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REVIEW

Combined Treatment of Radiotherapy and Immunotherapy for Urological Malignancies: Current Evidence and Clinical Considerations

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Abstract: Although it has always been believed that radiation has immunosuppressive effects, more and more preclinical and clinical trials have shown that the combination of radiotherapy and immunotherapy has a potential synergistic effect to treat cancers including urological malignancies. When radiotherapy is combined with immunotherapy, improved prognosis has been observed in different urinary tumors. However, there is no standard treatment, such as the optimal dose/fractionation and the sequence of immunotherapy and radiotherapy. In this review, we discussed the effects of radiotherapy on the cancer immune system and emphasized the synergy of radiotherapy combined with immunotherapy. Although it has significantly improved the prognosis of tumors, there are still some unresolved questions about how to best use this combination in clinical practice. Ongoing trials will provide further information on the interaction of radiotherapy combined with immunotherapy, and are expected to guide clinical practice and improve clinical outcomes.

Keywords: radiotherapy, immunotherapy, urological malignancies, abscopal effect

Introduction

Radiotherapy has a long history in the treatment of tumor. It has a significant effect in the treatment of unresectable diseases and the prevention of postoperative local recurrence. Historically, it is believed that radiation has immunosuppressive effects. Due to the limitation of treatment planning and radiotherapy technology, larger treatment areas were needed in the past, which led to a significant myelosuppression, thus strengthens the above-mentioned concept.[1] However, the emergence of advanced radiation therapy planning and delivery has made tremendous changes in the ability to treat tumors. Stereotactic radiosurgery and stereotactic ablation radiotherapy (also known as stereotactic body radiation therapy, SBRT) can provide radiotherapy with millimeter level accuracy and minimize the dose to the surrounding tissue structures.[2] These advances have greatly reduced the fields of radiotherapy and allowed a higher radiation dose. This essential change needs to re-examine the immunological effects of radiotherapy.

It is widely known that the classical mechanism of radiation-mediated cell death is the irreparable damage of DNA through two primary effects. Under the direct effect, photons destroy DNA and break its double strand, which leads to cell apoptosis. Under the indirect effect, hydroxyl free radicals produced by photon beams mediate DNA damage and subsequent cell death.[3] However, some studies have indicated that the

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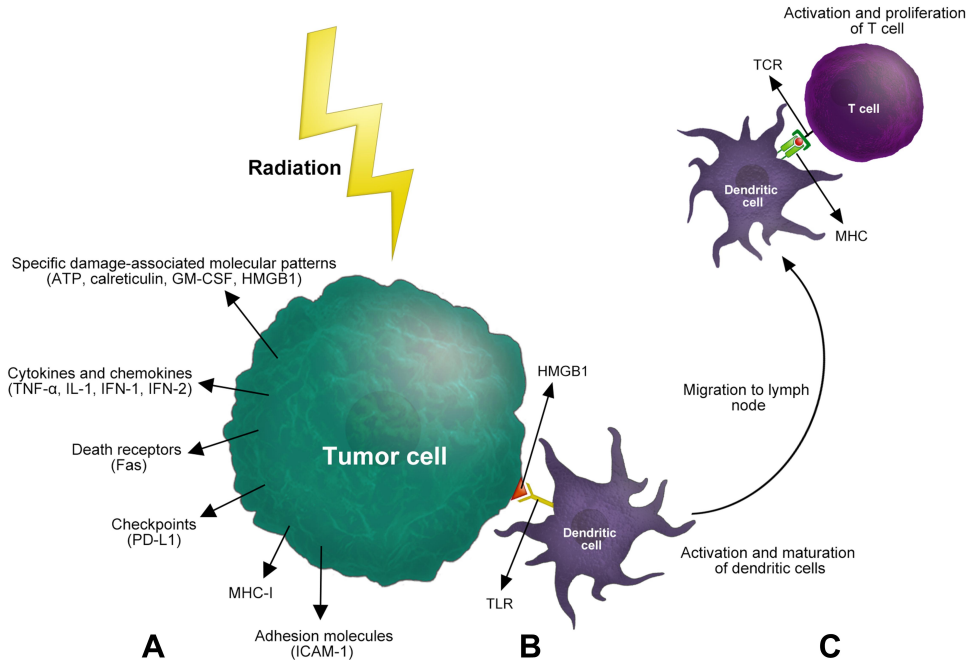

Figure 1 (**A**) Radiation induces changes to the tumor cell phenotype and release of cytokines; (**B**) HMGB1 activate dendritic cells by binding to Toll-like receptors; (**C**) The migration of dendritic cells to regional lymph nodes and the subsequent T-cell activation and proliferation. TLR, Toll-like receptors; TCR, T cell receptor.

immune system plays an important role in radiation therapy by promoting tumor cell death. Stone et al[4] reported some of the earliest data on the immune system related to the therapeutic efficacy of radiation. They used different doses of radiation to treat chemically induced fibrosarcoma in mice. The dose of radiation required to control the tumor was significantly reduced when they stimulated the immune system with crude bacterial preparation. Oppositely, when the mice receive immunosuppression before receiving systemic radiotherapy or thymectomy, higher doses of radiation were needed to control tumor growth.[4] These data indicate that the immune system can affect the therapeutic effects of radiation. In summary, available data suggest that radiotherapy can lead to immunogenic cell death and stimulate systemic antitumor immune response. The immunogenicity of radiotherapy has renewed interest in combining radiotherapy with immunotherapy to further increase systemic antitumor immune responses leading to improved prognosis.

