Received: 14 April 2023 | Revised: 3 July 2023 | Accepted: 2 August 2023

DOI: 10.1002/wnan.1924

FOCUS ARTICLE

WIREs NANOMEDICINE AND NANOBIOTECHNOLOGY WILEY

Nanotechnology-enhanced radiotherapy and the abscopal effect: Current status and challenges of nanomaterial-based radio-immunotherapy

Dhushyanth Viswanath[1] | **Jeehun Park**[2] | **Rahul Misra**[3] | **Vincenzo J. Pizzuti**[4] | **Sung-Ho Shin**[1] | **Junsang Doh**[2,5] | **You-Yeon Won**[1,6]

[1]Davidson School of Chemical Engineering, Purdue University, West Lafayette, Indiana, USA

[2]SOFT Foundry Institute, Seoul National University, Seoul, Republic of Korea

[3]Analytical Sciences, Sanofi, Toronto, Ontario, Canada

[4]Department of Medicine, University of Colorado, Anschutz Medical Campus, Aurora, Colorado, USA

[5]Department of Materials Science and Engineering, Institute of Engineering Research, BioMAX, Seoul National University, Seoul, Republic of Korea

[6]Purdue University Institute for Cancer Research, Purdue University, West Lafayette, Indiana, USA

Correspondence
Junsang Doh, SOFT Foundry Institute, Seoul National University, Seoul 08826, Republic of Korea.
Email: jsdoh@snu.ac.kr

You-Yeon Won, Davidson School of Chemical Engineering, Purdue University, West Lafayette, IN 47907, USA.
Email: yywon@purdue.edu

Funding information
National Science Foundation, Grant/Award Numbers: CBET-1803968, CBET-2211843; Purdue University Institute for Cancer Research (PU-ICR), Grant/Award Number: P30CA023168; Davidson School of Chemical Engineering at Purdue University; National Research Foundation of Korea under the Korean Ministry of Science and ICT (MSIT), Grant/Award Number: RS-2023-00208359; Seoul National University

Edited by: Andrew Wang, Associate Editor and Gregory M. Lanza, Co-Editor-in-Chief

Abstract

Rare but consistent reports of abscopal remission in patients challenge the notion that radiotherapy (RT) is a local treatment; radiation-induced cancer cell death can trigger activation and recruitment of dendritic cells to the primary tumor site, which subsequently initiates systemic immune responses against metastatic lesions. Although this abscopal effect was initially considered an anomaly, combining RT with immune checkpoint inhibitor therapies has been shown to greatly improve the incidence of abscopal responses via modulation of the immunosuppressive tumor microenvironment. Preclinical studies have demonstrated that nanomaterials can further improve the reliability and potency of the abscopal effect for various different types of cancer by (1) altering the cell death process to be more immunogenic, (2) facilitating the capture and transfer of tumor antigens from the site of cancer cell death to antigen-presenting cells, and (3) co-delivering immune checkpoint inhibitors along with radio-enhancing agents. Several unanswered questions remain concerning the exact mechanisms of action for nanomaterial-enhanced RT and for its combination with immune checkpoint inhibition and other immunostimulatory treatments in clinically relevant settings. The purpose of this article is to summarize key recent developments in this field and also highlight knowledge gaps that exist in this field. An improved mechanistic understanding will be critical for clinical translation of nanomaterials for advanced radio-immunotherapy.

This article is categorized under:

Dhushyanth Viswanath, Jeehun Park, Rahul Misra, and Vincenzo J. Pizzuti shared equally to co-first authorship.

Therapeutic Approaches and Drug Discovery > Nanomedicine for Oncologic Disease

KEYWORDS

abscopal effect, antigen capture/presentation, immune checkpoint inhibitors, nanoparticle radio-enhancers/radio-sensitizers, radiotherapy

1 | CURRENT STATUS OF THE UNDERSTANDING OF THE ABSCOPAL EFFECT

1.1 | History/clinical phenomenology

The term "abscopal effect" was first coined by Mole in 1953 to describe the effect of irradiation "at a distance from the irradiated volume, but within the same organism" (Mole, 1953). However, it was not widely used by the medical community until the mid-1970s due to a lack of concrete human evidence. Two independent case reports were published in 1973 and 1975 that described shrinkage of distal tumors secondary to irradiation of localized tumor, presumably mediated by the abscopal effect (Ehlers & Fridman, 1973; Kingsley, 1975). Since then, the abscopal effect has been consistently but rarely reported in radiotherapy (RT) case reports—only 46 cases were reported in 45 years (1969–2014; Abuodeh et al., 2016). The reported rate of cases increased dramatically to 47 cases in the 6-year period from 2012 to 2018, which coincides with the advent of combining RT with immunotherapy, in particular immune checkpoint inhibitors (ICIs; Dagoglu et al., 2019). The first case report demonstrating the abscopal effect in a human patient treated with a combination of RT and an ICI was published in 2012 (Postow et al., 2012). Importantly, this study demonstrated that this combination therapy could be synergistic and provided mechanistic justification for this treatment approach.

The combination of RT and ICIs has greatly raised the incidence of the abscopal effect, but it is still reported in only a small fraction of patients. Detailed analysis has revealed that higher CD8+ T cells, lower PD-1+ T cells, and lower regulatory T (Treg) cells in tumor tissues positively correlate with abscopal effect responses (Ji et al., 2020; Twyman-Saint Victor et al., 2015). These findings indicate that the immune cell populations inhabiting the tumor microenvironment (TME) are major determinants of the incidence of abscopal effects.

Radiation therapy treatment parameters are also important variables to control to maximize the abscopal effect, as they have a large effect on immune cell populations in tumor tissues. In preclinical mouse models, dose and fractionation of RT had a significant impact on immune cell profiles, including tumor-reactive CD8+ T cells and Tregs, which in turn alter tumor regression and the abscopal effect (Dewan et al., 2009; Morisada et al., 2018; Schaue et al., 2012). Stereotactic body radiation therapy (SBRT), a type of radiation therapy that delivers a high dose of radiation to a targeted area within the body, also enhanced immune response-mediated abscopal effect when combined with ICIs (Watanabe et al., 2020). Numerous clinical trials combining various immunotherapies with advanced radiotherapies are currently ongoing to identify optimal conditions (Kang et al., 2016).

1.2 | Mechanistic hypotheses

Since its initial observation, the abscopal effect has been hypothesized to be an immune-mediated phenomenon, but a detailed mechanistic study in human patients has been technically challenging. Consequently, it was first shown in a mouse model that the abscopal effect is mediated by immune responses. Intratumoral injection of flt3-ligand [flt3-L, a growth factor of dendritic cells (DCs)] prior to RT significantly increased abscopal effects whereas no abscopal effect was observed in T cell-deficient nude mice (Demaria et al., 2004). These results clearly indicate that the abscopal effect depends on antigen-specific immune responses mediated by DCs and T cells. Further study highlighted the significance of the cross-presentation of cancer neoantigens through DCs and the activity of type 1 interferon (IFN) for the abscopal effect (Burnette et al., 2011; Rodriguez-Ruiz et al., 2016). Collectively, the induction of immunogenic cell death (ICD) that initiates antigen-specific immune responses is crucial for the abscopal effect (summarized in Figure 1).

Irradiation causes diverse modes of cell death, including apoptosis, necrosis, autophagy, and mitotic catastrophe. The predominant modes of death depend on the cell type and on the dose, duration, and source of radiation

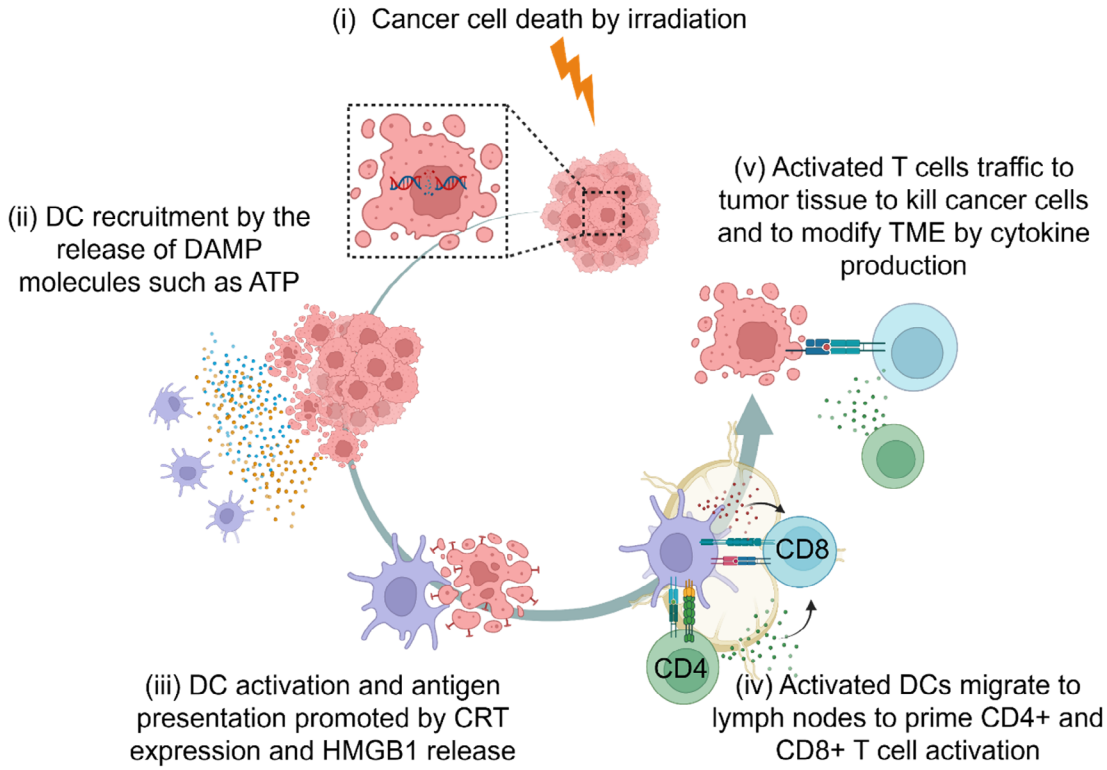

FIGURE 1 Mechanism of abscopal effect induced by radiotherapy. ATP, adenosine triphosphate; CRT, calreticulin; DAMP, danger-associated molecular pattern; DC, dendritic cell; HMGB1, high mobility group box 1.

[represented by Step (i) in Figure 1; Grass et al., 2016]. Radiation damages DNA, causing mutations, chromosomal aberrations, and other forms of genomic instability. Ionizing radiation damages DNA directly and via the production of reactive oxygen species (ROS) that can contribute to cell death by increasing oxidative stress. DNA damage is considered the major cause of cell death from irradiation because the pattern of damage that radiation induces occurs in clusters, such as inducing more than two modifications in 1–2 helical turns, single-strand breaks, and double-strand breaks (Sutherland et al., 2000). In this way, radiation disrupts the cell cycle to cause mitotic catastrophe and leads to multiple nuclei formation, abnormal chromosome segregation, and ultimately cell death. In order to adequately produce an abscopal effect, radiation-mediated cell death must initiate ICD pathways, which induce tumor-specific immune responses. Biochemical hallmarks for the induction of ICD, including the release of danger-associated molecular patterns (DAMPs) such as adenosine triphosphate (ATP) and high mobility group box 1 (HMGB1), and surface expression of calreticulin (CRT) were previously confirmed by in vitro irradiation experiments (Golden et al., 2014). Specifically, ATP released from dying cells is a representative "find-me" signal used to recruit DCs for the engulfment of dying cells [Step (ii) in Figure 1; Elliott et al., 2009]. CRT cell surface expression on tumor cells acts as an "eat-me" signal for DCs as well. HMGB1 released from dying cells activates DCs through various receptors, including receptor for advanced glycation endproducts (RAGE), Toll-like receptor 2 (TLR2), and TLR4 to promote pro-inflammatory gene expression and antigen cross-presentation [Step (iii) in Figure 1; Apetoh et al., 2007; Lotze & Tracey, 2005]. Activated DCs can subsequently migrate to lymph nodes via lymphatics to prime CD8+ T cells [Step (iv) in Figure 1]. These activated CD8+ T cells, in turn, can traffic to tumor tissues through blood vessels to exert cytotoxicity against cancer cells [Step (v) in Figure 1; Chen & Mellman, 2013]. While CD8+ T cells have traditionally been recognized as major contributors to anti-tumor immune responses due to their potent cytotoxic capabilities against tumor cells, the importance of CD4+ T cells in orchestrating anti-tumor immunity is now gaining prominence (Speiser et al., 2023). CD4+ T cells not only promote CD8+ T-cell activation in the lymph nodes (Ferris et al., 2020), but they also exhibit their own effector functions by producing cytokines that trigger diverse anti-tumor activities within the TME (Speiser et al., 2023) or directly engage in tumor cell killing (Cachot et al., 2021).

It is crucial to acknowledge that activated T cells have the ability to migrate not only to the irradiated primary tumor tissues but also to distal tumor sites, enabling them to engage in anti-tumor activities. As RT primarily influences

Steps (i)–(iii) and ICIs predominantly target Steps (iv)–(v), the combination of these therapies can result in synergistic effects. The subsequent sections delve into ongoing investigation of methods aimed at further enhancing one or more of these steps highlighted in Figure 1. Furthermore, the importance of considering these findings for future work and potential incorporation into clinical practice will be underscored.

We would like to note the comprehensive review conducted recently by Wang et al. (2020) on topics within this same general field. However, it is worth highlighting that our article delves into considerably greater detail regarding the subjects covered in Section 2.1 [nanoparticle radio enhancers promoting Steps (i) and (ii)] and Section 2.2 [antigen capturing nanoparticles enhancing Steps (ii)–(iv)], while also presenting more recent advancements in these technologies. Although there is some overlap in Section 2.3 [incorporation of immune checkpoint inhibition for promoting Step (v)] with the previous review, we have appropriately cited and mentioned it. Furthermore, in Section 2.3, we have provided updates in the form of newer articles and emphasized key outstanding questions that persist in the field at present.

