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Augmenting the therapeutic window of radiotherapy: A perspective on molecularly targeted therapies and nanomaterials

Moataz Reda^{a,b}, Alexander F. Bagley^c, Husam Y. Zaidan^b, Wassana Yantasee^{a,b,*}

^aDepartment of Biomedical Engineering, Oregon Health & Science University, Portland, OR 97239, United States

^bPDX Pharmaceuticals, Portland, OR 97239, United States

^cDepartment of Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, United States

Abstract

Radiation therapy is a cornerstone of modern cancer therapy alongside surgery, chemotherapy, and immunotherapy, with over half of all cancer patients receiving radiation therapy as part of their treatment regimen. Development of novel radiation sensitizers that can improve the therapeutic window of radiation therapy are sought after, particularly for tumors at an elevated risk of local and regional recurrence such as locally-advanced lung, head and neck, and gastrointestinal tumors. This review discusses clinical strategies to enhance radiotherapy efficacy and decrease toxicity, hence, increasing the overall therapeutic window. A focus is given to the molecular targets that have been identified and their associated mechanisms of action in enhancing radiotherapy. Examples include cell survival and proliferation signaling such as the EGFR and PI3K/AKT/mTOR pathways, DNA repair genes including PARP and ATM/ATR, angiogenic growth factors, epigenetic regulators, and immune checkpoint proteins. By manipulating various mechanisms of tumor resistance to ionizing radiation (IR), targeted therapies hold significant value to increase the therapeutic window of radiotherapy. Further, the use of novel nanoparticles to enhance radiotherapy is also reviewed, including nanoparticle delivery of chemotherapies, metallic (high- Z) nanoparticles, and nanoparticle delivery of targeted therapies – all of which may improve the therapeutic window of radiotherapy by enhancing the tumor response to IR or reducing normal tissue toxicity.

Keywords

Radiation therapy; Targeted therapy; Radiation sensitizer; Nanomedicine

*Corresponding author at: 3303 SW Bond Ave, CH13B, Portland, OR 97239, United States. yantasee@ohsu.edu (W. Yantasee).

Conflict of interest

OHSU, MR, and WY have a significant financial interest in PDX Pharmaceuticals, a company that may have a commercial interest in the results of this research and technology. This potential personal and institutional conflict of interest has been reviewed and managed by OHSU.

Radiation therapy remains an essential component of cancer therapy for millions of patients annually in the United States and globally. Radiation therapy involves the focal deposition of ionizing radiation (IR) to the tumor and surrounding at-risk tissue compartments such as regional lymph nodes. The most common treatment approaches include external beam radiation therapy (EBRT) using high-energy photons, electrons, or protons, as well as brachytherapy and radionuclide therapy. Significant technical advances in EBRT and diagnostic imaging over the past several decades have enabled more localized and precise dose deposition within tumors and greater sparing of adjacent normal tissues; these include intensity-modulated radiation therapy (IMRT), image-guided radiation therapy (IGRT), integration of MRI and PET imaging into CT-based treatment planning, and advanced motion management strategies during treatment. Brachytherapy, which involves interstitial or intraluminal implantation of radioisotopes, permits high dose delivery to tumors with steep dose gradients into surrounding tissue. Brachytherapy can be performed either as a permanent implant into tumors or transient delivery of radioisotopes via catheters or specialized applicators. Radionuclide therapy involves the systemic administration of radioisotopes such as iodine-131, radium-223, or lutetium-177, and conjugation of radionuclides to peptides that bind to tumor-specific receptors allows for tumor-targeted delivery.

Upon interaction with tissue, IR damages cellular DNA through direct effects (i.e., double-stranded breaks (DSB)) and indirect effects (i.e., formation of reactive oxygen species (ROS)) [1]. DNA damage results in cellular injury and death by multiple mechanisms including apoptosis, mitotic catastrophe, and cellular senescence, each contributing to the overall clonogenic survival of tumor cells [2]. The primary goals of radiation therapy are to achieve local and regional tumor control (for either curative or palliative intent) and to minimize early and late normal tissue toxicities. This is referred to as therapeutic ratio. Understanding of the radiobiology of tumors and normal tissues has led to treatment approaches and methods that enhance the therapeutic ratio. Some of these advances include: (1) developing image-guided radiation therapy to reduce intra- and inter-fraction uncertainty in the delivery of radiation, (2) exploring various dose and fractionation regimens to exploit radiobiological differences between tumors and normal tissues, and (3) administering agents (e.g., systemic radiation sensitizers or locally-administered metallic nanomaterials) which enhance IR effects on cancer cells.

Advances in image-guided radiation therapy have improved the ability to more accurately and reproducibly focus radiation to the tumor site, thereby decreasing the volume of irradiated normal tissue. Techniques such as IMRT, stereotactic body radiation therapy (SBRT), proton beam therapy (PBT), and brachytherapy permit highly conformal radiation therapy to tumors and further reduce radiation to surrounding normal tissues [3]. In certain clinical contexts, these modern radiation therapy approaches have shown to reduce toxicity and maintain disease outcomes [4–6]. However, radiation-related toxicity to normal tissues remains an important concern and further approaches to minimize toxicity are warranted.

Radiation dose and fractionation is selected based on multiple factors including the tumor type, tumor location and volume, the patient's performance status, and the treating center's capabilities for patient immobilization and image-guided therapy. Radiation is most

commonly delivered in fractions of 1.8–2 Gy per day, which is thought to reduce late tissue toxicities compared to larger daily fraction sizes [7]. The basis of fractionated radiation relies on the “4R’s” of radiation biology as described by H.R. Withers in 1975: repair, reoxygenation, reassortment, and repopulation [8]. Differences between normal tissue and tumors regarding the 4R’s leads to an increase in the therapeutic ratio with fractionated radiation [9]. With fractionated radiation, normal tissues are allowed to repair sub-lethal damage and repopulate more efficiently than cancer cells and thus can survive while the cancer cells do not. Reoxygenation and reassortment potentiate the effects of fractionated radiation on tumors. Reoxygenation renders tumors more sensitive to radiation as their hypoxic fraction decreases during treatment, while reassortment refers to the cycling of cancer cells from radioresistant (e.g. S-phase) to radiosensitive phases of the cell cycle (e.g., G2/M phase). In addition to the 4R’s of radiobiology, a 5th R, intrinsic radiosensitivity, also differs between different tumor types and normal tissues [10]. In particular, cancer stem cells are postulated to represent a radiation-resistant subpopulation within tumors contributing to relapse following radiation therapy [11].

Similar to fractionated radiation, biological modifiers to cancer cells can also enhance the therapeutic ratio [12]. For example, early efforts to enhance radiation sensitivity were aimed at increasing tumor oxygenation [13]. Another class of radiation sensitizers are chemotherapies, which can enhance radiation therapy by adversely affecting DNA repair or inducing cell cycle arrest (reassortment) [14]. More recently, efforts in precision medicine have motivated the use of molecularly targeted therapies to improve radiation response of cancer through a variety of mechanisms [15,16]. We review these classes of radiation sensitizers with a focus on the progress of molecularly targeted therapies in this setting. To further enhance the therapeutic ratio, the implementation of nanoparticle-based therapies has been explored [17]. For instance, metallic (high-Z) nanoparticles which augment the effective dose of IR within tissues are currently being explored as radioenhancers [18]. Moreover, nanoparticles provide the means to enhance tumor accumulation of various therapeutics including chemotherapies and molecularly targeted agents and thereby minimize normal tissue toxicity [19]. The application of novel nanoparticle-based therapies to enhance radiation sensitivity is reviewed.

Biological modifiers to enhance radiotherapy

Oxygen/oxygen mimics:

Oxygen enhances IR damage by reacting with free radicals to create peroxide species, which ‘fix’ (make permanent) damaged DNA [9,20]. Initially, to augment radiation with oxygen, the use of hyperbaric chambers was introduced [21]. However, hyperbaric chambers are cumbersome and difficult to implement in a clinical setting and their use has been limited. The use of hydrogen peroxide (H_2O_2) to produce oxygen radicals in the tumor has also been investigated [22], and intratumoral injection of H_2O_2 is currently under evaluation in a phase I/II breast cancer clinical trial ([NCT03946202](#)). An alternative strategy in this area has been the use of oxygen mimics rather than increasing oxygen in tumors directly. Oxygen mimics are nitrogen-containing compounds that act in the same manner as oxygen by fixing the damage induced by free radicals [23]. Misonidazole and nimorazole are two oxygen

mimics that have been evaluated in preclinical and clinical settings. Misonidazole showed promising radiation sensitizing effects in preclinical models, however, the results were not corroborated in clinical trials [23]. Likewise, nimorazole showed promising pre-clinical results but has limited adoption in clinical practice, such as the treatment of head and neck cancers in combination with radiation therapy in selected centers [23,24]. Collectively, despite the translational progress made in this area, treatment-related toxicities and lack of tumor specificity have limited the clinical adoption of this strategy to date.

