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Radiopharmaceuticals as combinatorial partners for immune checkpoint inhibitors

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Abstract

Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of multiple cancer types. However, only a fraction of patients with cancer responds to ICIs employed as standalone therapeutics, calling for the development of safe and effective combinatorial regimens to extend the benefits of ICIs to a larger patient population. Besides exhibiting a good safety and efficacy profile, targeted radionuclide therapy (TRT) with radiopharmaceuticals that specifically accumulate in the tumor microenvironment has been associated with promising immunostimulatory effects that (at least in preclinical cancer models) provide a robust platform for the development of TRT/ICI combinations. Here, we discuss preclinical and clinical findings suggesting that TRT stands out as a promising partner for the development of safe and efficient combinatorial regimens involving ICIs.

Keywords

external beam radiation therapy; immune checkpoint inhibitors; immunogenic cell death; inflammation; nivolumab; pembrolizumab

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Author contributions

JC, LG and JPP conceived the article. JC, LG and JPP wrote the first version of the manuscript, with constructive input from TAC. JC designed display items under supervision by LG and JPP. All authors approve the submitted version of the article.

Declaration of interests

LG holds research contracts with Lytix Biopharma, Promontory and Onxeo, has received consulting/advisory honoraria from Boehringer Ingelheim, AstraZeneca, OmniSEQ, Onxeo, The Longevity Labs, Inzen, Imvax, Sotio, Promontory, Noxopharm, EduCom, and the Luke Heller TECPR2 Foundation, and holds Promontory stock options. The remaining authors have no interests to declare.

Rationale

Immunotherapy (IT) with immune checkpoint inhibitors (ICIs) has transformed the clinical management of some tumors (e.g., melanoma, lung carcinoma and lymphoma) [1,2]. However, ICIs are only active in a fraction of patients when used as standalone therapeutics (ORR: 10–40%, depending on the specific drug and clinical setting), and they are not devoid of serious toxicities [3]. To circumvent these (and other) limitations [4] efforts have been devoted to the development of combinatorial regimens that would safely extend the clinical benefits of ICIs [5]. Among other treatments [6,7], external beam radiation therapy (EBRT) has attracted considerable attention as a modality to unlock the therapeutic potential of ICIs (Box 1). However, not all clinical studies testing EBRT-ICI combinations demonstrated a superiority for this approach as compared to either treatment modality alone [8]. Recent data suggest that targeted radionuclide therapy (TRT) – a radiation modality with expanding oncological applications (Table 1) – may constitute a valid alternative to EBRT as a combinatorial partner for ICIs, at least in some settings [9]. TRT is fundamentally different from EBRT as it consists of the systemic administration of radiolabeled molecules that specifically accumulate in the tumor microenvironment (TME) and irradiate cancer cells (Figure 1) [10]. That said, TRT also exhibit prominent cytotoxic activity against malignant cells, and hence may be harnessed as a debulking strategy prior to the administration of ICIs (which are less effective in patients with a high tumor burden) [11]. Moreover, similar to EBRT, TRT mediates immunostimulatory effects that may convert immunologically inactive (so-called “cold”) tumors into immunologically active (“hot”) lesions that respond to ICIs [9].

This narrative review discusses preclinical and clinical data suggesting that TRT represents a clinically actionable strategy to unlock the full therapeutic potential of ICIs, at least in some oncological indications.

Radiobiological properties of TRT

While EBRT is administered according to specific dose and fractionation regimens with linear accelerators that operate at a predetermined dose rate (the ratio between total dose and time of exposure), TRT is predicated on the use of radionuclides that deliver energy to cancer cells at a continuous low dose rate (CLDR), at least until radiolabeled molecules are metabolized and cleared [10]. This not only has important implications for dosimetry (Box 2), but also implies that TRT efficacy depends on at least three key factors: vector, radionuclide and tumor (Figure 2).

Vector

Some radionuclides have natural tropism for a tissue and hence can be injected as such to irradiate tumors with such anatomical location. For instance, this applies to ^{131}I, which is currently approved to the treatment of thyroid tumors [12], as well as to $^{223}RaCl_2$, which is currently employed for the treatment of bone metastases [13]. That said, TRT is more often based on vectors, including monoclonal antibodies (mAbs) or more often fragments thereof, that enable the accumulation of other non-specific radionuclides in the TME [10]. As an example, this applies to ^{90}Y-ibritumomab tiuxetan, a CD20-targeted

radiopharmaceutical approved by the Food and Drug Administration (FDA) and other regulatory agencies worldwide for the therapy of Non-Hodgkin's lymphoma [14]. A large panel of vectors is currently being tested in the clinic for their capacity to safely and effectively deliver radionuclides to the TME (Table 1). That said, recent efforts appear to favor mAb fragments including single-domain antibodies (sdAbs, 15 KDa), single-chain variable fragments (scFvs, 25 KDa), as well as single or double fragment antigen-binding regions (Fab and Fab´2, 50 and 100 KDa, respectively) over full-size mAbs (150 KDa) (Table 1). This reflects the facts that full-size mAbs circulate in the blood for 3–4 weeks after infusion, are associated with low and heterogeneous tumor uptake (<0.1% injected dose), and hence with a higher propensity to irradiate healthy tissues [15,16].