2 | NANOMATERIALS FOR ENHANCED ABSCOPAL EFFECT

2.1 | Enhancing radiation-induced cell death and immunogenicity

As discussed briefly in the previous section, RT is a stochastic process that involves direct and indirect interaction of x-ray photons with different organelles, leading to cellular damage, with DNA being the primary target (Hall & Giaccia, 2006). Radiation-induced cellular damage can result in cell death via different mechanisms such as mitotic catastrophe, apoptosis, necrosis, autophagy, and senescence (summarized in Figure 2; Castedo et al., 2004; Ouellette et al., 2022; Vakifahmetoglu et al., 2008). Alternatively, the damage can be repaired, resulting in cell survival. In conventional RT, the primary concern is the maximization of cancer cell death, reflected by the elimination of the irradiated tumor, while minimizing off-target toxicities. However, to initiate the abscopal effect, an additional consideration is to shift the pathway taken for cell death to be primarily immunogenic to enable the recruitment and activation of dendritic cells [Steps (i) and (ii) of Figure 1]. A recent clinical trial combining conventional RT with anti-PD-1 therapy achieved a 29% abscopal effect rate for patients with advanced cancers (Trommer et al., 2019). Although this study sets a proof-of-principle precedent, there remains a clear need for technologies that can sensitize cancer cells to not only result in enhanced cell death from RT, but also bolster the extent of ICD to more reliably initiate an abscopal response.

One such development toward this aim is the usage of high-Z metal-containing nanoparticles (NPs) that amplify energy deposition from ionizing radiation via localized production of electrons through photoelectric and Compton effects, yielding elevated intracellular ROS levels (Retif et al., 2015). At present, the most extensively studied platform is NBTXR3, a formulation of HfO_2 NPs, which is undergoing phase I trials for treatment of head and neck cancers in combination with anti-PD-1 ICI [clinical trial number: NCT03589339]. Preclinical investigations of intratumorally administered NBTXR3 have unveiled several key insights on the mode of action toward inciting abscopal responses. Irradiated NBTXR3 promotes cancer cell death more effectively than conventional RT by increasing the extent of DNA double-strand breaks (DSBs), as demonstrated by the γH2AX assay, which leads to early or late apoptosis via activation of the caspase cascade. Furthermore, there is increased formation of micronuclei which promotes DC recruitment via the cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) pathway (Marill et al., 2019). NBTXR3-treated cancer cells were also found to be more susceptible to ICD upon irradiation as observed by increased CRT expression and release of ATP and HMGB1 (Darmon et al., 2022). In addition, an immunopeptidome analysis of the MHC-I complex revealed a remarkable 4.7-fold increase in peptide abundance following treatment compared to the control group receiving only RT. Notably, this increase included several peptides with positive immunogenicity scores. It is speculated that this augmented peptide abundance may enhance the availability of weaker tumor epitopes, thereby facilitating a robust anti-tumor T cell regardless of the tumor-associated antigen epitope type. In vivo testing of NBTXR3 + RT demonstrated a significant increase in the infiltration of CD4+ T cells, CD8+ T cells, and macrophages within tumors compared to RT only. Moreover, when combined with anti-PD-1 ICI treatment, NBTXR3 + RT exhibited a substantial induction of significant abscopal responses (Hu, Paris, Barsoumian, Abana, He, Sezen, et al., 2021; the comprehensive discussion of these study results is provided in Section 2.3). Collectively, these studies strongly support the notion that NBTXR3 not only enhances the effects of RT through dose-enhancement, but also fundamentally alters and improves the immunogenicity of the treatment itself.

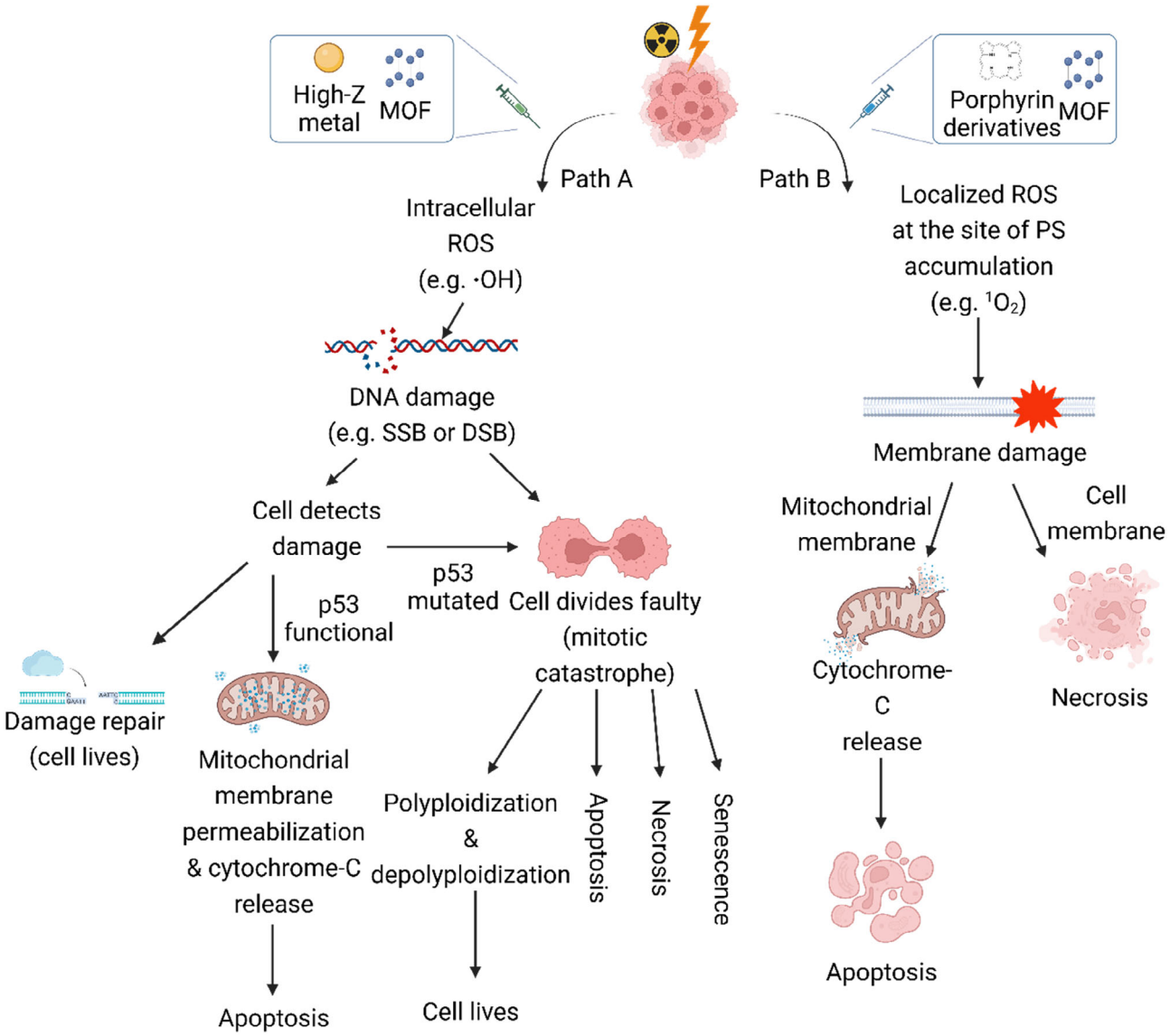

FIGURE 2 Schematic overview of dominant cancer cell death pathways caused by conventional RT with high-Z dose enhancement (Path A), and photodynamic therapy (Path B). Note that the above depiction is simplified and more detailed mechanisms have been covered in reviews by Ouellette et al. (2022), Vakifahmetoglu et al. (2008), Castedo et al. (2004), and Chilakamarthi and Giribabu (2017). DSB, double-strand break; MOF, metal–organic framework; ROS, reactive oxygen species; SSB, single-strand break.

Although the simplicity of the NBTXR3 formulation, speculated to be HfO_2 NPs with a coating of sodium trimetaphosphate/sodium hexametaphosphate [patent number: US 8845507 B2], is attractive from the perspective of clinical translation, this approach potentially limits the ability to co-load compounds for concomitant multi-modal treatment. Nevertheless, motivated by the promising results of NBTXR3, recent preclinical innovations have sought to sensitize cancer cells using high-Z NPs while enabling additional therapeutic benefits. These additional benefits include tumor-associated macrophage (TAM) depletion (Huang et al., 2021), concomitant systemic NP/ICI administration (Chen, Pan, et al., 2020), macrophage polarization (Qin et al., 2020), and hypoxia-independent sensitization (Li et al., 2021; Zhou et al., 2021), as summarized in Table 1 and discussed again in the context of combining NPs with ICIs in Table 3. From a mechanistic standpoint, all platforms operate predominantly via DNA DSB-initiated apoptosis mechanism as previously outlined for NBTXR3. Based on the pioneering mechanistic studies conducted by Nanobiotix, which elucidated the positive impact of high-Z dose-enhancement on augmenting the immunogenicity of cell death induced by RT (Darmon et al., 2022), it is reasonable to speculate that similar characteristics may be observed with these alternative platforms. However, case-specific experiments are necessary to confirm this hypothesis. Nevertheless,

TABLE 1 Summary of nanomaterial dose enhancers covered in Sections 2.1 and 2.3.

Radio-enhancer/ sensitizer	Coating	Cancer cell line(s)	Mode of cell death	References
HfO_2	Unconfirmed: potentially sodium trimetaphosphate/ sodium hexametaphosphate	CT26, 344SQ, 344SQR	Enhanced apoptosis, increased necrosis	(Hu, Paris, Barsoumian, Abana, He, Sezen, et al., 2021)
Zoledronic acid-gadolinium (Gd^{3+}) coordinated nanorods	Zoledronic acid (ZA)	CT26, 4T1	N/A	(Huang et al., 2021)
Hollow mesoporous TiO_2	PEG-Mal, PEG-NH_2	4T1	Enhanced apoptosis	(Chen, Pan, et al., 2020)
$Na_{0.2}Bi_{0.8}O_{0.35}F_{1.91}$:20%Yb, 2%Er UCNPs	PVP	H460	Enhanced apoptosis	(Qin et al., 2020)
$Fe_4Se_2W_{18}$ NCs	None	HeLa, 4T1	Enhanced apoptosis	(Zhou et al., 2021)
PbS/CdS QDs	RGD peptides, catalase, PEG	4T1	Enhanced apoptosis	(Li et al., 2021)
MnO_2	PEG	B16F10, CT26	Increased ICD	(Guan et al., 2022)
Snowflake Au-nanocarriers (composed of Ag + Au)	PEG	Tramp-C1	Enhanced apoptosis and ICD	(Choi et al., 2020)
Poly-(L-lysine) + iron oxide NP + CpG	Poly-(L-lysine)	B78, MyC-CaP, TC11	Enhanced ICD	(Zhang, Sriramaneni, et al., 2022)
MnO_2 NPs + temozolomide	PEG-PAE	G422	Enhanced ICD	(Meng et al., 2021)
AGuIX® NPs (chelated gadolinium)	Polysiloxane matrix	B16	Enhanced late apoptosis and ICD	(Song et al., 2022)
$WO_{2.9}$–WSe_2–PEG	OA-PEG	4T1	Increased late apoptosis and necrosis and ICD	(Dong et al., 2020)
Hafnium, Ce6, atovaquone, and sabutoclax NPs (AHSC NPs)	Ce6–PEG–polyphenols	4T1	Enhanced apoptosis and ICD	(Sang et al., 2021)
Au	MC38 cell-membrane encapsulated	MC38	Enhanced apoptosis and ICD	(Qin et al., 2021)

efficacy studies conducted using bilateral in vivo models have demonstrated the capacity of high-Z dose enhancers to effectively impede tumor growth in the primary treated tumors when compared to RT alone. The presence of ICD in the primary tumor suggests indications of a noticeable, although inconsistent and limited, abscopal effect on untreated secondary tumors compared to RT alone. However, when dose-enhanced RT is combined with ICI or macrophage polarization, there is a substantial improvement in the extent of abscopal effect observed on secondary tumors. This suggests that neither dose enhancement nor ICI treatment alone is likely to be capable of reliably initiating the abscopal response in clinical applications. Section 2.3 discusses the combination of the two treatment modalities in detail. Additionally, due to mechanistic overlap with conventional RT (Path A, Figure 2), it remains unclear whether these dose enhancement strategies would also suffer from common sources of radio-resistance, such as p53 mutations that can hinder DSB-initiated apoptosis, or epidermal growth factor receptor (EGFR) overexpression that can increase the probability of DSB repair (Hutchinson et al., 2020).

