Emerging Treatment Paradigms in Radiation Oncology
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Abstract
Rapid advancements in radiotherapy and molecularly targeted therapies have resulted in the development of potential paradigm-shifting use of radiotherapy in the treatment of cancer. In this review, we discuss some of the most promising therapeutic approaches in the field of radiation oncology. These strategies include the use of highly targeted stereotactic radiotherapy and particle therapy as well as combining radiotherapy with agents that modulate the DNA damage response, augment the immune response, or protect normal tissues.

Introduction
The combination of radiotherapy and cytotoxic chemotherapy has become the standard of care for many locally advanced cancers, including those in the brain, head and neck, and lung, and in the gastrointestinal, gynecologic, and genitourinary tracts. Chemotherapy, when given concomitantly with radiotherapy, can enhance radiation efficacy, thus serving as a radiosensitizer in many cases. Clinical trials have confirmed that concomitant chemoradiation (CRT) is superior to radiotherapy alone in several solid tumors, including head and neck, cervical, esophageal, and lung cancers (1–7). However, the addition of chemotherapy to radiotherapy has also resulted in a higher rate of acute and late toxicity, thereby limiting the use of this combination (8). Clearly, there is room to improve the efficacy of radiotherapy. Because the therapeutic index of radiotherapy is favorable if the response of the tumor is greater than the toxicity of the surrounding normal tissues, different strategies can be used to maximize this therapeutic index. The most common approach is to deliver ablative radiotherapy with large fractions or to develop novel radiosensitizers by targeting the DNA damage response (DDR), cell-cycle checkpoints, signaling or metabolic pathways, the tumor microenvironment, and immune checkpoints. More recently, strategies have emerged to protect normal tissues by using particle therapies or through manipulation of the DDR, mucosal barriers, and adult stem cell regeneration. Because of space limitations, this review focuses on novel radiation deliveries, targeting the DDR and the immune checkpoints, and normal tissue protection or regeneration after radiotherapy damage.

Novel Radiation Delivery Approaches
Figure 1 shows the progress of radiation technologies over the past 65 years. Due to the invention of the linear accelerator, radiation treatment has evolved from a static treatment approach with fixed photon beams delivered in two-dimensional space (conventional 2D) to multiple beams with an added volumetric dimension (3D) to modulation of the beam intensity during beam delivery (IMRT, intensity-modulated radiation treatment) to the introduction of heavy particle beam therapy. In addition, two other paradigm-shifting radiation technologies are discussed in greater detail: the use of stereotactic body radiotherapy (SBRT) or stereotactic ablative radiotherapy (SABR) and the use of particle-beam therapy.

Radiotherapy has conventionally been reserved for patients with localized disease. The tumor and adjacent nodal regions are treated to the normal tissue tolerance of irradiated areas. Although high-dose, precision radiotherapy has long been used to treat brain tumors (stereotactic radiosurgery, SRS), advances in imaging and radiotherapy targeting have allowed similar radiotherapy techniques to treat extracranial tumors (9–13). This approach, referred to as SBRT or SABR, challenges the paradigm that only patients with localized disease will benefit from radiotherapy. Many authors have suggested that an important subset of patients with oligometastatic disease may benefit from SBRT/SABR (14–17). SBRT/SABR compresses an entire course of radiotherapy to a few fractions, allowing for greater flexibility to integrate radiotherapy with other treatment modalities. Some investigators have suggested that above a threshold radiotherapy dose, there may be enhanced endothelial cell apoptosis (18). However, this hypothesis has been challenged as tumor cell killing may be explained purely by the increased biologic effective doses (BED) of larger radiotherapy fractions (19). Nevertheless, numerous preclinical and clinical studies have demonstrated that the tumor control probability is enhanced with SBRT/SABR approaches. Two excellent reviews on this topic were recently published in the Journal of Clinical Oncology (20, 21).

Compared with conventional radiotherapy (photons), particle-beam therapy using protons or carbon ions offers an increased...
therapeutic potential because of the lack of exit dose and higher BED (carbon ions). Although prospective randomized data comparing particle beams with conventional radiotherapy do not exist, there is considerable promise for these technologies because of their increased BED while shedding lower collateral radiation dose to normal tissues, resulting in potentially lower normal tissue toxicity, especially in the case of protons for pediatric malignancies (22, 23). Combining particle-beam therapy with other cancer therapies may offer additional clinical benefit. Ultimately, prospective studies will need to be completed to demonstrate the clinical benefit of particle therapy, especially because of the significantly higher investment cost compared with conventional radiotherapy (24).

