of these treatments—radiotherapy, immunotherapy, and nanotherapeutics—are shown in Figure 1. Clinical translations of these advances are already underway, with the nanoscale metal−organic framework (nMOF) RiMO-301 in a phase I clinical trial and Hf based NBXTR3 NPs already completing a phase I and phase II−III trial. In this review, we aim to review the scientific

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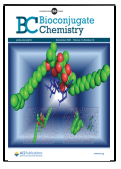

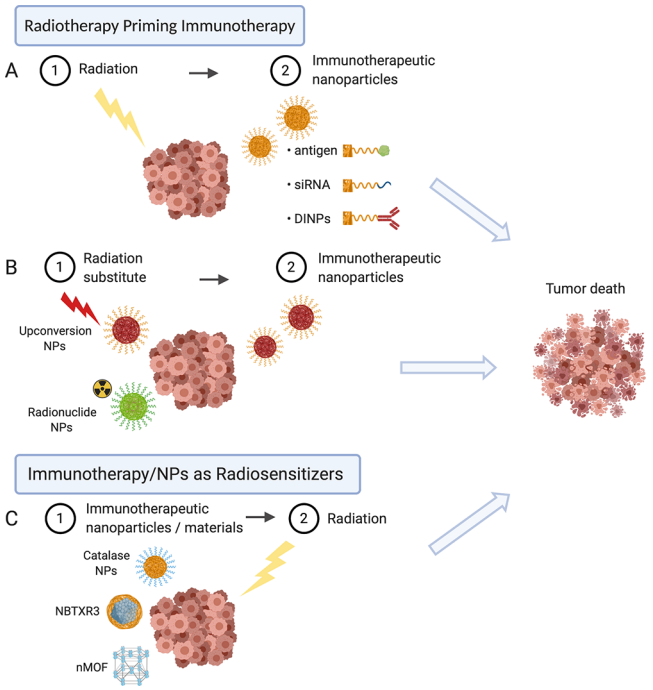

Figure 1. Application of nanotechnology to radiotherapy and immunotherapy has a high potential to improve existing clinical treatments. (A) Radiotherapy releases neoantigens, increases cytokine production, and modifies the tumor microenvironment, improving conditions for immunotherapeutic NPs to elicit an upregulated, tumor specific immune response. (B) Conventional radiotherapy alternatives such as radionuclide NPs or upconversion NPs can provide radiotherapy priming or radiation itself, and simultaneous delivery of immunotherapeutic agents. (C) Immunotherapeutic nanomaterials radiosensitize tumors, increasing DNA damage and tissue susceptibility to radiotherapy (made in ©BioRender - biorender.com).

evidence on the interface of radiotherapy, immunotherapy, and nanotechnology.

INTERACTIONS BETWEEN RADIOTHERAPY AND IMMUNOTHERAPY

There is clinical evidence that a combination of radiotherapy and immunotherapy may be more effective than either treatment alone. The synergy is bidirectional: there are cases in which immunotherapy acts as a radiosensitizer[25,26] and others in which radiation provides precursors or improved conditions for immunotherapy, bolstering its efficacy.[27] While radiation-induced cellular death has often been attributed exclusively as a result of DNA damage, there is increasing evidence that a coupled immune response is an important part of the process. The complementary nature of radiotherapy and the immune system has been observed in immunocompromised patients exhibiting inferior tumor control following radiotherapy.[28] This effect has also been demonstrated in immunocompromised mice where fibrosarcoma and head and neck squamous cell carcinoma models required more than double the radiation dose as immunocompetent mice to achieve local tumor control in 50% of mice.[29,30] These studies demonstrate that radiation is more effective when coupled with an immune response.

In an attempt to dampen this therapeutic immune response, cancer cells have a number of mechanisms through which they elude detection and attack from the immune system, such as downregulating MHC I expression, decreasing antigen presentation, targeting regulatory T cells, and producing immunosuppressive mediators.[31,32] Following radiotherapy, however, there is an increase in release of neoantigens which can be presented to the immune system for subsequent targeting in an immune response.[19,33,34] Radiatively damaged DNA can also lead to an increase in production of additional mutated antigens. These non-tumor specific antigens could help in the upregulation of immune surveillance.[35] An increase of cytokines is also detected after radiotherapy, such as type I interferons, which are upregulated through the stimulator of interferon genes (STING) pathway as it reacts to damaged DNA which has escaped into the cytosol.[36] IFN-γ is also increased as a result of an increase in CD8+ T cells.[26,34,37] MHC-1 molecules are more prevalent on the cell surface following radiotherapy, allowing increased antigen presentation to the simultaneously increasing number of T cells.[38] Hammerich et al. found that by combining FMS-like tyrosine kinase 3 ligand (Flt3L) with radiotherapy, intratumoral dendritic cells (DCs) acquired CD103 expression, while neither non-irradiated DCs nor irradiated DCs not in the presence of tumor cells acquired this expression.[39] They showed that radiotherapy leads to increased CD103+ DCs and increased antigen capture. Mice were then treated with anti-PD-1 (αPD-1), resulting in durable tumor remissions increasing from approximately 40% to 80% (P = 0.0001). These studies show that radiation leads to immune system activation, reversing some immune-eluding strategies of tumors and improving subsequent immunotherapy.