Radiotherapy is used in the clinical treatment for various urological malignancies, such as prostate cancer or bladder cancer. In addition, it can also be used for palliative treatment for bone metastases from urological malignancies to alleviate symptoms. In this review, we discuss the combination of radiotherapy and immunotherapy in urological malignancies and put forward the clinical considerations for future research.

Materials and Methods

A systematic literature search of PubMed was conducted in May 2020 without language restrictions. Several key terms were used, including "radiation therapy", "radiotherapy", "immunotherapy", "combined" and "combination". The inclusion criteria were treatment included both radiotherapy and immunotherapy combined.

Effects of Radiotherapy on the Tumor Cells

Radiotherapy causes cell death by irreparable double stranded DNA damage. Despite the belief that radiotherapy is immunosuppressive, radiotherapy has been shown to promote antitumor immune response, although how radiation interacts with the immune system is unclear.[5–7] There is evidence that radiotherapy can induce the expression of immune factors in tumor cells and tumor microenvironment, and stimulate innate and adaptive immunity resulting in a tumor-specific T-cell response, as well as leukocyte infiltration of tumor in the radiation field.[8]

Immune-Stimulating Effects of Radiation

Pre-clinical data suggest that radiotherapy has multiple immunogenic effects. The mechanism of radiotherapy leading to immunologic cell death has been explored by numerous reviews.[9–12] Overall, through the damage of

tumor cell and regulation of tumor microenvironment, radiotherapy releases specific damage-associated molecular patterns, such as adenosine triphosphate (ATP), calreticulin, granulocyte-macrophage colony-stimulating factor (GM-CSF) and high-mobility group box 1 protein (HMGB1), to induce the activation and maturation of dendritic cells and antigen-presenting cells (APCs).[13,14] Continuous dendritic cell activation plays an important role in the production of effective antitumor immune response. Radiotherapy has also shown to promote the migration of APCs to regional lymph nodes and the subsequent T-cell priming.[15] Gameiro et al[16] found that radiation can induce a remarkable increase in HMGB1 release and the surface expression of calreticulin in human prostate, breast, and lung cell lines. However, as a damage related molecular pattern, HMGB1 may activate dendritic cells by binding to Toll-like receptors to prime immune responses.[17]

Radiation damage also leads to the release of chemokines, chemokine ligands, CXCL16, which lead to the recruitment of CD8 T-cells in tumor microenvironment and vascular remodeling to maximize the migration of T-cells into tumor.[18,19] Radiotherapy also induces inflammatory cytokines, including interleukin (IL) 1b, tumor necrosis factor-α (TNF-α) and type 1 and 2 interferons (IFN-1 and IFN-2), through the STING pathway, to promote the anti-tumor T-cell response.[20]

Radiation damage also leads to the phenotype change of the remaining tumor cells after radiation. For example, the up-regulation of surface molecules, including major histocompatibility complexes (MHC) I, costimulatory T-cell signaling molecules, adhesion molecules (such as intercellular adhesion molecule 1, ICAM-1) and stress-induced ligands, contribute to the recognition and clearance of tumor cells by the immune system.[21] In addition, radiotherapy can also up-regulate the expression of Fas death receptors on tumor cells and induce the sensitivity of tumor cells to Fas-mediated killing that is unrelated to T-cell receptors.[22] Garnett et al[23] found that when delivering radiation to human colon, lung, and prostate cancer cells, the higher the radiation dose, the greater expression of stimulating immune signal and tumor antigen (Fas, MHC-I, ICAM-1, carcinoembryonic antigen [CEA], and mucin) on the surface of tumor cells, which led to more effective immune-mediated tumor killing. Conclusively, all of these effects lead to the formation of pro-inflammatory microenvironment, anti-tumor activation of the immune system, and increased cancer cell death.

