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Synergizing Radiation Therapy and Immunotherapy for Curing Incurable Cancers: Opportunities and Challenges

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Abstract

The combination of radiation therapy and immunotherapy holds particular promise as a strategy for cancer therapeutics. There is evidence that immunotherapy is most beneficial alone when employed early in the disease process or in combination with standard therapies (e.g., radiation) later in the disease process. Indeed, radiation may act synergistically with immunotherapy to enhance immune responses, inhibit immunosuppression, and/or alter the phenotype of tumor cells, thus rendering them more susceptible to immune-mediated killing. Furthermore, as monotherapies, both immunotherapy and radiation may be insufficient to eliminate tumor masses. However, following immunization with a cancer vaccine, the destruction of even a small percentage of tumor cells by radiation could result in cross-priming and presentation of tumor antigens to the immune system, thereby potentiating antitumor responses. Learning how to exploit radiation-induced changes to tumor-cell antigens, and how to induce effective immune responses to these cumulatively immunogenic stimuli, is an exciting frontier in cancer therapy research. This review examines a) mechanisms by which many forms of radiation therapy can induce or augment antitumor immune responses and b) preclinical systems that demonstrate that immunotherapy can be effectively combined with radiation therapy. Finally, we review current clinical trials where standard-of-care radiation therapy is being combined with immunotherapy.

Introduction

Radiation is often considered immunosuppressive, an activity that is most likely a result of the complex interplay of hormesis and the abscopal effect. The abscopal effect, also called the “distant bystander” effect, is a paradoxical effect of radiation on cellular systems whereby local radiation may have an antitumor effect on tumors distant from the site of radiation [1]. Indeed radiation’s ability to enhance distinct immune responses by inducing a “danger” signal that excites and activates the immune system has recently come under investigation. In the context of tumors, radiation has been hypothesized to cause tumor

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disruption, and cause a type of danger signal that could be successfully exploited to improve the effectiveness of immunotherapy [2].

Radiation therapy is conventionally used for local tumor control. Although local control of the primary tumor can usually prevent development of subsequent systemic metastases, tumor radiation fails to control pre-existing systemic disease, which may be present only as micrometastatic (and therefore undetectable) deposits. Combining radiation therapy with immunotherapy allows one to exploit 2 broad areas: a) radiation-induced tumor-cell death as a potential source of tumor antigens for immunotherapy, and b) post-radiation tumor-cell modulation that allows more efficient immune-cell access and increased sensitivity to T-cell killing. These tumor-specific T cells could arise endogenously or be induced from active vaccination strategies. Many clinical trials exploring the use of radiation and vaccines in the treatment of cancer are currently underway. As knowledge of the synergistic effects of radiation and immunotherapy increases, the translational use of this strategy for a variety of carcinomas will become more feasible.

Foundation: Combining Radiation Therapy and Immunotherapy

Local irradiation of tumor is the standard of care for many cancer types. Traditionally, it is employed to destroy tumor cells or to alter tumor/stroma architecture with either curative or palliative intent. However, it is often the case that not all tumor cells in a given mass receive a lethal dose of radiation due to dose constraints mandated by the need to limit damage to normal tissue. Nevertheless, even sublethal doses of radiation can generate potent immmune responses by altering tumor cells in a variety of ways.