Chemotherapy:

Chemotherapies can enhance IR effects by impairing DNA synthesis, DNA repair, and/or cell cycle progression. Types of chemotherapeutic agents that have been investigated include taxanes/alkaloids, alkylating agents, and antimetabolites (Table 1). Taxanes are anti-microtubule agents that can induce G2/M arrest and have been used in combination with radiation therapy [25]. Cisplatin, an alkylating agent that causes DNA cross-linking and impairs DNA repair, is used concurrently with radiation therapy in multiple settings including head and neck, thoracic, gastrointestinal, gynecological, and genitourinary malignancies. Antimetabolites, such as 5-FU and gemcitabine, interfere with DNA synthesis and have also been shown to sensitize cells to IR [26]. As such, concurrent chemoradiation has become widely adopted in clinical practice for multiple tumor types, with demonstrated improvements in locoregional control and survival rates. However, the toxicity associated with concurrent chemoradiation is of significant concern, and ultimately can limit the delivered doses of both radiation and chemotherapy, particularly in patients with diminished functional status [27].

Molecularly targeted radiation sensitizers:

In the current era of precision medicine, strategies to utilize molecularly targeted therapeutics to enhance IR effects are being investigated. In contrast to chemotherapies, molecularly-targeted therapeutics which can act specifically on cancer cells provide an avenue to improve the tumor response while limiting toxicity to normal cells. This has motivated efforts to develop targeted therapeutics to selectively enhance IR effects to cancer cells only [15,16]. Through molecular targeting of multiple pathways associated with radiation response and DNA damage repair (Fig. 1), investigators have sought to improve the therapeutic ratio of radiotherapy and thereby improve clinical outcomes (Fig. 2).

Table 2 summarizes molecularly targeted therapies that have been studied in oncology clinical trials with radiation therapy, and selected results are summarized using non-small cell lung cancer (NSCLC) as a representative site. In the following section, the most promising targets that are under investigation in clinical trials are reviewed in detail.

EGFR: The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase involved in cell survival/growth signaling that is overexpressed in several cancers [28]. EGFR mutations are present in various cancers including NSCLC, with a higher incidence of EGFR mutations seen in patients who are either female, nonsmokers, or of Asian ethnicity [29]. Following radiation, activation of EGFR has been reported, leading to downstream signaling which contributes to cancer cell survival [30]. Further, EGFR has

been shown to participate in mediating DNA repair following radiation. After radiation damage, EGFR is phosphorylated and translocated to the nucleus, where it has been shown to interact with DNA-dependent protein kinase, catalytic subunit (DNA-PKcs) and repair the damaged DNA [31]. Clinical trials investigating EGFR inhibition to enhance radiation sensitivity have been explored in a variety of cancer types, using both EGFR monoclonal antibodies (e.g., cetuximab) and small molecule inhibitors (e.g., erlotinib, gefitinib, afatinib, and osimertinib). The most promising initial results were in head and neck cancer, where cetuximab improved outcomes when combined with radiation therapy compared with radiation therapy alone [32]. In NSCLC, the addition of cetuximab to a chemoradiation regimen demonstrated improved survival in the subset of patients with high expression of EGFR [7]. To date, cetuximab remains the only FDA approved targeted therapy for concurrent use with radiation for head and neck cancer [15]. However, the RTOG 1016 clinical trial [33] demonstrated that treatment with cetuximab and radiation therapy is associated with worse overall and progression-free survival compared to the current standard treatment of radiation and cisplatin in patients with human papillomavirus (HPV)-positive oropharyngeal cancer. The treatment arms also had similar toxicity rates. Therefore, cisplatin and radiation therapy remains the standard of care, while cetuximab and radiation therapy is reserved for patients who cannot tolerate cisplatin.

PARP: Poly(ADP-ribose) polymerase (PARP) proteins represent another promising target to interfere with the DNA damage response after radiation. PARP inhibitors function as radiation sensitizers by blocking repair of single strand breaks (SSBs) in DNA following radiation, resulting in complex lesions that are difficult for tumor cells to repair [34]. PARP inhibitors have shown activity in a variety of cancers in pre-clinical studies [34], and the combination of radiation and PARP inhibitors (e.g., olaparib, veliparib, niraparib) are ongoing in clinical trials in several cancers including breast, glioblastoma, NSCLC, and head and neck cancers [34,35]. PARP inhibitors have also been administered in combination with chemotherapies [36]. Importantly, PARP inhibitors have been shown to induce synthetic lethality in cancers deficient in homologous recombination (HR) repair such as BRCA1/2-mutant breast, ovarian, and pancreatic cancer, and have recently been granted FDA approval as a single agent therapeutic for ovarian and metastatic breast cancer patients with BRCA1/2 mutations [37]. PARP inhibitors are more effective radiation sensitizers in BRCA1/2-mutant tumors than BRCA1/2-wildtype tumors [38]. Thus, it is likely that the role of PARP inhibitors as radiation sensitizers will be most beneficial for patients with deficiencies in DNA repair such as BRCA-mutant cancers.

HDAC: Histone deacetylase (HDAC) is an epigenetic regulator that alters chromatin by removing acetyl groups, in turn altering gene expression. Abnormal activity of HDAC has been demonstrated in cancer development and progression, resulting in transcriptional alteration of oncogenes and tumor suppressor genes [39]. Hence, HDAC inhibitors have been developed to control the epigenetic programming of cancer cells. In the context of radiation therapy, inhibitors of HDAC have shown promise in preclinical studies [40–42] and have advanced to clinical trials. Although the precise mechanisms of how targeting HDAC improves radiation response is an ongoing area of study, HDAC inhibition has been shown to reduce expression of several DNA repair genes such as ATM, BRCA1, Ku70, Rad51, and

DNA-PK [43–46]. Moreover, inhibiting HDAC relaxes chromatin and can result in more DNA damage following IR [47,48]. HDAC inhibitors including vorinostat, panobinostat, and valproic acid have entered clinical trials for various cancers including glioblastoma, NSCLC, and pancreatic cancer. However, HDAC inhibitors have shown limited efficacy in clinical trials to date [49], and due to a lack of specificity for tumors, off-target toxicities remain a concern [50].

ATM/ATR: The ataxia-telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR) proteins are two key mediators of DNA damage response. These proteins work together to facilitate DNA repair in response to cellular stress [51]. As such, inhibitors of these proteins have been developed in an effort to impair DNA repair and hence increase tumor radiation sensitivity [52]. This is highlighted by the finding that ATM-deficient patients are the most sensitive patients to radiation [53]. Pre-clinical findings using ATM or ATR inhibitors have supported targeting these regulators to enhance IR effects [54,55], which has led to initiation of clinical trials in glioblastoma ([NCT03423628](#)), brain metastasis ([NCT02589522](#)), head and neck cancer ([NCT02567422](#)), and solid tumors ([NCT02223923](#)). Results of these trials have not yet been reported.

PI3K/AKT/mTOR: The phosphatidylinositol 3-kinase (PI3K)/AKT pathway is a key signaling pathway promoting cancer cell survival and proliferation [56]. Previous studies have reported that activation of this pathway following radiation results in radiation resistance [57,58]. This has led to efforts to target this pathway to enhance radiation sensitivity. Nelfinavir was originally developed to treat HIV infection, but was also found to inhibit PI3K/AKT [59], and has been investigated in combination with radiation in NSCLC, glioblastoma, pancreatic, cervical, and rectal cancer. Results of nelfinavir with chemoradiation in locally advanced NSCLC showed median progression-free survival and overall survival of 12 months and 40 months, respectively [60]. While promising compared to historical controls [61], these results in a small population ($n = 35$ patients) warrant further study and validation in larger populations. Another target in the pathway is the mammalian target of rapamycin (mTOR), a master regulator coordinating the complex activities of nutrient and amino acid sensing, cellular growth, and cellular proliferation. mTOR inhibitors have demonstrated radiation-sensitizing properties in preclinical studies [62,63]. Everolimus, an mTOR inhibitor, has been tested in clinical trials in several cancers. However, results from a phase I trial in NSCLC demonstrated that concurrent thoracic radiation and everolimus was associated with an elevated risk of pulmonary toxicity, as 5 out of 26 patients experienced grade 3–4 interstitial pneumonitis [64].