Alternatively, immunotherapy prior to radiation can act to serve as a radiosensitizer. αPD-1 treatment with pembrolizumab given prior to radiation has been shown to increase T cell activation and may increase tumor response to radiation.[40] Another mechanism through which radiosensitization can occur involves the uncoordinated growth of tumor blood vessels causing a hypoxic and immunosuppressive local tumor microenvironment (TME).[41−43] This hypoxia leads to decreased metabolism and subsequent DNA damage with radiotherapy compared to a well oxygenated tumor.[44,45] Immunotherapy can normalize the dysfunctional tumor vasculature, increasing the effectiveness of subsequent radiotherapy.[25] One more mechanism of radiosensitization demonstrated by Cho et al. was that the Toll-like receptor 7 (TLR7) agonist imiquimod (IMQ) acts as a potent radiosensitizer. Mice bearing B16−F10 tumors were pretreated with IMQ, releasing reactive oxygen species (ROS) which enhanced the MAPK and NF-κB pathways, upregulating the autophagy process.[46]

Immune checkpoint blockade has also been shown to yield significant clinical responses when paired with radiation.[9,10,47] Multiple immune checkpoints can be blocked simultaneously to co-opt more than one pathway. Twyman-St. Victor et al. showed that radiotherapy coupled with dual checkpoint blockade using αCTLA-4 with either αPD-L1 or αPD-1 was significantly better than radiotherapy with αCTLA-4 alone.[27] After treatment with radiotherapy and only αCTLA-4, PD-L1 became more highly expressed, leading to T cell exhaustion. Subsequent addition of PD-L1 reinvigorated T cells, reversing exhaustion. Dual checkpoint blockade with radiotherapy in naïve tumors yielded a complete response rate of 80%, with 58% of those surviving past 90 days. An osteosarcoma model used by Takahashi et al. was shown to have significant tumor growth inhibition at local and distant tumors when treated with a combination of radiotherapy, αPD-L1, and αCTLA-4.[48]

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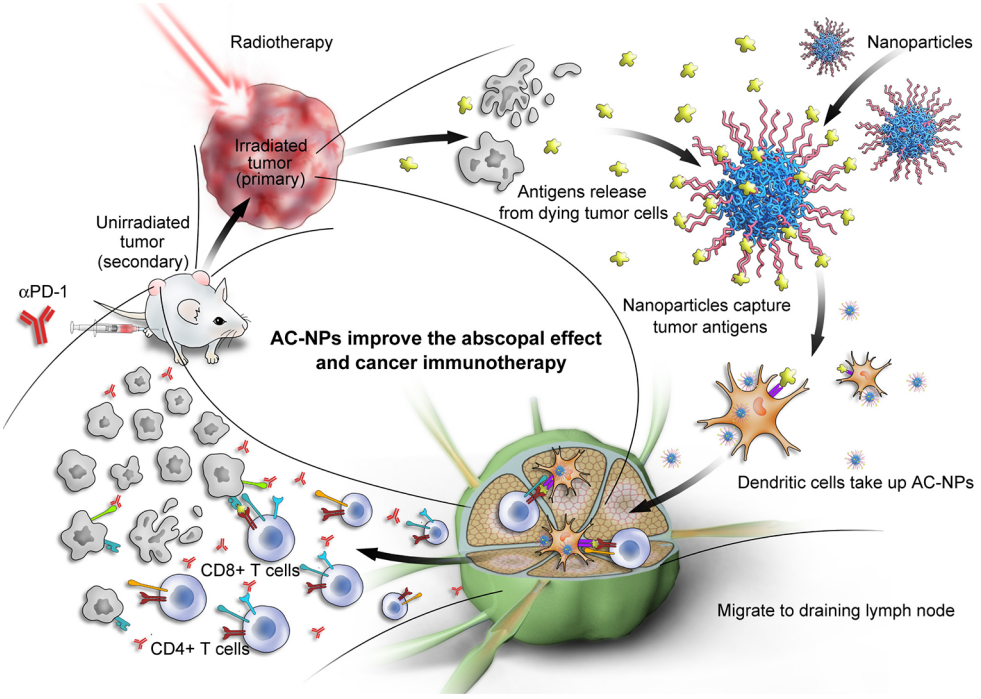

Figure 2. AC-NPs improve the abscopal response in mice by binding to tumor antigens released following radiotherapy and improving their presentation to dendritic cells. This increased immune activation is synergistic with αPD-1 treatment. Reprinted with permission.[19]

They found a significant increase in CD8+ T cells in mice treated with radiotherapy and αPD-L1/αCTLA-4 compared to αPD-L1/αCTLA-4 alone (9.5 ± 2.3% versus 8.5 ± 7.7%, P =.0118), particularly in CD8+ T cells that carried an increase in the cytotoxic protein GzmB. Lung metastases were reduced by 94% with αPD-L1/αCTLA-4 compared to no treatment, and by 98% when radiation was added (P = 0.0002 and P = 0.0005 respectively), with 3 of 7 mice surviving ≥60 days, compared to no mice surviving past 45 days in any other treatment group. A study by Belcaid et al. used an orthotopic glioblastoma model and anti-CTLA-4 (αCTLA-4) antibody prior to radiation to significantly prolong survival compared to radiotherapy alone (P < 0.05).[49] α-4-1BB was then also administered prior to radiotherapy as a triple therapy, extending medial survival from 24 days with radiotherapy alone to 67 days (P < 0.05) with 50% long term survival. This improved locoregional control and enhanced CD4+ and CD8+ T cells in the brain. Initial immunotherapy provided radiosensitization and improved radiotherapy in these studies, with either a single immunotherapeutic agent or multiple immune checkpoint inhibitors.