**Novel biologic approaches to improve the therapeutic index of radiotherapy**

Figure 2 provides a global view of the effect of radiation on the tumor cells, their microenvironment, and the surrounding normal tissues. Despite recent improvements in radiotherapy delivery techniques, radiotherapy affects not only tumor cells but also adjacent nontumor cells. To improve the therapeutic index, several strategies can be used, including...
Figure 2.
The effect of radiation on the tumor, its microenvironment and adjacent normal tissues. A, global view of radiation targeting a solid tumor, showing that it affects not only tumor cells but also neighboring nontumor cells and vascular structures. B, DNA-damaging effect of radiation on tumor cells, leading to DNA damage repair, cell-cycle arrest, transcriptional response, and eventual cell death. C, effect of radiation on the immune compartment, leading to upregulation of inhibitory immune checkpoints and recruitment of MDSCs, resulting in immune suppression and anergy. D, acute and late adverse radiation effects on surrounding normal tissues and strategies to counteract such an effect. BDMC, bone marrow-derived macrophage; EPC, endothelial precursor cell; MDSC, myeloid-derived suppressor cell; PHD, prolyl hydroxylase.
targeting the DDR to enhance tumor cell kill, activating the immune system during radiotherapy, protecting normal tissues from radiotherapy damage, or a combination of these approaches. The following sections discuss these strategies in detail.

**Targeting the DNA damage response**

The DDR represents a complex signaling network involving cell-cycle checkpoints, DNA repair, transcriptional programs, and apoptosis (25). When DNA damage occurs, checkpoint pathways are activated, which block S-phase entry (G1–S), delay S-phase progression, or prevent mitotic entry (G2–M; Fig. 3; ref. 26). This leads to phase-specific repair mechanisms. If repair fails, checkpoints are activated and unrepaired damage can trigger P53-dependent or -independent apoptosis (26, 27). Although much is known about DDR and DNA strand break repair, there has been limited success in translating this knowledge into clinical use. For example, the ATM gene, which is mutated in the ataxia telangiectasia syndrome, has been extensively characterized (28). Screening for ATM inhibitors has identified several small-molecule inhibitors, such as CP46672 and Ku55933 (29). Treatment of tumor and untransformed cells with ATM inhibitors results in significant radiosensitization. However, the clinical problem that arises is how to apply these drugs with radiotherapy without enhancing normal tissue toxicity. Systemic administration of ATM inhibitors is problematic because of the potential radiosensitization of normal tissues located within the radiation portals, especially when large fields are used for prophylactic nodal irradiation, as in head and neck or cervical cancers. The optimal use of these small-molecule inhibitors of ATM may be combined safely with SBRT/SABR, SRS, or particle therapy (30).

Another DDR target is CHK1, which is associated with cell-cycle checkpoints and is essential for genomic integrity. CHK1 functions in homologous recombination (HR) repair, stabilizing stalled replication forks, and inhibiting apoptosis (26, 31). Several CHK1 inhibitors have been identified, including UN001, MK8776, LY2603618, AZD7762, CBT501, PF00477736, SCH900776, and XL844 (26). Many CHK1 inhibitors exhibit radiosensitization in different preclinical cancer models (32, 33). Moreover, some enhance the effectiveness of chemoradiation, especially with antimetabolites, such as gemcitabine, cytarabine, and pemetrexed (34, 35). For example, the addition of MK8776 resulted in better sensitization of pancreatic tumors to radiation and gemcitabine combination than to radiation alone (36). By redistributing cells into S-phase, where HR is most active, antimetabolites synchronize cells and maximize the effects of CHK1 inhibitors on radiotherapy-induced DNA damage (31). CHK1 inhibitors are currently being evaluated in clinical trials for cancers of the breast, ovary, lung, head and neck, and anus (Supplementary Table S1). To date, there have been no trials combining CHK1 inhibitors with radiotherapy.