Conversely, mAb fragments and other small vectors are rapidly cleared from the circulation (in a few hours for sdAbs and scFvs) as they accumulate in the TME, resulting in superior cytotoxicity for malignant cells after a reduced number of administrations [15,17]. Small vectors have been successfully employed for ^{177}Lu-DOTA-TATE (1 KDa), which is currently employed in patients with gastroenteropancreatic neuroendocrine tumors expressing somatostatin receptor 2 (SSTR2) [18], ^{177}Lu-PSMA-617 (1KDa), which is successfully employed for the clinical management of patients with folate hydrolase 1 (FOLH1)-expressing castration-resistant prostate cancer (CRPC) [19], as well as promising fibroblast activation protein alpha (FAP)-targeting radiopharmaceuticals currently under clinical development [20]. (Table 1). Interestingly, vectors can also operate as agonists or antagonists for their receptors, generally offering improved tumor uptake and retention time [21]. As an example, this applies to investigational radiopharmaceuticals based on SSTR2 antagonists, like ^{161}Tb-DOTA-LM3 and ^{177}Lu-DOTA-JR11 [22].

In summary, various TRT vectors are being developed to enable rapid blood clearance in favor of high tumor to normal tissue ratio (and hence superior safety profile).

Radionuclide

Although there are still questions relative to chelation, radiolabeling methods and radionuclide availability, TRT currently offers the possibility to irradiate tumors with (1) electrons (β particles), which have a maximal tissue penetration of 1–2 mm; (2) helium nuclei (α particles), with a maximal range of 50–100 μm; and (3) Auger electrons (AEs), which penetrate living tissue for a maximal distance from the source of 75 μm [23]. Range determines the ability of a specific radiopharmaceutical to irradiate (and hence potentially kill) cells that failed to directly interact with the vector (so-called "cross-fire irradiation") [23]. This is particularly important for at least 2 reasons. On the one hand, a high range (such as that enabled by β particles) may counteract, at least to some degree, an elevated heterogeneity in the expression of the vector receptor, which would otherwise negatively impact efficacy [24]. On the other hand, an elevated range increases the risk for healthy tissue exposure (and hence for toxicity) [24]. The latter is especially relevant for bone marrow toxicities, at least in part reflecting the elevated vascularization of the bone marrow and the elevated radiosensitivity of hematopoietic cell precursors [25].

Radionuclides currently available for TRT also exhibit considerable differences in linear energy transfer (LET), which is a measure of energy deposited by radiation per unit of

length [26]. While radionuclides generally emit with heterogeneous LETs, α particles have a dominant LET in the range of 50–230 KeV/μm, AEs with low energy (<1 KeV) a dominant LET in the range of 4–26 KeV/μm, and β particles a dominant LET in the range of 0.2 KeV/μm, which resembles EBRT based on X- or γ rays [27]. LET directly correlates with cytotoxicity, reflecting the ability of high LET particles to elicit increased amount of macromolecular damage to target cells [28]. Specifically, while macromolecular damage as imposed by low LET TRT mainly originates from water radiolysis and consequent reactive oxygen species (ROS) generation, high LET radiation generally oxidizes macromolecules (including DNA and lipids) [29–31]. directly and in clusters, overall resulting in complex molecular aberrations that are difficult to repair and can occur even in absence of molecular oxygen (which instead is required for ROS generation by low LET particles) [32,33]. Of note, radiopharmaceuticals based on radionuclides such as ^{68}Ga, ^{89}Zr or ^{111}In can also be harnessed for imaging procedures including positron emission tomography (PET) and single-photon emission computerized tomography (SPECT) scans, as part of strategies that combine active treatment with biomarker assessment (so-called “theranostic” approaches) [34].

Thus, besides delivering cytotoxic irradiation to cancer cells interacting with the vector, depending on range and LET, radionuclides can promote the demise of bystander cells, an effect that factor into the safety and efficacy of common radiopharmaceuticals.