IMMUNOTHERAPEUTIC NANOPARTICLES WITH RADIATION

While many immunotherapeutic agents can work alone or in combination with radiotherapy, using NPs either as a vehicle with which to deliver these compounds or as an immunotherapeutic can further enhance treatment. NPs can improve cargo delivery by targeting tumor cells, increasing stability and solubility, and extending half-life.[50]

Erel-Akbaba et al. have shown that radiation followed by the administration of solid lipid NPs conjugated with immunotherapeutic small interfering RNAs (siRNAs) against epidermal growth factor receptor (EGFR) and PD-L1 leads to a significant decrease in glioblastoma growth and improved mouse survival.[51] Combining radiotherapy with targeted NPs without EGFR and PD-L1 siRNAs did not lead to a significant effect versus control (median survival of 21 and 22 days respectively). When employing immune checkpoint blockade via EGFR and PD-L1 siRNAs on non-targeted NPs without radiotherapy, they were able to show a moderate effect on tumor growth using bioluminescent luciferase imaging with total flux decreasing from (14.3 ± 0.8) × 10^7 in control to (9.1 ± 0.9) × 10^6 (P < 0.05) and mouse survival increasing from 21 to 24 days (P = 0.0072). When the NP was targeted using the cyclic peptide iRGD, they demonstrated the most significant reduction of tumor growth with a total flux of (1.1 ± 0.1) × 10^6 (P < 0.01 versus control), and an increased mouse survival of 38 days (P = 0.0001 versus control, P = 0.0040 versus radiation plus non-targeted NPs). A different type of NP used viral-like particles derived from the cowpea mosaic virus (CPMV) as an alternative to siRNAs to elicit an immune response.[52] Patel et al. used these NPs in combination with radiotherapy in an ovarian cancer mouse model. This caused an increase in tumor infiltrating lymphocytes and significantly delayed tumor growth, with tumor volumes in combination treated animals being 2−3× smaller than the next smallest group with radiotherapy alone (P < 0.05). Viral like NPs and tumor targeted siRNA NPs provided successful strategies to enhance radiotherapy with immunotherapeutic NPs.

Another approach to utilize NPs to improve the immune response post radiotherapy is through antigen-capturing NPs. The abscopal effect is a mechanism thought to be a part of many joint radiotherapy/immunotherapy treatments. This effect occurs when local tumor treatment causes a systemic regression of distant metastatic tumor burden, thought to be due to systemic immune effects.[14] Min et al. used maleimide-polyethylene glycol (PEG)-poly(lactic-co-glycolic acid) (PLGA) to form antigen capturing NPs (AC-NPs) to capture neoantigens from dying tumor cells post radiotherapy.[19] These NPs enhanced antigen presentation by APCs and resulted in

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increased CD8+ T cell activation as shown in Figure 2. These local treatments coupled with systemic αPD-1 delayed tumor growth and increased survival time, with up to a 20% cure rate using AC-NPs compared to 0% in treatments lacking AC-NPs. Coupling a previously reported STING-activating NP nanovaccine with local radiotherapy in two mouse models, Luo et al. saw a significant increase in CD8+ T cells via a STING dependent pathway following therapy.[53] In TC-1 and B16-OVA tumor models, 50% and 40% of treated mice were cancer free at 60 days, demonstrating improved therapy in distal tumors and enhanced outcomes in late stage solid cancers. AC-NPs proved useful in improving the radiotherapy/immunotherapy coupled abscopal effect and enhancing distal tumor control.

HIGH Z NANOPARTICLES AND NANOSCALE METAL−ORGANIC FRAMEWORKS

High atomic number (Z) elements have been shown to enhance radiotherapy through their high X-ray absorption, with Au and HfO_2 NPs yielding promising results.[54−57] A high Z NP that has been included in multiple clinical studies is the Hf based NBTXR3 (Nanobiotix).[58] This NP acts as a radiosensitizer, increasing DNA damage and cell destruction. A phase I dose-escalation, open-label, nonrandomized clinical trial was conducted in 22 patients with locally advanced soft tissue sarcoma (STS). NBTXR3 injections were given intratumorally 1 day prior to radiotherapy (50 Gy over 5 weeks) with resection following 6-8 weeks after completing radiotherapy. NBTXR3 was injected at a concentration of 53.3 g/L, with patients sequentially assigned to escalating dose levels of 2.5%, 5%, 10%, and 20% of baseline tumor volume. The recommended dose volume was determined to be 10% of baseline tumor volume, with dose limiting toxicities such as pain and necrosis at 20%. While this study was focused on dosing and safety profiles, the 10% recommended dosing provided median tumor shrinkage of 40% and showed this NP treatment to be technically feasible. A follow-up phase II−III trial compared radiotherapy alone to radiotherapy with intratumoral NBTXR3 in 176 randomized patients.[59] The previously found dosing of 10% tumor volume was used in those receiving NBTXR3, with all patients again receiving 50Gy radiation prior to resection. Pathological complete response was defined as "the presence of less than 5% residual malignant viable cells." They found that including NBTXR3 increased the percentage of patients with a pathological complete response from 8% to 16% ($P = 0.044$). These are promising initial clinical results that NPs given prior to radiation improve patient outcomes.