Another promising DDR target is the cell-cycle kinase WEE1, which is responsible for inhibiting phosphorylation of the tyrosine15 residue on CDK1/CDC2, thereby leading to G2 cell-cycle arrest in response to DNA damage (37). Furthermore, WEE1 regulates HR through modulation of CDK1 and the BRCA2–RAD51 interaction (38). Because radiotherapy-induced double-stranded breaks (DSB) use cell-cycle delay at the G2 checkpoint for repair through HR (39), inhibition of WEE1 impairs HR, thereby resulting in accumulation of irreparable lesions and eventual cell death through mitotic lethality (39). Preclinically, pharmacologic
inhibition or genetic downregulation of WEE1 results in formation of increased γH2AX foci (marker of DNA DSBs) in breast cancer and osteosarcoma cell lines when combined with radiotherapy (40, 41). MK-1775, a potent WEE1 inhibitor, radiosensitizes multiple human cancer cell lines, preferentially in P53-mutant lines, in clonogenic survival assays (42). The addition of MK-1775 to radiation resulted in significant tumor growth delay in multiple xenograft models (42–46). MK-1775 is being tested in clinical trials either as a single agent or in combination with chemotherapy, radiotherapy, or both in several solid tumors (Supplementary Table S1). Of particular interest are the trials combining MK-1775 with radiotherapy in patients with newly diagnosed glioblastoma, diffuse pontine glioma, and cervical cancer. These studies will help to determine the toxicity profile of combining this targeted drug with radiotherapy with or without chemotherapy and to establish a safe dose range for future trials.

The most logical schedule for sequencing CHK1 and WEE1 inhibitors is to deliver them before radiation to block early repair and for an extended period of time thereafter to inhibit late repair and the G2 checkpoint (47). However, this approach is difficult to translate into clinical practice. Preclinical studies suggest that delivery of these inhibitors after antimitabolite-based chemotherapy and just before radiation is most effective when given with CRT in preclinical models (31). An important aspect of combining WEE1 and CHK1 inhibitors with radiotherapy is their differential effect on normal and tumor cells based on the cell’s P53 status. P53 protein is often inactivated in tumor cells through either an inactivating mutation or other mechanism such as E6/E7 activation in cervical cancer and HPV+ oropharyngeal carcinoma. Cells without functional P53 protein lack an effective G1 checkpoint and are therefore highly dependent on the G2 checkpoint for DNA damage repair. These cells are therefore more sensitive to CHK1 and WEE1 inhibitors, and this concept of synthetic lethality has been confirmed in preclinical studies (42). In contrast, most normal cells possess functional P53 and would be less affected by CHK1 and WEE1 inhibition. Consistent with this hypothesis, CHK1 inhibition did not sensitize the small intestines to gemcitabine and radiation in a preclinical study (36).

Radiation and immunotherapy

An emerging paradigm in cancer is the use of radiotherapy to stimulate the immune system to attack metastatic disease (48). Within the past decade, a major advance in immunotherapy has been the identification of immune checkpoints, which act as rheostats for T-cell responses against cancer (49). These checkpoints comprise a series of costimulatory molecules such as CD28, which interacts with CD80 or CD86 on antigen-presenting cells to promote mitogenic and survival signals. Inhibitory receptors such as CTLA-4, PD-1, LAG-3, TIM-3, NKG2A, and KIRs downregulate T-cell function when an antigen is recognized by the T-cell receptor (TCR) (50). One common mechanism for tumors to induce tolerance is to express one or more of these inhibitory molecules. To date, the primary focus has been on targeting the CTLA-4 and PD-1 pathways with blocking antibodies; ipilimumab (targeting the CTLA-4) and pembrolizumab (targeting PD-1) have been approved by the FDA for the treatment of metastatic melanoma.

Although radiotherapy can promote the release of tumor antigens, which can stimulate the immune system (51), it can also induce the expression of certain chemokines, including CXCL9, CXCL10, and CXCL16, which promote the recruitment of T cells into the tumor microenvironment (52, 53). Moreover, radiotherapy can increase the expression of death receptors (54, 55), MHC class I proteins (56, 57), costimulatory molecules (58), and stress-induced ligands (59, 60) on tumor cells that enhance their recognition and killing by T lymphocytes. This response can lead to systemic induction of antitumor immunity, causing tumor shrinkage in distant sites from the irradiated areas, known as the abscopal effect.