Antigen release from dying tumor cells can activate immune responses

On their own, tumor cells do not generate potent antitumor immune responses due to their inefficient expression of molecules important for antigen processing and presentation. Tumor cells frequently do not express the antigen transporter gene product TAP-2 and class I MHC molecules [3], and they lack T-cell costimulatory molecules such as B7-1 (CD80). Irradiation can induce recognition and phagocytosis signals for dendritic cells (DCs), such as membrane-bound calreticulin, as well as release “danger” signals for DC activation [2], such as various heat shock proteins (HSP) and high-mobility group protein B1 (HMGB1). Antigens released by dying tumor cells can activate the immune system to induce immunogenic cancer cell death, thus contributing to the eradication of residual tumor cells (Figure 1) [1,4,5]. In order to induce this immune response, dying tumor cells need to provide 2 signals for DCs. First, a specific phagocytosis/recognition signal is presented by the translocation of cytoplasmic calreticulin to the cell membrane, which allows DCs to engulf dying tumor cells [6]. Second, a specific “danger” signal is released by the dying cell that activates DCs and stimulates antigen processing and presentation to T cells. It was recently demonstrated that irradiated, dying tumor cells release the nuclear nonhistone protein HMGB1, which binds to Toll receptor 4 (TLR4), thereby providing a “danger” signal to DCs for TLR4-dependent antigen processing (Figure 3) [7]. In addition, several groups have demonstrated that one class of endogenous “danger” signals is provided by stress proteins, or HSPs, which are released from dying tumor cells and actively taken up by DCs for cross-presentation via HSP receptors (CD91 for gp96, calreticulin, HSP70, and HSP90; CD14 for HSP70) [8–11]. In other experiments, Sozzani *et al.* [12] demonstrated that hepatocellular carcinoma cells switched chemokine receptor expression from CCR1 to CCR7 following irradiation. This chemokine switching acted as a homing receptor for activated DCs and facilitated the DC migration to draining lymph nodes. The immunologic consequences of radiation therapy-induced tumor-cell death are thus 2-fold: providing a source of tumor antigens for presentation by circulating DCs and providing “danger” signals for DC activation (Figure 1). Radiation-treated tumor cells would thus serve as an *in situ*

autologous tumor vaccine [13], inducing a strong tumor-specific immune response that could eradicate residual tumor cells in primary tumors and distant micrometastases (Figure 1).

Irradiation modulates tumor-cell phenotype and increases immune recognition

Neoplastic cells may evade the adaptive immune system by altering expression of specific molecules such as tumor-associated antigens (TAAs) or MHC molecules. Studies investigating the mechanism by which local tumor irradiation enhances therapeutic response to immunotherapy have established that nonlethal doses of radiation may alter the phenotype of target tissue by upregulating some gene products and making tumor cells more susceptible to T cell-mediated immune attack (Figure 2 and Figure 3). MHC class I is responsible for direct presentation of tumor antigen peptides to cytotoxic T lymphocytes (CTLs) via peptide-MHC complexes. ICAM-1 and other cell adhesion molecules enhance T cells' ability to kill target cells by improving cell-to-cell adhesion [14,15]. Studies have demonstrated that nonlethal doses of radiation induce a 2-phase, dose-dependent increase in MHC class I presentation in human tumor cells [16]. MHC class I molecules present endogenous peptides to CTLs. Many of these peptides are generated by the proteasome from newly synthesized but rapidly degraded proteins (RDPs). Within 4 hours after exposure, the protein degradation triggered by radiation damage leads to an increased peptide pool (Figure 2 and Figure 3). During the latter phase of ionizing radiation (> 4 hours after exposure) the mTOR (molecular target of rapamycin) pathway is activated, resulting in translation of proteins and increased generation of peptides from the RDPs of these new proteins. At each of these stages, unique proteins are expressed and upregulated in response to ionizing radiation, resulting in novel peptide presentation (Figure 2) [16]. These novel peptides could cause activation of resting T cells specific for these epitopes, leading to an antitumor immune response.

Radiation has also been shown to alter the cell-surface expression of a variety of immunomodulatory molecules. Garnett *et al.* [17] examined 23 human carcinoma cell lines (12 colon, 7 lung, and 4 prostate) for their response to nonlytic doses of radiation (10 or 20 Gy). They examined changes in surface expression of Fas and other molecules involved in T cell-mediated immune attack, such as the adhesion molecule ICAM-1, TAAs such as carcinoembryonic antigen (CEA) and mucin-1 (MUC-1), and MHC class I. Radiation upregulated at least one of these surface molecules in 21 of 23 (91%) cell lines studied. Furthermore, all 5 irradiated $CEA^+/A2^+$ colon tumor-cell lines demonstrated significantly enhanced killing by CEA-specific HLA-A2-restricted $CD8^+$ CTLs compared to nonirradiated cell lines. Microarray analysis of gene expression changes revealed that many additional genes had been modulated by irradiation. These upregulated gene products may further enhance the tumor cells' susceptibility to T cell-mediated immune attack or serve as additional targets for immunotherapy. Taken together, these results suggest that nonlethal doses of radiation render human tumor cells more amenable to immune system recognition and attack.