In addition to acting as a radiosensitizer, NBTXR3 coupled with radiotherapy also activates an immune response with increased CD8+ T cell infiltrates present in tumors in a CT26 tumor mouse model, and increased CD8+ T cells and PD1 in human patients with STS compared to radiotherapy alone.[60] These findings indicate that the local TME becomes more immunogenic with NBTXR3 and concurrent treatment with a checkpoint blockade agent such as αPD-1 could yield improved therapy, which is currently under investigation.[61]

nMOFs are another type of nanomaterial which can incorporate high Z elements and are comprised of organometallic polymers with metal ions linked by organic molecules. nMOFs can be used in a variety of applications, such as Gd^{3+} and Mn^{2+} nMOFs as T_1-weighted contrast agents or a Tb^{3+} nMOF to deliver a chemotherapeutic cisplatin prodrug.[62] Ni et al. have shown that Hf based nMOFs can further improve the sensitization of tumors to radiotherapy.[63,64] Their initial work showed that these nMOF X-ray absorbers improved ROS generation and increased hydroxyl radical formation by up to 55.3% compared to water. They also used the radioluminescent anthracene-based bridging ligand DBAn to show that Hf_{12}-DBAn had a radioluminescence slope of 1.36 ± 0.05 compared to Hf_6-DBAn's of 0.86 ± 0.04, indicating that Hf_{12}-DBAn had approximately 1.5 times greater X-ray absorption efficiency. In a CT26 colorectal adenocarcinoma mouse model, mice treated with radiotherapy and Hf nMOFs had greater tumor regression than standard radiotherapy with HfO_2 treatment, even when a 3.2-fold dose of HfO_2 was given. In a separate experiment in which mice had both a primary tumor treated with radiotherapy and a distant non-irradiated tumor, the immune checkpoint inhibitor αPD-L1 was added with the nMOF. This study showed that joint treatment significantly inhibited distant tumor growth through the abscopal effect and induced systemic antitumor immunity. An increase in tumor-specific T cells was noted, with CD8+ T cells increasing from 0.17 ± 0.07% and 0.06 ± 0.02% (primary and distant tumors respectively) in the PBS group without radiotherapy, to 0.98 ± 0.20% and 0.85 ± 0.30% in the Hf nMOF with αPD-L1 group with radiotherapy ($P < 0.001$ primary, $P < 0.05$ distant). This combination treatment also provided a memory effect for a group of mice that demonstrated complete primary tumor regression when rechallenged 1 month later on the contralateral flank, remaining tumor free for 60 days. Further work showed that nMOFs also catalyze the decomposition of H_2O_2 in hypoxic tumor environments to generate hydroxyl radicals and O_2.[64] These varying mechanisms show how nMOFs can be coupled with radiotherapy to improve tumor control.

Additional incorporation of photosensitizing ligands in the nMOF improves radiotherapeutic efficacy through the radiotherapy-radiodynamic therapy effect. While photodynamic therapy has a limited penetration depth, ionizing radiation can instead be used to excite photosensitizers—called radiodynamic therapy.[65] The hydroxyl radicals chemodynamically provide antitumor activity while O_2 attenuates the hypoxic environment, allowing radiodynamic therapy to permanently fix DNA damage, thereby enhancing radiotherapy.[66,67] These combined effects provide an immunogenic TME which can assist a systemic immune response through the use of αPD-L1. Initial phase I trials have begun with RiMO-301, an intratumorally injected nMOF used for radiotherapy-radiodynamic therapy to produce ROS and mediate DNA damage, and may also contain an immunomodulating agent to induce a tumor-associated antigen immune response.[68] This is another exciting clinical application of combined treatment with nanomaterials and radiotherapy.

NANOPARTICLES TO PROVIDE RADIATION OR IMMUNE BLOCKADE

NPs can provide other benefits as well, such as carrying photosensitizers, acting as complex delivery vehicles for antibodies, or transporting radioactive isotopes for direct radiation delivery.[23,69] NPs can be activated by alternative means, such as in photothermal or photodynamic therapy, or be upconverted to label and stimulate DCs, allowing precise tracking after injection into animals.[70−72] Photodynamic and upconversion methods rely on lower frequency, less energetic photons than X-rays, and only minimally penetrate tissue.