To address this potential limitation, an alternate approach is to use RT as a stimulus for initiating photodynamic therapy (PDT) with the aid of energy-transducing NPs (RT-PDT; Viswanath & Won, 2022). Photodynamic therapy, conventionally potentiated in clinic using type-II photosensitizers (PS), operates via the production of $^1O_2$ which can damage cell or organelle membranes as opposed to DNA. This is due to the hydrophobic nature of commonly used photosensitizers, which leads to their accumulation in membranes (Lucky et al., 2015). However, the limited penetration depth associated with conventional PDT has restricted its application to surface-level lesions (Viswanath & Won, 2022). To address this challenge, energy-transducing NPs offer a solution by utilizing x-rays from RT to either generate visible

light photons in situ or directly activate photosensitizers through the inelastic scattering of photoelectrons (Lu et al., 2018). Pioneering work on combining RT-PDT with immune checkpoint inhibition (ICI) to initiate abscopal responses has been reported by Lin and co-workers. Their formulation, RiMO-301, is speculated to be nano-metal organic frameworks (nMOFs) comprising Hf clusters bridged with 5,15-di(p-benzoato)porphyrin photosensitizer and loaded with an indoleamine 2,3-dioxygenase inhibitor (IDOi@DBP-Hf; Lu et al., 2018), is currently undergoing phase I trials for the treatment of advanced cancers amenable to intratumoral injections (clinical trial number: NCT03444714). Although the presence of Hf-clusters enables dose-enhancement via the DNA DSB mechanism outlined above (Path A, Figure 2), the predominant cell-killing effect arises from the energy transfer between x-ray-activated Hf and the bridging photosensitizers, leading to the production of $^1O_2$. Subsequently, this causes membrane damage, which results in cell death via apoptosis, and necrosis to a lesser extent (Path B, Figure 2). Cell death was shown to be immunogenic as denoted by CRT expression, resulting in a significant abscopal response mediated by CD8+ T cells in bilateral tumor models established with TUBO and CT26 cell lines. However, similar to the previously discussed high-Z dose enhancers, the abscopal effect was negligible in the absence of the ICI (IDOi), further emphasizing the synergistic effects of both components. One potential limitation of the RT-PDT platform is the dependence of PDT on oxygen (O_2), which may reduce treatment efficacy for hypoxic tumors. To address this limitation, Ni et al. reduced the dimensionality of nMOFs from 3D to 2D, which improves the diffusion of ROS out of the particle, and therefore maximizes the therapeutic potential of available oxygen in the tumor (Ni et al., 2019). Alternatively, Sang et al. co-loaded oxygen-bound hemoglobin in their formulation to alleviate hypoxia (Sang et al., 2021). While the studies mentioned above are pioneering investigations into combining RT-PDT with ICIs for initiating abscopal responses, there are several unique RT-PDT platforms have been developed to effectively suppress primary tumor growth upon irradiation. Some of these platforms possess cancer-specific or even organelle-specific targeting capability upon systemic administration (Cline et al., 2019). Additionally, previous investigations have explored the combination of conventional light-activated PDT with ICI, which has shown impressive results (Lou et al., 2023; Xia & Wang, 2022). Taken together, future studies integrating these technologies could lead to promising results for depth-independent RT-PDT-mediated abscopal responses, addressing current technology limitations.

As previously noted in other reviews, finding an optimal treatment schedule for combining RT with ICI presents several challenges (Buchwald et al., 2018). It has been hypothesized that the timing of dead/apoptotic cell uptake is critical, as cells that are taken up at the peak of immune response can incite ICD, whereas cells that are taken up as immune response wanes can yield no response or potentially even induce tolerogenic cell death (TCD; Green et al., 2009). When using sensitizing NPs, these considerations become more complex as the NPs not only promote cancer cell death, but also accelerate it. This is reflected in the preclinical studies discussed thus far, which unanimously report increased extents of early and late apoptosis as well as necrosis relative to conventional RT. Additionally, radioresistant tumors that incur DNA damage tend to primarily undergo mitotic catastrophe, which occurs over a significantly longer timescale than necrosis and direct apoptosis (Erenpreisa & Cragg, 2001; Hutchinson et al., 2020). Previous discussions have attempted to establish a correlation between the final mode of cancer cell death and its immunogenicity, suggesting that apoptosis is nonimmunogenic or tolerogenic while necrosis is immunogenic. However, numerous examples, including those highlighted in this section, clearly contradict this hypothesis (Green et al., 2009). Furthermore, it is crucial to recognize that the pathway leading to cell death (as illustrated in Figure 1) can influence the release of immunogenic markers, such as DAMPs, subsequently altering the timescale and characteristics of the immune response (Li, 2018). Therefore, attaining a more comprehensive understanding of the intricate interplay between the kinetics of cell killing, mode of cell death, and the timescale of immune response is vital for developing optimized treatment protocols. Initial studies suggest that dose fractionation may be more beneficial than single-dose RT in stimulating immune responses because the latter can have adverse effects on immune cells present in the TME at the time of radiation (Buchwald et al., 2018). As radiosensitizers can provide dose-enhancement effects, a lower incident x-ray dose can be applied, thereby mitigating the issue. Lastly, questions remain over the optimal delivery route for radiosensitizers and ICIs. Most of the studies reviewed in this section utilized direct intratumoral injections for radiosensitizers, and separate intraperitoneal (IP) injections for ICIs. Lu et al. conducted an in vivo study demonstrating that intratumorally administered nMOFs loaded with IDOi resulted in a better abscopal response than when IDOi was delivered separately via IP injection (Lu et al., 2018). However, current clinical trials with NBTXR3 utilize the latter strategy to deliver anti-PD-1. Therefore, systematic studies are needed to investigate the synergistic effects of co-delivered radiosensitizers and ICIs with the goal of attaining the optimal prognosis using each respective platform. Initial progress toward addressing this knowledge gap is discussed in Section 2.3.

2.2 | Increasing antigen presentation

Radio-enhancement/sensitization-induced ICD enhances the release of tumor-derived protein antigens (TDPAs) from dying cancer cells and thus the probability of inducing immune responses through their interactions with a series of receptors expressed on DCs as discussed in Sections 1.2 and 2.1. Unfortunately, the combined radio-immune responses from RT + radio-enhancer/sensitizer combination treatments are typically still insufficient to elicit abscopal effects. The incidence of abscopal effects can further be improved by additionally facilitating the capture and presentation of released tumor antigens to immune cells [Steps (ii)–(iv) of Figure 1]. There have been relatively few studies exploring how to enhance antigen capture and presentation in the context of RT. However, useful insights can be borrowed from similar studies involving photothermal therapy (PTT). ICD induced by RT or PTT triggers the release of TDPAs. In theory, the exposure and recognition of TDPAs by immune cells elicit abscopal immune responses even in distal tumors (Suek et al., 2019). In reality, such incidents are very rare (Janopaul-Naylor et al., 2021). Researchers have proposed possible reasons for this that include (1) rapid clearance of protein antigens, (2) inefficient recognition by and recruitment of immune cells, and (3) development of immune resistance.

To the best of our knowledge, Wang and coworkers were the first who demonstrated the concept of using antigen-capturing nanoparticles (ACNPs) to enhance the capture and presentation of released tumor antigens to immune cells (Min et al., 2017). Since this work, many similar approaches have been demonstrated for the purpose of enhancing the immunogenicity of RT and PTT. Common ACNP designs include (i) a radio/photosensitizer core (for enhancing antigen release from cancer cells), (ii) a surface functional group/ligand (for antigen capture), and (iii) an immunomodulator and/or a targeting moiety (for enhancing recognition and interactions of NPs with immune cells). Figure 3 presents a schematic illustration of the concept of using ACNPs for enhancing tumor antigen release and capture/presentation to immune cells as a way of boosting abscopal immune responses.

Surface chemistry plays a critical role in the selection and capture of tumor antigens by NPs. Previous studies have explored this either by functionalizing ACNPs with specific surface ligands or by using ACNP materials that have a nonspecific affinity to bind with proteins (Liu et al., 2021; Min et al., 2017; M. Wang, Song, et al., 2019; R. Wang, He, et al., 2019; Yang et al., 2021). Wang and coworkers conducted combinatorial testing on four different variations of poly(lactic-co-glycolic acid) (PLGA) NPs: (a) PLGA-PEG-NH_2 NPs, (b) 1,2-dioleoyloxy-3-(trimethylammonium)propane-functionalized PLGA NPs (PLGA-DOTAP NPs), (c) maleimide-PEG-coated PLGA NPs (PLGA-PEG-mal NPs), and (d) unmodified PLGA NPs (Min et al., 2017). They found that the amount and composition of proteins captured by the NPs are dependent upon the surface chemistries of the NPs, and only PLGA-PEG-mal NPs were found to bind to proteins through formation of stable covalent (thioether) bonds. All other NPs were found to bind to protein antigens via weaker non-covalent hydrophobic and/or ionic interactions. Other more recent studies also examined PEGylated NPs with amine and maleimide end groups (Liu et al., 2021; Min et al., 2017; M. Wang, Song, et al., 2019; R. Wang, He, et al., 2019; Yang et al., 2021). Among common PEG end groups, the order of antigen capture efficiency was found to be PEG-OCH_3 < PEG-OH < PEG-NH_2 < PEG-mal (R. Wang, He, et al., 2019). All studies (Min et al., 2017; M. Wang, Song, et al., 2019; R. Wang, He, et al., 2019) appear to support that PEG-mal is thus far the most suitable surface chemistry for antigen capture applications. This is likely accomplished via the sulfhydryl group of maleimide reacting with the thiol group of a protein's cysteine residue.

However, it remains a question whether PEGylation is the best approach because non-PEGylated NPs were able to capture a greater total number and wider variety of antigens (Min et al., 2017). Because all types of neoantigens contribute to arising adaptive immunity to cancer, the capability of non-PEGylated PLGA NPs to absorb a diverse range of antigens is an advantage, although this is at the expense of losing specificity due to the possibly of nonspecific binding of several other nonimmunogenic proteins. Non-PEGylated PLGA NPs were also found to effectively accumulate in lymph nodes and become taken up by antigen-presenting cells (APCs). Their immune activation efficacy was comparable to that of PLGA-PEG-mal NPs, and unmodified PLGA NPs were better than other types of PLGA NPs tested (i.e., PLGA-PEG-NH_2 and PLGA-DOTAP NPs). Unfortunately, at this point, it is unknown how the in vivo pharmacokinetic and biodistribution characteristics of ACNPs are influenced by PEGylation. There have also been reports of other types of non-PEGylated ACNPs, such as those made of natural polysaccharides (Guibin Pang et al., 2019). PEGylated PLGA NPs loaded with $Al(OH)_3$ nanoclusters (NCs) have also been explored as ACNPs (Zhong et al., 2022). $Al(OH)_3$ NCs capture antigens via electrostatic, hydrophobic as well as metal–ligand exchange interactions. Hollow NP structures, such as mesoporous silica NPs (Yang et al., 2021) and mesoporous TiO_2 NPs (Y. Chen, Pan, et al., 2020), have also been explored. These studies demonstrated the potential advantage of these hollow NPs—their large internal surface areas available for antigen adsorption.

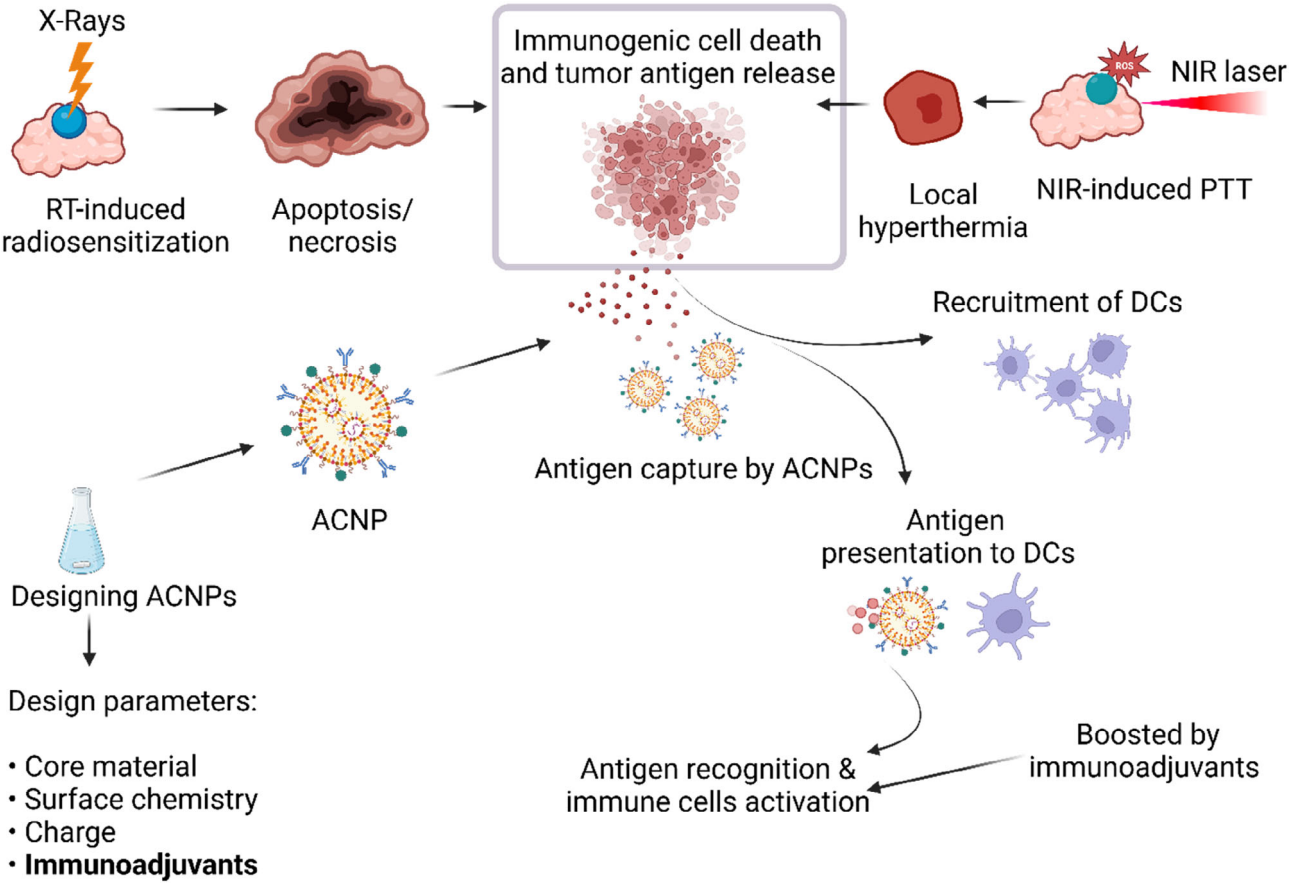

FIGURE 3 Schematic representation of tumor antigen release, its capture, and presentation by ACNPs. ACNP, antigen capturing nanoparticle; DC, dendritic cell; NIR, near-infrared; PTT, photothermal therapy; RT, radiotherapy.

