Combining Targeted Radionuclide Therapy and Immune Checkpoint Inhibition for Cancer Treatment

Simone C. Kleinendorst[1], Egbert Oosterwijk[2], Johan Bussink[3], Harm Westdorp[4,5], Mark W. Konijnenberg[1], and Sandra Heskamp[1]

ABSTRACT

The development of immunotherapy, in particular immune checkpoint inhibitors (ICI), has revolutionized cancer treatment in the past decades. However, its efficacy is still limited to subgroups of patients with cancer. Therefore, effective treatment combination strategies are needed. Here, radiotherapy is highly promising, as it can induce immunogenic cell death, triggering the release of pro-inflammatory cytokines, thereby creating an immunogenic phenotype and sensitizing tumors to ICI. Recently, targeted radionuclide therapy (TRT) has attained significant interest for cancer treatment. In this approach, a tumor-targeting radiopharmaceutical is used to specifically deliver a therapeutic radiation dose to all tumor cells, including distant metastatic lesions, while limiting radiation exposure to healthy tissue. However, fundamental differences between TRT and conventional radiotherapy make it impossible to directly extrapolate the biological effects from conventional radiotherapy to TRT. In this review, we present a comprehensive overview of studies investigating the immunomodulatory effects of TRT and the efficacy of combined TRT-ICI treatment. Preclinical studies have evaluated a variety of murine cancer models in which α- or β-emitting radionuclides were directed to a diverse set of targets. In addition, clinical trials are ongoing to assess safety and efficacy of combined TRT-ICI in patients with cancer. Taken together, research indicates that combining TRT and ICI might improve therapeutic response in patients with cancer. Future research has to disclose what the optimal conditions are in terms of dose and treatment schedule to maximize the efficacy of this combined approach.

Introduction

The development of immune checkpoint inhibitors (ICI) revolutionized cancer treatment (1). Key immune checkpoints are inhibitory T-cell regulators cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1; refs. 2, 3). Antibodies blocking these checkpoints, such as ipilimumab (anti–CTLA-4), pembrolizumab and nivolumab (anti–PD-1), atezolizumab and durvalumab (anti–PD-L1), have shown remarkable efficacy in subsets of patients with cancer (1). However, a significant number of patients do not respond because they fail to generate an effective antitumor immune response (4). To increase the efficacy in non-responding patients, combinations with other therapies that create a more immunogenic tumor microenvironment (TME) and thereby sensitize tumors to ICI, are needed.

There is considerable evidence that ionizing radiation can boost antitumor immunity, mostly originating from studies with external beam radiation therapy (EBRT). However, with EBRT only a limited number of tumor lesions are irradiated. In targeted radionuclide therapy (TRT), a radionuclide is either linked to a carrier molecule, such as a small molecule or a monoclonal antibody directed towards a TME-associated antigen, or accumulates in lesions of interest by physiologic uptake (5). Therefore, TRT results in specific irradiation of all tumor lesions, regardless of location, while sparing healthy tissue. This makes TRT an attractive approach to treat patients with metastatic disease or tumors present in close proximity to radiosensitive organs, to which EBRT would cause severe toxicity (6). TRT has grown significantly over recent years, exemplified by the NETTER-1 trial (7) and subsequent clinical approval of lutetium-177-DOTATATE ([^{177}Lu]Lu-DOTATATE) for the treatment of neuroendocrine tumors, the phase III VISION trial with [^{177}Lu]Lu-PSMA-617 for prostate cancer treatment (NCT03511664), and the development of agents directed to TME-associated targets (e.g., fibroblast activation protein), which are not limited to treat one specific cancer type (8, 9).

The biological effects of radiation depend on many factors including total absorbed dose, absorbed dose heterogeneity, dose rate, and fractionation, which are different between EBRT and TRT and also differ for various types of TRT (6). Definition of these and other common radiation terms are included in **Table 1** (10). EBRT involves a homogeneous beam of X-rays with a low linear energy transfer (LET). EBRT is given to the tumor at a high dose rate often in a fractionated manner. In TRT the absorbed dose rate is 100 to 1,000 times lower, but the exposure time of the tumor is much longer. Furthermore, in TRT, the radiation exposure is heterogeneous, consisting of α-, β-, or Auger particles accompanied or not by X or γ rays. These particles have variable LET, path-length, and half-life, all depending on the radionuclide. β-particles generally have a multicellular range (1–10 mm) and high energy (0.1–1 MeV), α-particles have a cellular range (50–80 μm), and very high energy (5–8 MeV), and Auger electrons have a very short, subcellular range (1–1,000 nm) and low energy (<25 keV; refs. 5, 8, 11). Due to these different physical properties, findings