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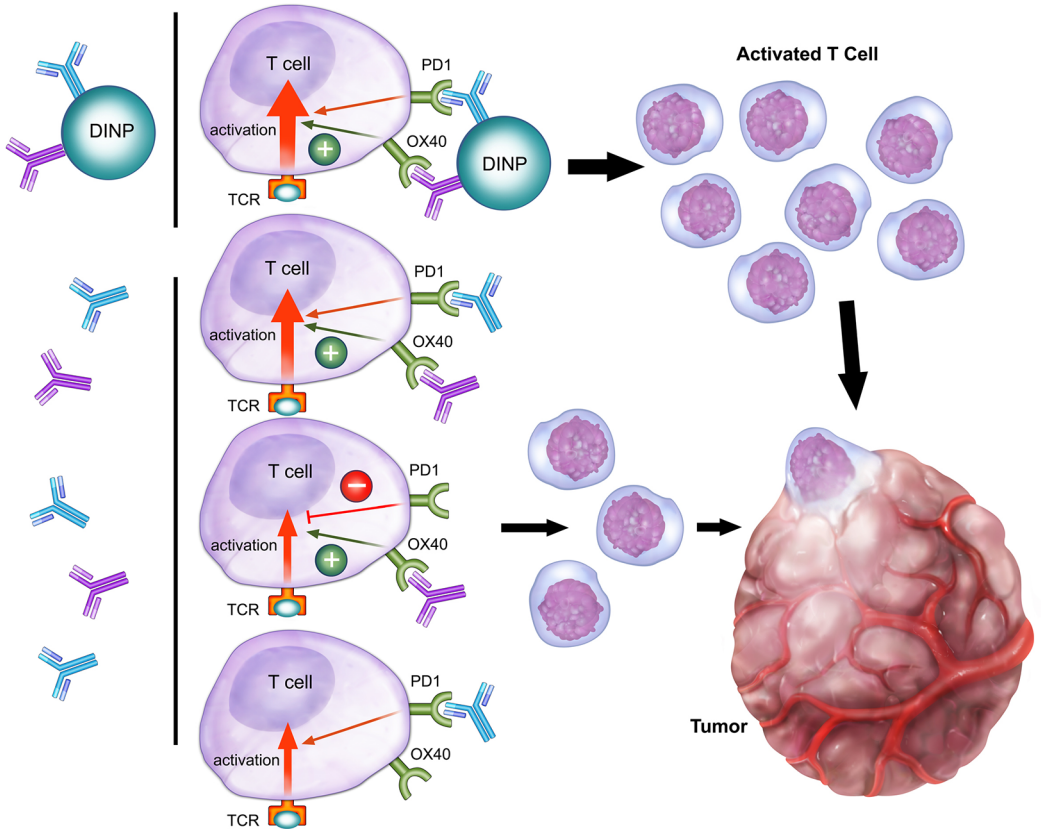

Figure 3. A dual immunotherapy nanoparticle (DINP) conjugated to both αPD-1 and αOX40 is able to bind both target proteins simultaneously, facilitating the enhancement of combination immunotherapy. αPD-1 blocks the PD1 inhibition of T cell activation (red arrow), while αOX40 stimulates OX40 mediated T cell activation (green arrow). Delivery of dual free antibodies usually result in sub-optimal single binding events, with only a small subset being co-stimulated, while DINPs provide spatiotemporal codelivery of antibodies, resulting in a greater number of dual binding events and maximizing T cell activation. Reprinted with permission.[69]

While this significantly limits their clinical applications to superficial tissue or areas where a light emitting source could be inserted, near-infrared light has been shown to penetrate tissue up to 3 cm at biologically beneficial levels.[73] Using NPs to deliver photosensitizers, immunotherapeutics, or radiation provides alternative methods to combine radiotherapy and immunotherapy.

Photodynamic Therapy. Photodynamic therapy can be used in place of radiotherapy to locally kill cells. This is accomplished by exposing photosensitizers to specific wavelengths of light to form ROS and kill nearby cells.[74] Another study which demonstrated the abscopal effect did so using natural killer cell membrane cloaked NPs (NK-NPs) to target tumors, which were loaded with a photosensitizer 4,4′,4′′,4′′′-(porphine-5,10,15,20-tetrayl) tetrakis (benzoic acid) (TCPP), in a 4T1 mouse model.[71] Photodynamic therapy at 660 nm was used and led to enhanced M1-macrophage polarization for antitumor immunity, increased tumor-infiltrating T cells, inhibited distal tumor growth, and prolonged mouse survival. This shows that photodynamic therapy can provide a substitute for radiotherapy in shallow use cases, with a similar tumor response.