TABLE 2 Change in diameter of ACNPs pre- and post-antigen capture and the amount of captured antigens in different studies.

Core material	ACNP	Surface functionalization	Diameter before antigen capture (nm)	Diameter after antigen capture (nm)	Amount of antigens captured	Reference
$NaYF_4$	UCNP/ICG/RB-mal	Maleimide	44	241	340 μg/mg	(M. Wang, Song, et al., 2019)
	UCNP/ICG/RB-PEG	PEG coating	38.7	79.8	~50 μg/mg	
PLGA	PLGA	Unmodified	150	1000	~550 μg/mg	(Min et al., 2017)
	PLGA-PEG-mal	PEG-Maleimide	100	200	~450 μg/mg	
	PLGA-DOTAP	DOTAP Ligand	200	8500	~550 μg/mg	
	PLGA-NH_2	NH_2	100	150	~300 μg/mg	
Manganese (Mn)	ONc-Mn-A-$MalF^{127}$	Maleimide	180	800	~940 μg/mg	(Li et al., 2022)
Copper sulfide (CuS)	CuS-PEG-NH_2	PEG-NH_2	13.4	2739	0.55 mg/mL	(R. Wang, He, et al., 2019)
	CuS-PEG-mal	PEG-Maleimide	15.4	5007	0.69 mg/mL	
PLGA-PEG	PPIAO	PEG, Aluminum hydroxide	333	400	0.51 mg/mL	(Zhong et al., 2022)

Antigen capture is often confirmed by changes in the hydrodynamic size and surface charge (zeta potential) characteristics of ACNPs in vitro. Some ACNPs, such as PLGA-PEG-mal NPs (Min et al., 2017), showed relatively minor increases in NP diameter of about 100–200 nm post exposure to antigens, whereas other ACNPs, such as unmodified PLGA NPs (Min et al., 2017), showed dramatic NP size increases of about 1000–8000 nm (Table 2). In the latter

situation, the size increase signifies severe agglomeration of ACNPs upon exposure to antigens. Interestingly, the agglomeration did not significantly reduce the amount of antigens captured (Table 2), as the same was observed for PLGA-DOTAP NPs as well (Table 2). These results demonstrate the potential disadvantage of non-PEGylated ACNPs. Large agglomerated ACNPs would be unable to diffuse through the TME and would face more difficulty in being internalized by DCs. The question of how antigen-induced aggregation influences immune cell interactions and transport kinetics of the ACNPs deserves more detailed study.

In some instances, the NP core material serves additional functions beyond acting as a substrate for antigen adsorption or for attaching end-functionalized PEG chains. Manganese NPs coated with maleimide-functionalized Pluronic F127 surfactants are an example (Li et al., 2022). Upon phagocytosis of antigen-coated NPs by DCs, the Mn^{2+} ions released from the NP core function as a cofactor together with ABZI (amidobenzimidazole, a STING agonist) for the phosphorylation of TBK1 and p65 enzymes. As a result, cGAS-STING signaling is activated. Presentation of antigens captured by the surface maleimide groups to DCs and cGAS-STING signaling activated by Mn^{2+} ions promote Type 1 IFN production in DCs, which causes the activation of CD8+ T and NK cells. This dual mechanistic approach to immune activation was shown to be able to significantly enhance the abscopal effect in this study. Natural polysaccharides are another core material that has been explored for their inherent protein binding and immunomodulatory characteristics. Polysaccharide NPs have been shown to be effective in inducing abscopal responses by promoting CD40, CD80, and CD86 expression (activation markers for T cells) on DCs, which induce innate and adaptive immune responses (Guibin Pang et al., 2019).

The abscopal effect can further be enhanced by providing additional stimulation to immune cells within the tumor site. This can be achieved by adding additional surface functionalization to ACNPs with immunoadjuvants, such as toll-like receptor (TLR) agonists [e.g., CpG and poly(I:C)] and STING agonists (e.g., cyclic-di-GMP). These immunoadjuvants stimulate DCs and thus facilitate antigen recognition and uptake by DCs. NPs can even be produced from immunoadjuvant materials themselves. For instance, poly(imidazoquinoline) [poly(IMDQ)] nanogel-type immunoadjuvants have been used to activate the TLR7/8 pathway in DCs, enhance the maturation of DCs after antigen capture, and induce significant upregulation of CD86 and CD40 expression on DCs (Nuhn et al., 2016). Another example is PC7A, a pH-sensitive polymeric immunoadjuvant whose repeat unit contains a seven-membered ring and a tertiary amine. PC7A activates the STING pathway in DCs and promotes maturation and migration of DCs and the subsequent activation of T cells. Antigen-loaded PEGylated PC7A NPs have been shown to be able to deliver antigens to DCs and significantly increase the activity of CD8+ cytotoxic T cells via a STING-dependent pathway following RT (Min Luo et al., 2019). Concurrent administration of immunoadjuvants (such as TLR and STING agonists) and antigen capture moieties within a single NP regimen is expected to produce a synergistic effect toward enhanced abscopal effects and warrant further future exploration (Figure 4).

2.3 | Considerations for the preclinical study of combining ICIs with nanoparticle-enhanced RT

ICIs have become an increasingly important component of standard care therapy in a variety of cancer subtypes. ICIs in current clinical use target common immune-suppressive signaling pathways facilitated by programmed-death ligand 1 (PD-L1) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) ligand interactions by inhibiting binding and downstream signaling (Marin-Acevedo et al., 2021). Treatment algorithms that incorporate ICIs for different tumor subtypes vary in the timing of ICI therapy and depend upon the tumor mutational status of each patient's disease (Vaddepally et al., 2020). For example, ICI therapy is frequently utilized in the treatment of non-small cell lung cancers (NSCLCs) without targetable driver mutations [e.g., EGFR, anaplastic lymphoma kinase (ALK), etc.] or as a second-line therapy after treatment with platinum-based chemotherapy (Xiong et al., 2021). Additionally, ICIs are combined with RT routinely, and data suggest that ICIs improve progression-free and overall survival when combined with RT compared to RT or ICI therapy alone, possibly through an abscopal response (Samstein et al., 2017; Shulman et al., 2022).

Motivated by this recent clinical success, preclinical research studies have begun to further explore combinations of ICIs with RT. An area of particular interest has been nanomaterial-enhanced/modified RT combined with ICIs, which have incorporated ICIs into their therapeutic strategy via systemic delivery or co-delivery within the nanomaterial constructs to enhance radio-immunotherapy responses [Step (v) of Figure 1; Cremolini et al., 2021; Han et al., 2020]. This topic was previously discussed in a review by Wang et al., and the following discussion aims to expand upon points raised in that article and provide pertinent updates in this area (Wang et al., 2020). In a recent article by Guan et al.,

the authors devised hollow MnO_2 NPs loaded with a PI3Kγ inhibitor (IPI549) that, when exposed to x-ray RT, significantly reduced immunosuppressive myeloid cells, increased cytotoxic T lymphocyte infiltration, and enhanced antitumor efficacy and survival in post-surgical resection models of colon carcinoma and melanoma at primary tumor sites (Guan et al., 2022). Further, they explored combining this technology with systemically delivered ICIs in the form of anti-PD-L1 antibodies and examined how this intervention compared to controlling tumor growth at both primary and modeled metastatic secondary sites. In mice receiving IPI549-loaded NPs + RT + anti-PD-L1, there was a robust response in controlling distant tumor growth that was significantly better compared to RT, anti-PD-L1, and IPI549-loaded NPs ± RT. The mechanism of this improvement was further studied, and it was found that CD8+ T lymphocytes were increased in the tumor site, M1/M2 macrophage ratios were increased, and immunosuppressive myeloid cells were decreased in the combination treatment in both primary and secondary metastatic tumor sites. They also found that tumor hypoxia was somewhat relieved by utilizing these NPs, which has been identified as a limiting factor for radio-immunotherapy/abscopal responses (Mudassar et al., 2022). Of note, MnO_2 NPs have previously been used to catalyze the breakdown of hydrogen peroxide into molecular oxygen, which increases the efficacy of RT by increasing the production of tumoricidal ROS and relieving tumor hypoxia (Prasad et al., 2014). The improved antitumor responses demonstrated by the work of Guan and colleagues were also found to be durable in inducing a vaccine-like effect by preventing tumor formation in re-inoculation, which was mediated by memory CD8+ effector T lymphocytes and was only observed in the IPI549-loaded NP + RT + anti-PDL1 group. These results underscore the importance of driving pro-inflammatory changes in the form of disinhibition (anti-PD-L1), enhanced antigen presentation and inflammatory cell recruitment (RT), and reduction in immunosuppressive cell tumor-site infiltration to shift toward M1 macrophage versus M2 predominant cell populations (PI3Kγ inhibition via IPI549). Meng and colleagues devised a similar MnO_2 NP system that contained a HIF-1 inhibitor (acriflavine) that relieved tumor hypoxia, relieved T-cell exhaustion in a fashion similar to PD-L1 axis blockade, improved the effectiveness of RT, and induced abscopal responses, as was discussed at length in a review by Wang et al. (Meng et al., 2018; Wang et al., 2020).

Additional preclinical studies have explored combinations of nanomaterial radio-enhancers/sensitizers with systemically administered ICIs. In a preclinical model of melanoma, Zhang and colleagues combined systemic anti-CTLA-4 antibodies with an iron oxide + poly-(L-lysine) + CpG oligodeoxynucleotide in the setting of RT with significant immune-mediated antitumor responses and improved overall mouse survival, along with anti-tumor vaccine-like effects (Y. Zhang, Sriramaneni, et al., 2022). Chen et al. also studied systemic anti-CTLA-4 therapy in concert with RT-enhancing PLGA-coated NPs, with successful induction of an abscopal response, as has been previously reviewed by Wang et al. (Chen et al., 2019; Wang et al., 2020). In another article exploring NBTXR3 radio-enhancers/sensitizers with systemic anti-PD-1 and anti-CTLA-4 antibodies in an anti-PD-1 inhibitor-resistant model of lung cancer, Hu et al. found that combined high-dose RT to primary and low-dose RT to secondary tumors in conjunction with ICIs and NBTXR3 significantly improved overall survival and tumor growth control in primary/secondary tumors, reduced spontaneous lung metastases, and induced a vaccine-like response in preventing tumor growth/metastasis in a cancer cell re-challenge (Y. Hu, Paris, Barsoumian, Abana, He, Wasley, et al., 2021). Several other preclinical explorations of RT-enhancing nanomaterials and systemically administered ICIs displayed similar trends of improved outcomes and anti-tumor immune responses and are summarized in Table 3 (Dong et al., 2020; Huang et al., 2021; Li et al., 2021; Meng et al., 2021; Qin et al., 2021; Sang et al., 2022; Song et al., 2022; Zhou et al., 2021). In total, these preclinical studies provide significant motivation for further exploring the impact of systemic anti-PD-1/PD-L1 and anti-CTLA-4 inhibitors in the setting of RT-enhancing nanomaterials, especially those that can reduce immunosuppressive mechanisms within tumor milieu, enhance anti-tumor responses through ICI mechanisms, and increase overall anti-tumor efficacy and survival.

In contrast with the previously mentioned studies, other researchers have focused on the co-delivery of ICIs with nanomaterials and purposefully avoided systemic administration (G. Chen, Chen, et al., 2020). The central rationale behind this approach posits that because immune-related adverse events (IRAEs) are directly correlated with anti-tumor responses in general (Freeman-Keller et al., 2016), co-delivery of ICIs in radio-enhancing/sensitizing nanomaterials may reduce off-target IRAEs while preserving anti-tumor immunity induced via the nanomaterial-RT-ICI combination (G. Chen, Chen, et al., 2020). Recently, Choi et al. developed and evaluated snowflake-like gold nanocarriers (Au NCs) loaded with anti-PD-L1 antibodies in the setting of RT (Choi et al., 2020). Their work demonstrated increased effectiveness of radio-enhancement/sensitization and enhanced tumor growth-suppression effects for RT + anti-PD-L1-loaded Au NCs when compared to RT only and RT + systemic anti-PD-L1 + Au NCs in a mouse model of prostate adenocarcinoma. This provided direct in vivo evidence of enhanced tumor suppressive effects with co-delivered ICI therapy compared to systemic administration. Additionally, the authors evaluated both immune cell response and IRAEs between these different groups and demonstrated that the anti-PD-L1-loaded Au NCs both

TABLE 3 Summary of nanomaterial combinations with systemic ICIs covered in Section 2.3 and previously detailed in Section 2.1.