[1]Department of Medical Imaging, Nuclear Medicine, Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Nijmegen, the Netherlands. [2]Department of Urology, Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Nijmegen, the Netherlands. [3]Department of Radiation Oncology, Radboud University Medical Center, Nijmegen, the Netherlands. [4]Department of Tumor Immunology, Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Nijmegen, the Netherlands. [5]Department of Medical Oncology, Radboud University Medical Center, Nijmegen, the Netherlands.

Corresponding Author: Sandra Heskamp, Radboud University Medical Center, PO Box 9101, 6500 HB, Nijmegen, the Netherlands. Phone: 243-614-511; E-mail: sandra.heskamp@radboudumc.nl

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Table 1. Definition of radiation terms.

Radiation term	Definition	Derived SI units
Absorbed dose rate	Absorbed dose delivered by ionizing radiation per unit of time	Gray per second (Gy s^{-1})
Fractionation	Exposure to radiation in several small fractions	
LET	Amount of energy deposited by ionizations per unit distance as it traverses matter	Kiloelectronvolt per micrometer (keV μm^{-1})
Radioactive half-life	Time required for the radioactive atomic nuclei of a specific radionuclide to decay to one half of their initial activity	Depending on its magnitude: seconds (s) or hours (h)
Range	Average distance that a charged particle travels from its source through matter	α-particles: μm β-particles: mm or cm Auger-electrons: nm
Total absorbed dose	Mean amount of energy deposited per mass in tissue of interest	Gray (Gy), equivalent to J/kg

Note: Data from ICRU report 96 (ref. 10).

regarding immunologic effects of radiation cannot be directly extrapolated to TRT (12).

This review aims to (1) describe the immunomodulatory effects of TRT (2), present an overview of the studies on combined TRT and ICI to date, and (3) provide directions for future research.

Immunomodulatory Effects of External Beam Irradiation

EBRT generates local tumor control through DNA damage–induced cell death, but in rare clinical cases regression of distant unirradiated lesions has been observed: the "abscopal effect" (13, 14). Preclinical studies have shown the role of the adaptive immune system in these abscopal responses. An important mechanism involved is immunogenic cell death (ICD). ICD can be triggered by reactive oxygen species, which are formed upon ionizing radiation (15, 16). To induce ICD-based antitumor immune responses, four steps are required. First, radiation increases the presence of neoantigens and tumor-associated antigens resulting in a so-called *in situ* vaccination effect (17). Second, cytokines and DAMP, such as ATP, high mobility group box 1 (HMGB1), calreticulin, and annexin-A1 are released. In addition, the cytoplasmic DNA-sensing cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) signaling pathway can be activated by cytosolic DNA (18–20). The resulting type I interferon response is essential for dendritic cell (DC) function and is therefore a central player in activation of adaptive immune responses (21–23). Third, professional antigen-presenting cells (APC), such as DCs are recruited into the tumor (24, 25). Fourth, tumor-specific effector T cells are activated and infiltrate the tumor, resulting in a long-lasting antitumor immune response (26). Opposed to these immunostimulatory effects, radiation also induces suppressive signaling, directly or indirectly through activation of the cGAS-STING-IFN1 axis (27). This is characterized by the recruitment of immunosuppressive cells, like regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC; refs. 28, 29), and PD-L1 upregulation (28, 30). In addition, immune cells are inherently radiosensitive, thus radiation can also deplete tumor-infiltrating lymphocytes. Whether radiation induces immunostimulatory or -suppressive effects can be dose-dependent. Taken together, the irradiated TME can be chronically inflamed, but also strongly immunosuppressive (**Fig. 1**). In this regard, a combination of EBRT and ICI could improve treatment efficacy. Various preclinical studies have shown that the combination of EBRT with ICI can improve treatment outcome. A large phase III trial in patients with unresectable stage III non–small cell lung cancer (PACIFIC) demonstrated improved progression-free survival and overall survival (31) and many clinical studies are ongoing [reviewed in (16, 32, 33)]. Nevertheless, the response to EBRT-ICI combination therapy highly depends on tumor type and many open questions remain about the optimal dose, fractionation, and treatment schedule for EBRT-ICI combinations (16).