Immune-Suppressive Effects of Radiation

Interestingly, the effects of radiation on tumor microenvironment and its interaction with the immune system is a complex balance of stimulating and suppressing. In addition to the immune-stimulating effects, radiotherapy can also inhibit the immune response of tumor cells. Radiotherapy has been shown to increase regulatory T-cells (Treg) in the tumor microenvironment, resulting in the inherent higher radiosensitivity of these cells,[10] the down-regulation of immune response and the secretion of transforming growth factor-β (TGF-β).[24] Kachikwu et al[25] reported that radiation-promoted tumor regression was enhanced in Treg-deficient prostate cancer model. Besides, radiotherapy can also stimulate myeloid-derived suppressive cells, which support tumor progression by promoting tumor cell survival, angiogenesis, tumor cell invasion and metastasis to healthy tissues.[26] Recent report by Wu et al demonstrated that radiotherapy could up-regulate programmed death ligand 1 (PD-L1) of bladder cancer mice temporarily.[27] Similarly, radiotherapy can up-regulate the expression of cytotoxic T lymphocyte antigen-4 (CTLA-4) in Treg cells.[28] In addition, Twyman-Saint Victor et al suggested that radiation enhances the diversity of T-cell receptor repertoire in tumors. After local high dose irradiation, the higher expression of PD-L1 and CTLA-4 was also observed in tumor cells.[29] Although these are disadvantageous to the immunogenicity of radiotherapy, this provided a strong theoretical basis for the combination of radiotherapy and immune checkpoint blockade, especially in the case of tumor resistant to immunotherapy.

Abscopal Effect

Radiation-mediated cell death and its effect on tumor microenvironment not only cause local effects, but may also induce “abscopal effect”. Abscopal effect refers to the partial or complete remission of distant involved regions outside the radiation field. The term “abscopal effect” was imported by R.H. Mole in 1952 before he introduced drug immunotherapy into oncology. It described radiation effects outside the radiation field but in the same body for the first time.[30] However, the effect is an ambiguous phenomenon. A systematic review found that in the 35 years from 1969 to 2014, only 46 clinical cases reported the abscopal effect. And most cases were traditionally considered immunogenic, including 7 cases of renal cell carcinoma.[31] This phenomenon lacks repeatability and its

mechanism is not clear. However, the combination of radiotherapy and immunotherapy has the potential to impel such abscopal effect repeatable and produce long-term remission for people with metastatic diseases.[32] Some cases about the abscopal effect with radiotherapy published in the recent literature are based on the treatment of immune checkpoint inhibitor therapy. Park et al[33] used preclinical melanoma and renal cell carcinoma models to show the abscopal effect of radiotherapy combined with anti-PD-1 therapy. In addition, Dewan et al[34] found that radiotherapy induces the abscopal effect when in combination with anti-CTLA-4 antibody in preclinical breast and colon carcinoma models. This indicates that the combination of radiotherapy and immune checkpoint inhibitor therapy may make the abscopal effect occur more frequently.

Combining Radiotherapy with Immunotherapy

The term "abscopal effect" and its definition described the non-radiation oncology response caused by the stimulation of the immune system by radiation, which can be supported and intensified by the use of immunologic drugs recently. With the great success of single immunotherapy in the treatment of multiple solid tumors, including melanoma, lung cancer, and urological tumors, it becomes clear that there is an inherently effective immune response without any radiological effects, which can be stimulated by pharmacological stimulation or inhibition of T-cells and/or antigen-presenting cells. However, data from in vitro/in vivo trials and clinical studies have been accumulating, showing a significant synergy between immunotherapy and radiotherapy. Lee et al[35] found that 20–25Gy was effective at tumor control in melanoma mice, while it does not work in mice with CD8 T-cells depletion. Thus, there is a hypothesis that enhancing the function of T-cells may lead to radiosensitization and improve local control. Several pre-clinical data supported this hypothesis.[27,36] However, we must remember that the purpose of animal models is to simplify extremely complex syndromes, usually caused by multiple pathogenies, into manageable research problems. These animal models are in marked contrast to humans with relatively different genetic composition and exposure to a range of environmental stresses. Therefore, animal research results need to be further verified in humans.

Combining Radiotherapy with Immunotherapy in Urological Malignancies

Some ongoing trials are evaluating the combination of radiotherapy and immunotherapy for urological malignancies. Tables 1–3 describe the clinical trials of patients with prostate cancer, renal cell carcinoma, and urothelial cancer, respectively. The results of these trials are expected to elucidate the potential synergistic effect of radiotherapy and immunotherapy in patients with urological malignancies.

Sipuleucel-T is an active cellular immunotherapy approved by FDA for the treatment of asymptomatic or mild symptoms in patients with metastatic castration-resistant prostate cancer (mCRPC).[37] Currently, a number of randomized clinical trials (NCT01807065, NCT01818986, NCT02232230, NCT01833208, NCT02463799) are underway to evaluate the efficacy of sipuleucel-T combined with radiotherapy in the treatment of mCRPC.