Radio-enhancer/ sensitizer NPs	ICI (delivery route)	Cancer cell line(s)	Key findings of NP + ICI treatments	References
HfO_2 NPs	Anti-PD-1 and anti-CTLA-4 (systemic)	CT26, 344SQ, 344SQR	Increased immune-cell infiltration, better tumor growth control, and effective vaccine-like response to rechallenge	(Hu, Paris, Barsoumian, Abana, He, Sezen, et al., 2021)
Zoledronic acid-gadolinium (Gd^{3+}) coordinated nanorods	Anti-PD-L1 (systemic)	CT26, 4T1	Increased CD4+/CD8+ T cells in primary and secondary tumors, prevented metastases, and found CD8+ T cells as crucial effector cells	(Huang et al., 2021)
$Fe_4Se_2W_{18}$ NCs	Anti-PD-L1 (systemic)	HeLa, 4T1	Effectively inhibited primary and secondary tumor growth	(Zhou et al., 2021)
PbS/CdS QDs	Anti-PD-1 (systemic)	4 T1	Improved primary and secondary tumor growth control, increased CD8+ T cells, and increased IFN-γ and TNF-α	(Li et al., 2021)
MnO_2 NPs	Anti-PD-L1 (systemic)	B16F10, CT26	Remarkable local and distant control	(Guan et al., 2022)
Snowflake Au-nanocarriers (composed of Ag + Au)	Anti-PD-L1 (systemic vs. local)	Tramp-C1	Improved tumor control, increased CD8+ T cells, and reduced Treg in primary and secondary tumors	(Choi et al., 2020)
Poly-(L-lysine) + iron oxide NP + CpG	Anti-CTLA-4 (systemic)	B78, MyC-CaP, TC11	Increased M1/M2 ratio and CD8+ T cells in tumor. NP-RT + anti-CTLA-4 served as anti-cancer vaccine	(Y. Zhang, Sriramaneni, et al., 2022)
MnO_2 NPs + temozolomide	Anti-PD-L1 (systemic)	G422	Tumor burden decreased and increased CD8+ T cells in glioblastoma tumors after BBB-opening US treatment	(Meng et al., 2021)
AGuIX® NPs (chelated gadolinium)	Anti-PD-1 (systemic)	B16	Improved tumor control, increased CD8+ and CD4+ T cells, and increased CD8+/Treg ratio, and vaccine-like effects	(Song et al., 2022)
$WO_{2.9}$–WSe_2–PEG	Anti-PD-L1 (systemic)	4T1	Reduced primary and secondary tumor volume, increased CD4+ and CD8+ T cells, and had vaccine-like effects	(Dong et al., 2020)
Hafnium, Ce6, atovaquone, and sabutoclax NPs	Anti-CTLA-4 ± anti-PD-L1 (systemic)	4T1	Increased CD8+ T cells, increased tumor control, and facilitated immune memory-cell formation	(Sang et al., 2021)
Membrane-encapsulated Au NPs	Anti-PD-1 (systemic)	MC38	Improved tumor control, improved prevention of tumor metastasis, and increased CD4+ and CD8+ T cells	(Qin et al., 2021)

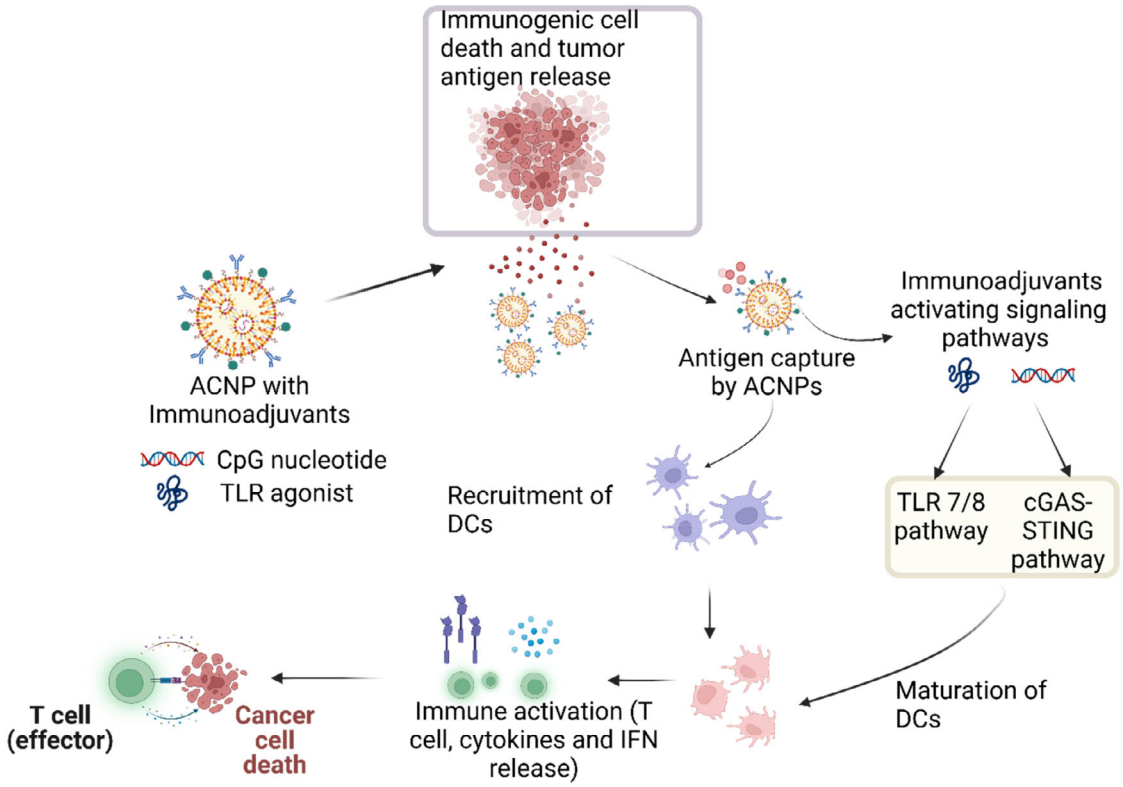

FIGURE 4 Graphical depiction of immunoadjuvant-loaded ACNPs for enhancing ICD. ACNP, antigen capturing nanoparticle; cGAS-STING, cyclic GMP-AMP synthase-stimulator of interferon genes; DC, dendritic cell; IFN, interferon; TLR, toll-like receptor.

increased the ratio of cytotoxic T lymphocytes to Treg lymphocytes much more effectively than other treatment combinations while maintaining a low induction of systemic Th17 lymphocyte response. Th17 lymphocyte responses within tumors can help to enhance anti-tumor immunity, but when present systemically, can lead to IRAEs. Because this study directly compared the systemic delivery of anti-PD-L1 antibodies to co-delivery within nanocarriers, it serves as a useful proof-of-principle for justifying the local, co-delivered ICI approach over the systemic delivery. While there are limitations of this single preclinical study, the results suggest an interesting strategy for future investigations; that is, to directly compare systemic versus co-delivered ICIs in the nanoparticle-RT treatment setting.

Another interesting question that arises from this topic concerns the timing of ICI therapy relative to RT administration, as was briefly mentioned in Section 2.1. This has been previously studied in a preclinical melanoma model by Dovedi et al., who found that the concurrent administration of ICI therapy with RT is essential for anti-tumor immune response via CD8+ T lymphocytes and subsequent mouse survival (Dovedi et al., 2014). These authors also determined that the likely reason for this is the reduced expression of PD-L1 in tumor cells at 7 days post-RT compared to the first-day post-RT. These preclinical results are consistent with several observations in clinical studies of a variety of cancers. As was reviewed recently by Zhang et al., several clinical trials and retrospective cohort analyses support the concurrent administration of RT + ICIs (Z. Zhang, Liu, et al., 2022). Furthermore, sequential administration has been found to be effective, but progression-free and overall survival decrease when the administration of ICI therapy is delayed beyond a few-week period post-RT (Z. Zhang, Liu, et al., 2022). It should be noted, however, that the optimal timing of ICIs with respect to RT may vary according to tumor type and the specific ICI therapy employed, as another recent review highlights (Wang et al., 2018). The one clear consensus from these individual studies and reviews is that further exploration of the timing of ICI treatment relative to RT should be conducted to optimize treatment algorithms.

Taken together, there is an abundance of preclinical evidence that supports the use of nanomaterial-enhanced RT in combination with ICI therapy to improve anti-tumor responses and survival. As discussed previously, multiple variations in approach have been attempted with predominantly positive results. However, there are a few key questions that remain and warrant further exploration with respect to nanotechnology-enhanced radio-immunotherapy. First, more investigation is warranted in comparing systemic ICI administration with co-delivery of ICI therapy within nanomaterials. Specific comparisons between these treatments with respect to anti-tumor and survival efficacy would

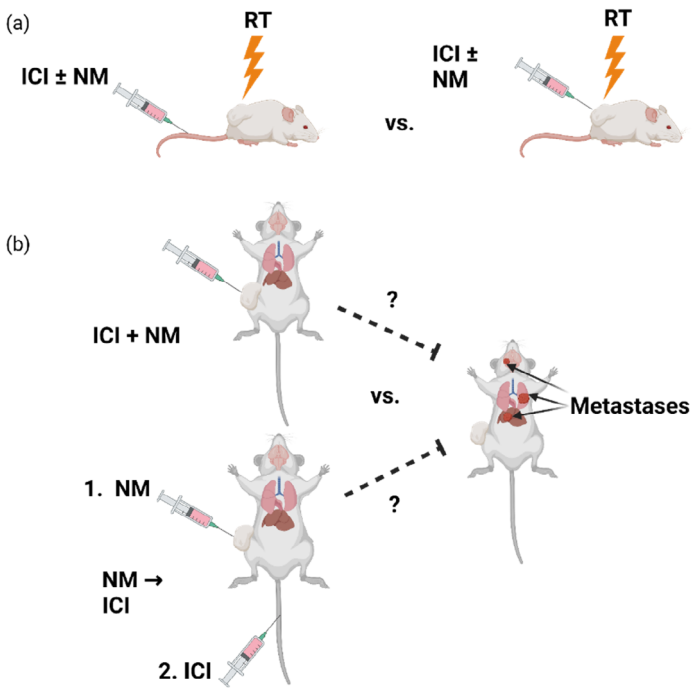

FIGURE 5 Schematic representation of knowledge gaps in combining ICI with nanoparticle-bolstered RT. (a) Is intra-tumoral co-delivery of ICIs with nanomaterials superior to systemic delivery of ICIs with NMs? (b) Does the sequence or timing of NM versus ICI administration affect treatment outcomes? ICI, immune checkpoint inhibitor; NM, nanomaterial; RT, radiotherapy.

be helpful in guiding future translation of these nanotechnologies into clinical use. Second, evaluation of potential off-target IRAEs in systemic versus locally co-delivered ICIs should be examined, as this comparison will provide further motivation for attempting one approach or the other in future technologies. Third, it remains to be seen if the timing of ICI therapy with respect to nanomaterial-enhanced RT has a long-term effect on treatment outcomes, so future studies can consider comparing concurrent versus delayed administration of ICI therapy. These key remaining questions are summarized graphically in Figure 5.

3 | CONCLUSIONS AND OUTLOOK

As demonstrated in the studies discussed above, nanomaterials have great potential to be used to enhance the abscopal effect of RT. Specifically, nanomaterials can be useful in three important ways. The first is, as discussed in Section 2.1, to use NPs to enhance ICD following RT. The basic hypothesis behind this approach is that an increased release of DAMPs (ATP, HMGB1, CRT, etc.) leads to an increase in systemic immune response (Zhou et al., 2019). Radiation can cause cancer cell death by a few different mechanisms, including some that are nonimmunogenic or even immunosuppressive (Weichselbaum et al., 2017). One outdated but popular theory is that necrosis is immunogenic whereas apoptosis is non-immunogenic/tolerogenic. Unfortunately, this simplistic theory has been found to be indefensible; the mode of cell death itself does not necessarily correlate with the degree of immunogenicity (Green et al., 2009). The examples discussed in Section 2.1, in fact, support this more modern view. We think that ICD-enhancing NPs reported to date (summarized in Section 2.1) can be categorized into two types: (1) RT enhancers and (2) photodynamic therapy (PDT) transducers. RT enhancers enhance the DNA damaging/cell-killing effectiveness of RT by locally depositing x-ray energy (i.e., by producing secondary electrons under x-ray irradiation; Retif et al., 2015). Preclinical data support that RT-enhancer NPs are able to enhance ICD, which is interesting because RT kills cancer cells predominantly by apoptosis, even under the influence of RT enhancers. A potential limitation of RT enhancers, however, is the similarity in the mechanism of cell killing relative to normal RT; therefore, their efficacy might be limited for radio-resistant (P53/EGFR-mutant) tumors (Hutchinson et al., 2020). PDT transducers are NPs that convert ionizing radiation (x-rays) into light and thus potentiate radiation-induced PDT (Viswanath & Won, 2022). As discussed in Section 2.1, photosensitizers used in tandem are typically hydrophobic and thus preferentially accumulate in cell/organelle membranes, and as a result, cause localized damage to cellular

membranes and thus an increase in necrosis upon photo-activation. PDT transducers have been shown to be highly effective in enhancing DAMP signaling and have potential to be effective even against radio-resistant tumors because of the multiple modes of action that they make possible. At the fundamental level, however, we should note that although the (hypothetical) link between DAMP release and immunogenicity has been widely cited (Galluzzi et al., 2017), there have also been reports in the literature that are indicative of the opposite. DAMP molecules can also cause immunosuppressive (and even pro-metastatic) effects (Hou et al., 2017). Therefore, it is fair to state that the status of this DAMP-induced immunogenicity hypothesis remains conjectural. For this reason, it is important to keep in mind that, in exploring nanomaterials for enhancing radiation-induced ICD, it cannot be automatically assumed that increased levels of DAMPs (especially, those observed in in vitro biochemical studies) will always result in increased antitumor immune responses and thus higher chances of abscopal cancer remission.