Radiation effects on the immune system

Radiotherapy that involves high doses to multiple lymph node chains can decrease nonspecific immune system responses and these responses may remain suppressed for several months following radiation [18]. In addition, there may be decreases in T-cell subsets following radiation therapy, largely in the naive T-cell populations [19]. However, for radiation that does not involve high doses to multiple lymph node chains within the standard treatment ports, the potential benefits of combining radiation with immunotherapy remain very appealing. In examining the potential mechanisms of the combination of radiation therapy with immunotherapy, it has recently been reported in murine systems that

lymphodepletion from 5 Gy total-body irradiation followed by adoptive transfer of tumor-specific T cells resulted in significantly improved antitumor activity [20]. There, Gattinoni *et al.* found that the irradiation removed endogenous cell populations that acted as sinks for the needed IL-7 and IL-15 cytokine supporting the tumor reactive T cells. Another factor being examined is the removal of regulatory T cells (Tregs) by irradiation [4,21] and the role of Toll-like receptor-4 from radiation-injured gut on the activation of immune cells [22,23]. These mechanisms are also being seen in patients. Improved understanding of the intertwined mechanisms involved in the augmentation of antitumor immunity by the addition of immunotherapy and radiation can further optimize this combination.

Reduction To Practice: Preclinical Studies

External beam radiation and cancer immunotherapy

In vivo preclinical studies have demonstrated that the combination of tumor-focused external beam radiation and immunotherapy can facilitate antitumor immunity better than either modality alone. Younes *et al.* [24] studied endogenous T-cell responses to renal carcinoma with bilateral pulmonary metastases in a murine model and observed that local irradiation to the left lung in combination with systemic IL-2 therapy led to greater tumor reduction in both lungs than was achieved by either modality alone. For active specific immunotherapy, therapeutic cancer vaccines are being actively investigated. TAAs such as CEA and MUC-1, which are overexpressed on a wide variety of tumor cells *in vivo* [25], are being studied as targets for vaccine-mediated immunotherapies [26–29]. Chakraborty *et al.* [30] have focused on the combination of low-dose radiation (8 Gy) delivered directly to the tumor, and active therapeutic vaccination for the treatment of subcutaneous murine tumors. There, the vaccine was composed of poxviral vectors that express CEA and 3 T-cell costimulatory molecules: B7-1, ICAM-1, and LFA-3 (CEA/TRICOM). Although either modality alone was ineffective, the combination of radiation and vaccine was not only curative in 50% of mice bearing CEA-expressing tumors, but also imparted protection from subsequent tumor challenge. Interestingly, mice cured of tumors demonstrated “antigen cascade,” that is, they developed $CD4^+$ and $CD8^+$ T-cell responses not only for CEA, but also for other tumor antigens not encoded in the vaccine, such as p53 and gp70. The immune response to gp70 was much greater than that seen to the vaccine-encoded CEA, suggesting that the immune response to the cascade antigen may play an important role in antitumor activity. This antigen cascade phenomenon has also been observed in patients enrolled in clinical trials (see below).

Bone-seeking radionuclide and cancer immunotherapy

In advanced stages, many primary human carcinomas such as thyroid, breast, kidney, prostate, and multiple myeloma typically involve painful bone metastases that require palliative therapy. Strontium-89 (^{89}Sr) and samarium-153 (^{153}Sm lexidronam) are bone-seeking radiopharmaceuticals used to relieve the pain of metastasis to bone. The calculated dose of palliative radiation delivered to bone metastases by ^{153}Sm lexidronam is between 18 and 80 Gy [31,32]—doses that have been associated with phenotypic modulation of human tumor cells. In one study, 10 human tumor-cell lines representing classes of tumors that metastasize to bone (4 prostate, 2 breast, and 4 lung) were exposed to clinically relevant palliative levels of ^{153}Sm lexidronam for 4 days, then examined by flow cytometry for modulation of several cell-surface molecules. Of the 10 cell lines, 100% upregulated Fas and CEA, 70% upregulated MUC-1, 40% upregulated MHC class I, and 30% upregulated ICAM-1. Upregulation of any of these surface molecules could potentially render tumor cells more susceptible to killing by CTLs [33]. Exposure of the human prostate cancer cell line LNCaP to ^{153}Sm lexidronam also resulted in upregulation of prostate-specific antigen (PSA), prostate-specific membrane antigen, and prostatic acid phosphatase. LNCaP cells

express PSA, MUC-1 and CEA. When incubated with CTLs specific for each of these target, LNCaP cells treated with ^{153}Sm lexidronam were killed significantly better than untreated tumor cells. Thus exposure of human tumor cells to ^{153}Sm lexidronam rendered them more susceptible to killing by a variety of antigen-specific CTLs. These data suggest that ^{153}Sm lexidronam may work synergistically with immunotherapy to increase the susceptibility of tumor cells to CTL killing and have formed the basis for an ongoing clinical trial (see below).