Immunomodulatory Effects of TRT

TRT with β-emitting radionuclides

Several immunomodulatory effects of irradiation described for EBRT have also been observed for β-emitting TRT. In a murine melanoma model, treatment with iodine-131-anti-melanin ([^{131}I]I-anti-melanin) increased the presence of DAMPs annexin-A1 and calreticulin (34), demonstrating that β-irradiation may induce ICD. In two recent preclinical studies with yttrium-90-NM600 ([^{90}Y]Y-NM600) activation of the STING-IFN1 pathway and production of pro-inflammatory cytokines were observed (35, 36). Modulation of the immune system by β-emitter TRT is also exemplified by changes in immune-cell infiltration. Several studies have observed an increased infiltration of immunostimulatory immune cells into the TME after TRT, for example $CD4^+$ and/or $CD8^+$ T cells (34, 36–39), APCs, natural killer cells (36, 40), and other innate immune cells (34, 36). In addition, the amount of immunosuppressive immune cells, like Tregs or macrophages, was reported to decrease after TRT (37, 39). However, these events are not always consistent between all treatments or models. In a recent study using [^{177}Lu]Lu-albumin the numbers of $CD4^+$ and $CD8^+$ T cells in the TME were reduced and fewer circulating B cells and DCs were found (39). β-irradiation can also trigger immunosuppressive features: two independent studies with [^{177}Lu]Lu-anti–PD-L1 and [^{177}Lu]Lu-anti–integrin-$\alpha_v\beta_3$ have shown upregulation of PD-L1 and increased infiltration of PD-$L1^+$ immune cells to the TME (38, 41). In addition, increased expression of Treg-regulated genes and genes involved in immune tolerance were found after [^{131}I]I-anti-melanin (34). So far, evidence for abscopal effects of radionuclide therapy is limited to one case, where ^{90}Y-radioembolization resulted in complete regression of an untargeted lesion (42).

TRT with α-emitting radionuclides

Although the number of in-depth studies with α-emitter TRT is relatively limited, there is substantial evidence that α-irradiation can induce ICD. Radium-223-dichloride ([^{223}Ra]Ra-dichloride) treatment resulted in calreticulin upregulation and *in vitro* activation of $CD8^+$ T cells (43). Similarly, studies with thorium-227 (^{227}Th)-conjugates targeted to mesothelin (44, 45) and bismuth-213-albumin ([^{213}Bi]Bi-albumin; ref. 46) observed upregulation of DAMPs and DC activation *in vitro*. When mice were vaccinated with [^{213}Bi]Bi-albumin-irradiated cancer cells prior to injection of non-irradiated cancer cells, immunocompetent but not immunodeficient mice were protected from tumor growth for at least two