Upconversion Nanoparticles. Nanoparticles have been engineered by some groups into upconversion NPs (UCNPs), which are able to perform upconversion luminescence (UCL), absorbing two or more low energy photons and emitting a single higher energy photon.[72] This distinguishes them from more common fluorescent or downconverting probes which typically emit a photon at a lower energy than absorbed. Infrared light is most often used for excitation, offering resistance to photobleaching from high power excitation light sources and minimization of background autofluorescence, but with limited penetration depth.[75−77] There is growing interest in UCNPs for their applications in sensing and imaging, especially for in vivo models.[78] Xiang et al. formed a PEG and polyethylene imine (PEI) dual-polymer-coated UCNP-PEG-PEI (UPP).[72] The antigen chicken egg ovalbumin (OVA) was bound to the UPP, and treatment with this UPP@OVA stimulated DC maturation, leading to increased cytokine secretions and cellular immunity. Mature DC levels increased from 27.72 ± 0.34% in the control to 50.47 ± 3.22% with UPP@OVA, while free OVA only increased mature DC levels to 41.9 ± 3.08% ($P < 0.05$ versus UPP@OVA). UCL imaging could then be performed to show the migration of UPP labeled DCs from peripheral tissues to draining lymph nodes, with as few as 50 DCs in a mouse being detectable. This was a significantly lower detection limit than other nanoprobes, such as quantum dots or magnetic NPs, which typically require a few thousand cells in vivo.[79,80] Wang et al. also used a UCNP, but one which was triggered photodynamically and used to capture antigens in order to elicit the abscopal effect.[20] Their UCNP was coated with DSPE-PEG-maleimide and the photodynamic enhancer indocyanine green, followed by loading with the photosensitizer rose bengal. These UCNPs

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were excited intratumorally with near-IR light, which the UCNPs converted to visible light, and subsequently activated the rose bengal photosensitizer to generate ROS. Indocyanine green enhances the UCL of the UCNP to also achieve local heat and photothermal therapy. The maleimide coated UCNPs also act as an antigen binding nanoplatform and deliver bound antigens to APCs, causing the abscopal effect. These UCNPs increased DC maturation levels by 3.01-fold compared to PBS with light and 1.55-fold compared to similar UCNPs without a surface maleimide coating. When this treatment was coupled with systemic αCTLA-4 treatment in a 4T1 mouse model, 84% of mice survived long term, with 34% developing tumor-specific immunity. UCNPs provide unique photosensitization techniques to improve imaging, or provide local tumor control or initiate antigen capture to improve therapy.

Nanoparticle Delivery of Immune Adjuvants. As previously discussed, local tumor hypoxia decreases the effectiveness of radiation. Chen et al. showed that local hypoxia could be ameliorated through the use of dual loaded core-shell PLGA NPs containing water-soluble catalase. These NPs were able to relieve local tumor hypoxia, enhancing radiotherapy.[81] They also conjugated the TLR7 agonist IMQ to the PLGA shell to locally deliver an immune adjuvant, and CTLA-4 checkpoint blockade was administered systemically for a synergistic whole-body response. When a primary fLuc-4T1 tumor was treated with radiotherapy and this dual loaded NP, tumor metastasis following IV injection of fLuc-4T1 cells was strongly inhibited and led to a 60% survival rate 60 days post therapy compared to 0% survival for all other groups after 35 days. Water-soluble catalase NPs improved radiotherapy through the delivery of an immune adjuvant and relief of TME hypoxia.

Nanoparticles Enable Combination Immunotherapy. To broaden the use of non-redundant immune checkpoints through dual checkpoint blockade,[27] Mi et al. developed an improved mechanism for immunotherapy checkpoint inhibitor delivery by creating a dual immunotherapy NP (DINP).[69] These DINPs consisted of maleimide-PEG-PLGA NPs with both αOX40 and αPD-1 conjugated to the surface. This allowed a precise spatiotemporal codelivery of antibodies to simultaneously block both pathways, as shown in Figure 3. The combined effects of αPD-1 blocking T cell inhibition and αOX40 increasing activation led to significantly upregulated T cell activity and numbers of CD8+ tumor infiltrating T cells (85.2%) compared to codelivery of free antibodies (68.5%), and an increase in the ratio of effector memory T cells to central memory T cells in DINP treated mice versus free antibody mice (54.4 versus 23.0). In this study, radiation was used to prime T cells, and DINP treatment resulted in a 20% increase in survival time compared to any other treatment and a 30% cure rate ($P < 0.001$), with 83% surviving a tumor rechallenge. Engineered DINPs effectively codeliver multiple checkpoint receptors concurrently for improved immunotherapy.

Another DINP, called an immunoswitch NP, was synthesized by Kosmides et al. by conjugating αPD-1 and α-4-1BB to iron-dextran NPs. They saw significant in vivo tumor growth inhibition in multiple murine models, including MC38-OVA and B16-SIY. In the B16-SIY model, tumors treated with intratumoral injection of immunoswitch NPs were only 19 mm^2 on day 36, compared to 158 and 126 mm^2 tumors in untreated and isotype NP treated tumors, respectively. Immunoswitch treated mice also had a 70% survival rate at day 55 compared to 10% in untreated mice. In the MC38-OVA model, 5 of 10 mice had complete tumor regression. The importance of the administration route was also investigated with B16-SIY bearing mice receiving intravenous immunoswitch NPs instead of intratumoral. This delayed tumor growth at least 13 days compared to no treatment or treatment with soluble intravenously injected αPD-1 and α-4-1BB ($P < 0.01$). They also showed that immunoswitch NPs demonstrate prolonged particle retention at the injection site with a local retention half life of 84.5 h compared to 15.2 h for soluble antibodies, allowing significantly longer interaction times when administered intratumorally. Immunoswitch NPs not only induce immune checkpoint inhibition but also prolong these effects due to their ability to remain localized.