Radiolabeled monoclonal antibodies and cancer immunotherapy

Systemic radiotherapy, which delivers therapeutic radionuclides to cancer cells via monoclonal antibodies (mAb), has been shown to target tumor cells precisely and preferentially and can seek out micrometastases that are not observable by current imaging technology and thus cannot be targeted by external beam radiation. A recent report [34] cited the ability of radiolabeled mAb to alter tumor-cell phenotype and enhance immunologic targeting of tumor cells, as well as a therapeutic synergy between radiolabeled antibody and vaccine therapy. There, mice transgenic for human CEA were transplanted with a CEA-expressing murine carcinoma cell line. Radioimmunotherapy consisted of yttrium-90 (Y-90)-labeled anti-CEA mAb, used either alone or in combination with CEA-targeted vaccine therapy. A single dose of Y-90-labeled anti-CEA mAb, in combination with vaccine therapy, resulted in a statistically significant increase in survival in tumor-bearing mice over vaccine or mAb alone. Mice receiving the combination therapy also showed a significant increase in the percentage of viable tumor-infiltrating CEA-specific $CD8^+$ T cells compared to vaccine alone. Here, as seen above with external beam radiation, mice cured of tumors demonstrated an antigen cascade resulting in $CD4^+$ and $CD8^+$ T-cell responses not only for CEA, but also for other tumor antigens not encoded by the vaccine (p53 and gp70). Surprisingly, the tumor-infiltrating T cells were shown to be unaffected by the residential radiation source presented by the radiolabeled antibody. This finding was consistent with a preclinical study by Grayson *et al.* [35] which found that murine memory T cells were more resistant to apoptosis than naïve T cells after whole-body irradiation.

In the Field: Radiation and Immunotherapy in the Clinic

A recent clinical study reported on the use of a recombinant cancer vaccine combined with standard definitive radiotherapy in patients with localized prostate cancer [36]. The purpose of this trial was to determine if vaccine could induce an immune response in the presence of tumor irradiation. Because radiation alone can generate an inflammatory reaction, the trial was designed as a randomized phase II study, with patients receiving local definitive radiation with or without vaccine [36]. The primary endpoint of the trial was immunologic response, with secondary endpoints of safety and clinical response. Nineteen patients received vaccine plus radiation and 11 patients were given radiation alone. Patients in the combination arm received a priming vaccine of recombinant vaccinia (rV) expressing PSA (rV-PSA) admixed with rV expressing the costimulatory molecule B7-1 (rV-B7-1), followed by monthly booster vaccines with recombinant fowlpox (rF)-PSA. The vaccines were given with local GM-CSF and low-dose systemic IL-2. Patients received standard external beam radiation therapy between the fourth and sixth vaccinations.

Of 17 patients in the combination arm who completed all 8 vaccinations, 13 (76%) had increases of at least 3-fold in circulating PSA-specific T cells. No detectable increases in PSA-specific T cells were seen in the radiotherapy-only arm ($p < 0.0005$). Patients in the combination arm who had blood drawn at baseline and then after 3 vaccines (but before radiation) also showed evidence of *de novo* generation of T cells to prostate-associated antigens not present in the vaccine (antigen cascade), providing indirect evidence of immune-mediated tumor killing.

This regimen was well tolerated, with no grade 3 toxicities attributed to vaccine. However, many patients did develop transient toxicities to IL-2 (given at 4 MIU/M^2 daily from day 8 to 14), resulting in IL-2 dose reductions in 82% of the patients. Because of this, another cohort of 18 patients were enrolled and treated with the same dose and schedule of vaccine/GM-CSF but with metronomic-dose IL-2 (0.6 MIU/M^2 daily from day 8 to 21) [37]. This was much better tolerated, with dose reductions in IL-2 only in 4 (22%) patients largely due to transient asymptomatic laboratory abnormalities (e.g., hyperglycemia). A similar proportion of patients mounted an immune response, with 5 of 8 patients tested (63%) having a 3-fold or greater increase in PSA-specific T cells following vaccine.