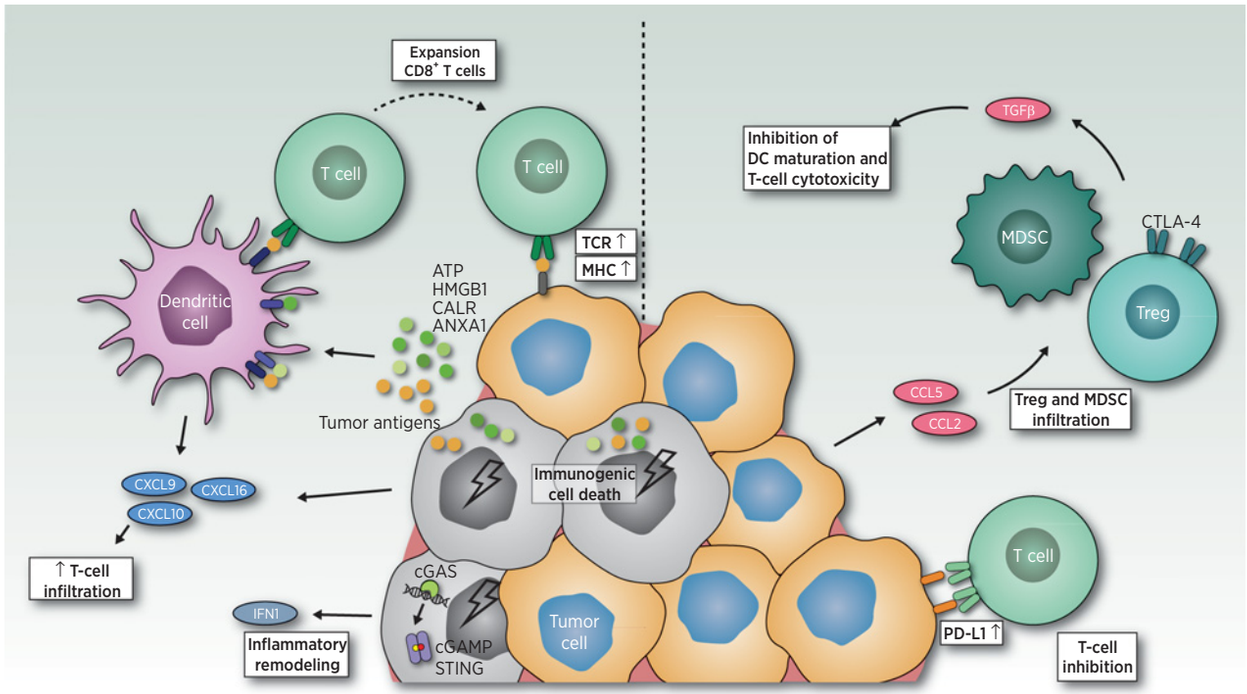

Figure 1.

Immunostimulatory (left) and immunosuppressive (right) effects of radiation. ICD results in the release of DAMPs such as ATP, HMGB1, calreticulin (CALR), and annexin A1 (ANXA1). This release promotes cross-presentation of tumor antigens by DCs, resulting in expansion of $CD8^+$ T cells. The diversity of the T-cell receptor (TCR) repertoire on $CD8^+$ T cells and expression of MHC on tumor cells are increased. The presence of cytosolic DNA triggers cGAS-STING signaling, resulting in IFN1-induced inflammatory remodeling. Release of chemokine (C-X-C motif) ligand 9 (CXCL9), CXCL10, and CXCL16 by tumor cells and DCs promotes T-cell infiltration. On the other hand, tumor cells release immunosuppressive C-C motif chemokine 5 (CCL5) and CCL2, which promote infiltration of Tregs and myeloid-derived suppressor cells (MDSC). Production of TGFβ inhibits DC maturation and T-cell cytotoxicity. PD-L1 overexpression on tumor cells results in T-cell inhibition.

months (46). A similar protective effect after vaccination was found with lead-212 (^{212}Pb) targeted to the melanocortin 1 receptor (47) and radium-224 (^{224}Ra; ref. 48). In the latter study, the protective effect was more pronounced in a highly immunogenic tumor model compared with a weakly immunogenic model. In addition, changes in immune-cell infiltration upon α-irradiation have been reported. One preclinical study showed that melanocortin 1 receptor-targeted ^{212}Pb-TRT increased the number of tumor-infiltrating lymphocytes (47) and increased neutrophil blood counts were found after astatine-211-anti-PARP ([^{211}At]At-anti-PARP) therapy (49). However, a decrease of $CD8^+$ T cells in the TME initially decreased lymphocyte blood counts, and increased infiltration of macrophages and $CD4^+$ T cells were also observed in the latter study (49). In patients with prostate cancer, ^{223}Ra-treatment induced changes in circulating immune cells and immune checkpoint expression. For example, ^{223}Ra can reduce the number of PD-1 expressing CTLs, increase CTLs expression of co-stimulatory or inhibitory (PD-L1, PD-1, and TIM-3) checkpoint molecules, and increase expression of PD-L1 on plasma-derived exosomes (50–52). In addition, solid tumor biopsies of ^{223}Ra-treated patients showed PD-L1 upregulation (52).

Preclinical Studies on Combined TRT and ICI

Combination of β-emitter TRT with ICI

Various preclinical studies have examined combination therapy of ^{177}Lu-TRT with ICI (38, 39, 41, 53–56). For example, Chen and colleagues evaluated a combination of [^{177}Lu]Lu-EB-RGD, targeting integrin $\alpha_v\beta_3$, with anti–PD-L1 in a colorectal cancer model (41). Mice were responsive to both TRT and ICI monotherapy, but combination therapy was superior in delaying tumor growth and prolonging survival. After combined treatment tumors demonstrated reduced glucose metabolism, a lower vascular density, increased apoptosis, earlier necrosis, and reduced tumor cell proliferation, compared with monotherapy. Furthermore, the number of tumor-infiltrating $CD8^+$ T cells significantly increased, while the number of Tregs did not change. Finally, combination therapy generated immunologic memory, as recovered mice rechallenged with cancer cells, rejected the tumors. Other studies have confirmed the superior efficacy of ^{177}Lu-TRT-ICI combination therapy in various tumor models and for different types of ICIs (38, 39, 53–55). Occasionally, efficacy could be explained by increased infiltration of T cells or decreased infiltration of immunosuppressive cells into the TME (38, 39), while others did not observe these changes (54). Furthermore, immunosuppressive features like enhanced PD-L1 expression were reported (38). Efficacy of TRT-ICI combination therapy has also been reported for the β-emitters ^{131}I and ^{90}Y in various tumor models (34–36, 57). Again, the immunologic effects of combined treatment were diverse and sometimes contradictory.