VEGF: Vascular endothelial growth factor (VEGF) is a major regulator of angiogenesis. Given the role of VEGF in modulating tumor vascularity and oxygenation and the importance of oxygen species in mediating radiation responses, VEGF inhibitors have been evaluated in combination with radiation therapy [65,66]. The irregular angiogenic vascular network results in hypoxic regions harboring radiation-resistant cells; VEGF inhibition to normalize the tumor vasculature has been proposed as a strategy to improve tumor perfusion and assist both drug delivery and radiation therapy [66]. Bevacizumab, a monoclonal antibody targeting circulating VEGF-A, has been evaluated with radiation

therapy in large clinical studies [67]. While encouraging results have been reported in some settings, increased Grade 3 or greater adverse events involving bleeding, thromboembolic events, and gastrointestinal perforation were higher with the combination of bevacizumab and radiation therapy [68,69]. Furthermore, the concept that vascular normalization reduces tumor hypoxia has been challenged, as prolonged exposure of anti-angiogenic agents may cause a reduction of tumor vascularity that would result in an increase in tumor hypoxia [70]. Further studies are needed to evaluate the effects of VEGF inhibition on tumor hypoxia. Optimizing the timing and duration of anti-angiogenic agents may be key to garnering clinical benefit with radiation therapy.

Immune checkpoints (PD-1, PD-L1, CTLA-4): Recently, the development of immune checkpoint inhibitors has transformed the treatment paradigms for multiple cancers. Immune checkpoint proteins dampen anti-tumor immune responses of tumors. Clinical trials to date have primarily focused on three immune checkpoint targets: PD-1, PD-L1, and CTLA-4. PD-1 is expressed on cytotoxic T-cells and when bound to its ligand, PD-L1, on cancer cells, T-cells are unable to mount an effective anti-tumor immune response allowing cancer cells to avoid immune-mediated death [71]. CTLA-4 is expressed on T-cells and inhibits activation by dendritic cells [72]. Immune checkpoint inhibitors are now widely used in the treatment of cancers. The application of these therapies with radiation has been studied since radiation in certain contexts may promote immunogenic cell death of tumor cells and lead to the release of damage-associated molecular patterns (DAMPs) and tumor-associated antigens. DAMPs and tumor-associated antigens may then prime immune cells and stimulate an anti-tumor response [73]. Radiation can also promote an immunosuppressive state, and understanding how radiation can modulate the balance between immunogenicity and immunosuppression is an area of active study. Interest in combining immunotherapy with radiation was motivated in part by the abscopal effect, which refers to regression of distant, unirradiated tumors following radiation of an index lesion. While the abscopal effect has been described in numerous case reports [74], methods to consistently obtain such responses in larger patient populations have not been described at this time. Clinical trials in many solid tumors are currently evaluating immune checkpoint inhibitors with radiation therapy in both sequential and concurrent regimens [75]. For locally advanced NSCLC, the PACIFIC trial demonstrated improved progression-free survival and overall survival in patients receiving durvalumab (anti-PD-L1) following chemoradiation compared to observation after chemoradiation; rates of grade 3–4 toxicity were similar between treatment groups [61,76]. This trial led to the FDA approval of durvalumab in the adjuvant setting for patients with locally advanced, unresectable NSCLC who have not progressed following chemoradiation. Optimization of timing, duration of therapy, sequencing of therapy, identification of potential responders, and minimization of adverse events remain active areas of study to achieve the greatest benefit of immune checkpoint inhibitors combined with radiation therapy.

Nanoparticles for cancer treatment

The application of nanotechnology in cancer has become a central focus in biomedical research. Nanoparticles (NPs) possess unique properties which allow them to overcome the

limitations associated with traditional cancer therapies, and they have been developed as targeted drug delivery vehicles to deliver therapeutics such as chemotherapies and nucleic acids [77]. First, the increased size of NPs (>10 nm) can eliminate fast renal clearance of small molecules. In addition, nanoparticles can render non-soluble drugs water soluble, which is more suitable for parenteral administration. Nanoparticles also reduce toxicity to normal tissues by localizing therapeutic agents to tumors due to the enhanced permeability and retention (EPR) effect. The EPR effect is enabled by the physiological nature of tumors, which upregulate pro-angiogenic factors, leading to formation of immature and leaky vasculature which can increase nanoparticle accumulation in tumors [78]. Moreover, poor lymphatic drainage increases nanoparticle retention within the tumors. This abnormal vasculature is known to allow for the accumulation of NPs with diameters of 30–200 nm in tumors compared with normal tissues [77]. However, EPR is thought to be largely diverse among tumors, and may not exist in some tumors at all [78]. Additionally, the use of steric hindrance via molecules such as polyethylene glycol (PEG) as a nanoparticle coating can avoid opsonization and rapid clearance by the mononuclear phagocytic system, which prolongs nanoparticle half-life and increases tumor accumulation. To further enhance targeting and reduce systemic toxicity, nanoparticles can also be decorated with targeting agents (e.g., antibodies) to specifically target cancer cells. Nanoscale materials have high surface area-to-volume ratios, enabling loading with high concentrations of therapeutic payloads or diagnostic imaging agents [79]. Furthermore, delivery of multiple therapeutics in well-defined molar ratios can be achieved in a single nanoparticle delivery system, which may enhance potential synergistic effects of the therapeutics [80]. Nanoparticles can also deliver agents that would otherwise be non-functional or degrade under normal physiological conditions. For instance, siRNA therapies hold great promise and can be designed to target any oncogene with great specificity, including genetic lesions previously considered difficult to target with small molecules or antibodies. Consequently, nanoparticles that enable delivery of therapeutic concentrations of siRNA to tumors are sought after. Lastly, in addition to serving as drug delivery vehicles, nanoparticles can also have direct effects (e.g., photothermal, ROS scavenging, ROS generating) on cancer cells depending on their chemical composition.

Nanoparticle-based radiation sensitizers

This versatility of nanoparticles has been utilized in numerous cancer treatment settings. Herein we review nanoparticle-based radiation sensitizers including nanoparticle delivery of chemotherapies, high-atomic number nanoparticles, and nanoparticle delivery of targeted therapies.

Nanoparticle delivery of chemotherapies.

A number of studies have investigated delivery of chemotherapies with nanoparticles in combination with radiation therapy. As previously discussed, certain chemotherapies can potentiate IR effects, and concurrent chemoradiation therapy is now the standard of care in a variety of cancers. However, the combination of chemotherapy and radiation therapy also results in earlier and more severe toxicity, which may limit potential synergistic benefits and impact quality of life. Thus, attempts to improve chemotherapy delivery with nanoparticles

have been explored. Abraxane, a nanoformulation of albumin-bound paclitaxel, improves solubility of the hydrophobic drug and can reduce systemic toxicity of standard paclitaxel. Several clinical trials have investigated Abraxane in combination with chemoradiotherapy for NSCLC, pancreatic, and head and neck cancers. In locally advanced NSCLC, Abraxane plus carboplatin and concurrent radiotherapy led to a median PFS of 11.8 months in 56 patients [81], which is a promising result compared to the historical control [61]. However, the treatment regimen resulted in greater hematologic toxicities, and nearly 40% of patients skipped weekly Abraxane/carboplatin at least once due to leukopenia. CRXL101 is a cyclodextrin-PEG polymeric nanoparticle formulation with the chemotherapy drug camptothecin. A phase I/II clinical trial of CRLX101 in combination with capecitabine and radiation therapy in rectal cancers ([NCT02010567](#)) demonstrated that the combination was well tolerated with promising pathological complete response (pCR) rates [82].

Metallic (high-Z) nanoparticles.