As discussed in Section 2.2, the second area where nanomaterials are expected to play an important role is the use of NPs for facilitating the capture and presentation of tumor antigens by antigen-presenting cells (APCs, such as dendritic cells). Since the seminal work by Wang and co-workers, which demonstrated the suitability of poly(lactide-co-glycolide) (PLGA)-based NPs for this application (Min et al., 2017), several other nanomaterial chemistries, both organic and inorganic, have been investigated for the possibility of using them for the purpose of collecting and delivering tumor-generated antigens to APCs (summarized in Section 2.2). Results from these studies now allow us to define desirable attributes for ACNPs—(1) biocompatible and nontoxic, (2) resistant to nonspecific protein adsorption, (3) selective affinity to DAMPs and tumor-associated and neo-antigens, (4) favorable interaction with (i.e., efficient uptake by) APCs, (5) nonself-aggregating, and so forth. These stringent requirements mean that an optimal ACNP system might have to be a highly engineered construct that integrates multiple functional elements (for instance, in order to satisfy the seemingly conflicting requirements of protein adsorption resistance and antigen affinity). An obvious downside of using a complex, multifunctional nanoparticle design is, of course, the risks associated with scale-up and quality control in manufacturing, if one really intends to translate ACNPs into clinical use. In this regard, the original finding of Wang and co-workers that simple, nonsurface-coated PLGA nanoparticles exhibit outstanding performance in facilitating antigen capture/presentation (Min et al., 2017) is quite interesting and inspiring. It is believed that tumor antigens adsorb to the surfaces of the PLGA nanoparticles due to hydrophobic and/or hydrogen-bonding interactions; neither of these interactions is molecule specific. Therefore, it is reasonable to expect that, in this approach, there exists a competition between tumor antigens and nonimmunogenic biomolecules for adsorption sites. It is therefore further expected that the best performance in antigen capture is achieved only when PLGA nanoparticles are injected into the tumor immediately prior to RT. We envision that further studies clarifying the effect of the relative timing of ACNP administration on the antigen capture efficiency (and the degree of abscopal effect) will be extremely informative.

Section 2.3 of this article discusses the topic of introducing immune checkpoint inhibitors (ICIs) on top of nanoparticle-enhanced RT. As discussed in that section, because of the multiple therapeutic agents/mechanistic stages involved in going from the process of ICD all the way to cancer cell kill by activated lymphocytes (explained in Section 1), it becomes an important factor how those different therapies are combined to produce maximum synergistic effects. There are a series of relevant questions that should be addressed as we explore new ways of combining radio-enhancing/sensitizing nanomaterials with ICIs: (1) Does codelivery of ICIs with nanomaterials improve therapeutic outcomes compared with systemic delivery of ICIs? (2) Does codelivery of ICIs within nanomaterials prevent or lower the risk of immune-related adverse events in immunotherapy treatment? (3) Does the timing of ICI therapy have a significant effect on post-RT outcomes, specifically with respect to concurrent versus sequential dosing? There are initial clinical (Z. Zhang, Liu, et al., 2022) and preclinical (Choi et al., 2020; Han et al., 2020; Wilhelm et al., 2016) data that suggest that concurrent or, at least, early post-RT sequential delivery of ICIs is more effective in terms of both increased efficacy and reduced side effects. Answering the above questions is a daunting task because of the vastness of the parameter space, and it will require extensive, systematic investigation in both preclinical and clinical settings.

Translating nanoparticle cancer therapeutics from the laboratory to the clinic has been found to be extremely difficult (Hare et al., 2017). One inevitable limitation of nanomedicine is the extremely low efficiency of delivery to the tumor (typically <1% of the injected amount), especially when administered via a systemic route (Wilhelm et al., 2016). Fortunately, however, this issue is not a concern for radio-enhancing/sensitizing nanomaterials, because, for the purpose of RT enhancement, NPs can actually be delivered by direct intra-tumoral injection. Although cancer is a systemic disease, local delivery of NPs will be clinically justifiable as long as this route of administration is still capable of producing systemic anti-cancer immune responses.

Numerous recent studies, taken together, clearly support that nanomaterials have great potential for radio-immunotherapy applications, and thus, further, more extensive exploration is warranted. We expect that the next several years

will see even more increased research in these areas. Newer materials and designs will continue to be explored, while gaps in mechanistic knowledge will continue to be addressed.

AUTHOR CONTRIBUTIONS

Dhushyanth Viswanath: Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (supporting). **Jeehun Park:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); visualization (equal); writing – original draft (equal). **Rahul Misra:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); visualization (equal); writing – original draft (equal). **Vincenzo Pizzuti:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (supporting). **Sung-Ho Shin:** Conceptualization (supporting); data curation (equal); formal analysis (equal); investigation (supporting); visualization (equal); writing – original draft (supporting). **Junsang Doh:** Conceptualization (equal); data curation (supporting); formal analysis (supporting); funding acquisition (equal); investigation (supporting); project administration (equal); resources (equal); supervision (equal); writing – original draft (supporting); writing – review and editing (supporting). **You-Yeon Won:** Conceptualization (lead); data curation (supporting); formal analysis (supporting); funding acquisition (lead); investigation (equal); project administration (lead); resources (lead); supervision (lead); visualization (supporting); writing – original draft (equal); writing – review and editing (lead).

ACKNOWLEDGMENTS

Funding for this research was provided by Purdue University Institute for Cancer Research (PU-ICR) (P30CA023168) (Phase I Concept Award, Challenge Research Award, and DDMS Collaborative Ideas Project Award), and the Davidson School of Chemical Engineering at Purdue University (Ihlenfeld Fund). You-Yeon Won is also grateful for funding from NSF (CBET-1803968 and CBET-2211843). Dhushyanth Viswanath is thankful for the Leslie Bottorff Fellowship from Purdue University College of Engineering/Indiana University School of Medicine. Junsang Doh acknowledges funding from the National Research Foundation of Korea under the Korean Ministry of Science and ICT (MSIT) (RS-2023-00208359) and Seoul National University (Creative Pioneering Researchers Program).

CONFLICT OF INTEREST STATEMENT

The authors have declared no conflicts of interest for this article.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Vincenzo J. Pizzuti https://orcid.org/0000-0002-1708-3776
You-Yeon Won https://orcid.org/0000-0002-8347-6375

RELATED WIREs ARTICLES

Mechanisms of nanoparticle radiosensitization
Radiotherapy reimagined: Integrating nanomedicines into radiotherapy clinical trials

REFERENCES

Abuodeh, Y., Venkat, P., & Kim, S. (2016). Systematic review of case reports on the abscopal effect. *Current Problems in Cancer*, *40*(1), 25–37. https://doi.org/10.1016/j.currproblcancer.2015.10.001

Apetoh, L., Ghiringhelli, F., Tesniere, A., Obeid, M., Ortiz, C., Criollo, A., Mignot, G., Maiuri, M. C., Ullrich, E., Saulnier, P., Yang, H., Amigorena, S., Ryffel, B., Barrat, F. J., Saftig, P., Levi, F., Lidereau, R., Nogues, C., Mira, J.-P., ... Zitvogel, L. (2007). Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nature Medicine*, *13*(9), 1050–1059. https://doi.org/10.1038/nm1622

Buchwald, Z. S., Wynne, J., Nasti, T. H., Zhu, S., Mourad, W. F., Yan, W., Gupta, S., Khleif, S. N., & Khan, M. K. (2018). Radiation, immune checkpoint blockade and the abscopal effect: A critical review on timing, dose and fractionation. *Frontiers in Oncology*, *8*, 612. https://doi.org/10.3389/fonc.2018.00612

Burnette, B. C., Liang, H., Lee, Y., Chlewicki, L., Khodarev, N. N., Weichselbaum, R. R., Fu, Y. X., & Auh, S. L. (2011). The efficacy of radiotherapy relies upon induction of type I interferon-dependent innate and adaptive immunity. *Cancer Research*, *71*(7), 2488–2496. https://doi.org/10.1158/0008-5472.CAN-10-2820

Cachot, A., Bilous, M., Liu, Y.-C., Li, X., Saillard, M., Cenerenti, M., Rockinger, G. A., Wyss, T., Guillaume, P., & Schmidt, J. (2021). Tumor-specific cytolytic CD4 T cells mediate immunity against human cancer. *Science Advances*, *7*(9), eabe3348. https://doi.org/10.1126/sciadv.abe3348

Castedo, M., Perfettini, J.-L., Roumier, T., Andreau, K., Medema, R., & Kroemer, G. (2004). Cell death by mitotic catastrophe: A molecular definition. *Oncogene*, *23*(16), 2825–2837. https://doi.org/10.1038/sj.onc.1207528

Chen, D., & Mellman, I. (2013). Oncology meets immunology: The cancer-immunity cycle. *Immunity*, *39*(1), 1–10. https://doi.org/10.1016/j.immuni.2013.07.012

Chen, G., Chen, Z., Wen, D., Wang, Z., Li, H., Zeng, Y., Dotti, G., Wirz, R. E., & Gu, Z. (2020). Transdermal cold atmospheric plasma-mediated immune checkpoint blockade therapy. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(7), 3687–3692. https://doi.org/10.1073/pnas.1917891117

Chen, Q., Chen, J., Yang, Z., Xu, J., Xu, L., Liang, C., Han, X., & Liu, Z. (2019). Nanoparticle-enhanced radiotherapy to trigger robust cancer immunotherapy. *Advanced Materials*, *31*(10), 1802228. https://doi.org/10.1002/adma.201802228

Chen, Y., Pan, W., Gao, P., Shi, M., Wu, T., Li, N., & Tang, B. (2020). Boosting the abscopal effect of radiotherapy: A smart antigen-capturing radiosensitizer to eradicate metastatic breast tumors. *Chemical Communications*, *56*(71), 10353–10356. https://doi.org/10.1039/D0CC01080D

Chilakamarthi, U., & Giribabu, L. (2017). Photodynamic therapy: Past, present and future. *The Chemical Record*, *17*(8), 775–802. https://doi.org/10.1002/tcr.201600121

Choi, B., Choi, H., Yu, B., & Kim, D. H. (2020). Synergistic local combination of radiation and anti-programmed death ligand 1 immunotherapy using radiation-responsive splintery metallic nanocarriers. *ACS Nano*, *14*(10), 13115–13126. https://doi.org/10.1021/acsnano.0c04701

Cline, B., Delahunty, I., & Xie, J. (2019). Nanoparticles to mediate X-ray-induced photodynamic therapy and Cherenkov radiation photodynamic therapy. *WIREs Nanomedicine and Nanobiotechnology*, *11*(2), e1541. https://doi.org/10.1002/wnan.1541

Cremolini, C., Vitale, E., Rastaldo, R., & Giachino, C. (2021). Advanced nanotechnology for enhancing immune checkpoint blockade therapy. *Nanomaterials*, *11*(3), 661. https://doi.org/10.3390/nano11030661

Dagoglu, N., Karaman, S., Caglar, H. B., & Oral, E. N. (2019). Abscopal effect of radiotherapy in the immunotherapy era: Systematic review of reported cases. *Cureus*, *11*(2), e4103. https://doi.org/10.7759/cureus.4103

Darmon, A., Zhang, P., Marill, J., Mohamed Anesary, N., da Silva, J., & Paris, S. (2022). Radiotherapy-activated NBTXR3 nanoparticles modulate cancer cell immunogenicity and TCR repertoire. *Cancer Cell International*, *22*(1), 208. https://doi.org/10.1186/s12935-022-02615-w

Demaria, S., Ng, B., Devitt, M. L., Babb, J. S., Kawashima, N., Liebes, L., & Formenti, S. C. (2004). Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. *International Journal of Radiation Oncology Biology Physics*, *58*(3), 862–870. https://doi.org/10.1016/j.ijrobp.2003.09.012

Dewan, M. Z., Galloway, A. E., Kawashima, N., Dewyngaert, J. K., Babb, J. S., Formenti, S. C., & Demaria, S. (2009). Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. *Clinical Cancer Research*, *15*(17), 5379–5388. https://doi.org/10.1158/1078-0432.CCR-09-0265

Dong, X., Cheng, R., Zhu, S., Liu, H., Zhou, R., Zhang, C., Chen, K., Mei, L., Wang, C., Su, C., Liu, X., Gu, Z., & Zhao, Y. (2020). A heterojunction structured WO. *ACS Nano*, *14*(5), 5400–5416. https://doi.org/10.1021/acsnano.9b08962

Dovedi, S. J., Adlard, A. L., Lipowska-Bhalla, G., McKenna, C., Jones, S., Cheadle, E. J., Stratford, I. J., Poon, E., Morrow, M., Stewart, R., Jones, H., Wilkinson, R. W., Honeychurch, J., & Illidge, T. M. (2014). Acquired resistance to fractionated radiotherapy can be overcome by concurrent PD-L1 blockade. *Cancer Research*, *74*(19), 5458–5468. https://doi.org/10.1158/0008-5472.CAN-14-1258

Ehlers, G., & Fridman, M. (1973). Abscopal effect of radiation in papillary adenocarcinoma. *The British Journal of Radiology*, *46*(543), 220–222. https://doi.org/10.1259/0007-1285-46-543-220

Elliott, M. R., Chekeni, F. B., Trampont, P. C., Lazarowski, E. R., Kadl, A., Walk, S. F., Park, D., Woodson, R. I., Ostankovich, M., Sharma, P., Lysiak, J. J., Harden, T. K., Leitinger, N., & Ravichandran, K. S. (2009). Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. *Nature*, *461*(7261), 282–286. https://doi.org/10.1038/nature08296

Erenpreisa, J., & Cragg, M. S. (2001). Mitotic death: A mechanism of survival? A review. *Cancer Cell International*, *1*(1), 1. https://doi.org/10.1186/1475-2867-1-1

Ferris, S. T., Durai, V., Wu, R., Theisen, D. J., Ward, J. P., Bern, M. D., Davidson Iv, J. T., Bagadia, P., Liu, T., & Briseño, C. G. (2020). cDC1 prime and are licensed by CD4+ T cells to induce anti-tumour immunity. *Nature*, *584*(7822), 624–629. https://doi.org/10.1038/s41586-020-2611-3

