

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 will 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 radiation (RT) and cytotoxic chemotherapy has become the standard of care for many locally advanced cancers including those in the brain, head and neck (HN), lung, gastrointestinal (GI), gynecologic (GYN) and genitourinary (GU) tracts. Chemotherapy, when given concomitantly with RT, can enhance radiation efficacy, thus serving as radiosensitizers in many cases.

Clinical trials have confirmed that concomitant chemoradiation (CRT) is superior to RT alone in several solid tumors including HN, cervical, esophageal and lung cancers(1-7). However, the addition of chemotherapy to RT 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.

Since the therapeutic index of RT is favorable if the response of the tumor is greater than the toxicity of the surrounding normal tissues, there are two different strategies to maximize this therapeutic index. The most common approach is to deliver ablative RT 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 are emerging to protect normal tissues by utilizing particle therapies or through manipulation of the DDR, mucosal barriers and adult stem cell regeneration. Due to space limitations, this review will focus on novel radiation deliveries, targeting the DDR and the immune checkpoints, and normal tissue protection or regeneration after RT damage.

Novel Radiation Delivery Approaches

Fig. 1 shows the progress of radiation (RT) technologies over the last 65 years. Since 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) to the introduction of heavy particle beam therapy. In addition, there are two additional paradigm shifting radiation technologies to discuss in greater detail: the use of stereotactic body radiotherapy (SBRT) or stereotactic ablative radiotherapy (SABR) and the use of particle beam therapy.

RT 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 RT has long been used to treat brain tumors (stereotactic radiosurgery, SRS), advances in imaging and RT targeting have allowed similar RT techniques to treat extracranial tumors(9-14). This approach, referred to SBRT or SABR, challenges the paradigm that only patients with localized disease will benefit from RT. Many have suggested that an important subset of patients with oligometastatic disease may benefit from SBRT/SABR(15-18). SBRT/SABR compresses an entire course of RT to a few fractions, allowing for greater flexibility to integrate RT with other treatment modalities. Some investigators have suggested that above a threshold RT dose, there may be enhanced endothelial cell apoptosis(19). However, this hypothesis has been challenged as tumor cell killing may be explained purely by the increased biological effective doses (BED) of larger RT fraction(20).

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*(21, 22).

Compared with conventional RT (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 RT do not exist, there is considerable promise for these technologies because of its 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(23, 24). 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 to conventional RT(25).

Novel Biological Approaches to Improve the Therapeutic Index of Radiotherapy

Fig. 2 provides a global view of the effect of radiation on the tumor cells, its microenvironment and the surrounding normal tissues. Despite recent improvements in RT delivery techniques, RT impacts not only tumor cells but also adjacent non-tumor cells. To improve the therapeutic index, several strategies can be used, including targeting the DDR to enhance tumor cell kill,

activating the immune system during RT, protecting normal tissues from RT damage, or a combination of these approaches. The following sections will discuss these strategies in details.

Targeting the DNA Damage Response (DDR)

The DDR represents a complex signaling network involving cell cycle checkpoints, DNA repair, transcriptional programs, and apoptosis(26). 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)(27) (Fig. 3). This leads to phase-specific repair mechanisms. If repair fails, checkpoints are activated and unrepaired damage can trigger P53-dependent or -independent apoptosis(27, 28). 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(29). Screening for ATM inhibitors has identified several small molecule inhibitors such as CP46672 and Ku55933(30). Treatment of tumor and untransformed cells with ATM inhibitors results in significant radiosensitization. However, the clinical problem arises of 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 such as in HN or cervical cancers. The optimal use of these inhibitors may be in conjunction with brachytherapy, where most of the dose is localized to the tumor.

Alternatively, these small molecule inhibitors of ATM may be combined safely with SBRT/SABR, SRS, or particle therapy(31).