Another strategy for nanoparticle radioenhancement is the use of high-Z metallic particles. These are promising materials for use with radiation as they increase secondary electron and free radical production in the tumor microenvironment, and thus can amplify the radiobiological effects on DNA (Fig. 1). Gold nanoparticles are the most studied and among the most potent metallic nanomaterials for radiation enhancement [83–86]. Favorable properties of gold for radiosensitization include biological inertness, ease in manufacturing, and scalability. Zhang et al. reported on 2 nm gold nanoclusters covered with a glutathione (GSH) shell (final size in water about 3 nm) that could accumulate in tumors via EPR effects, and significantly enhance IR effects in a U14 mouse cervical cancer model [87]. While this material relied on EPR to accumulate the gold particles in tumors, Liang et al. developed gold nanoclusters with cyclic RGD peptide shell to target $\alpha_v\beta_3$ integrin positive cancer. The targeted nanoclusters with radiation were shown to improve tumor control compared with non-targeted nanoclusters with radiation, or radiation alone in a 4T1 mouse breast tumor model (Fig. 3) [88]. Another promising candidate is the gadolinium-based nanoparticle, AGuIX, which is a <5 nm nanoparticle consisting of gadolinium-DOTA chelates covalently attached to a polysiloxane core. In pre-clinical studies, AGuIX was shown to serve dual purpose as an MRI contrast agent to enhance tumor contrast in T1-weighted images and as a radiation sensitizer in several tumor models [89,90]. Moreover, intravenous administration of AGuIX showed a favorable toxicity profile in both mice and non-human primates [89]. Based on these findings, two phase I clinical trials using AGuIX in combination with radiation therapy have been initiated. The NanoRAD phase Ib trial ([NCT02820454](#)) will investigate a single intravenous injection of AGuIX at escalating doses prior to radiotherapy to treat brain metastases from four different cancer types. Early results from this trial have demonstrated the ability of AGuIX to pass the blood–brain barrier and selectively accumulate in brain metastases 2-hr post intravenous injection [89]. The NanoCOL clinical trial ([NCT03308604](#)) will investigate the combination of AGuIX with chemoradiotherapy (cisplatin and external beam radiotherapy – 45 Gy in 5 weeks) followed by brachytherapy (15 Gy in 2 weeks) [90]. Similar to gold and gadolinium-based nanoparticles, hafnium oxide nanoparticles are electron-dense materials that enhance the effects of IR in tissues. Hafnium oxide nanoparticles have shown promising pre-clinical results, and are under clinical investigation for soft tissue sarcomas (phase II/III) and head

and neck cancers (phase I), where they are administered intratumorally prior to radiation therapy [91]. In a phase II/III trial, patients with locally advanced soft tissue sarcoma were administered a single intratumoral injection of hafnium oxide nanoparticles, NBTXR3, combined with pre-operative radiation therapy. The combination led to a doubling of the pathological complete response rate (16% versus 8%) compared to pre-operative radiation therapy alone, but longer-term results on tumor control and survival outcomes remain to be reported [92].

Nanoparticle delivery of molecularly targeted therapies

More recently, efforts have shifted to developing molecularly targeted nanoparticles as radiation sensitizers. Wang et al. delivered HDAC inhibitors, vorinostat and quisinostat, with nanoparticles consisting of PLGA/inhibitor core coated with layer of DSPE-PEG [50]. Nanoparticle delivery of each inhibitor was shown to be superior to the inhibitor alone, and could enhance radiation effects in colorectal and prostate cancers as demonstrated by increased cH2ax foci formation [50]. Karve et al. utilized a similar nanoparticle construct to deliver wortmannin, a PI3K pathway inhibitor, which is a highly toxic agent when delivered alone. Delivery of wortmannin on the NP was shown to provide radiation sensitivity while also significantly enhancing the maximum tolerated dose of the inhibitor [93].

In addition to small molecule inhibitors, siRNA-based therapeutics hold great promise for cancer treatment. While several siRNAs have shown potential to combine with radiation *in vitro*, few studies have shown *in vitro* radiation sensitizing effects using siRNA due to limitations of *in vivo* delivery. In one study, an iron oxide nanoparticle coated with chitosan, PEI, and PEG polymers delivered siRNA against APE1, an enzyme involved in base excision repair of DNA, and was shown to be effective as a radiation sensitizer in glioblastoma mouse models [94]. Our group has recently reported [95] on EGFR-targeted mesoporous silica nanoparticles delivering PLK1 siRNA to enhance radiation sensitivity of NSCLC (Fig. 4A). In this study, the EGFR antibody coated on the nanoparticle served as both a targeting agent and as a radiation sensitizer. PLK1, a highly conserved mitotic protein, is a promising therapeutic target to enhance radiation sensitivity and its inhibition results in mitotic arrest, where cells are most sensitive to IR. Further, PLK1 inhibition has intrinsic DNA damaging ability, and reduces DNA repair capacity following IR [95]. Importantly, PLK1 signaling also regulates numerous cancer molecular pathways. For instance, we and others have shown that PLK1 inhibition leads to reduced activity of the PI3K/AKT pathway, which is a mediator of radiation resistance as discussed. Delivery of PLK1 siRNA by EGFR-targeted nanoparticles led to radiation sensitivity in NSCLC cells *in vitro* (Fig. 4B), and was shown to be more effective than monotherapy (nanoparticle or radiation alone) in a NSCLC tumor model (Fig. 4C and D).

Conclusions and future directions

We review various targeted therapies and nanoparticle-based radiation sensitizers that have recently been investigated to improve clinical outcomes in patients receiving radiotherapy. Despite advances in our understanding of molecular pathways governing radiation responses and resistance, the overall progress in implementing molecularly targeted radiation

sensitizers has been slow partly due to the complexities in the clinical development pathway and clinical trial designs [15,96,97]. Future translational studies should provide a strong rationale and biological mechanism for the combination, along with identifying optimal treatment scheduling, reduction of toxicity to normal tissues, and a well-defined patient population. Collaborations between academic researchers, clinicians, and industry are often critical, and researchers should seek guidance early from regulatory agencies to help accelerate the development pathway. Clinical trials must be well-designed to measure their primary endpoint and answer their primary objective, which should be selected based on factors including the treatment intervention, tumor type, and patient population. For example, biomarker-driven patient selection for molecularly targeted therapies may increase the probability of achieving a positive clinical trial result in a more well-defined population. For combinations of targeted therapy and radiotherapy, it is important to note that the maximum tolerated dose (MTD) of the drug may not necessarily lead to improved efficacy, but rather a lower dose of either drug or radiation may provide greater synergistic activity while mitigating toxicity [96]. The complex interactions of molecularly-targeted agents and radiation in the tumor microenvironment may indeed require novel biologically-defined endpoints beyond the classically defined MTD used for cytotoxic therapies.

In addition to the complexity in the clinical development pathway, increased risks of targeted therapy and radiotherapy combinations compared to administering drugs or radiotherapy alone remains a significant limitation. For instance, the combination of BRAF inhibitors (vemurafinib, dabrafenib) with radiotherapy can cause severe radiation recall (severe skin rash in irradiated regions due to acute inflammatory reaction) in melanoma patients [98]. Ultimately, the clinical utility of combining targeted therapies and radiotherapy requires validation in specific clinical contexts and well-defined patient populations.

Precision medicine benefits cancer patients by providing targeted therapies both in the upfront treatment-naïve and recurrent/treatment-refractory settings. With an improved mechanistic understanding of how tumors respond to IR, precision medicine approaches may also benefit radiotherapy in a similar manner. Further, radiation enhancers that not only improve local tumor control, but also promote tumor immunogenicity are highly desirable. This is being aided by a greater understanding how IR interacts with tumor- and immune cell populations in the tumor microenvironment. As these multifaceted interactions are deciphered in the coming years, nanoparticles that enable targeted delivery or co-delivery of therapeutics and immunomodulatory agents will provide additional opportunities to augment the therapeutic window of radiotherapy.