Combination immunotherapy is not limited to dual therapy, with Au et al. multifunctionalizing NPs into trispecific nanoengagers. These nanoengagers are functionalized with the α-EGFR antibody cetuximab to target EGFR expressing tumors and the NK activating agents αCD16 and α-4-1BB to elicit an innate immune response. They first showed that DINPs with αCD16 and α-4-1BB reduced murine in vivo B16−F10 tumor growth by 40% compared to no treatment ($P = 0.0479$) and prolonged survival by 3 days ($P = 0.0156$). A similar experiment was performed with tumors first receiving 5Gy of radiation to enable NK targeting of tumor cells prior to NP treatment, resulting in even greater tumor growth reduction of 60% compared to radiotherapy alone. In addition, administration of these DINPs demonstrated more significant reduction than delivery of a combination of NPs which had only one of the antibodies each ($P < 0.05$), showing again that dual delivery improves therapy through simultaneous spatiotemporal delivery. They finally incorporated epirubicin for local chemotherapeutic release and cetuximab for EGFR targeting. Utilizing EGFR targeting to enable NK recognition in lieu of radiation allows for systemic over local tumor targeting. In an A431 murine model, they showed that EGFR targeting with no other treatment provided no benefit over no treatment ($P = 0.6217$) but when αCD16/α-4-1BB DINPs were added there was a delay in tumor growth over no treatment ($P = 0.0046$ with free α-EGFR, $P = 0.0061$ with α-EGFR NPs). Treatment using their trispecific nanoengagers with α-EGFR/ αCD16/α-4-1BB all delivered on the same NPs provided the greatest treatment response compared to no treatment, delaying tumor growth by 24 days and prolonging survival by 18 days ($P = 0.0018$). Trispecific nanoengagers enhanced tumor suppression through targeted delivery of multiple chemo- and immunotherapeutics.

Nanoparticle Delivery of Radionuclides. As an alternative to radiotherapy, recent studies have attached radionuclides directly to NPs for tumor delivery.[22,23] Petriev et al. conjugated rhenium-188 (^{188}Re) with PEG coated Si NPs, which, when injected intravenously, reached all organs and target tumors, whereas the ^{188}Re salt accumulated primarily in the thyroid.[22] When intratumorally injected, ^{188}Re concentration in the tumor was always over 30% during the first 3 h when conjugated to an NP, whereas it decreased to only 6% in the first 3 h in the case of free ^{188}Re. They achieved a 72% survival rate at 30 days compared to 0% of control in a cholangioma RS-1 Wistar rat model. Using a combinatorial approach, Au et al. used pretargeted radioimmunotherapy (PRIT), which consists of an initial tumor targeting antibody-based compound followed by a second radionuclide containing effector.[23] This technique has previously shown promising in

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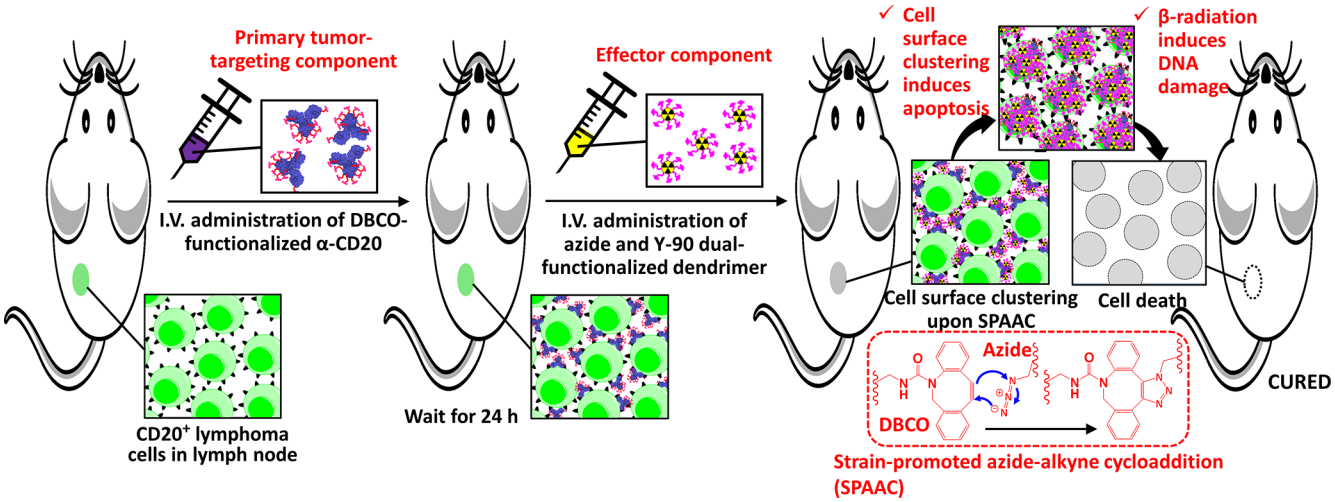