Recently, with the development of many clinical studies, immune checkpoint inhibitors have received extensive attention. Ipilimumab is the first human monoclonal antibody approved by FDA in the field of cancer. It can specifically block the binding of CTLA-4 and its ligand, thus enhancing the activation and proliferation of T cells and mediating the anti-tumor effect.[38] There is a Phase I randomized clinical trial (NCT03477864) determining the safety and tolerability for ipilimumab with SBRT in patients with locally advanced prostate cancer. In addition, pembrolizumab is a humanized antibody, which can block the inhibitory ligand of programmed cell death 1 receptor (PD-1). Several trials (NCT02662062, NCT03419130, NCT02621151, NCT02560636, NCT03287050) investigating the safety, tolerability, and effectiveness of pembrolizumab combined with radiotherapy in muscle-invasive bladder cancer. The study by Twyman-Saint Victor and his colleagues[29] exploring radiotherapy with dual checkpoint blockage (anti-CTLA-4 and anti-PD-L1 antibodies) in nonurological cancer showed encouraging results. A few ongoing trials (NCT03065179 and NCT03149159) assessing the efficacy of ipilimumab + nivolumab with SBRT in the management of patients with metastatic clear cell renal cell carcinoma.

Clinical Evidence of Combination in Urological Malignancies

At the ASCO GU in 2020, two studies on SBRT combined with immunotherapy attracted the attention of

Table 1 Clinical Trials Combining Immunotherapy with Radiotherapy in Prostate Cancer

Trial	Condition	Aims	Phase	Intervention	Institution/Group
NCT01436968	Intermediate-high risk localized PCa	The purpose of this study is to evaluate the effectiveness of ProstAtak immunotherapy in combination with RT for patients with intermediate-high risk localized PCa	Phase III	RT + valacyclovir ± AdV-tK	Advantagene, Inc. d. b.a. Candel Therapeutics
NCT02107430	High risk localized PCa	To determine whether DCVAC/PCa added after radical primary prostatectomy can improve PSA progression times within 5 years for patients with high risk localized PCa	Phase II	RT ± dendritic cells (DCVAC/PCa)	Sotio a.s. (Czech Republic)
NCT01807065	mCRPC	To study how well giving sipuleucel-T with or without RT works in treating patients with mCRPC	Phase II	RT followed by sipuleucel-T	City of Hope Medical Center
NCT01818986	mCRPC	Sipuleucel-T and SABR for patients with mCRPC	Phase II	SABR + sipuleucel-T	University of Texas Southwestern Medical Center
NCT01303705	Metastatic PCa	To examine a novel combination of anti-OX40 to induce proliferation of memory and effector T-cells in conjunction with cyclophosphamide (CTX) and radiation to induce tumour antigen release with the overall goal of promoting an immune response against prostate cancer	Phase I/II	RT + cyclophosphamide + anti-OX40	Providence Portland Medical Center
NCT02232230	mCRPC	To assess the effect of RT to augment antitumor responses from immune therapy with Provenge	Phase II	RT + sipuleucel-T	21st Century Oncology
NCT03477864	Locally advanced prostate cancer	To study the side effects of anti-PD-1 monoclonal antibody REGN2810 and/or ipilimumab when given together with SBRT before surgery in treating participants with progressive advanced or oligometastatic PCa	Phase I	SBRT + anti-PD-1 ± ipilimumab before radical prostatectomy	Sidney Kimmel Cancer Center at Thomas Jefferson University
NCT03007732	Hormone-naïve oligometastatic PCa	SBRT and pembrolizumab with or without intratumoral SD-101 in patients with newly diagnosed hormone-naïve oligometastatic PCa	Phase II	SBRT + ADT + pembrolizumab ± TLR9 agonist (SD-101)	Lawrence Fong, University of California
NCT01833208	mCRPC	Impact of radiation therapy on the immunogenicity of sipuleucel-T	Pilot study	RT + sipuleucel-T	Roswell Park Cancer Institute
NCT02463799	mCRPC	To study the effect of radium-223 when added to sipuleucel-T for treating castrate-resistant prostate cancer that has spread to the bone	Phase II	Radium-223 + sipuleucel-T	Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins

Abbreviations: PCa, prostate cancer; mCRPC, metastatic castrate-resistant prostate cancer; RT, radiation therapy; PSA, prostate-specific antigen; SABR, stereotactic ablative radiosurgery; SBRT, stereotactic body radiation therapy; Anti-PD-1, antibody against programmed cell death protein 1; ADT, androgen deprivation therapy; AdV-tK, adenoviral vector expressing the herpes thymidine kinase gene; TLR9, toll-like receptor 9.

participants. NIVES is a Phase II multicenter study (NCT03469713)[47] in Italy, which explored the safety and efficacy of nivolumab combined with radiotherapy for metastatic renal cell carcinoma (mRCC) after failure of targeted therapy. Besides, this is the first prospective clinical study of nivolumab combined with radiotherapy in the treatment of mRCC. Nivolumab was given as flat dose of 240 mg in intravenous infusion beginning on day 1 every 14 days for 6 months, and SBRT (30 Gy/3 fractions) was administered 7 days after the first infusion