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References

- [1]. Wang J-s, Wang H-j, Qian H-l. Biological effects of radiation on cancer cells. *Mil Med Res* 2018;5:20. [PubMed: 29958545]
- [2]. Surova O, Zhivotovsky B. Various modes of cell death induced by DNA damage. *Oncogene* 2013;32:3789–97. [PubMed: 23208502]
- [3]. Diwanji TP, Mohindra P, Vyfhuis M, Snider JW III, Kalavagunta C, Mossahebi S, et al. Advances in radiotherapy techniques and delivery for non-small cell lung cancer: benefits of intensity-modulated radiation therapy, proton therapy, and stereotactic body radiation therapy. *Transl Lung Cancer Res* 2017;6:131–47. [PubMed: 28529896]
- [4]. Coles CE, Griffin CL, Kirby AM, Titley J, Agrawal RK, Alhasso A, et al. Partial-breast radiotherapy after breast conservation surgery for patients with early breast cancer (UK IMPORT LOW trial): 5-year results from a multicentre, randomised, controlled, phase 3, non-inferiority trial. *Lancet (London, England)* 2017;390:1048–60.
- [5]. Klopp AH, Yeung AR, Deshmukh S, Gil KM, Wenzel L, Westin SN, et al. Patient-reported toxicity during pelvic intensity-modulated radiation therapy: NRG oncology-RTOG 1203. *J Clin Oncol* 2018;36:2538–44. [PubMed: 29989857]
- [6]. Brown PD, Jaeckle K, Ballman KV, Farace E, Cerhan JH, Anderson SK, et al. Effect of radiosurgery alone vs radiosurgery with whole brain radiation therapy on cognitive function in patients with 1 to 3 brain metastases: a randomized clinical trial. *JAMA* 2016;316:401–9. [PubMed: 27458945]
- [7]. Bradley JD, Paulus R, Komaki R, Masters G, Blumenschein G, Schild S. Standard-dose versus high-dose conformal radiotherapy with concurrent and consolidation carboplatin plus paclitaxel with or without cetuximab for patients with stage IIIA or IIIB non-small-cell lung cancer (RTOG 0617): a randomized, two-by-two factorial phase 3 study. *Lancet Oncol* 2015;16.
- [8]. Oehler C, Dickinson DJ, Broggin-Tenzer A, Hofstetter B, Hollenstein A, Riesterer O, et al. Current concepts for the combined treatment modality of ionizing radiation with anticancer agents. *Curr Pharm Des* 2007;13:519–35. [PubMed: 17348847]
- [9]. Hall EJ. *Radiobiology for the radiologist*. 7th ed. Lippincott Williams & Wilkins; 2012.
- [10]. Steel GG, McMillan TJ, Peacock JH. The 5Rs of radiobiology. *Int J Radiat Biol* 1989;56:1045–8. [PubMed: 2574214]
- [11]. Pajonk F, Vlashi E, McBride WH. Radiation resistance of cancer stem cells: the 4 R's of radiobiology revisited. *Stem Cells (Dayton, Ohio)* 2010;28:639–48.
- [12]. Citrin DE, Mitchell JB. Altering the response to radiation: sensitizers and protectors. *Semin Oncol* 2014;41:848–59. [PubMed: 25499642]
- [13]. Révész L, Palcic B. Radiation dose dependence of the sensitization by oxygen and oxygen mimic sensitizers. *Acta Radiol Oncol* 1985;24:209–17. [PubMed: 2994369]
- [14]. Seiwert TY, Salama JK, Vokes EE. The concurrent chemoradiation paradigm—general principles. *Nat Clin Pract Oncol* 2007;4:86–100. [PubMed: 17259930]
- [15]. Ahmad SS, Crittenden MR, Tran PT, Kluetz PG, Blumenthal GM, Bulbeck H, et al. Clinical development of novel drug-radiotherapy combinations. *Clin Cancer Res* 2019;25:1455–61. [PubMed: 30498095]
- [16]. Morris ZS, Harari PM. Interaction of radiation therapy with molecular targeted agents. *J Clin Oncol* 2014;32:2886–93. [PubMed: 25113770]
- [17]. Chatterjee DK, Wolfe T, Lee J, Brown AP, Singh PK, Bhattarai SR, et al. Convergence of nanotechnology with radiation therapy—insights and implications for clinical translation. *Transl Cancer Res* 2013;2:256–68. [PubMed: 25279336]
- [18]. Retif P, Pinel S, Toussaint M, Frochot C, Chouikrat R, Bastogne T, et al. Nanoparticles for radiation therapy enhancement: the key parameters. *Theranostics* 2015;5:1030–44. [PubMed: 26155318]
- [19]. Tran S, DeGiovanni P-J, Piel B, Rai P. Cancer nanomedicine: a review of recent success in drug delivery. *Clin Transl Med* 2017;6:44-. [PubMed: 29230567]

- [20]. Chang DS, Lasley FD, Das IJ, Mendonca MS, Dynlacht JR. Oxygen effect, relative biological effectiveness and linear energy transfer. Basic radiotherapy physics and biology. Cham: Springer International Publishing; 2014. p. 235–40.
- [21]. Daruwalla J, Christophi C. Hyperbaric oxygen therapy for malignancy: a review. *World J Surg* 2006;30:2112–31. [PubMed: 17102915]
- [22]. Ogawa Y, Kubota K, Ue H, Tadokoro M, Matsui R, Yamanishi T, et al. Safety and effectiveness of a new enzyme-targeting radiosensitization treatment (KORTUC II) for intratumoral injection for low-LET radioresistant tumors. *Int J Oncol* 2011;39:553–60. [PubMed: 21667020]
- [23]. Wang H, Mu X, He H, Zhang XD. Cancer radiosensitizers. *Trends Pharmacol Sci* 2018;39:24–48. [PubMed: 29224916]
- [24]. Bentzen J, Toustrup K, Eriksen JG, Primdahl H, Andersen LJ, Overgaard J. Locally advanced head and neck cancer treated with accelerated radiotherapy, the hypoxic modifier nimorazole and weekly cisplatin. Results from the DAHANCA 18 phase II study. *Acta Oncol (Stockholm, Sweden)* 2015;54:1001–7.
- [25]. Golden EB, Formenti SC, Schiff PB. Taxanes as radiosensitizers. *Anticancer Drugs* 2014;25:502–11. [PubMed: 24335716]
- [26]. Kvols LK. Radiation sensitizers: a selective review of molecules targeting DNA and non-DNA targets. *J Nucl Med* 2005;46(Suppl 1):187s–90s. [PubMed: 15653668]
- [27]. Schae D, McBride WH. Opportunities and challenges of radiotherapy for treating cancer. *Nat Rev Clin Oncol* 2015;12:527–40. [PubMed: 26122185]
- [28]. Seshacharyulu P, Ponnusamy MP, Haridas D, Jain M, Ganti AK, Batra SK. Targeting the EGFR signaling pathway in cancer therapy. *Expert Opin Ther Targets* 2012;16:15–31. [PubMed: 22239438]
- [29]. Zhang Y-L, Yuan J-Q, Wang K-F, Fu X-H, Han X-R, Threapleton D, et al. The prevalence of EGFR mutation in patients with non-small cell lung cancer: a systematic review and meta-analysis. *Oncotarget* 2016;7:78985–93. [PubMed: 27738317]
- [30]. Lee HC, An S, Lee H, Woo SH, Jin HO, Seo SK, et al. Activation of epidermal growth factor receptor and its downstream signaling pathway by nitric oxide in response to ionizing radiation. *Mol Cancer Res: MCR*. 2008;6:996–1002. [PubMed: 18567803]
- [31]. Chen DJ, Nirodi CS. The epidermal growth factor receptor: a role in repair of radiation-induced DNA damage. *Clin Cancer Res* 2007;13:6555–60. [PubMed: 18006754]
- [32]. Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N Engl J Med* 2006;354:567–78. [PubMed: 16467544]
- [33]. Gillison ML, Trotti AM, Harris J, Eisbruch A, Harari PM, Adelstein DJ, et al. Radiotherapy plus cetuximab or cisplatin in human papillomavirus-positive oropharyngeal cancer (NRG Oncology RTOG 1016): a randomised, multicentre, non-inferiority trial. *Lancet (London, England)* 2019;393:40–50.
- [34]. Lesueur P, Chevalier F, Austry J-B, Waissi W, Burckel H, Noël G, et al. Poly-(ADP-ribose)-polymerase inhibitors as radiosensitizers: a systematic review of pre-clinical and clinical human studies. *Oncotarget* 2017;8:69105–24. [PubMed: 28978184]
- [35]. Kunz-Schughart LA, Dubrovskaya A, Peitzsch C, Ewe A, Aigner A, Schellenburg S, et al. Nanoparticles for radiooncology: mission, vision, challenges. *Biomaterials* 2017;120:155–84. [PubMed: 28063356]
- [36]. Kamel D, Gray C, Walia JS, Kumar V. PARP inhibitor drugs in the treatment of breast, ovarian, prostate and pancreatic cancers: an update of clinical trials. *Curr Drug Targets* 2018;19:21–37. [PubMed: 28699513]
- [37]. Lord CJ, Ashworth A. PARP inhibitors: synthetic lethality in the clinic. *Science (New York, NY)* 2017;355:1152–8.
- [38]. Bi Y, Verginadis II, Dey S, Lin L, Guo L, Zheng Y, et al. Radiosensitization by the PARP inhibitor olaparib in BRCA1-proficient and deficient high-grade serous ovarian carcinomas. *Gynecol Oncol* 2018;150:534–44. [PubMed: 30025822]
- [39]. Li Y, Seto E. HDACs and HDAC Inhibitors in Cancer Development and Therapy. *Cold Spring Harbor perspectives in medicine*.6:a026831.