Freeman-Keller, M., Kim, Y., Cronin, H., Richards, A., Gibney, G., & Weber, J. S. (2016). Nivolumab in resected and Unresectable metastatic melanoma: Characteristics of immune-related adverse events and association with outcomes. *Clinical Cancer Research*, *22*(4), 886–894. https://doi.org/10.1158/1078-0432.CCR-15-1136

Galluzzi, L., Buque, A., Kepp, O., Zitvogel, L., & Kroemer, G. (2017). Immunogenic cell death in cancer and infectious disease. *Nature Reviews Immunology*, *17*(2), 97–111. https://doi.org/10.1038/nri.2016.107

Golden, E. B., Frances, D., Pellicciotta, I., Demaria, S., Barcellos-Hoff, M. H., & Formenti, S. C. (2014). Radiation fosters dose-dependent and chemotherapy-induced immunogenic cell death. *OncoImmunology*, *3*(4), e28518. https://doi.org/10.4161/onci.28518

Grass, G. D., Krishna, N., & Kim, S. (2016). The immune mechanisms of abscopal effect in radiation therapy. *Current Problems in Cancer*, *40*(1), 10–24. https://doi.org/10.1016/j.currproblcancer.2015.10.003

Green, D. R., Ferguson, T., Zitvogel, L., & Kroemer, G. (2009). Immunogenic and tolerogenic cell death. *Nature Reviews Immunology*, *9*(5), 353–363. https://doi.org/10.1038/nri2545

Guan, X., Sun, L., Shen, Y., Jin, F., Bo, X., Zhu, C., Han, X., Li, X., Chen, Y., Xu, H., & Yue, W. (2022). Nanoparticle-enhanced radiotherapy synergizes with PD-L1 blockade to limit post-surgical cancer recurrence and metastasis. *Nature Communications*, *13*(1), 2834. https://doi.org/10.1038/s41467-022-30543-w

Guibin Pang, C. C., Liu, Y., Jiang, T., Huan, Y., Yanxian, W., Wang, Y., Wang, F.-J., Liu, Z., & Zhang, L. W. (2019). Bioactive polysaccharide nanoparticles improve radiation-induced abscopal effect through manipulation of dendritic cells. *ACS Applied Materials & Interfaces*, *11*, 42661–42670. https://doi.org/10.1021/acsami.9b16814

Hall, E. J., & Giaccia, A. J. (2006). *Radiobiology for the radiologist* (Vol. 6). Lippincott Williams and Wilkins.

Han, X., Li, H., Zhou, D., Chen, Z., & Gu, Z. (2020). Local and targeted delivery of immune checkpoint blockade therapeutics. *Accounts of Chemical Research*, *53*(11), 2521–2533. https://doi.org/10.1021/acs.accounts.0c00339

Hare, J. I., Lammers, T., Ashford, M. B., Puri, S., Storm, G., & Barry, S. T. (2017). Challenges and strategies in anti-cancer nanomedicine development: An industry perspective. *Advanced Drug Delivery Reviews*, *108*, 25–38. https://doi.org/10.1016/j.addr.2016.04.025

Hou, J. J., Greten, T. F., & Xia, Q. (2017). Immunosuppressive cell death in cancer. *Nature Reviews Immunology*, *17*(6), 401. https://doi.org/10.1038/nri.2017.46

Hu, Y., Paris, S., Barsoumian, H., Abana, C. O., He, K., Sezen, D., Wasley, M., Masrorpour, F., Chen, D., Yang, L., Dunn, J. D., Gandhi, S., Nguyen, Q. N., Cortez, M. A., & Welsh, J. W. (2021). A radioenhancing nanoparticle mediated immunoradiation improves survival and generates long-term antitumor immune memory in an anti-PD1-resistant murine lung cancer model. *Journal of Nanobiotechnology*, *19*(1), 416. https://doi.org/10.1186/s12951-021-01163-1

Hu, Y., Paris, S., Barsoumian, H., Abana, C. O., He, K., Wasley, M., Younes, A. I., Masrorpour, F., Chen, D., & Yang, L. (2021). Radiation therapy enhanced by NBTXR3 nanoparticles overcomes anti-PD1 resistance and evokes abscopal effects. *International Journal of Radiation Oncology, Biology, Physics*, *111*(3), 647–657. https://doi.org/10.1016/j.ijrobp.2021.06.041

Huang, Z., Yao, D., Ye, Q., Jiang, H., Gu, R., Ji, C., Wu, J., Hu, Y., & Yuan, A. (2021). Zoledronic acid–gadolinium coordination polymer nanorods for improved tumor radioimmunotherapy by synergetically inducing immunogenic cell death and reprogramming the immunosuppressive microenvironment. *ACS Nano*, *15*(5), 8450–8465. https://doi.org/10.1021/acsnano.0c10764

Hutchinson, M.-K. N. D., Mierzwa, M., & D'Silva, N. J. (2020). Radiation resistance in head and neck squamous cell carcinoma: Dire need for an appropriate sensitizer. *Oncogene*, *39*(18), 3638–3649. https://doi.org/10.1038/s41388-020-1250-3

Janopaul-Naylor, J. R., Shen, Y., Qian, D. C., & Buchwald, Z. S. (2021). The abscopal effect: A review of pre-clinical and clinical advances. *International Journal of Molecular Sciences*, *22*(20), 11061. https://doi.org/10.3390/ijms222011061

Ji, D., Song, C., Li, Y., Xia, J., Wu, Y., Jia, J., Cui, X., Yu, S., & Gu, J. (2020). Combination of radiotherapy and suppression of Tregs enhances abscopal antitumor effect and inhibits metastasis in rectal cancer. *Journal for Immunotherapy of Cancer*, *8*(2), e000826. https://doi.org/10.1136/jitc-2020-000826

Kang, J., Demaria, S., & Formenti, S. (2016). Current clinical trials testing the combination of immunotherapy with radiotherapy. *Journal for ImmunoTherapy of Cancer*, *4*(1), 51. https://doi.org/10.1186/s40425-016-0156-7

Kingsley, D. P. E. (1975). An interesting case of possible abscopal effect in malignant melanoma. *British Journal of Radiology*, *48*(574), 863–866. https://doi.org/10.1259/0007-1285-48-574-863

Li, H., Wang, M., Huang, B., Zhu, S.-W., Zhou, J.-J., Chen, D.-R., Cui, R., Zhang, M., & Sun, Z.-J. (2021). Theranostic near-infrared-IIb emitting nanoprobes for promoting immunogenic radiotherapy and abscopal effects against cancer metastasis. *Nature Communications*, *12*(1), 7149. https://doi.org/10.1038/s41467-021-27485-0

Li, J. X., Ren, H., Qiu, Q., Yang, X. Y., Zhang, J. Y., Zhang, C., Sun, B. Y., Lovell, J. F., & Zhang, Y. M. (2022). Manganese coordination micelles that activate stimulator of interferon genes and capture in situ tumor antigens for cancer metalloimmunotherapy. *ACS Nano*, *16*, 16909–16923. https://doi.org/10.1021/acsnano.2c06926

Li, X. (2018). The inducers of immunogenic cell death for tumor immunotherapy. *Tumori Journal*, *104*(1), 1–8. https://doi.org/10.5301/tj.5000675

Liu, X., Su, Q., Song, H., Shi, X., Zhang, Y., Zhang, C., Huang, P., Dong, A., Kong, D., & Wang, W. (2021). PolyTLR7/8a-conjugated, antigen-trapping gold nanorods elicit anticancer immunity against abscopal tumors by photothermal therapy-induced in situ vaccination. *Biomaterials*, *275*, 120921. https://doi.org/10.1016/j.biomaterials.2021.120921

Lotze, M. T., & Tracey, K. J. (2005). High-mobility group box 1 protein (HMGB1): Nuclear weapon in the immune arsenal. *Nature Reviews Immunology*, *5*(4), 331–342. https://doi.org/10.1038/nri1594

Lou, J., Aragaki, M., Bernards, N., Chee, T., Gregor, A., Hiraishi, Y., Ishiwata, T., Leung, C., Ding, L., & Kitazawa, S. (2023). Repeated photodynamic therapy mediates the abscopal effect through multiple innate and adaptive immune responses with and without immune checkpoint therapy. *Biomaterials*, *292*, 121918. https://doi.org/10.1016/j.biomaterials.2022.121918

Lu, K., He, C., Guo, N., Chan, C., Ni, K., Lan, G., Tang, H., Pelizzari, C., Fu, Y.-X., & Spiotto, M. T. (2018). Low-dose X-ray radiotherapy–radiodynamic therapy via nanoscale metal–organic frameworks enhances checkpoint blockade immunotherapy. *Nature Biomedical Engineering*, *2*(8), 600–610. https://doi.org/10.1038/s41551-018-0203-4

Lucky, S. S., Soo, K. C., & Zhang, Y. (2015). Nanoparticles in photodynamic therapy. *Chemical Reviews*, *115*(4), 1990–2042. https://doi.org/10.1021/cr5004198

Marill, J., Anesary, N. M., & Paris, S. (2019). DNA damage enhancement by radiotherapy-activated hafnium oxide nanoparticles improves cGAS-STING pathway activation in human colorectal cancer cells. *Radiotherapy and Oncology*, *141*, 262–266. https://doi.org/10.1016/j.radonc.2019.07.029

Marin-Acevedo, J. A., Kimbrough, E. O., & Lou, Y. (2021). Next generation of immune checkpoint inhibitors and beyond. *Journal of Hematology & Oncology*, *14*(1), 45. https://doi.org/10.1186/s13045-021-01056-8

Meng, L., Cheng, Y., Tong, X., Gan, S., Ding, Y., Zhang, Y., Wang, C., Xu, L., Zhu, Y., Wu, J., Hu, Y., & Yuan, A. (2018). Tumor oxygenation and hypoxia inducible factor-1 functional inhibition via a reactive oxygen species responsive nanoplatform for enhancing radiation therapy and Abscopal effects. *ACS Nano*, *12*(8), 8308–8322. https://doi.org/10.1021/acsnano.8b03590

Meng, L., Wang, C., Lu, Y., Sheng, G., Yang, L., Wu, Z., Xu, H., Han, C., & Han, F. (2021). Targeted regulation of blood-brain barrier for enhanced therapeutic efficiency of hypoxia-modifier nanoparticles and immune checkpoint blockade antibodies for glioblastoma. *ACS Applied Materials & Interfaces*, *13*(10), 11657–11671. https://doi.org/10.1021/acsami.1c00347

Min Luo, Z. L., Zhang, X., Han, C., Samandi, L. Z., Dong, C., Sumer, B. D., Lea, J., Yang-Xin, F., & Gao, J. (2019). Synergistic STING activation by PC7A nanovaccine and ionizing radiation improves cancer immunotherapy. *Journal of Controlled Release*, *300*, 154–160. https://doi.org/10.1016/j.jconrel.2019.02.036

Min, Y., Roche, K. C., Tian, S., Eblan, M. J., McKinnon, K. P., Caster, J. M., Chai, S., Herring, L. E., Zhang, L., Zhang, T., DeSimone, J. M., Tepper, J. E., Vincent, B. G., Serody, J. S., & Wang, A. Z. (2017). Antigen-capturing nanoparticles improve the abscopal effect and cancer immunotherapy. *Nature Nanotechnology*, *12*(9), 877–882. https://doi.org/10.1038/nnano.2017.113

Mole, R. H. (1953). Whole body irradiation; radiobiology or medicine? *The British Journal of Radiology*, *26*(305), 234–241. https://doi.org/10.1259/0007-1285-26-305-234

Morisada, M., Clavijo, P. E., Moore, E., Sun, L., Chamberlin, M., Van Waes, C., Hodge, J. W., Mitchell, J. B., Friedman, J., & Allen, C. T. (2018). PD-1 blockade reverses adaptive immune resistance induced by high-dose hypofractionated but not low-dose daily fractionated radiation. *OncoImmunology*, *7*(3), e1395996. https://doi.org/10.1080/2162402X.2017.1395996

Mudassar, F., Shen, H., Cook, K. M., & Hau, E. (2022). Improving the synergistic combination of programmed death-1/programmed death ligand-1 blockade and radiotherapy by targeting the hypoxic tumour microenvironment. *Journal of Medical Imaging and Radiation Oncology*, *66*(4), 560–574. https://doi.org/10.1111/1754-9485.13416

Ni, K., Lan, G., Chan, C., Duan, X., Guo, N., Veroneau, S. S., Weichselbaum, R. R., & Lin, W. (2019). Ultrathin metal-organic-layer mediated radiotherapy-radiodynamic therapy. *Matter*, *1*(5), 1331–1353. https://doi.org/10.1016/j.matt.2019.06.007

Nuhn, L., Vanparijs, N., de Beuckelaer, A., Lybaert, L., Verstraete, G., Deswarte, K., Lienenklaus, S., Shukla, N. M., Salyer, A. C. D., Lambrecht, B. N., Grooten, J., David, S. A., de Koker, S., & de Geest, B. G. (2016). pH-degradable imidazoquinoline-ligated nanogels for lymph node-focused immune activation. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(29), 8098–8103. https://doi.org/10.1073/pnas.1600816113

Ouellette, M. M., Zhou, S., & Yan, Y. (2022). Cell signaling pathways that promote radioresistance of cancer cells. *Diagnostics*, *12*(3), 656. https://doi.org/10.3390/diagnostics12030656