Tumor

As for many other therapeutic modalities, the success of TRT heavily relies on tumor-intrinsic features, including (but not limited to) disease burden (which is also associated with limited sensitivity to ICIs) [11] as well as the expression levels and intratumoral distribution of the vector target [35]. Specifically, while tumor volume remains a key parameter in the efficacy of radiotherapy [36], a purely ballistic view according to which short-range emitters should only be used for the management of micrometastases (< 1 mm in diameter) no longer holds. Indeed, the α emitter ^{225}Ac-PSMA-617 has demonstrated remarkable clinical activity in patients with macrometastatic CRPC lesions [37]. This can be explained not only by the improved distribution of radiopharmaceuticals based on small vectors [15], but also (1) by the existence of bystander effects (including immunological effects, see below) between irradiated and non-irradiated components of the TME [38,39 31061069,40], and (2) by the impact of parameters other than disease burden and vector target expression on the efficacy of TRT, including genetic configuration and microenvironmental features. Moreover, it is tempting to speculate that a heterogeneous dose distribution may be beneficial from an immunological perspective as it appears to be the case for spatially fractionated EBRT [41]. Approaches combining short- plus long-range, as well as low- and high-LET particles are currently being clinically evaluated in the attempt to maximize the control of large and heterogeneous lesions in the context of manageable toxicity (Table 1).

In summary, multiple features intrinsic to the tumor have a major impact of the ability of TRT to efficiently control disease progression, but rational TRT design may be envisioned to overcome some of such obstacles.

Immunological effects of TRT

TRT has been shown to elicit a number of immunological effects that (1) may factor into therapeutic efficacy and (2) may be harnessed in combinatorial regimens involving ICIs. These effects emerge from direct interactions between TRT and target malignant cells, bystander effects driven by cellular toxicity as well as by the ability of TRT to minimize exposure of normal tissues and circulating immune cells (Figure 2).

Immunostimulatory signals elicited by TRT

DNA damage as induced by irradiation has been consistently associated with the accumulation of ectopic nucleic acid species in the cytosol of cancer cells, effectively eliciting type I interferon (IFN) response via cytosolic DNA [42], or RNA [43] sensors. In line with this notion, ^{90}Y-NM600 (a radiolabeled alkylphosphocholine that preferentially accumulates in most tumor types), has been shown to unlock tumor-targeting immunity in mouse models of immunologically cold melanomas, neuroblastomas, oral squamous cell carcinomas and breast carcinomas, via a mechanism that depends on the cyclic GMP-AMP synthase (CGAS) signal transducer stimulator of interferon response cGAMP interactor 1 (STING1) [44–46]. Similar results have been obtained in immunocompetent syngeneic models of colorectal carcinoma treated with artificially targeted ^{227}Th conjugates [47], as well as in immunocompetent syngeneic models of fibrosarcoma receiving FAP-targeted ^{177}Lu [48]. Moreover, AEs have been associated with mitotic derangements linked to the formation of micronuclei [49,50], which are potent drivers of type I IFN secretion [51,52]. Finally, at least some radiopharmaceuticals appear to consistently mediate DNA damage upon binding membrane-associated targets [30,39], or via specific mitochondrial targeting [49], as mitochondrial permeabilization has also been associated with multipronged immunostimulatory effects [53]. Whether mitochondrial DNA (mtDNA) or other mitochondrial components that have immunostimulatory effects once released in the cytosol are mechanistically involved in the ability of TRT to activate immune signaling, however, remains to be formally investigated.

It is tempting to speculate that the immunogenicity of TRT may also emerge – at least in part – from bystander effects. Specifically, extracellular vesicles (EVs) released by cancer cells succumbing to radiation have previously been shown to be efficiently taken up by tumor-resident dendritic cells (DCs), culminating in DC-driven type I IFN secretion via STING1 and the activation of tumor targeting immunity [54–56]. In support of this possibility, EVs released by mouse melanoma B16 cells exposed to AE-based TRT have been shown to contain increased levels of potentially interferogenic double-stranded DNA molecules as compared to EVs from untreated B16 cells [57]. Moreover, circulating EVs from patients with metastatic CRPC receiving $^{223}RaCl_2$ reportedly are enriched in transcripts that code for components of the DNA damage response (e.g., *ATM*, *BRCA1*, *CHEK1*) as compared to pretreatment EVs [58]. This observation is particularly relevant given the intimate link between the DNA damage response in malignant cells and inflammation/immunity [59]. Interestingly, the same EVs also appeared to exhibit increased levels of surface-exposed CD274 (best known as PD-L1) [58], a potently immunosuppressive protein that is upregulated by malignant cells exposed to an ongoing immune response [60]. A

similar upregulation of PD-L1 has been documented on the surface of mouse multiple myeloma cells responding to a ^{225}Ac conjugate in immunocompetent syngeneic hosts, a process that was accompanied by increased tumor infiltration by $CD8^+$ T cells [61], as well as on the surface of myeloid cells infiltrating mouse melanomas upon treatment with a ^{177}Lu conjugate [62].