- [40]. Konsoula Z, Velena A, Lee R, Dritschilo A, Jung M. Histone deacetylase inhibitor: antineoplastic agent and radiation modulator. *Adv Exp Med Biol* 2011;720:171–9. [PubMed: 21901627]
- [41]. Seo SK, Jin HO, Woo SH, Kim YS, An S, Lee JH, et al. Histone deacetylase inhibitors sensitize human non-small cell lung cancer cells to ionizing radiation through acetyl p53-mediated c-myc down-regulation. *J Thoracic Oncol* 2011;6:1313–9.
- [42]. Chinnaiyan P, Vallabhaneni G, Armstrong E, Huang SM, Harari PM. Modulation of radiation response by histone deacetylase inhibition. *Int J Radiat Oncol Biol Phys* 2005;62:223–9. [PubMed: 15850925]
- [43]. Tsai CL, Liu WL, Hsu FM, Yang PS, Yen RF, Tzen KY, et al. Targeting histone deacetylase 4/ubiquitin-conjugating enzyme 9 impairs DNA repair for radiosensitization of hepatocellular carcinoma cells in mice. *Hepatology (Baltimore, Md)*. 2017.
- [44]. Kachhap SK, Rosmus N, Collis SJ, Kortenhorst MS, Wissing MD, Hedayati M, et al. Downregulation of homologous recombination DNA repair genes by HDAC inhibition in prostate cancer is mediated through the E2F1 transcription factor. *PLoS ONE* 2010;5:e11208. [PubMed: 20585447]
- [45]. Ren J, Chu Y, Ma H, Zhang Y, Zhang X, Zhao D, et al. Epigenetic interventions increase the radiation sensitivity of cancer cells. *Curr Pharm Des* 2014;20:1857–65. [PubMed: 23888958]
- [46]. Thurn KT, Thomas S, Raha P, Qureshi I, Munster PN. Histone deacetylase regulation of ATM-mediated DNA damage signaling. *Mol Cancer Ther* 2013;12:2078–87. [PubMed: 23939379]
- [47]. Shabason JE, Tofilon PJ, Camphausen K. Grand rounds at the National Institutes of Health: HDAC inhibitors as radiation modifiers, from bench to clinic. *J Cell Mol Med* 2011;15:2735–44. [PubMed: 21362133]
- [48]. Johnstone RW. Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. *Nat Rev Drug Discovery* 2002;1:287–99. [PubMed: 12120280]
- [49]. Suraweera A, O'Byrne KJ, Richard DJ. Combination therapy with histone deacetylase inhibitors (HDACi) for the treatment of cancer: achieving the full therapeutic potential of HDACi. *Front Oncol* 2018;8:92-. [PubMed: 29651407]
- [50]. Wang EC, Min Y, Palm RC, Fiordalisi JJ, Wagner KT, Hyder N, et al. Nanoparticle formulations of histone deacetylase inhibitors for effective chemoradiotherapy in solid tumors. *Biomaterials* 2015;51:208–15. [PubMed: 25771011]
- [51]. Maréchal A, Zou L. DNA damage sensing by the ATM and ATR kinases. *Cold Spring Harbor perspectives in biology*.5:a012716.
- [52]. Weber AM, Ryan AJ. ATM and ATR as therapeutic targets in cancer. *Pharmacol Ther* 2015;149:124–38. [PubMed: 25512053]
- [53]. Taylor AMR, Harnden DG, Arlett CF, Harcourt SA, Lehmann AR, Stevens S, et al. Ataxia telangiectasia: a human mutation with abnormal radiation sensitivity. *Nature* 1975;258:427–9. [PubMed: 1196376]
- [54]. Teng P-n, Bateman NW, Darcy KM, Hamilton CA, Maxwell GL, Bakkenist CJ, et al. Pharmacologic inhibition of ATR and ATM offers clinically important distinctions to enhancing platinum or radiation response in ovarian, endometrial, and cervical cancer cells. *Gynecol Oncol* 2015;136:554–61. [PubMed: 25560806]
- [55]. Dillon MT, Barker HE, Pedersen M, Hafsi H, Bhide SA, Newbold KL, et al. Radiosensitization by the ATR inhibitor AZD6738 through generation of acentric micronuclei. *Mol Cancer Ther* 2017;16:25–34. [PubMed: 28062704]
- [56]. Fresno Vara JA, Casado E, de Castro J, Cejas P, Belda-Iniesta C, Gonzalez-Baron M. PI3K/Akt signalling pathway and cancer. *Cancer Treat Rev* 2004;30:193–204. [PubMed: 15023437]
- [57]. Schuurbijs OC, Kaanders JH, van der Heijden HF, Dekhuijzen RP, Oyen WJ, Bussink J. The PI3-K/AKT-pathway and radiation resistance mechanisms in non-small cell lung cancer. *J Thoracic Oncol* 2009;4:761–7.
- [58]. Li H-F, Kim J-S, Waldman T. Radiation-induced Akt activation modulates radioresistance in human glioblastoma cells. *Radiat Oncol (London, England)* 2009;4:43-.
- [59]. Plastaras JP, Vapiwala N, Ahmed MS, Gudonis D, Cerniglia GJ, Feldman MD, et al. Validation and toxicity of PI3K/Akt pathway inhibition by HIV protease inhibitors in humans. *Cancer Biol Ther* 2008;7:628–35. [PubMed: 18285707]

- [60]. Rengan R, Mick R, Pryma DA, Lin LL, Plastaras J, Simone CB II, et al. Long-term results of a phase I/II trial of nelfinavir with concurrent chemoradiotherapy for locally advanced non-small cell lung cancer. *Int J Radiat Oncol Biol Phys* 2018;102:S19.
- [61]. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, et al. Overall survival with durvalumab after chemoradiotherapy in stage III NSCLC. *N Engl J Med* 2018;379:2342–50. [PubMed: 30280658]
- [62]. Nagata Y, Takahashi A, Ohnishi K, Ota I, Ohnishi T, Tojo T, et al. Effect of rapamycin, an mTOR inhibitor, on radiation sensitivity of lung cancer cells having different p53 gene status. *Int J Oncol* 2010;37:1001–10. [PubMed: 20811722]
- [63]. Schiewer MJ, Den R, Hoang DT, Augello MA, Lawrence YR, Dicker AP, et al. mTOR is a selective effector of the radiation therapy response in androgen receptor-positive prostate cancer. *Endocrine-related cancer*.19:1–12. [PubMed: 21903859]
- [64]. Deutsch E, Le Pechoux C, Faivre L, Rivera S, Tao Y, Pignon JP, et al. Phase I trial of everolimus in combination with thoracic radiotherapy in non-small-cell lung cancer. *Annals Oncol* 2015;26:1223–9.
- [65]. Goel S, Duda DG, Xu L, Munn LL, Boucher Y, Fukumura D, et al. Normalization of the vasculature for treatment of cancer and other diseases. *Physiol Rev* 2011;91:1071–121. [PubMed: 21742796]
- [66]. Willett CG, Kozin SV, Duda DG, di Tomaso E, Kozak KR, Boucher Y, et al. Combined vascular endothelial growth factor-targeted therapy and radiotherapy for rectal cancer: theory and clinical practice. *Semin Oncol* 2006;33:S35–40. [PubMed: 17145523]
- [67]. Schmidt B, Lee H-J, Ryeom S, Yoon SS. Combining bevacizumab with radiation or chemoradiation for solid tumors: a review of the scientific rationale, and clinical trials. *Curr Angiogenesis* 2012;1:169–79.
- [68]. Chinot OL, Wick W, Mason W, Henriksson R, Saran F, Nishikawa R, et al. Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. *N Engl J Med* 2014;370:709–22. [PubMed: 24552318]
- [69]. Wozniak AJ, Moon J, Thomas CR Jr, Kelly K, Mack PC, Gaspar LE, et al. A pilot trial of cisplatin/etoposide/radiotherapy followed by consolidation docetaxel and the combination of bevacizumab (NSC-704865) in patients with inoperable locally advanced stage III non-small-cell lung cancer: SWOG S0533. *Clin Lung Cancer* 2015;16:340–7. [PubMed: 25703100]
- [70]. Murata R, Nishimura Y, Hiraoka M. An antiangiogenic agent (TNP-470) inhibited reoxygenation during fractionated radiotherapy of murine mammary carcinoma. *Int J Radiat Oncol Biol Phys* 1997;37:1107–13. [PubMed: 9169820]
- [71]. Zou W, Wolchok JD, Chen L. PD-L1 (B7–H1) and PD-1 pathway blockade for cancer therapy: mechanisms, response biomarkers, and combinations. *Sci Transl Med* 2016;8:328rv4–rv4.
- [72]. Buchbinder EI, Desai A. CTLA-4 and PD-1 pathways: similarities, differences, and implications of their inhibition. *Am J Clin Oncol* 2016;39:98–106. [PubMed: 26558876]
- [73]. Patel SA, Minn AJ. Combination cancer therapy with immune checkpoint blockade: mechanisms and strategies. *Immunity* 2018;48:417–33. [PubMed: 29562193]
- [74]. Dagoglu N, Karaman S, Caglar HB, Oral EN. Abscopal effect of radiotherapy in the immunotherapy era: systematic review of reported cases. *Cureus* 2019;11: e4103–e. [PubMed: 31057997]
- [75]. Asna N, Livoff A, Batash R, Debbi R, Schaffer P, Rivkind T, et al. Radiation therapy and immunotherapy-a potential combination in cancer treatment. *Curr Oncol* 2018;25:e454–60. [PubMed: 30464697]
- [76]. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, et al. Durvalumab after chemoradiotherapy in stage III non-small-cell lung cancer. *N Engl J Med* 2017;377:1919–29. [PubMed: 28885881]
- [77]. Rosenblum D, Joshi N, Tao W, Karp JM, Peer D. Progress and challenges towards targeted delivery of cancer therapeutics. *Nat Commun* 2018;9:1410. [PubMed: 29650952]
- [78]. Golombek SK, May J-N, Theek B, Appold L, Drude N, Kiessling F, et al. Tumor targeting via EPR: strategies to enhance patient responses. *Adv Drug Deliv Rev* 2018;130:17–38. [PubMed: 30009886]