Another DDR target is CHK1, which is associated with cell cycle checkpoints and essential for genomic integrity. CHK1 functions in homologous recombination (HR) repair, stabilizing stalled replication forks and inhibiting apoptosis(27, 32). Several CHK1 inhibitors have been identified including UN001, MK8776, LY2603618, AZD7762, CBT501, PF00477736, SCH900776 and XL844 (27). Many CHK1 inhibitors exhibit radiosensitization in different preclinical cancer models(33, 34). Moreover, some enhance the effectiveness of chemoradiation, especially with antimetabolites such as gemcitabine, cytarabine, pemetrexed(35, 36). For example, the addition of MK8776 resulted in better sensitization of pancreatic tumors to radiation and gemcitabine combination than to radiation alone(37). By redistributing cells into S-phase, where HR is most active, antimetabolites synchronize cells and maximize the effects of CHK1 inhibitors on RT-induced DNA damage(32). CHK1 inhibitors are currently being evaluated in clinical trials for cancers of the breast, ovary, lung, HN 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(38). Furthermore, WEE1 regulates homologous recombination (HR) through modulation of CDK1 and the BRCA2–RAD51 interaction(39). Because

RT-induced double stranded breaks (DSBs) utilize cell cycle delay at the G2 checkpoint for repair through HR (40), inhibition of WEE1 impairs HR, thereby resulting in accumulation of irreparable lesions and eventual cell death through mitotic lethality(40). Preclinically, pharmacologic inhibition or genetic down-regulation of WEE1 results in formation of increased γ H2AX foci (marker of DNA DSBs) in breast cancer and osteosarcoma cell lines when combined with RT(41, 42). MK-1775, a potent WEE1 inhibitor, radiosensitizes multiple human cancer cell lines, preferentially in *P53* mutant lines, in clonogenic survival assays(43). The addition of MK-1775 to radiation resulted in significant tumor growth delay in multiple xenograft models(43-47). 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 GBM, diffuse pontine glioma and cervical cancer. These studies will help to determine the toxicity profile of combining this targeted drug with RT +/- 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 prior to radiation to block early repair and for an extended period time thereafter to inhibit late repair and the G2 checkpoint(48). However, this approach is difficult to translate into clinical practice. Preclinical studies suggest that delivery of these inhibitors after antimetabolite-based chemotherapy and just before radiation is most effective when given with CRT in preclinical models(32). An important aspect of combining WEE1 and CHK1 inhibitors with RT 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 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 (43). 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(37).

Radiation and Immunotherapy

An emerging paradigm in cancer is the use of RT to stimulate the immune system to attack metastatic disease(49). Within the last decade, a major advance in immunotherapy has been the identification of immune checkpoints, which act as rheostats for T cell responses against cancer(50). 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 down regulate T cell function when an antigen is recognized by the T cell receptor(51). One common mechanism for tumors to induce tolerance is to express one or more of these inhibitory molecules. To date, 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.

While RT can promote the release of tumor antigens, which can stimulate the immune system(52), 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(53, 54). Moreover, RT can increase the expression of death receptors(55, 56), MHC class I proteins(57, 58), costimulatory molecules(59) and stress induced ligands(60, 61) 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 to the above pro-immunogenic effects, RT 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 transforming growth factor- β (62) and enhances the immunosuppressive effect of macrophages(63, 64). Experimentally, RT can enhance Galectin-1 expression, leading to systemic lymphopenia, which has been associated with worse prognosis(65). Finally, RT can upregulate PD-L1 in the tumor microenvironment, triggering a tumor escape mechanism from T cells(66).

These opposing effects of RT on the immune system have made it difficult to synergize the immunologic response of RT. However, with the recent

development of immune checkpoint inhibitors, exploiting RT induced abscopal effects has become a realistic goal. With the irradiated tumor functioning as an in-situ vaccine, the combination of RT and anti CTLA-4 or anti PD-1 antibodies have resulted in successful T cell mediated immune response and inhibition of metastases outside of RT fields in several preclinical models, including breast, colorectal, lung cancer and melanoma(66-69). Fig. 4 shows how RT can be used with either anti-PD-1 or anti-PD-L1 antibody to enhance RT 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(70-72). Similarly provocative observations have been made with SBRT/SABR and interleukin-2 in patients with metastatic melanoma and renal cell cancer(73).