The combination of radiation and vaccine is now being studied in patients with castration-resistant prostate cancer metastatic to bone. In a randomized phase II study at the National Cancer Institute (NCI), patients are receiving ^{153}Sm lexidronam (Quadramet®; Cytogen, Princeton, NJ), the bone-seeking radionuclide, alone or in combination with vaccines containing PSA-TRICOM based on the preclinical data discussed above. The NCI study is designed to determine if radionuclide plus vaccine can delay time to progression over radionuclide alone.

Another vaccine approach discussed above that has been tested in clinical trials is to make use of dying tumor cells as a rich environment in which to prime DCs. A phase I clinical trial was conducted in 14 patients with refractory hepatoma [38]. Patients received 8 Gy of external beam radiation therapy to the tumor followed by intratumoral injection of immature DCs (day 2 and day 24). Cells treated with 8 to 10 Gy are killed predominantly by apoptosis over 30 to 60 hours [39]. These conditions are thought to optimally allow antigen processing and presentation by DCs. Two patients had partial responses and 8 of 10 evaluable patients had increases in alpha-fetoprotein (AFP)-specific immune responses by cytokine release assay, with 7 of 10 having increases in AFP-specific immune responses by ELISPOT.

Recent research has further studied radiation-induced tumor-cell death as a means of inducing tumor-specific immune responses. Nesslinger *et al.* studied immune responses before and after either radiation or surgery in patients with localized prostate cancer [40]. A higher proportion of patients who were treated with external beam radiation therapy (4 of 29, 13.8%) and brachytherapy (5 of 20, 25%) mounted tumor-specific immune responses (new antibody formation measured by Western blot) than those treated with radical prostatectomy (0 of 14) or controls (2 of 36, 5.6%). Another ongoing clinical trial utilizing radiation to generate a tumor-specific immune response in patients with metastatic castration-resistant prostate cancer is studying the combination of external beam radiation therapy with an anti-CTLA4 antibody (ipilimumab), which potentiates immune responses.

The results of the above clinical data demonstrate that immune responses can be mounted to vaccine despite radiation therapy, and indeed that radiation therapy alone may induce immune responses. Ongoing clinical endpoint studies should determine whether the promise of synergy offered by the combination of immunotherapy and radiotherapy can translate into clinical benefit for patients.

Synergy in Our Future

The synergy of radiation therapy and immunotherapy underlies a novel strategy with the potential to better target local radiated/unkilled tumor cells, and to provide better control of nonradiated systemic disease. Systemic metastatic disease requires systemic therapy, which has traditionally been chemotherapy. However, the resultant systemic immunosuppression makes chemotherapy less desirable for combination with immunotherapeutic regimens. While radiation therapy has traditionally been considered only as local therapy, it is clear that it has systemic effects, many of which lead to improvements in antitumor immunity.

Furthermore, the absence of systemic immunosuppression makes radiation therapy an attractive adjuvant regimen for combination with immunotherapy and tumor-specific vaccine strategies. These strategies are applicable not only for overt metastatic disease, but also for occult metastatic disease. Learning how to exploit radiation-induced changes to tumor-cell antigens and how to induce effective immune responses to these cumulatively immunogenic stimuli will be an ongoing challenge. As knowledge of the synergistic effects of radiation and immunotherapy increases, the translational use of this strategy for a variety of carcinomas will become more feasible and more available to patients.