- [79]. Thakor AS, Gambhir SS. Nanooncology: the future of cancer diagnosis and therapy. *CA Cancer J Clin* 2013;63:395–418. [PubMed: 24114523]
- [80]. Lancet JE, Uy GL, Cortes JE, Newell LF, Lin TL, Ritchie EK, et al. CPX-351 (cytarabine and daunorubicin) liposome for injection versus conventional cytarabine plus daunorubicin in older patients with newly diagnosed secondary acute myeloid leukemia. *J Clin Oncol* 2018;36:2684–92. [PubMed: 30024784]
- [81]. Kawano Y, Sasaki T, Yamaguchi H, Hirano K, Horiike A, Satouchi M, et al. Phase I/II study of carboplatin plus nab-paclitaxel and concurrent radiotherapy for patients with locally advanced non-small cell lung cancer. *Lung Cancer (Amsterdam, Netherlands)* 2018;125:136–41.
- [82]. Sanoff HK, Moon DH, Moore DT, Boles J, Bui C, Blackstock W, et al. Phase I/II trial of nano-camptothecin CRLX101 with capecitabine and radiotherapy as neoadjuvant treatment for locally advanced rectal cancer. *Nanomed Nanotechnol Biol Med* 2019;18:189–95.
- [83]. Hainfeld JF, Slatkin DN, Smilowitz HM. The use of gold nanoparticles to enhance radiotherapy in mice. *Phys Med Biol* 2004;49:N309–15. [PubMed: 15509078]
- [84]. Haume K, Rosa S, Grellet S, mialek MA, Butterworth KT, Solov'yov AV, et al. Gold nanoparticles for cancer radiotherapy: a review. *Cancer Nanotechnol* 2016;7:8-. [PubMed: 27867425]
- [85]. Schuermann J, Bagley A, Berbeco R, Bromma K, Butterworth KT, Byrne H, et al. Roadmap for metal nanoparticles in radiation therapy: current status, translational challenges, and future directions. *Phys Med Biol* 2020.
- [86]. Schuermann J, Berbeco R, Chithrani DB, Cho SH, Kumar R, McMahon SJ, et al. Roadmap to clinical use of gold nanoparticles for radiation sensitization. *Int J Radiat Oncol Biol Phys* 2016;94:189–205. [PubMed: 26700713]
- [87]. Zhang X-D, Luo Z, Chen J, Song S, Yuan X, Shen X, et al. Ultrasmall glutathione-protected gold nanoclusters as next generation radiotherapy sensitizers with high tumor uptake and high renal clearance. *Sci Rep* 2015;5:8669. [PubMed: 25727895]
- [88]. Liang G, Jin X, Zhang S, Xing D. RGD peptide-modified fluorescent gold nanoclusters as highly efficient tumor-targeted radiotherapy sensitizers. *Biomaterials* 2017;144:95–104. [PubMed: 28834765]
- [89]. Verry C, Sancey L, Dufort S, Le Duc G, Mendoza C, Lux F, et al. Treatment of multiple brain metastases using gadolinium nanoparticles and radiotherapy: NANO-RAD, a phase I study protocol. *BMJ Open* 2019;9:e023591–e.
- [90]. Lux F, Tran VL, Thomas E, Dufort S, Rossetti F, Martini M, et al. AGuIX[®] from bench to bedside-transfer of an ultrasmall theranostic gadolinium-based nanoparticle to clinical medicine. *Br J Radiol* 2019;92:20180365-. [PubMed: 30226413]
- [91]. Bobo D, Robinson KJ, Islam J, Thurecht KJ, Corrie SR. Nanoparticle-based medicines: a review of FDA-approved materials and clinical trials to date. *Pharm Res* 2016;33:2373–87. [PubMed: 27299311]
- [92]. Bonvalot S, Rutkowski PL, Thariat J, Carrere S, Ducassou A, Sunyach MP, et al. NBTXR3, a first-in-class radioenhancer hafnium oxide nanoparticle, plus radiotherapy versus radiotherapy alone in patients with locally advanced soft-tissue sarcoma (Act. In.Sarc): a multicentre, phase 2–3, randomised, controlled trial. *Lancet Oncol* 2019;20:1148–59. [PubMed: 31296491]
- [93]. Karve S, Werner ME, Sukumar R, Cummings ND, Copp JA, Wang EC, et al. Revival of the abandoned therapeutic wortmannin by nanoparticle drug delivery. *PNAS* 2012;109:8230–5. [PubMed: 22547809]
- [94]. Kievit FM, Wang K, Ozawa T, Tarudji AW, Silber JR, Holland EC, et al. Nanoparticle-mediated knockdown of DNA repair sensitizes cells to radiotherapy and extends survival in a genetic mouse model of glioblastoma. *Nanomed Nanotechnol Biol Med* 2017;13:2131–9.
- [95]. Reda M, Ngamcherdtrakul W, Gu S, Bejan DS, Siriwon N, Gray JW, et al. PLK1 and EGFR targeted nanoparticle as a radiation sensitizer for non-small cell lung cancer. *Cancer Lett* 2019;467:9–18. [PubMed: 31563561]
- [96]. Sharma RA, Plummer R, Stock JK, Greenhalgh TA, Ataman O, Kelly S, et al. Clinical development of new drug-radiotherapy combinations. *Nat Rev Clin Oncol* 2016;13:627–42. [PubMed: 27245279]

- [97]. Falls KC, Sharma RA, Lawrence YR, Amos RA, Advani SJ, Ahmed MM, et al. Radiation-drug combinations to improve clinical outcomes and reduce normal tissue toxicities: current challenges and new approaches: report of the symposium held at the 63rd Annual Meeting of the Radiation Research Society, 15–18 October 2017; Cancun, Mexico. *Radiat Res* 2018;190:350–60. [PubMed: 30280985]
- [98]. Anker CJ, Grossmann KF, Atkins MB, Suneja G, Tarhini AA, Kirkwood JM. Avoiding severe toxicity from combined BRAF inhibitor and radiation treatment: consensus guidelines from the Eastern Cooperative Oncology Group (ECOG). *Int J Radiat Oncol Biol Phys* 2016;95:632–46. [PubMed: 27131079]

Fig. 1.

Multiscale tissue, cellular, and molecular mechanisms of tumor resistance to ionizing radiation (IR). (top right) Radiotherapy in the kilovoltage (KV) to megavoltage (MV) range is localized to tumors in various locations and surrounding tissues. (top left) Resistance to IR at the cellular level mediated by intracellular signaling pathways related to cellular survival and proliferation including the MAPK (RAS/RAF/MEK/ERK) and PI3K/AKT/mTORC pathways. Epigenetic regulation by HDAC and DNMT induces chromatin remodeling and altered gene expression leading to enhanced DNA repair and survival following IR. DNA repair genes including DNA-PKcs, PARP, ATM, and ATR can efficiently repair IR-induced single-strand and double-strand breaks. (center) Multiple factors in the tumor microenvironment can hinder the response to IR. Angiogenesis induced by VEGF secretion promotes tumor growth and irregular vasculature which can result in radiation-resistant hypoxic regions. Local immunosuppressive factors including 'exhausted' effector T cells, regulatory T cells, and myeloid-derived suppressor cells (MDSCs) lead to ineffective priming of adaptive immunity following IR. Metallic nanoparticles combined with IR may modulate the tumor stroma to counteract these inhibitory mechanisms. (bottom right) Metallic nanoparticles present in the cytoplasm and nucleus combined with radiotherapy amplify both indirect and direct DNA damage through increased secondary electron

production and generation of free radicals, leading to increased immunological cell death and release of tumor-associated neoantigens.