Figure 4. Two-step pretargeted radioimmunotherapy (PRIT) first targets the tumor with DBCO functionalized αCD20. Dual functionalized NPs carrying both azide and ^{90}Y cluster at the tumor through azide and DBCO SPAAC, delivering the radionuclide to induce cell damage and promote apoptosis through CDC. Reprinted with permission.[23]

vivo results;[82] however, bispecific antibody immunogenicity and competitive binding of effectors led to inferior treatments.[83] In order to minimize these deleterious effects, Au utilized bioorthogonal ligation reactions consisting of an αCD20 tumor targeting component functionalized with dibenzylcyclooctyne (DBCO) and an azide and yttrium-90 (^{90}Y) dual functionalized dendrimer NP effector as shown in Figure 4. The azide and DBCO undergo a strain-promoted azide−alkyne cycloaddition (SPAAC) to deliver the ^{90}Y to the tumor and also activate a compliment-dependent cytotoxicity (CDC) mechanism. The CDC forms a membrane attack complex to kill cancer cells, and the ^{90}Y damages tumor DNA. At the study end point of 90 days, 100% of the PRIT treated RAJI xenograft tumor-bearing mice remained alive compared to 33% in the next best group treated with pretargeted immunotherapy but a non-radioactive ^{89}Y. The PRIT treated mice either had no tumors remaining (67%) or tumors that were smaller than baseline (33%). A more aggressive disseminated lymphoma model was also evaluated through tail vein injection of Raji-luc cells. Treatment with ^{90}Y NPs without pretargeted immunotherapy somewhat delayed lymphoma propagation, increasing median survival by 20 days (P = 0.0795), and treatment with non-radioactive ^{89}Y NPs with pretargeted immunotherapy increased survival time to 81 days (P = 0.0090). PRIT treated mice had a 100% survival rate at the end point of 150 days (P = 0.0002 vs no treatment, P = 0.0098 vs pretargeted immunotherapy alone) and exhibited similar bioluminescence to tumor-free mice 46 days after treatment, demonstrating complete tumor eradication. Combination radionuclide and immunotherapeutic NP delivery resulted in 100% mouse survival in multiple models, providing an effective alternative to typical radiotherapy.

CONCLUSION AND OPPORTUNITIES FOR FUTURE RESEARCH

As many studies have shown, combined therapy provides superior treatment efficacy. Radiotherapy can not only treat local disease but also enhance immunotherapy through mechanisms such as MHC I upregulation,[38] neoantigen availability,[19,27,33,34] and increased cytokine release.[36] Immunotherapy can provide mutually derived benefits such as radiosensitizing the TME prior to radiotherapy[25,26,44−46] or eliciting an immune reaction to changes brought upon by radiation.[19,27,33,34] Radiotherapy and immunotherapy are synergistic, and nanotherapeutics can enhance both to further improve treatment effects. We have reviewed a number of innovative approaches to utilize nanotechnology to improve both radiotherapy and immunotherapy. However, it is important to note that a number of challenges remain to bring these approaches to clinical practice. These challenges include toxicity,[84] aggregation and in vivo clearance of particles,[85] and sequential or simultaneous timing of varying treatments.[86,87] On the other hand, many opportunities remain. One possibility for clinical translation of NP based combination therapy is the combined treatment of αPD-1 with stereotactic body radiotherapy (SBRT). A recent phase II clinical trial evaluated whether free αPD-1 coupled with SBRT was better than αPD-1 alone.[88] The dual treatment arm and αPD-1 only arm had an overall response rate of 36% and 18% (P = 0.07), median progression-free survival of 6.6 and 1.9 months (P = 0.19), and median overall survival of 15.9 and 7.6 months (P = 0.16) respectively. These results did not meet criteria for meaningful clinical benefit. This study did not include NPs so offers a potential opportunity to couple NP delivered immunotherapy with SBRT to increase radiosensitization and attain meaningful clinical benefit in future studies. In addition, the biology of cancer immunotherapy and the effect of radiation on the immune response are not fully understood and offer prospects for further research. As we learn more, there are likely many new opportunities to apply nanotechnology to improve both treatments. Such opportunities include improving NK and B cell responses in addition to T cell response, and engineering cells and agents that can improve the immune response to multiple neoantigens. Thus, the interface between cancer immunotherapy, radiotherapy, and nanotechnology is an exciting area of science. With more research focus and effort, advances in this area can bring significant clinical impact.

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Notes

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ABBREVIATIONS

AC-NP, antigen capturing NP; APC, antigen presenting cell; CDC, compliment-dependent cytotoxicity; CRT, chemo-radiotherapy; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DBCO, dibenzylcyclooctyne; DC, dendritic cell; DINP, dual immunotherapy NP; EGFR, epidermal growth factor receptor; IMQ, imiquimod; NK-NP, natural killer cell membrane cloaked NP; nMOF, nanoscale metal organic framework; OVA, ovalbumin; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PEG, polyethylene glycol; PLGA, poly(lactic-co-glycolic acid); PRIT, pretargeted radioimmunotherapy; ROS, reactive oxygen species; SBRT, stereotactic body radiotherapy; siRNA, small interfering RNA; SPAAC, strain-promoted azide−alkyne cycloaddition; STING, stimulator of interferon genes; STS, soft tissue sarcoma; TLR7, Toll-like receptor 7; TME, tumor microenvironment; UCL, upconversion luminescence; UCNP, upconversion NP; UPP, UCNP-PEG-PEI

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