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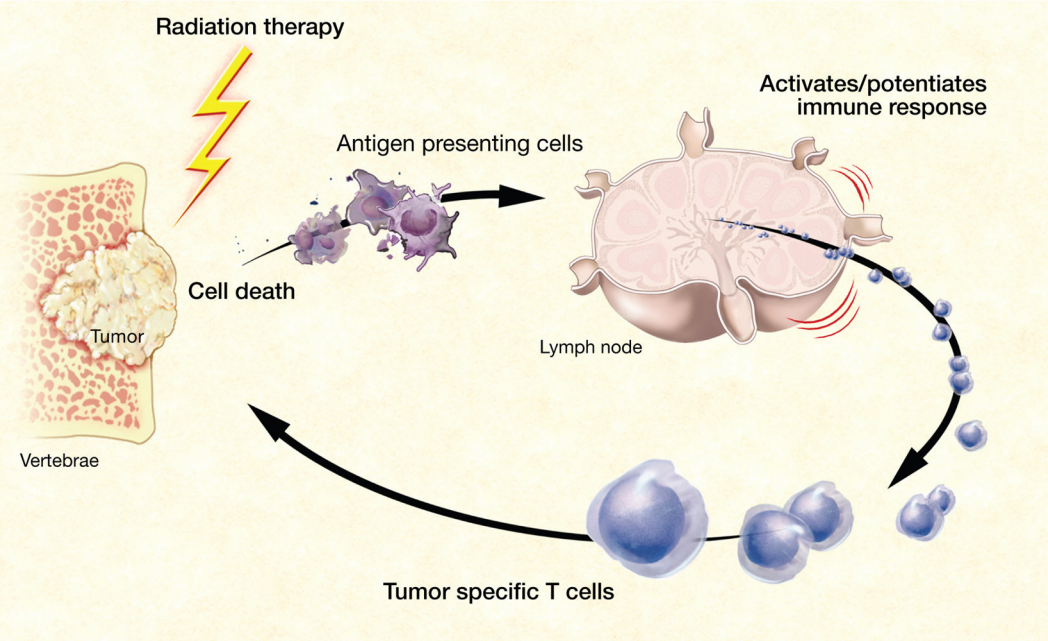

Figure 1.

Antigen release from dying tumor cells can activate immune responses. Irradiation induces death of cancer cells. As these cells die, they are taken up by scavenger cells called antigen-presenting cells. These antigen-presenting cells then travel to regional lymph nodes where they present antigen to T cells, initiating or potentiating an antitumor immune response. Activated tumor-specific T cells can then traffic to areas of tumor to participate in immune-mediated tumor killing.

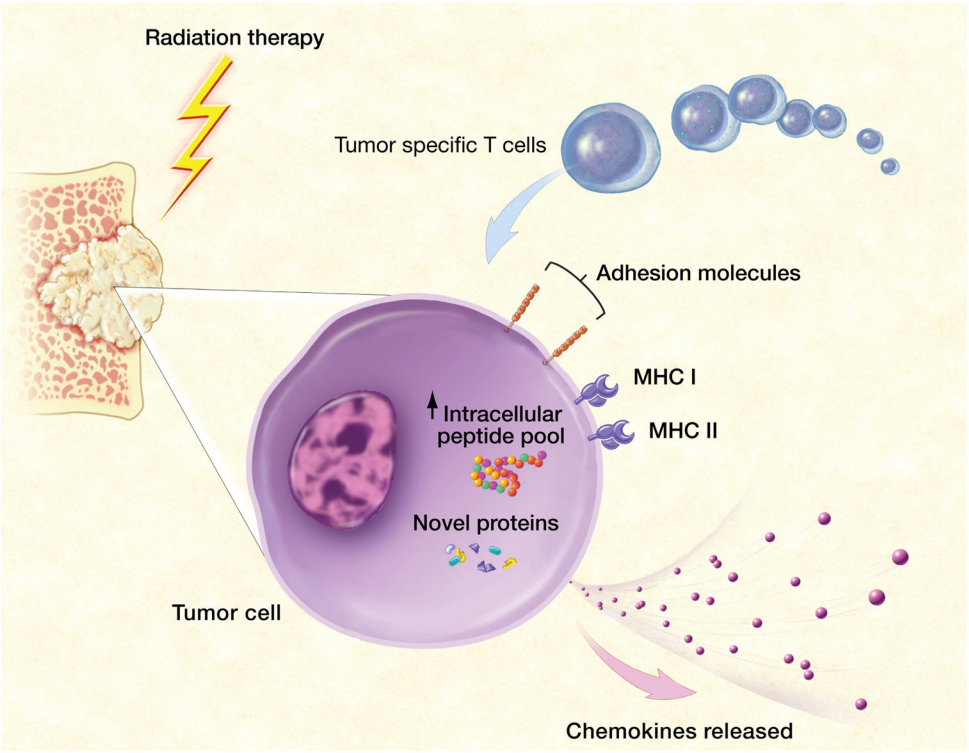

Figure 2.
Irradiation modulates tumor-cell phenotype and increases immune recognition. Irradiation can cause (a) upregulation of chemokines and adhesion molecules, providing signals for T cells to come to areas of tumor, (b) upregulation of MHC molecules and tumor-associated antigens, making it easier for T cells to recognize tumor, and (c) upregulation of Fas and downregulation of regulatory T cells, making it easier for cytotoxic tumor-specific T cells to kill tumor.