In contrast with the above proimmunogenic effects, radiotherapy also has immunosuppressive effects. It can alter the inflammatory tumor microenvironment, leading to stimulation of inhibitory immune cells such as Tregs and myeloid-derived suppression cells that in turn suppress T-cell activation and encourage tumor growth. It can also activate latent TGFβ (61) and enhance the immunosuppressive effect of macrophages (62, 63). Experimentally, radiotherapy can enhance Galectin-1 expression, leading to systemic lymphopenia, which has been associated with worse prognosis (64). Finally, radiotherapy can upregulate PD-L1 in the tumor microenvironment, triggering a tumor escape mechanism from T cells (65).

These opposing effects of radiotherapy on the immune system have made it difficult to synergize the immunologic response of radiotherapy. However, with the recent development of immune checkpoint inhibitors, exploiting radiotherapy-induced abscopal effects has become a realistic goal. With the irradiated tumor functioning as an in situ vaccine, the combination of radiotherapy and anti–CTLA-4 or anti–PD-1 antibodies has resulted in successful T-cell–mediated immune response and inhibition of metastases outside of radiotherapy fields in several preclinical models, including breast cancer, colorectal cancer, lung cancer, and melanoma (65–68). Figure 4 shows how radiotherapy can be used with either anti–PD-1 or anti–PD-L1 antibody to enhance radiotherapy effectiveness. These preclinical observations were further corroborated by sporadic clinical case reports describing remarkable abscopal effects with ipilimumab and SBRT/SABR in patients with metastatic melanoma (69–71). Similarly provocative observations have been made with SBRT/SABR and IL2 in patients with metastatic melanoma and renal cell cancer (72).

These anecdotal observations have led to a number of clinical trials to study this effect in a prospective manner and to develop a thorough comprehension of the underlying mechanisms behind the abscopal effect (51). More recently, a prospective clinical study revealed that melanoma patients with high PD-L1 tumor expression did not respond to radiation and anti–CTLA-4 therapy. As predicted by preclinical models, these patients showed T-cell exhaustion and progressed rapidly (73). The model suggested that for tumors with high PD-L1 expression, the combination of radiotherapy, CTLA-4, and PD-L1 blockade may be most effective because anti–CTLA-4 inhibits Treg, leading to a higher CD8/Treg ratio, whereas radiotherapy diversifies the TCR repertoire and PD-L1 blockade reinvigorates exhausted T cells.

Moving forward, the lack of complete understanding of this immune mechanism, the paucity of biomarkers except for myeloid-derived suppressor cells (MDSC), and the uncertain optimal radiotherapy dosing schedule suggest opportunities for additional preclinical testing in animal models. Our goals should be to identify tumors that will respond to checkpoint inhibition alone versus those that respond to combination therapy, to develop
ways to optimize antigen stimulation through radiotherapy exposure, and to investigate in more depth any other factors that are antagonizing the immune response.

Normal tissue protection or regeneration after radiation damage

The concept of therapeutic index takes into account radiotherapy response of both tumor and normal tissues. Even with significant technical advances in radiotherapy delivery, normal tissue toxicity still poses a significant clinical problem. Figure 2D shows the damaging effects of radiotherapy on normal tissues and potential strategies to counteract these effects. One strategy to improve the therapeutic index is to protect normal tissue from radiotherapy damage. Theoretically, radioprotection is easier to achieve than radiosensitization because only a fraction of normal cells needs to be protected to regenerate or maintain tissue function. Radioprotectors may be categorized into broad groups: free radical scavengers (e.g., amifostine; refs. 74, 75), inhibitors of DNA damage–induced signaling (e.g., the p53 tumor suppressor gene; refs. 76, 77), Toll-like receptor agonists (78); and ceramide pathway inhibitors (79). The only approved radioprotector is amifostine, which has not been widely adopted because of its adverse effects and marginal normal tissue protection (75).

Another therapeutic strategy is to target pathways involved in the underlying physiologic changes induced by radiation. For example, studies have shown that activating pathways to promote epithelial integrity in preclinical models can increase the survival of animals after lethal abdominal radiotherapy (80). These studies showed that activation of the HIF-2 transcription factor in the gastrointestinal tract either genetically or pharmaceutically (through inhibition of prolyl hydroxylases, a family of enzymes that hydroxylate HIF proteins and target them for degradation under aerobic conditions) could provide long-term protection against exposure to lethal irradiation. Moreover, this approach did not affect tumor radiosensitivity. Currently, several small molecules that inhibit prolyl hydroxylases are in clinical trials for the treatment of anemia caused by renal insufficiency (81, 82). These molecules appear to be safe and potent HIF inducers and represent a potential therapeutic path to testing this concept in humans.