Postow, M. A., Callahan, M. K., Barker, C. A., Yamada, Y., Yuan, J., Kitano, S., Mu, Z., Rasalan, T., Adamow, M., Ritter, E., Sedrak, C., Jungbluth, A. A., Chua, R., Yang, A. S., Roman, R. A., Rosner, S., Benson, B., Allison, J. P., Lesokhin, A. M., ... Wolchok, J. D. (2012). Immunologic correlates of the abscopal effect in a patient with melanoma. *New England Journal of Medicine*, *366*(10), 925–931. https://doi.org/10.1056/NEJMoa1112824

Prasad, P., Gordijo, C. R., Abbasi, A. Z., Maeda, A., Ip, A., Rauth, A. M., DaCosta, R. S., & Wu, X. Y. (2014). Multifunctional albumin-MnO_2 nanoparticles modulate solid tumor microenvironment by attenuating hypoxia, acidosis, vascular endothelial growth factor and enhance radiation response. *ACS Nano*, *8*(4), 3202–3212. https://doi.org/10.1021/nn405773r

Qin, X., Liu, J., Xu, Y., Li, B., Cheng, J., Wu, X., Zhang, J., Liu, Z., Ning, R., & Li, Y. (2020). Mesoporous Bi-containing radiosensitizer loading with DOX to repolarize tumor-associated macrophages and elicit immunogenic tumor cell death to inhibit tumor progression. *ACS Applied Materials & Interfaces*, *12*(28), 31225–31234. https://doi.org/10.1021/acsami.0c08074

Qin, X., Yang, C., Xu, H., Zhang, R., Zhang, D., Tu, J., Guo, Y., Niu, B., Kong, L., & Zhang, Z. (2021). Cell-derived biogenetic gold nanoparticles for sensitizing radiotherapy and boosting immune response against cancer. *Small*, *17*(50), e2103984. https://doi.org/10.1002/smll.202103984

Retif, P., Pinel, S., Toussaint, M., Frochot, C., Chouikrat, R., Bastogne, T., & Barberi-Heyob, M. (2015). Nanoparticles for radiation therapy enhancement: The key parameters. *Theranostics*, *5*(9), 1030–1044. https://doi.org/10.7150/thno.11642

Rodriguez-Ruiz, M. E., Rodriguez, I., Garasa, S., Barbes, B., Solorzano, J. L., Perez-Gracia, J. L., Labiano, S., Sanmamed, M. F., Azpilikueta, A., Bolaños, E., Sanchez-Paulete, A. R., Aznar, M. A., Rouzaut, A., Schalper, K. A., Jure-Kunkel, M., & Melero, I. (2016). Abscopal effects of radiotherapy are enhanced by combined immunostimulatory mAbs and are dependent on CD8 T cells and crosspriming. *Cancer Research*, *76*(20), 5994–6005. https://doi.org/10.1158/0008-5472.CAN-16-0549

Samstein, R., Rimner, A., Barker, C. A., & Yamada, Y. (2017). Combined immune checkpoint blockade and radiation therapy: Timing and dose fractionation associated with greatest survival duration among over 750 treated patients. *International Journal of Radiation Oncology, Biology, Physics*, *99*(2), S129–S130. https://doi.org/10.1016/j.ijrobp.2017.06.303

Sang, W., Xie, L., Wang, G., Li, J., Zhang, Z., Li, B., Guo, S., Deng, C. X., & Dai, Y. (2021). Oxygen-enriched metal-phenolic X-ray nanoprocessor for cancer radio-radiodynamic therapy in combination with checkpoint blockade immunotherapy. *Advanced Science*, *8*(4), 2003338. https://doi.org/10.1002/advs.202003338

Sang, W., Zhang, Z., Wang, G., Xie, L., Li, J., Li, W., Tian, H., & Dai, Y. (2022). A triple-kill strategy for tumor eradication reinforced by metal-phenolic network nanopumps. *Advanced Functional Materials*, *32*(21), 2113168. https://doi.org/10.1002/adfm.202113168

Schaue, D., Ratikan, J. A., Iwamoto, K. S., & McBride, W. H. (2012). Maximizing tumor immunity with fractionated radiation. *International Journal of Radiation Oncology Biology Physics*, *83*(4), 1306–1310. https://doi.org/10.1016/j.ijrobp.2011.09.049

Shulman, R. M., Bagnyukova, T., Chen, L., Chen, X., Borghaei, H., & Meyer, J. E. (2022). Synergistic expression of the abscopal effect in a murine tumor model treated with combined radiation and single or dual-immune checkpoint blockade. *International Journal of Radiation Oncology, Biology, Physics*, *114*(3), e524–e525. https://doi.org/10.1016/j.ijrobp.2022.07.2119

Song, H., Sun, H., He, N., Xu, C., Wang, Y., Du, L., Liu, Y., Wang, Q., Ji, K., Wang, J., Zhang, M., Gu, Y., Zhang, Y., Feng, L., Tillement, O., Wang, W., & Liu, Q. (2022). Gadolinium-based ultra-small nanoparticles augment radiotherapy-induced T-cell response to synergize with checkpoint blockade immunotherapy. *Nanoscale*, *14*(31), 11429–11442. https://doi.org/10.1039/d2nr02620a

Speiser, D. E., Chijioke, O., Schaeuble, K., & Münz, C. (2023). CD4+ T cells in cancer. *Nature Cancer*, *4*(3), 317–329. https://doi.org/10.1038/s43018-023-00521-2

Suek, N., Campesato, L. F., Merghoub, T., & Khalil, D. N. (2019). Targeted APC activation in cancer immunotherapy to enhance the abscopal effect. *Frontiers in Immunology*, *10*, 604. https://doi.org/10.3389/fimmu.2019.00604

Sutherland, B. M., Bennett, P. V., Sidorkina, O., & Laval, J. (2000). Clustered DNA damages induced in isolated DNA and in human cells by low doses of ionizing radiation. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(1), 103–108. https://doi.org/10.1073/pnas.97.1.103

Trommer, M., Yeo, S. Y., Persigehl, T., Bunck, A., Gruell, H., Schlaak, M., Theurich, S., von Bergwelt-Baildon, M., Morgenthaler, J., & Herter, J. M. (2019). Abscopal effects in radio-immunotherapy—Response analysis of metastatic cancer patients with progressive disease under anti-PD-1 immune checkpoint inhibition. *Frontiers in Pharmacology*, *10*, 511. https://doi.org/10.3389/fphar.2019.00511

Twyman-Saint Victor, C., Rech, A. J., Maity, A., Rengan, R., Pauken, K. E., Stelekati, E., Benci, J. L., Xu, B., Dada, H., Odorizzi, P. M., Herati, R. S., Mansfield, K. D., Patsch, D., Amaravadi, R. K., Schuchter, L. M., Ishwaran, H., Mick, R., Pryma, D. A., Xu, X., … Minn, A. J. (2015). Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. *Nature*, *520*(7547), 373–377. https://doi.org/10.1038/nature14292

Vaddepally, R. K., Kharel, P., Pandey, R., Garje, R., & Chandra, A. B. (2020). Review of indications of FDA-approved immune checkpoint inhibitors per NCCN guidelines with the level of evidence. *Cancers*, *12*(3), 738. https://doi.org/10.3390/cancers12030738

Vakifahmetoglu, H., Olsson, M., & Zhivotovsky, B. (2008). Death through a tragedy: Mitotic catastrophe. *Cell Death and Differentiation*, *15*(7), 1153–1162. https://doi.org/10.1038/cdd.2008.47

Viswanath, D., & Won, Y.-Y. (2022). Combining radiotherapy (RT) and photodynamic therapy (PDT): Clinical studies on conventional RT-PDT approaches and novel nanoparticle-based RT-PDT approaches under preclinical evaluation. *ACS Biomaterials Science & Engineering*, *8*(9), 3644–3658. https://doi.org/10.1021/acsbiomaterials.2c00287

Wang, J., Li, Z., Wang, Z., Yu, Y., Li, D., Li, B., & Ding, J. (2020). Nanomaterials for combinational radio-immuno oncotherapy. *Advanced Functional Materials*, *30*(30), 1910676. https://doi.org/10.1002/adfm.201910676

Wang, M., Song, J., Zhou, F., Hoover, A. R., Murray, C., Zhou, B., Wang, L., Qu, J., & Chen, W. R. (2019). NIR-triggered phototherapy and immunotherapy via an antigen-capturing Nanoplatform for metastatic cancer treatment. *Advanced Science*, *6*(10), 1802157. https://doi.org/10.1002/advs.201802157

Wang, R., He, Z., Cai, P., Zhao, Y., Gao, L., Yang, W., Zhao, Y., Gao, X., & Gao, F. (2019). Surface-functionalized modified copper sulfide nanoparticles enhance checkpoint blockade tumor immunotherapy by Photothermal therapy and antigen capturing. *ACS Applied Materials & Interfaces*, *11*(15), 13964–13972. https://doi.org/10.1021/acsami.9b01107

Wang, Y., Deng, W., Li, N., Neri, S., Sharma, A., Jiang, W., & Lin, S. H. (2018). Combining immunotherapy and radiotherapy for cancer treatment: Current challenges and future directions. *Frontiers in Pharmacology*, *9*, 185. https://doi.org/10.3389/fphar.2018.00185

Watanabe, T., Firat, E., Scholber, J., Gaedicke, S., Heinrich, C., Luo, R., Ehrat, N., Multhoff, G., Schmitt-Graeff, A., Grosu, A. L., Abdollahi, A., Hassel, J. C., von Bubnoff, D., Meiss, F., & Niedermann, G. (2020). Deep abscopal response to radiotherapy and anti-PD-1 in an oligometastatic melanoma patient with unfavorable pretreatment immune signature. *Cancer Immunology, Immunotherapy*, *69*(9), 1823–1832. https://doi.org/10.1007/s00262-020-02587-8

Weichselbaum, R. R., Liang, H., Deng, L., & Fu, Y.-X. (2017). Radiotherapy and immunotherapy: A beneficial liaison? *Nature Reviews. Clinical Oncology*, *14*(6), 365–379. https://doi.org/10.1038/nrclinonc.2016.211

Wilhelm, S., Tavares, A. J., Dai, Q., Ohta, S., Audet, J., Dvorak, H. F., & Chan, W. C. W. (2016). Analysis of nanoparticle delivery to tumours. *Nature Reviews Materials*, *1*(5), 1–12. https://doi.org/10.1038/natrevmats.2016.14

Xia, Y., & Wang, H. (2022). Engineered nanomaterials trigger abscopal effect for immunotherapy of metastatic cancers. *Frontiers in Bioengineering and Biotechnology*, *10*, 890257. https://doi.org/10.3389/fbioe.2022.890257

Xiong, W., Zhao, Y., Du, H., & Guo, X. (2021). Current status of immune checkpoint inhibitor immunotherapy for lung cancer. *Frontiers in Oncology*, *11*, 704336. https://doi.org/10.3389/fonc.2021.704336

Yang, K., Choi, C., Cho, H., Ahn, W. G., Kim, S. Y., Shin, S. W., Kim, Y., Jang, T., Lee, N., & Park, H. C. (2021). Antigen-capturing mesoporous silica nanoparticles enhance the radiation-induced abscopal effect in murine hepatocellular carcinoma Hepa1-6 models. *Pharmaceutics*, *13*(11), 1811. https://doi.org/10.3390/pharmaceutics13111811

Zhang, Y., Sriramaneni, R. N., Clark, P. A., Jagodinsky, J. C., Ye, M., Jin, W., Wang, Y., Bates, A., Kerr, C. P., Le, T., Allawi, R., Wang, X., Xie, R., Havighurst, T. C., Chakravarty, I., Rakhmilevich, A. L., O'Leary, K. A., Schuler, L. A., Sondel, P. M., … Morris, Z. S. (2022).

Multifunctional nanoparticle potentiates the in situ vaccination effect of radiation therapy and enhances response to immune checkpoint blockade. *Nature Communications*, *13*(1), 4948. https://doi.org/10.1038/s41467-022-32645-x

Zhang, Z., Liu, X., Chen, D., & Yu, J. (2022). Radiotherapy combined with immunotherapy: The dawn of cancer treatment. *Signal Transduction and Targeted Therapy*, *7*(1), 258. https://doi.org/10.1038/s41392-022-01102-y

Zhong, X. W., Li, C. Y., Zhao, G. Z., Li, M. M., Chen, S. N., Cao, Y., Wang, Q., Sun, J. C., Zhu, S. Y., & Chang, S. F. (2022). Photoacoustic mediated multifunctional tumor antigen trapping nanoparticles inhibit the recurrence and metastasis of ovarian cancer by enhancing tumor immunogenicity. *Journal of Nanobiotechnology*, *20*(1), 468. https://doi.org/10.1186/s12951-022-01682-5

Zhou, J., Wang, G., Chen, Y., Wang, H., Hua, Y., & Cai, Z. (2019). Immunogenic cell death in cancer therapy: Present and emerging inducers. *Journal of Cellular and Molecular Medicine*, *23*(8), 4854–4865. https://doi.org/10.1111/jcmm.14356

Zhou, R., Yan, L., Dong, X., Zhu, S., Chen, K., Wu, Y., Xiang, H., Li, L., Zhang, G., & Gu, Z. (2021). Fractionated regimen-suitable immunoradiotherapy sensitizer based on ultrasmall Fe4Se2W18 nanoclusters enable tumor-specific radiosensitization augment and anti-tumor immunity boost. *Nano Today*, *36*, 101003. https://doi.org/10.1016/j.nantod.2020.101003

How to cite this article: Viswanath, D., Park, J., Misra, R., Pizzuti, V. J., Shin, S.-H., Doh, J., & Won, Y.-Y. (2023). Nanotechnology-enhanced radiotherapy and the abscopal effect: Current status and challenges of nanomaterial-based radio-immunotherapy. *WIREs Nanomedicine and Nanobiotechnology*, e1924. https://doi.org/10.1002/wnan.1924