Table 2 Clinical Trials Combining Immunotherapy with Radiotherapy in Renal Cell Carcinoma

Trial	Condition	Aims	Phase	Intervention	Institution/ Group
NCT01896271	Metastatic ccRCC	To evaluate the RR in patients with mRCC after treatment with high-dose IL-2 immediately following SABR to multiple metastatic sites	Phase II	SABR + high-dose IL-2	University of Texas, Southwestern
NCT03065179	Metastatic ccRCC	To determine whether the combination of nivolumab plus ipilimumab and SBRT yields a clinically compelling antitumor activity measured as ORR	Phase II	SBRT + nivolumab + ipilimumab	University of Texas, Southwestern
NCT02306954	ccRCC	To compare the RR among renal cell cancer (RCC) patients of high dose IL-2 to SBRT + IL-2 in patients with metastatic renal cancer	Phase II	SBRT + high-dose IL-2	Providence Health
NCT02781506	Metastatic ccRCC	To increase the RR of treatment with Nivolumab by the concurrent administration of SABR	Phase II	SABR + nivolumab	University of Texas, Southwestern
NCT01884961	Metastatic RCC or melanoma	Radiotherapy as an immunological booster in patients with metastatic melanoma or renal cell carcinoma treated with high-dose IL-2	Phase II	RT boost + high-dose IL-2	Istituto Scientifico Romagnolo (Italy)
NCT02855203	Metastatic ccRCC	To examine the safety, efficacy and biological effects of combining pembrolizumab (MK-3475) an antibody targeted against anti-(PD-1), with SABR for oligometastatic RCC	Phase I/II	SABR + pembrolizumab	Peter MacCallum Cancer Centre (Australia)
NCT03050060	Metastatic RCC, melanoma, or NSCLC	To study how well image guided hypofractionated radiation therapy works with nelfinavir mesylate, pembrolizumab, nivolumab, and atezolizumab in treating patients with metastatic RCC, melanoma, or NSCLC	Phase II	IGRT + nelfinavir + (pembrolizumab or nivolumab or atezolizumab)	University of Washington
NCT02318771	Recurrent/metastatic H&N, RCC, melanoma, or lung cancer	To study RT and pembrolizumab (MK-3475) in treating patients with head and neck cancer, RCC, melanoma, or lung cancer that has returned, has spread to other parts of the body, or cannot be removed by surgery	Phase I	RT + pembrolizumab	Thomas Jefferson University
NCT02599779	Metastatic RCC	To investigate if a treatment strategy where SBRT is given with pembrolizumab is sufficiently active to warrant further investigation in randomized phase II or III studies	Phase II	SBRT + pembrolizumab	Sunnybrook Health Sciences Centre
NCT03149159	Metastatic ccRCC	To see if continued nivolumab with the addition of ipilimumab plus hypo-fractionated SBRT of a single lesion results in partial or complete responses in patients with metastatic ccRCC who fail initial treatment with single agent nivolumab	Phase II	SBRT + nivolumab + ipilimumab	Medical University of South Carolina

(*Continued*)

Table 2 (Continued).

Trial	Condition	Aims	Phase	Intervention	Institution/ Group
NCT03115801	Metastatic RCC or UC	To examine the overall response rates of combining immunotherapy (nivolumab/ atezolizumab) with RT for metastatic RCC or UC	Phase II	Nivolumab + atezolizumab ± RT	Weill Medical College of Cornell University
NCT02864615	Metastatic RCC	To evaluate safety and preliminary efficacy of stereotactic body radiation therapy in patients with metastatic renal cell carcinoma treated with VEGFR, mTOR or immune checkpoint inhibitors	Phase Ib	SBRT + (VEGFR inhibitor or mTOR inhibitor or checkpoint inhibitor)	Kidney Cancer Research Bureau
NCT03469713	Metastatic RCC	Combining SBRT with nivolumab in patients with metastatic RCC	Phase II	SBRT + nivolumab	Gruppo Oncologico Italiano di Ricerca Clinica (Italy)
NCT03474497	Metastatic NSCLC, RCC, or HNSCC after failed PD-1/ PD-L1 therapy	To evaluate the safety and toxicity of pembrolizumab and intralesional IL-2 in combination with hypofractionated radiotherapy in patients with metastatic NSCLC, RCC, or HNSCC after failed PD-1/ PD-L1 therapy	Phase I/II	RT + IL-2 + pembrolizumab	University of California, Davis
NCT03511391	NSCLC, UC, melanoma, RCC, H&N or HNSCC cancer	To investigate whether the addition of SBRT to checkpoint inhibitor treatment in patients with NSCLC, UCC, melanoma, RCC, H&N or HNSCC cancer can improve progression-free survival as compared to checkpoint inhibitor monotherapy.	Phase II	SBRT ± (pembrolizumab or nivolumab)	University Hospital, Ghent (Belgium)
NCT03226236	Metastatic RCC	To evaluate the ORR in patients with mRCC after treatment with IL-2+dendritic cell vaccine following RT	Phase II	RT + IL-2 + dendritic cell vaccine	UO Immunoterapia e Laboratorio TCS, IRCCS IRST (Italy)
NCT03693014	Metastatic cancer, melanoma cancer, lung cancer, bladder cancer, renal cancer, head/ neck cancers	Combining SBRT with checkpoint inhibitors in patients with solid tumors	Phase II	SBRT+ (ipilimumab, nivolumab, pembrolizumab or atezolizumab)	Memorial Sloan Kettering Cancer Center