In contrast, several studies do not support the potential efficacy of β-TRT-ICI. For example, combined [^{90}Y]Y-NM600-ICI therapy could not control primary tumor growth in a Lewis lung carcinoma model, while in various other cancer models it could (57). In addition, melanin-targeted ^{177}Lu-TRT combined with anti–PD-1 or anti–PD-L1 in a

melanoma model did not improve therapeutic efficacy compared with ICI monotherapy, while combination with anti–CTLA-4 therapy was successful (34). The authors suggest that immunogenic tolerance dominates the observed immune escape after TRT, as T-cell exhaustion was absent. Finally, one study even reported a negative effect of combining TRT with anti–PD-1 in a breast cancer model, where VEGF-targeted ^{177}Lu-TRT monotherapy was very effective, but the addition of anti–PD-1 diminished the therapeutic effect (56).

Combination of α-emitter TRT with ICI

Combined α-emitter TRT and ICI has shown diverse results. For example, [^{212}Pb]Pb-VMT01 targeting melanocortin 1 receptor induced immunogenicity in an otherwise immunotolerant murine melanoma model (47). In addition, when cancer cells were irradiated *in vitro* before injection, the tumor was sensitized to ICI treatment. Also, combined [^{212}Pb]Pb-VMT01 and ICI (anti–CTLA-4 and anti–PD-1) more effectively inhibited tumor growth compared with TRT or ICI monotherapy. Rechallenge of mice that showed complete response with cancer cells resulted in very slow or absent tumor growth, indicating the presence of adaptive antitumor immunity. Superior efficacy of combined α-emitter TRT and ICI has also been reported for ^{211}At, ^{213}Bi, ^{225}Ac, ^{227Th}, and ^{223}Ra directed to various targets (45, 49, 52, 58, 59) and in the latter studies, T-cell activation was observed (45, 52).

In contrast to these findings, melanin-targeted or PD-L1–targeted ^{225}Ac-TRT-ICI combination therapy was not superior to monotherapies in a melanoma (54) and breast cancer model (60), respectively. In the latter study, combination therapy even reduced survival significantly compared with monotherapies, although it was not reported whether this effect was due to progressive tumor growth or treatment toxicity (60). In addition, the therapeutic efficacy of combined [^{213}Bi]Bi-anti-melanin and anti–PD-1 in murine melanoma was dependent on the treatment schedule, with the most effective growth inhibition when ICI was sandwiched between two doses of TRT or when TRT was administered after ICI (58). Finally, combined [^{213}Bi]Bi-anti-melanin and anti–CTLA-4 therapy did not improve TRT monotherapy in a metastatic melanoma model (61).

Clinical Studies on Combined TRT and ICI

Several trials with combined TRT and ICI are currently ongoing (NCT03658447, NCT03805594, NCT04261855, NCT03457948, NCT03325816). So far, results are only available from the [^{177}Lu]Lu-DOTATATE (Luthathera) phase I study, which showed that combined Lutathera and nivolumab treatment was safe and led to antitumor activity in some patients (62). In addition, two recent case studies with [^{177}Lu]Lu-DOTATATE-ICI therapy in a patient with an aggressive pituitary tumor and [^{177}Lu]Lu-DOTATOC-ICI in a patient with metastatic Merkel cell carcinoma showed safety and antitumor activity (63, 64). Finally, two phase II trials with [^{177}Lu]Lu-TLX250 in combination with ICI for the treatment of metastatic clear cell renal cell carcinoma, are awaited (STARLITE-1 and STARLITE-2).

Discussion and Future Perspectives

Several preclinical studies provide proof-of-concept that TRT-ICI combination therapy can initiate powerful antitumor immune responses. On the other hand, a number of studies have reported negative or contradictory findings. Therefore, a better understanding of radiobiological and immunologic effects is crucial to optimize TRT-ICI combination therapy for clinical translation.