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Fig. 2.

Increasing the therapeutic window of radiotherapy. At any given dose, there is a probability of tumor control or adverse event, the separation between tumor control and adverse event is the therapeutic window (or therapeutic ratio). (A) Narrow therapeutic window can be increased by sensitizing tumors to radiation via various mechanisms, shifting tumor control curve to the left. (B) Increased therapeutic window after treatment with sensitizing drug.

Fig. 3.

In vivo AuNCs-enhanced radiotherapy of tumor-bearing mice [81]. (A) c(RGDyC)-AuNCs accumulate in $\alpha_v\beta_3$ integrin-positive cancer cells and interact with incident radiation intensively, generating secondary radiation, and leading to radiation enhancement effect. Radiotherapy was performed at 4 h after i.v. injection of saline, c(RADyC)-AuNCs or c(RGDyC)-AuNCs. At $0.1 \text{ mmol Au kg}^{-1}$. (B) Tumor volume growth curves of the mice after various treatments ($n = 3$). (C) *Ex vivo* weight of the tumors at 14 days after the treatments. Error bars, mean $\pm$ SD; * $P < 0.05$ (two-tailed Student's *t*-test). Reprinted with permission from Elsevier.

Fig. 4. EGFR-targeted (cetuximab) mesoporous silica nanoparticle (NP) platform for PLK1 siRNA (siPLK1) delivery (C-siPLK1-NP) [86]. (A) C-siPLK1-NPs bind to EGFR receptors and are internalized, resulting in the loss of EGFR and phosphorylated EGFR, which can normally reach the nucleus to repair DNA. This reduces the cell's ability to repair the damage caused by radiation. Simultaneously, siPLK1 on the nanoparticles is released in the cytosol and incorporated in the RNA induced silencing complex (RISC) to mediate PLK1 mRNA cleavage, which reduces PLK1 protein expression and arrests the cells in G2/M where they are most sensitive to radiation damage. Therefore, the platform serves a dual role (by targeting PLK1 and EGFR) to sensitize NSCLC cells to radiation. (B) A549 cells were treated with C-siSCR-NP or C-siPLK1-NP (50 nM as siRNA) for 72 hr followed by 2–6 Gy irradiation and re-plated for clonogenic survival assay. (C and D) 5 million A549 tumor cells were inoculated in both flanks of SCID mice. Treatments (0.3 nmol siRNA per tumor, once a week) and radiation (2 Gy to the left tumor only; 72 hr post treatments with nanoparticles) were administered for 6 weeks ($n = 7$). Growth of (C) non-irradiated tumors and (D) irradiated tumors. Data presented as mean $\pm$ SEM; ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Reprinted with permission from Elsevier.

Table 1

Types of chemotherapies used in combination with IR.

Type	Drug
Alkylating Agents	carboplatin, cisplatin, cyclophosphamide, oxaliplatin, temozolomide
Plant Alkaloids	paclitaxel, docetaxel, etoposide, vincristine, irinotecan
Antimetabolites	5-Fluorouracil, capecitabine, gemcitabine, methotrexate, hydroxyurea
Antitumor Antibiotics	doxorubicin, daunorubicin, epirubicin, bleomycin
Topoisomerase Inhibitors	etoposide, topotecan

Table 2

Molecularly targeted therapies in clinical trials with radiation therapy.

Target	Drug	Mechanism of action	Cancers investigated	Result in NSCLC
EGFR	* cetuximab, panitumumab, nimotuzumab, erlotinib, gefitinib, osimertinib, afatinib	DNA damage response/Cell survival signaling	NSCLC, HNC, CRC, GBM, pancreatic, prostate, skin, esophageal	Cetuximab + CRT in stage III: no benefit in overall survival. Higher rate of grade 3 or worse toxic effects (86% vs. 70%). 2-year OS benefit in patients with high EGFR (544 patients) [NCT00533949] Panitumumab + CRT in stage III: MNS rates were 68.2% and 50.0%, for CRT and CRT + panitumumab. Higher toxicity in experimental arm (60 patients) [NCT00979212] Erlotinib + CRT in stage III: median time to progression was 14.0 months. 1-, 2-, and 5-year OS rates were 82.6%, 67.4%, and 35.9%. Acceptable toxicity (0 grade 4–5, 11 grade 3 adverse events. 12 patients had no progression and 34 had local and/or distant failure (46 patients) [NCT00563784]
PARP	olaparib, veliparib	DNA damage response	NSCLC, HNC, breast, GBM, ovarian	Olaparib Dose Escalating Trial + Concurrent RT with or without Cisplatin in locally advanced NSCLC (Recruiting) [NCT01562210]
ATM	AZD1390	DNA damage response	GBM	NA
ATR	AZD6738, M6620	DNA damage response	NSCLC brain metastasis, HNC, solid tumors	M6620 and whole brain RT for brain metastases from NSCLC, SCLC, or neuroendocrine tumors (Recruiting) [NCT02589522]
PI3K/AKT	nelfinavir	cell survival signaling	NSCLC, GBM, CRC, pancreatic, cervical	Combination CRT and nelfinavir in stage III NSCLC: PFS 12 month, OS 40 month, 5 year OS was 37 % (35 patients) [NCT00589056]
mTOR	everolimus	cell survival signaling	NSCLC, HNC, prostate, GBM	Everolimus + RT followed by consolidation chemotherapy in locally advanced or oligometastatic untreated NSCLC: 24% of patients experienced grade 3–4 pneumonitis (26 patients) [NCT01167530]
VEGF	bevacizumab	angiogenesis	NSCLC, CRC, GBM, pancreatic	(1) Terminated (toxicity) [NCT005331076]. (2) Combination CRT and bevacizumab in unresectable stage III NSCLC. Of 29 patients, 17 did not complete study (5 due to adverse events, 2 treatment related death) [NCT00334815]
HDAC	vorinostat, panobinostat, valproic acid	chromatin modification	NSCLC, GBM, HNC, pancreatic, prostate	Palliative radiotherapy with vorinostat in advanced or metastatic NSCLC phase I: maximum tolerated dose of vorinostat given concurrently with thoracic radiotherapy is 400 mg per fraction (17 patients) [NCT00821951]
PD-1	pembrolizumab, nivolumab	immune checkpoint blockade	NSCLC, HNC, CRC, GBM, breast, cervical, pancreatic, prostate, melanoma, esophageal	Consolidation pembrolizumab following CRT in stage III: median PFS was 15.4 months. 16 (17.2%) patients developed grade 2 pneumonitis, 5 (5.4%) had grade 3–4 pneumonitis. 1 pneumonitis-related death. Improvement over historical controls (92 patients) [NCT02343952]
PD-L1	atezolizumab, durvalumab	immune checkpoint blockade	NSCLC, HNC, CRC, GBM, breast, cervical, pancreatic, prostate, melanoma, esophageal	Durvalumab consolidation post CRT: (473 patients received durvalumab and 236 received placebo). 24-month OS rate of 66.3% in durvalumab group vs. 55.6 in the placebo group. Median PFS 17.2 months vs. 5.6 months in placebo group. Median time to death or distant metastasis was 28.3 months vs. 16.2 months in placebo group. Grade 3–4 adverse events 30.5% vs. 26.1% in placebo group; 15.4% and 9.8% of the patients, respectively, discontinued due to adverse events [NCT02125461]
CTLA-4	ipilimumab	immune checkpoint blockade	NSCLC, HNC, CRC, cervical, pancreatic, prostate, melanoma, esophageal	Two patients experienced dose-limiting toxicity and 12 (34%) grade 3 toxicity. Response outside the radiation field was assessable in 31 patients. Three patients (10%) exhibited partial response outside irradiated field and 7 (23%) experienced clinical benefit (i.e. partial response or stable disease lasting 6 months) (35 patients) [NCT02239900]

* FDA approved with radiation, CRC: colorectal cancer, CRT: chemoradiotherapy, GBM: glioblastoma, HNC: head and neck cancer, MNS: Mediastinal nodal sterilization, NSCLC: non-small cell lung cancer, OS: overall survival, PFS: progression free survival, RT: radiation therapy.