Taken together, these observations suggest that – at least in some settings – TRT can efficiently elicit STING1 signaling in the TME, ultimately resulting in the activation of therapeutically relevant anticancer immune responses. To which extent cross-firing and the consequent killing of bystander, radiopharmaceutical-unbound, cells contribute to the immunogenicity of TRT has not been established yet.

Continuous low dose rate and normal tissue sparing

One of the limitations of current clinical EBRT approaches is that – because of dose and fractionation schedules and/or target volumes – they can result in the unwarranted irradiation of a large number of (circulating or lymph node-resident) immune cells, which obviously hinders beneficial interactions with ICIs [8]. Importantly, similar considerations do not entirely apply to TRT, for at least two reasons. First, especially with the use of small vectors, radiopharmaceuticals are very rapidly cleared from the circulation [15], and their very short emission range generally limits the unwarranted irradiation of healthy tissues [23]. Second, TRT is predicated on the concept of CLDR (see above), with dose rates of 0.1–0.2 Gy h^{-1} (500–1000 lower than those generally used for EBRT). Combined with the delivery of increased TRT activities per administration and with an increase in the number of TRT injections (up to 3–6), these dose rates generate a broad therapeutic window largely enabling DNA repair in healthy cells exposed to TRT, which is expected to complete within 24 hours from damage [63], but not in malignant cells (which invariably exhibit DNA repair defects) [64].

Such a window is obviously not perfect, as demonstrated by (1) the detection of DNA damage in the leukocytes of patients treated with TRT [65,66], or undergoing 18F-FDG PET/CT examinations [67], which demonstrates that TRT exposes at least some leukocytes to irradiation, as well as (2) by the increased therapeutic effects achieved by combining TRT with DNA repair inhibitors [68], which suggests that malignant cells preserve some capacity to repair TRT-elicited DNA lesions in support of survival. That said, TRT is associated with a low incidence of lymphopenia and circulating lymphocyte dysfunction [69–71], which instead can occur and is associated with poor clinical outcome in at least some patient populations receiving EBRT [8]. Finally, the use of a CLDR does not ensure error-free DNA repair, neither in malignant [50,57], nor in normal [72,73] cells. This is particularly important from an immunological perspective as defective DNA repair has been shown to mediate a multitude of immunostimulatory effects of therapeutic relevance beyond STING1 signaling [59]. In line with this notion, no less than ten different human cancer cell lines have been shown to respond to the radiopharmaceutical ^{153}Sm-EDTMP with DNA repair coupled to the upregulation of MHC Class I molecules, which render cancer cells more visible to the immune system, and Fas cell surface death receptor (FAS), which renders them more susceptible to killing by immune effectors [74]. Moreover both ^{131}I and ^{177}Lu

conjugates reportedly elicit a particularly immunostimulatory variant of cell death (i.e., immunogenic cell death, ICD) [75] that is associated with the exposure of eat-me signals like calreticulin (CALR) on the surface of dying cells and the release of immunostimulatory molecules like ATP in the microenvironment, resulting in the initiation of tumor-targeting immunity [62,76]. Supporting the ability of at least some radiopharmaceuticals to elicit *bona fide* ICD, mouse colorectal carcinoma MC38 cells succumbing *in vitro* to ^{213}Bi have been shown to confer prophylactic protection to immunocompetent syngeneic hosts against a subsequent challenge with living cells of the same type [77].

Taken together, these observations suggest that TRT mediates immunostimulatory effects that may provide a robust mechanistic foundation for the development of TRT/ICI combinations. Preclinical and clinical data supporting this possibility have begun to emerge, as further discussed below.