Abbreviations: ccRCC, clear cell renal cell carcinoma; NSCLC, non-small cell lung carcinoma; H&N, head and neck; UC, urothelial carcinoma; HNSCC, head and neck squamous cell carcinoma; SABR, stereotactic ablative radiosurgery; SBRT, stereotactic body radiation therapy; RT, radiation therapy; IL-2, interleukin-2; mTOR, mammalian target of rapamycin; VEGFR, vascular endothelial growth factor receptor; RR, response rate; ORR, objective response rate.

of nivolumab. A total of 69 patients were enrolled, of which 2 failed to undergo radiotherapy and 4 did not complete the first cycle of treatment, that is, 69 patients in intention-to-treat (ITT) group and 63 patients in per-protocol (PP) group. Among them, clear cell carcinoma accounts for about 80%, and the proportion of lung metastases is more exposed, accounting for 37.7%. In terms of efficacy, after a median follow-up of 15 months, the objective response rate (ORR) of the ITT group and the PP group were 17.4% and 19%, and the tumor control

Table 3 Clinical Trials Combining Immunotherapy with Radiotherapy in Urothelial Carcinoma

Trial	Condition	Aims	Phase	Intervention	Institution/Group
NCT02891161	UC of bladder	To evaluate the safety and efficacy of combining durvalumab with RT followed by adjuvant durvalumab for patients with UC of bladder	Phase Ib/II	RT + durvalumab	Big Ten Cancer Research Consortium
NCT03317158	NMIBC	To establish the safety of durvalumab monotherapy and durvalumab in combination with BCG and EBRT in NMIBC patients	Phase I/II	Durvalumab alone, durvalumab + EBRT, or durvalumab + BCG	Hoosier Cancer Research Network
NCT02662062	MIBC	To assess the safety and feasibility of combining pembrolizumab with chemoradiotherapy for patients with MIBC	Phase II	RT + cisplatin + pembrolizumab	Australian and New Zealand Urogenital and Prostate Cancer Trials Group
NCT03171025	MIBC	To evaluate the rate of failure free survival at 2 years after start of chemoradiation with adjuvant nivolumab in adult subjects who undergo chemoradiation for localized bladder cancer	Phase II	Chemoradiation followed by nivolumab	University of Utah
NCT03419130	MIBC	How well radiation therapy and pembrolizumab work in treating patients with urothelial bladder cancer that is restricted to the site of origin, without evidence of spread	Phase II	Pembrolizumab + (conventional RT or hypofractionated RT)	University of California
NCT02621151	MIBC	To assess the efficacy of pembrolizumab (MK3475) added to concurrent radiation and gemcitabine in the management of patients with muscle-invasive urothelial cancer who are not candidates for or decline radical cystectomy	Phase II	RT + gemcitabine + pembrolizumab	NYU Langone Health
NCT02560636	MIBC	To investigate the safety, tolerability and effectiveness of an immunotherapy drug called pembrolizumab used in combination with radiotherapy	Phase I	RT + pembrolizumab	Royal Marsden NHS Foundation Trust

(*Continued*)

Table 3 (Continued).

Trial	Condition	Aims	Phase	Intervention	Institution/Group
NCT03421652	MIBC ineligible for chemotherapy	How well nivolumab works with radiation therapy in treating patients with urothelial bladder cancer that has spread from its original site of growth to nearby tissues or lymph nodes and are ineligible for chemotherapy	Phase II	RT + nivolumab	Barbara Ann Karmanos Cancer Institute
NCT03287050	Metastatic UC	To investigate the feasibility of anti-PDL1/PD1 (pembrolizumab) and SBRT in patients with advanced, platinum-refractory urothelial carcinoma	Phase I	SBRT + pembrolizumab	University of Michigan Rogel Cancer Center
NCT03529890	Locally advanced UC of bladder	To assess safety and efficacy of preoperative RT before radical cystectomy combined with immunotherapy in locally advanced urothelial carcinoma of the bladder	Phase II	RT + nivolumab followed by radical cystectomy	Technische Universität München (Germany)
NCT03115801	Metastatic UC or RCC	To examine the overall response rates of combining immunotherapy with RT for mUC or RCC	Phase II	Atezolizumab ± RT	Weill Medical College of Cornell University