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(52). 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(74). The model suggested that for tumors with high PD-L1 expression, the combination of RT, CTLA-4 and PD-L1 blockade may be most effective because anti-CTLA-4 inhibits Treg, leading to a higher CD8/Treg ratio, while RT 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, and uncertain optimal RT 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 RT exposure, and to investigate in more depth what other factors are antagonizing the immune response.

Normal Tissue Protection or Regeneration after Radiation Damage

The concept of therapeutic index takes into account RT response of both tumor and normal tissues. Even with significant technical advances in RT delivery, normal tissue toxicity still poses a significant clinical problem. Fig. 2D shows the damaging effects of RT on normal tissues and potential strategies to counteract these effects. One strategy to improve the therapeutic index is to protect normal tissue from RT 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)(75, 76), inhibitors of DNA damage–induced signaling (e.g., the p53 tumor suppressor gene)(77, 78), toll-like receptor agonists(79) and ceramide pathway inhibitors(80). The only approved radioprotector is amifostine, which has not been widely adopted due to its adverse effects and marginal normal tissue protection(76).

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 RT(81). These studies showed that activation of the HIF-2 transcription factor in the GI tract either genetically or pharmacologically (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(82, 83). 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 activate stem cells *in-situ* within the irradiated organ to restore function after radiotherapy, a topic that has recently been reviewed extensively(83). An intriguing preclinical model is the transplantation of healthy unirradiated hepatocytes as a means of recovery from RT-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 hepatectomy(84). Interestingly, RT to a partial liver volume enhances the rate of successful engraftment of transplanted hepatocytes(85). 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(86, 87). 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(87-89). Intriguingly, some pathways, such as GDNF and ROBO1/SLIT2 are involved in kidney and neuronal development and have been shown to protect the GI tract from radiation damage(90).

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 RT 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(91, 92). Preclinical studies have shown that saliva gland damage is more accentuated when the cranial area containing the excretory ducts and the neurovascular structures were irradiated(92). Histologic studies suggested collateral damage in the un-irradiated parts of the parotid gland when the ducts and neurovascular bundle were included in the field. Since saliva stem cells are located around the striated ducts, they may represent critical sparing structures during RT(91).

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(93). Emerging evidence suggests that the pathogenesis of RT-induced neurocognitive deficit may involve RT injury to these proliferating neuronal

progenitor cells in the subgranular zone of the hippocampi(94, 95). In addition, relatively small RT doses can cause apoptosis in the subgranular zone of young rats and mice(95, 96), resulting in a sharp, prolonged decline in neurogenesis in the subgranular zone(96-100). Clinical studies suggest that RT-induced damage to the hippocampus plays a considerable role in the cognitive decline in patients(97, 101). 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(102). Based on 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 to historical control, conformal avoidance of the hippocampus during whole brain radiation was associated with significant memory preservation up to 6-month follow-up. In addition, this was associated with an excellent preservation of quality of life in these patients(103). Based on 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 CC001). They 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.

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Figure 1. Summary of the progress of radiation technologies over the last 65 years. A, The top row, from left to right, shows the following:

- Picture of the first linear accelerator that was employed for clinical use in the Western hemisphere, treating a 7-month old boy suffering from retinoblastoma with subsequent tumor control (Stanford 1955).
- Fluoroscopic x-ray simulation of a lung cancer for conventional 2-dimensional (2D) radiotherapy
- Picture of a modern linear accelerator with a 360 degree rotating gantry to treat deep-seated tumors.
- Dose distribution of a 3-dimensional (3D) radiation treatment plan superimposed on an axial computed tomography (CT) image of a thoracic tumor.
- Depiction of intensity modulated radiation treatment (IMRT) of a thoracic tumor using inhomogenous beam intensity from multiple directions.

The bottom row, from left to right, shows the following:

- Dose distribution of an IMRT plan superimposed on an axial CT image of a thoracic tumor, showing much lower dose to the adjacent spinal cord.
- Depiction of stereotactic body or ablative radiation treatment (SBRT/SABR) of hepatic tumors using non-coplanar multiple narrow beams from multiple directions.
- Dose distribution of the SBRT/SABR plans for two hepatic tumors superimposed on a coronal CT image.