Another innovative strategy to overcome normal tissue toxicities is the prospect of transplanting stem cells or activating stem cells in situ within the irradiated organ to restore function after radiotherapy, a topic that has recently been reviewed extensively (82). An intriguing preclinical model is the transplantation of healthy unirradiated hepatocytes as a means of recovery from radiotherapy-induced liver disease (RILD). In a rodent model of RILD, intrasplenic or intraportal infusion of adult primary hepatocytes reversed RILD and improved survival of rats treated with high-dose liver radiotherapy after partial heptectomy (83). Interestingly, radiotherapy to a partial liver volume enhances the rate of successful engraftment of transplanted hepatocytes (84). Similarly, preclinical studies have shown that salivary stem cells exist in adult murine salivary glands, and these cells can be used to restore function when transplanted into irradiated recipient glands (85, 86). Systematic analyses of these cells have identified several pathways, including the GDNF and ALDH3 pathways, that when activated, can enhance stem cell survival after radiation and minimize loss of function in irradiated glands (86–88). Intriguingly, some pathways, such as GNDF and ROBO1/SLIT2, are involved in kidney and neuronal development and have been shown to protect the gastrointestinal tract from radiation damage (89).

Finally, a better understanding of the normal stem cell niche and their physical location can be used to sculpt the radiation dose to protect normal stem cells from radiotherapy damage and subsequently allow them to restore function. For example, saliva stem cells are located along the striated and intercalated ducts of the major saliva glands (90, 91). Preclinical studies have shown that saliva gland damage is more accentuated when the cranial area containing the excretory ducts and the neurovascular structures are irradiated (91). Histologic studies suggested collateral damage in the unirradiated parts of the parotid gland when the ducts and neurovascular bundle were included.
in the field. Because saliva stem cells are located around the striated ducts, they may represent critical sparing structures during radiotherapy (90).

A similar stem cell niche sparing approach is likewise being explored in cranial irradiation. The hippocampal precursor cells that generate new neurons represent a "neurogenic reserve" that retains plasticity in hippocampal learning (92). Emerging evidence suggests that the pathogenesis of radiotherapy-induced neurocognitive deficit may involve radiotherapy injury to these proliferating neuronal progenitor cells in the subgranular zone of the hippocampi (93, 94). In addition, relatively small radiotherapy doses can cause apoptosis in the subgranular zone of young rats and mice (94, 95), resulting in a sharp, prolonged decline in neurogenesis in the subgranular zone (95–99). Clinical studies suggest that radiotherapy-induced damage to the hippocampus plays a considerable role in the cognitive decline in patients (96, 100). In a prospective study of benign or low-grade adult brain tumors treated with fractionated stereotactic radiotherapy, biologically equivalent doses in 2-Gy fractions exceeding 7.3 Gy to 40% of the total hippocampal volume was associated with long-term impairment in list-learning recall (101). On the basis of these data, the Radiation Therapy Oncology Group (RTOG) launched a phase II trial of hippocampal avoidance during whole-brain therapy for brain metastases (RTOG 0933). Of the 113 enrolled patients, 100 patients were included in the analysis. Compared with historical controls, conformal avoidance of the hippocampus during whole-brain radiation was associated with significant memory preservation up to 6-month follow-up. In addition, this finding was associated with an excellent preservation of quality of life in these patients (102). On the basis of these results, the RTOG has launched a phase III trial to validate these findings and to evaluate the combined neuroprotective effect of hippocampal avoidance in addition to prophylactic memantine during whole-brain radiotherapy for brain metastases (NRG C001). The researchers will also test the neuroprotective effect of hippocampal avoidance during prophylactic cranial irradiation for small-cell lung cancer in a phase II–III trial (NRG CC1432).

Conclusions

The application of novel radiation-modifying strategies and highly targeted radiotherapy will allow for increasing indications for radiotherapy, especially in the management of patients with large tumor volumes or multiple metastatic sites with less associated acute and late toxicity. The strategies described in this review hold great promise to increase the long-term survival and quality of life for cancer patients with solid tumors.

Disclosure of Potential Conflicts of Interest

A.J. Giaccia has ownership interest (including patents) in and is a consultant/advisory board member for Ruga Corporation. No potential conflicts of interest were disclosed by the other authors.

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