Abbreviations: UC, urothelial carcinoma; NMIBC, non-muscle-invasive bladder cancer; MIBC, muscle-invasive bladder cancer; RCC, renal cell carcinoma; RT, radiation therapy; BCG, bacillus Calmette-Guérin vaccine; EBRT, external beam radiation therapy; SBRT, stereotactic body radiation therapy.

rate was 58% and 63.5%. The median progression-free survival (PFS) of the whole group was 4.1 months, and the 1-year PFS rate was 32.6%; the median overall survival (OS) period was 22.07 months, and the 1-year OS rate was 73.4%. In this study, the ORR indicators of clear cell carcinoma were better than those of non-clear cell carcinoma (p=0.01). This may be due to the different driver mutation genes in non-clear cell carcinoma and clear cell carcinoma, which led to different immunotherapy responses. In terms of safety, severe treatment-related toxicities accounted for 24.6%, and all related toxicities were outside the range of radiotherapy, indicating that radiotherapy itself did not seem to increase the therapeutic toxicity. In short, for mRCC that have failed targeted therapy, nivolumab combined with radiotherapy is generally tolerable. Although it did not reach the expected ORR (40%), the overall tumor control rate and survival rate of combined radiotherapy were high.

Another multi-center study, RADVAX RCC (NCT03065179),[48] analyzed whether the dual immunotherapy (nivolumab + ipilimumab) combined with radiotherapy could bring a more satisfactory effect against mRCC on the basis of acceptable toxicity. Under the aforementioned mechanism of radiotherapy to activate immunity, CTLA-4 inhibitors could proliferate T cells, and PD-1 inhibitors could reverse suppressed T cells. Based on this, the research was divided into two phases: induction phase and maintenance phase. In the induction phase, dual immunotherapy (nivolumab + ipilimumab) was administrated, and radiotherapy (10 Gy×5 f in the tumor center, 8 Gy×5 f around the tumor, qod) started immediately after the first infusion of nivolumab. Subsequently, during the maintenance phase, only nivolumab treatment was performed. A total of 25 patients were enrolled, and 30% of patients had positive PD-L1 expression. In terms of radiotherapy, 92% of patients irradiated

only one lesion, and the median volume of the lesion was 18.7 cm3. After a median follow-up of 24 months, none of the lesions that received radiotherapy had progressed, and the lung metastases were most significantly reduced. The median PFS of all patients was 8.21 months, and the 1-year PFS rate was 36%. Compared with the previous study, such a high local control rate is due to the increase in radiotherapy dose and the dual immunotherapy.

Analyzing the NIVES and the RADVAX RCC, the single dose was 10 Gy, while the former only in 3 fractions (biological equivalent dose was 110 Gy), and the latter in 5 fractions (biological equivalent dose was 190 Gy). Therefore, for the lesions receiving radiotherapy, the latter has a better local control rate than the former (2-year LC: 100% vs 1-year LC: 82%). However, the appropriate dose of radiotherapy and the fractionation remain to be discussed. The number of radiotherapy lesions in NIVES and the RADVAX RCC was almost only one, and the selected tumor volume was too small to achieve full coverage treatment. In addition, the inconsistency between the gene mutation of the primary lesion and the metastasis might cause the antigen released by radiotherapy of a single lesion not suitable for other lesions, which makes it unable to entirely exert the immune effect induced by radiotherapy.

Clinical Considerations of Combining Radiotherapy with Immunotherapy

Although the combination of radiotherapy and immunotherapy is becoming a promising method, there are many questions about the clinical application of the combination which remain unanswered. The optimal sequence of radiotherapy and immunotherapy, and the optimal immunotherapy agent and its duration need to be further clarified. In addition, details of radiotherapy, such as optimal dose/fractionation, are unclear. Thirdly, it is necessary to clarify the possible acute and late toxicities of combined treatment.