- Profile of a particle beam covering the cancer at depth without exit dose behind the tumor.
- Dose distribution of particle beam (proton) therapy covering the entire cranio-spinal axis in a child with medulloblastoma, showing no exit dose to the lung or abdomen.

B, The graph reflects the progress of radiation delivery over time, starting with conventional 2-dimensional (2D) radiotherapy in ~1950's to the most recent introduction of particle beam therapy in ~2010.

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 non-tumor 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. The effect of radiation on the immune compartment, leading to upregulation of inhibitory immune checkpoints and recruitment of myeloid derived suppressor cells (MDSC), resulting in immune suppression and anergy
- D. Acute and late adverse radiation effects on surrounding normal tissues and strategies to counteract such an effect. PHD stands for prolyl hydroxylase

BDMC, Bone marrow derived macrophage; EPC, Endothelial precursor cell;
TCR, T-cell receptor.

Figure 3. Schematics of DNA damage response, leading to G1 arrest, S-phase delay and G2 arrest, providing the rationale for targeting CHK1, CHK2 and WEE1.

Figure 4. An example of how an immune check point inhibitor such as anti-PD-1 or anti-PD-L1 antibody can be used together with radiotherapy to enhance the effect of radiation locally and distantly.

MDSC, myeloid derived suppressor cells; TCR, T-cell receptor.

Figure 1:

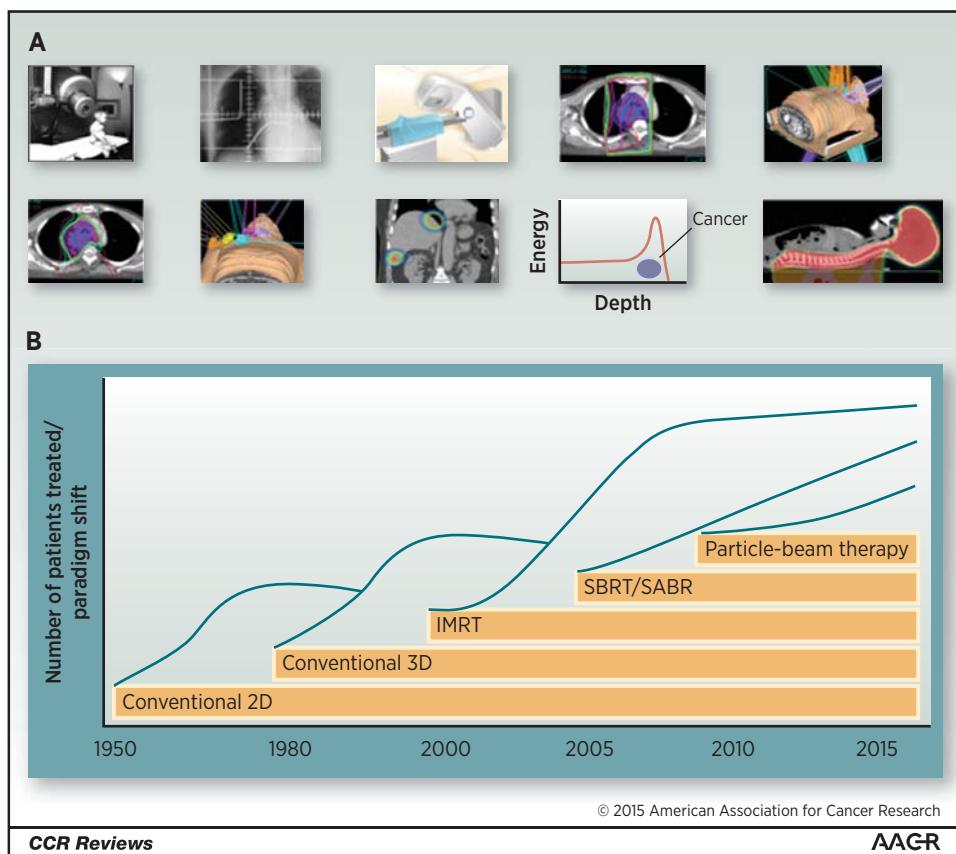


Figure 2:

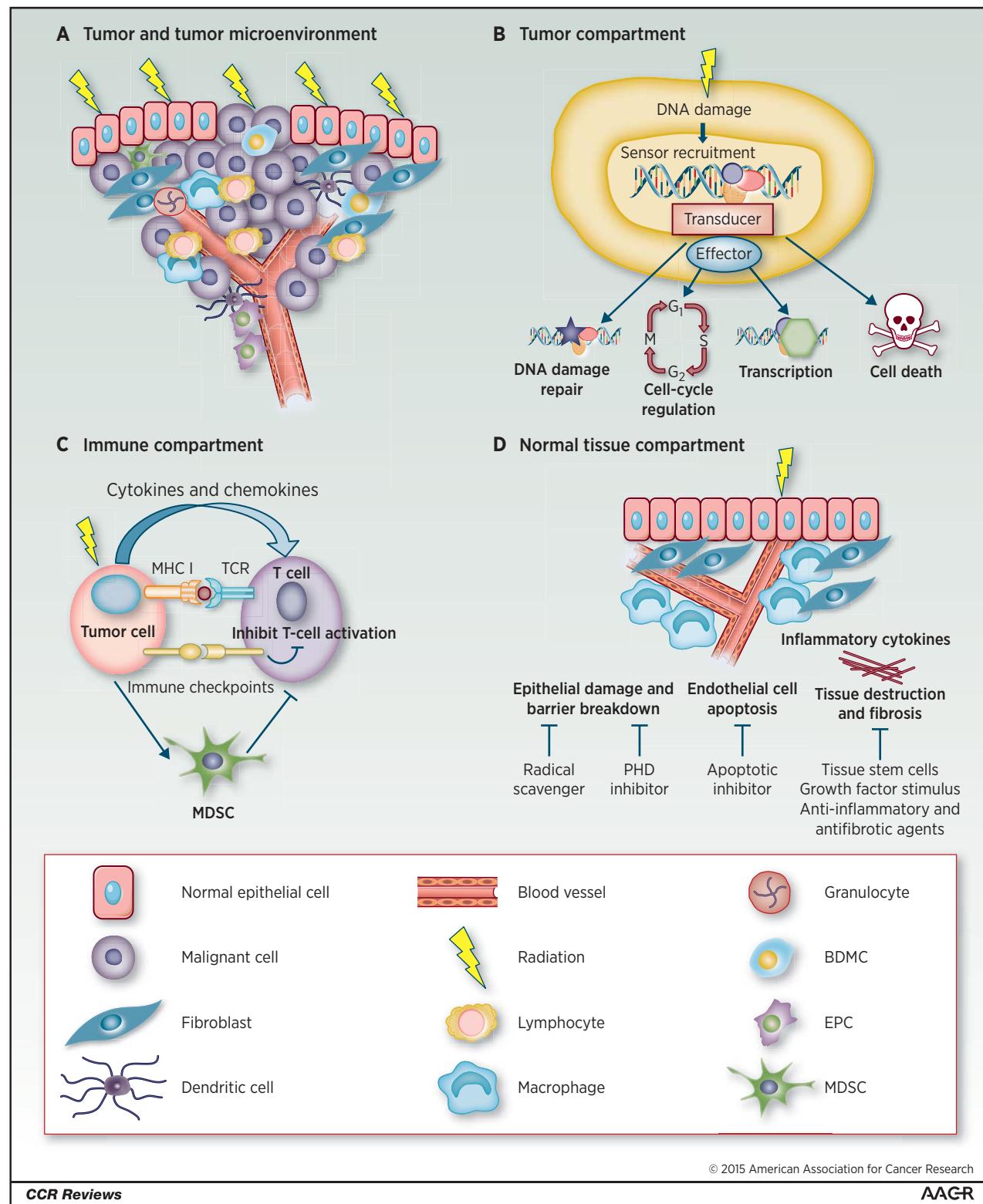


Figure 3:

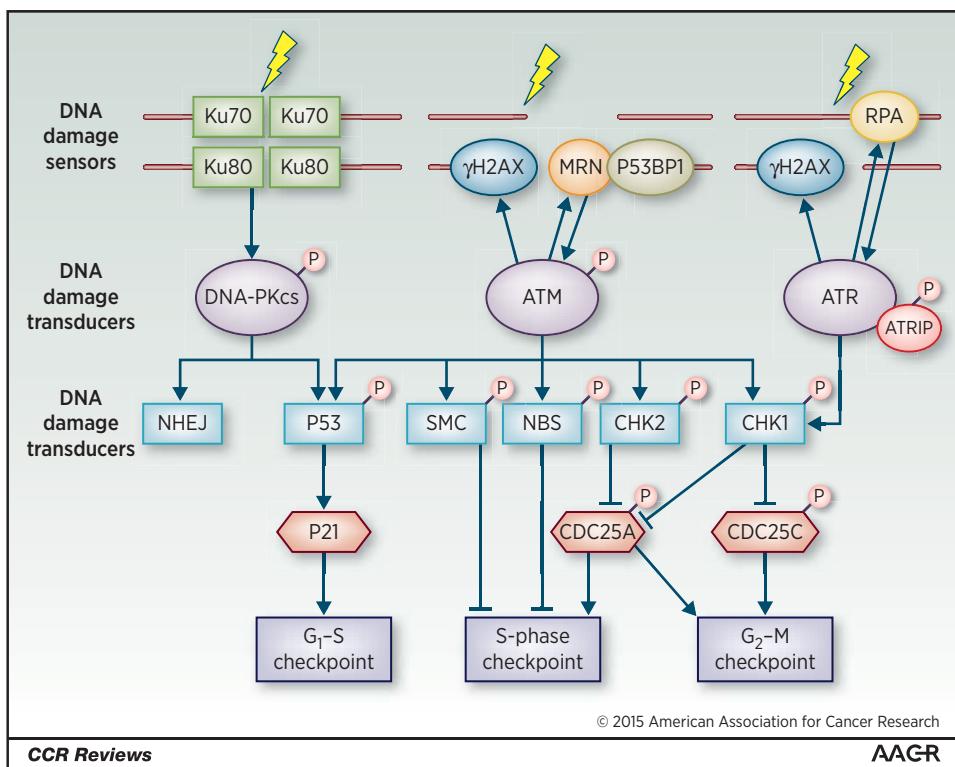
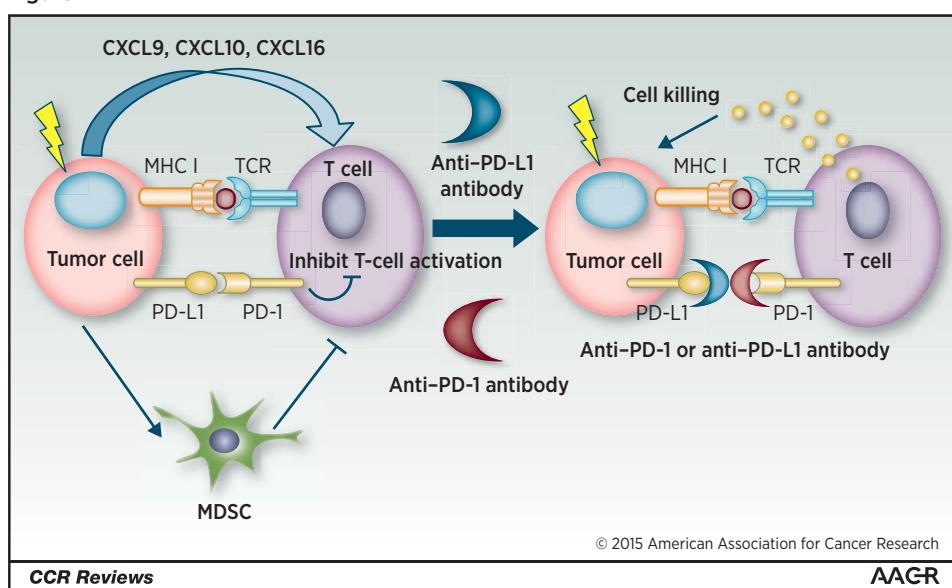


Figure 4:



Clinical Cancer Research

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