Absorbed dose to the tumor

How cancer cells die and modulate the TME in response to radiation depends on the radiation dose (22). With TRT, the tumor absorbed dose is determined by the administered activity and tracer accumulation and retention in the tumor. The latter is determined by several factors, such as target expression and tumor perfusion. Dosimetry can be used to accurately estimate the absorbed dose in the tumor. However, the absence of dosimetry in most of the published studies makes it impossible to directly relate tumor dose to immune effects and to compare findings between different studies. Furthermore, the extent of immune modulation may depend on the characteristics of the radionuclide used. For example, α-particles contain much higher energies than β-particles and deposit this energy over a much shorter range at different dose rates. In-depth studies investigating the relation between the physical properties of the radionuclide and the radiobiological and immunologic effects are lacking. Therefore, future preclinical TRT studies should include dosimetry to assess dose-effect relationships to elucidate which dose, particle type, or dose rate is preferred for immune activation.

Tumor characteristics

Various characteristics of the tumor and its microenvironment determine the response or resistance to TRT, including tumor solidity, intra-tumor heterogeneity, and mutations of specific cellular mechanisms such as apoptotic pathways (65). First, the intrinsic radiosensitivity of cancer cells is highly heterogeneous both within and between tumor types and depends on the genetic background (66, 67). This affects the type of cell death induced by radiation and, therefore, the extent of ICD and tumor-associated antigen release (15). Second, TME characteristics, such as tumor metabolism, perfusion, hypoxia, and immunogenicity determine responsiveness to both radiation and immunotherapy, and therefore, most likely also affect radiation-induced immune modulation (22, 68). With regards to TRT, hypoxia is of particular interest as the effects of α-radiation are less dependent on oxygen, which potentially makes α-TRT a fitting treatment for hypoxic tumors that are otherwise resistant to conventional EBRT and ICI (5). Taken together, resistance to TRT is determined by various tumor characteristics, which most likely also affect TRTs immunologic effects.

Finally, it remains to be elucidated which types of targets are preferred to enhance immune responses. For example, targeting vasculature did not synergize with ICI therapy (56), and further research should examine how targeting tumor stroma or other components of the TME would impact immunomodulation. Future research should investigate the relation between tumor characteristics and immunologic response to TRT, as these insights are essential to understand which tumor types will benefit from TRT-ICI combination therapies in the clinical setting. Elaborate tumor characterization and careful selection of tumor model would, therefore, improve preclinical studies on TRT-ICI combination therapy.

Treatment schedule

Mechanistically, sequential TRT followed by ICI is expected to be most effective, as TRT will induce ICD resulting in an immunogenic TME followed by ICI to release the brakes of the immune system. Notably, TRT generally exposes the tumor to a low dose rate over a long period, which may result in irradiation of infiltrating immune cells potentially hampering the antitumor immune response. Comparison

of different treatment schedules within one study showed that concurrent administration was more effective than sequential administration (41, 45, 58), but only a limited number of studies included this comparison and elaborated on the underlying mechanism. In all studies, different readouts to measure immune effects were used, such as cytokine release and IHC analysis of tumor tissue, but selecting the appropriate time-window for these methods is challenging. *In vivo* molecular imaging of immune cells and other relevant immune-related targets could help to elucidate the immune effects of TRT in living animals or patients. In addition, only one study examined the effect of fractionation and found that the efficacy of combined TRT-ICI was decreased (63). Future studies are warranted to directly compare treatment schedules and fractionation regimens and to investigate the underlying mechanisms (16). Finally, whether hematologic toxicity upon TRT might limit ICI efficacy remains unexplored. Therefore, ICI dose-finding for TRT combination strategies remains an important issue for future research to reduce ICI toxicity without compromising efficacy (69, 70).

Conclusion

TRT is a rapidly expanding field aiming to treat patients with metastatic cancer and its ionizing effects can potentially boost antitumor immunity. This review provides an up-to-date overview of the immunomodulatory effects of TRT and its efficacy in combination with ICI. However, the results discussed in this review are dispersed and the optimal conditions for clinical translation remain largely unknown. Therefore, in-depth preclinical studies are warranted, to elucidate how absorbed dose, fractionation, and tumor characteristics are related to neoantigen release, ICD induction, and long-lasting antitumor immune responses. These studies will have essential implications for the design of future clinical trials on combined TRT and ICI.

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