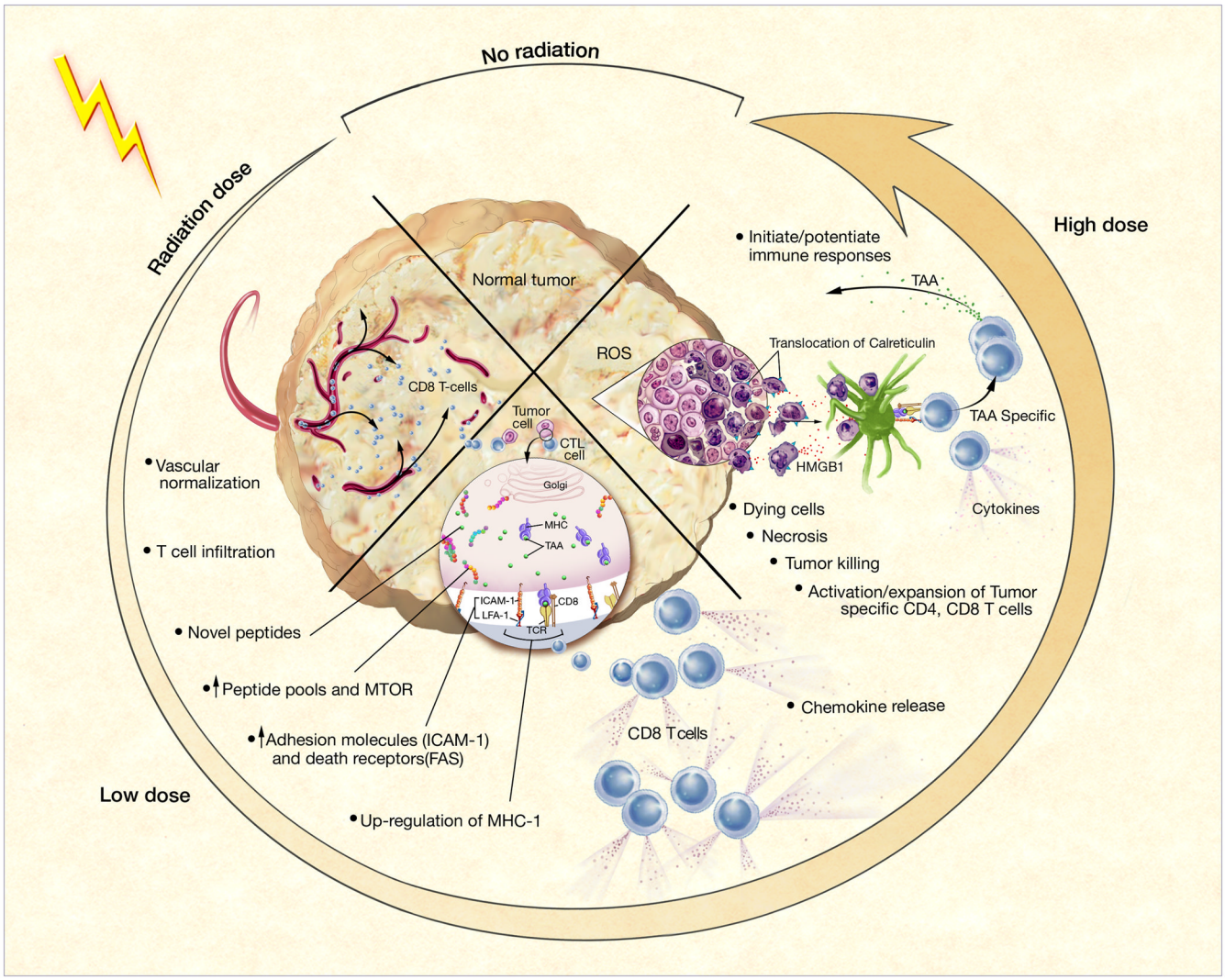

Figure 3.
Multiple mechanisms of synergy of radiation therapy and immunotherapy.