Dose/Fractionation

So far, a series of techniques and schedules have been used to investigate the combination of radiotherapy and immunotherapy in preclinical studies. However, the optimal dose/fractionation of radiation to induce the optimal immune response or to interact with immunotherapy is still controversial.[39] Some studies have shown that multiple fractionation radiation is superior to single dose radiation, while other studies have reported similar results for both, or multiple fractionation radiation is inferior. Tsai et al[40] reported selective up-regulation of IFN-related genes by fractionated dose (2Gy × 5) but not single dose (10 Gy×1) in human breast, prostate, and glioma tumor cells. Consistent with this, John-Aryankalayil et al[41] showed that genes regulating immune and stress response, cell cycle, and apoptosis were significantly up-regulated by multi-fractionated radiation (2 Gy×5) compared to single dose (10 Gy×1) in human prostate cancer cells. Besides, a preclinical study of breast carcinoma cells receiving both ipilimumab and radiotherapy found that compared with a single dose (20 Gy×1), fractionated dose (8 Gy×3 or 6 Gy×5) resulted in upregulation of tumor-specific T-cells, leading to significant responses in both primary tumor and the tumors outside the radiation field.[34] Conversely, Lugade et al[42] found that single dose (15 Gy×1) results in great numbers of immune cells than fractionated dose (5 Gy×3) in mice with B16 melanoma tumors. However, Lee et al[35] found comparable progressive growth of B16 melanoma tumors irrespective of being treated with single dose (20 Gy×1) or fractionated dose (5 Gy×4). This variability may be caused by a variety of different radiation technologies and energies, each with different scattering and dosimetry. In addition, the effects of fractionation could depend on the type of tumor or model system used. Therefore, further dose/fractionation comparison trials are needed to determine the optimal radiotherapy regimen.

Sequencing

Different from dose/fractionation, there is a relative consistency in preclinical studies on the sequence of immunotherapy and radiotherapy, and various studies have shown that simultaneous radiotherapy and immunotherapy are better than sequential therapy. Dewan et al[34] showed that delaying the administration of anti-CTLA-4 antibody after radiation reduced the therapeutic effect. Dovedi et al[36] found that the tumor cells of mice with colon cancer can be induced to express PD-L1 by the radiation dose of 10Gy directly. If anti-PD-L1 antibody is used at the same time, rather than after radiation, the survival rate of mice can be improved. Mechanistically, the optimum time of immunotherapy and radiation-induced cell death, antigen presentation, transport, and T-cell engagement may depend on the type of immunotherapy used. Meanwhile, the efficacy of immunotherapy alone before radiotherapy may be limited due to the reduction of inflammatory cell death and the reduction of antigen targets of the immune system.[43]

Young et al[44] compared the efficacy of anti-OX40 (a costimulatory signal for T-cell activation) and anti-CTLA4 with 20 Gy in a single fraction in mice with colorectal cancer. It was found that radiotherapy and anti-OX40 had the best survival rate if immunotherapy was carried out 1 day after radiotherapy, while radiotherapy and anti-CTLA4 had the best survival rate if immunotherapy was carried out 7 days before radiotherapy. This indicates that perhaps the specific mechanism of each immunotherapy may play a role in the optimal timing.[44] To sum up, the current preclinical data support the concurrent administration of immunotherapy with radiotherapy but need further clinical data to confirm.

Toxicities

Due to the potential toxicities of radiotherapy combined with immunotherapy, caution should be exercised when combining these two therapies. In addition to complications of conventional radiotherapy, such as nausea, fatigue, skin damage, and hemopenia, the combination of radiotherapy and immunotherapy could put patients at risk of serious complications. Furthermore, immune side-effects associated with specific sites might increase, such as immunotherapy combined with lung irradiation, resulting in an increase in pneumonia, the same as liver irradiation resulting in hepatitis. A recent phase I trial of pembrolizumab and hypofractionated radiation therapy in bladder cancer reported a high risk for severe toxicity. In this study, patients received pembrolizumab and urinary bladder radiation to a dose of 36 Gy in 6 fractions.[45] The trial was suspended after dose-limiting toxicity was observed in 5 patients. Three patients experienced grade 3 urinary toxicities and one patient experienced grade 4 intestinal perforation. However, Kwon et al[46] reported no significant increase in intestinal toxicity when ipilimumab was combined with pelvic radiation, which suggested that immunotherapy could also be safely combined with radiotherapy to specific sites. Therefore, more clinical trials are needed to assess the risks and toxicity of radiotherapy combined with immunotherapy.

Conclusion

Immunotherapy has produced substantial and enduring clinical responses in a series of studies and is becoming the fourth backbone of cancer treatment after surgery, chemotherapy, and radiotherapy. In addition, a series of published studies have shown that radiation enhances many steps required to generate an antigen-specific immune response, including tumor cell death, antigen cross-presentation, and cytotoxic T cell activation and proliferation. The combination of immunotherapy and radiotherapy can lead to local and systemic enduring responses of urological malignancies, which have been confirmed by increasing preclinical and clinical evidence. However, there are still many outstanding questions and trials in progress, which are expected to clarify appropriate patient selection and practical considerations, such as dose/fractionation, sequencing for delivery of therapy, and treatment-related toxicity, to maximize the treatment effect. Furthermore, whether other systemic therapies such as neoadjuvant chemotherapy can enhance the synergistic effect of radiotherapy and immunotherapy also provides more ideas and possible options for urological malignancy treatment in the future.

Disclosure

The authors report no conflicts of interest in this work.

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