Combining TRT with ICIs: preclinical findings and clinical perspectives

In line with the ability of TRT to drive immunostimulation and hence potentially convert immunologically cold, ICI-resistant tumors into immunologically active neoplasms with restored ICI sensitivity, TRT has been shown to synergize with ICIs in a variety of immunocompetent syngeneic mouse tumor models. These include models of: (1) oral squamous cell carcinoma and melanoma treated with ^{90}Y-NM600 plus dual PD-L1 and cytotoxic T lymphocyte-associated protein 4 (CTLA4) blockage; [44,46], (2) melanoma exposed to a ^{131}I derivative together with a CTLA4 inhibitor [76]; or to a ^{213}Bi conjugate plus a programmed cell death 1 (PDCD1, best known as PD-1) inhibitor [78]; (3) colorectal carcinoma receiving ^{227}Th conjugates combined with a PD-L1 blocker [47], a PD-L1 targeted radiopharmaceutical plus a PD-L1 blocker [79], or a ^{177}Lu conjugate plus a PD-L1 inhibitor; [80]; (4) FAP-expressing fibrosarcoma treated with ^{177}Lu-FAP-2287 along with a PD-1 inhibitor; [48]; (5) melanoma receiving a melanocortin 1 receptor (MC1R)-targeted radiopeptide (^{212}Pb-VMT01) plus dual CTLA4 and PD-1 blockage [81]; (6) mammary carcinoma exposed to ^{177}Lu-DOTA-folate along with a CTLA4 blocker or to a ^{177}Lu derivative plus dual CTLA4 and PD-L1 inhibition [82,83].

Similar signs of cooperative/synergistic interactions have also been documented for TRT and other forms of immunotherapy, like peptide-based vaccination (in models of colorectal carcinoma) [84], adoptive cell transfer (in models of multiple myeloma) [85], and intratumoral cytokine administration (in a model of multifocal melanoma) [86]. Of note, in this latter study, the most efficient treatment to establish systemic disease control was systemic TRT with ^{90}Y-NM600 plus EBRT in a single dose of 12 Gy (to a single lesion) and intratumoral immunocytokine administration (to the same lesion) [86]. These findings delineate scenarios where TRT and EBRT may be rationally combined with immunotherapy in support of superior disease control. Importantly, some studies have highlighted scenarios in which TRT and ICIs fail to cooperate at tumor control. For instance, PD-1 inhibition reportedly fails to ameliorate the antineoplastic effects of ^{90}Y-NM600 in two immunocompetent syngeneic models of prostate carcinoma, likely reflecting the compensatory activation of immunosuppressive $CD4^+CD25^+FOXP3^+$ regulatory T (T_{REG}) cells [87].

An expanding number of clinical studies are testing TRT in oncological indications (Fig. 3), with increasing attention on local and systemic immunological changes imposed by TRT [69–71]. In 11 patients with metastatic CRPC receiving one course of $^{223}RaCl_2$ (standard activity of 50 Bq/Kg body weight), lymphocyte responsiveness to *ex vivo* stimulation remained unchanged within 28 days from treatment, and no patients developed infections in this time frame [69]. Similar results have been obtained in an independent study enrolling 15 patients with metastatic CRPC treated as above and analyzing multiple lymphocyte subsets [70]. Specifically, one course of $^{223}RaCl_2$ at 50 Bq/Kg failed to alter overall frequencies of circulating $CD8^+$ T cells and their subsets (including the frequency of CD27-, CD28- and CTLA4-expressing cells), although it decreased the relative proportion of $CD8^+$ T cells expressing markers of exhaustion like PD-1 [70]. Conversely, up to six courses of $^{223}RaCl_2$ at 55 Bq/Kg resulted in decreased circulating $CD8^+$ and $CD4^+$ T cells in patients with metastatic CPRC, a phenomenon that was accompanied by a relative increase in the proportion of T cells expressing exhaustion markers like PD-1 and hepatitis A virus cellular receptor 2 (HAVCR2, best known as TIM-3), as well as in the proportion of circulating T_{REG} cells [71].

Results from clinical trials testing TRT in combination with ICIs are also beginning to emerge. In a Phase 1b study enrolling 45 men with metastatic CRPC, the addition of the PD-L1 blocker atezolizumab to $^{223}RaCl_2$ resulted in greater toxicity than either agent alone, irrespective of treatment schedule, and with no clear evidence of additional clinical benefit [88]. In this setting, PD-L1 appeared to be upregulated only in the TME of patients receiving atezolizumab prior to TRT, but no significant differences in tumor infiltration by $CD8^+$ T cells were documented [88]. Conversely, the addition of the PD-1 blocker nivolumab to ^{177}Lu-DOTA-TATE generated no concerning safety signals (only one dose-limiting toxicity in the cohort receiving ^{177}Lu-DOTA-TATE at 7.4 GBq every 8 weeks) as compared to either agent alone in 9 patients with neuroendocrine tumors of the lung [89]. Moreover, among 7 patients with measurable disease, one patient exhibited a partial response to this therapeutic approach [89], warranting further investigation in larger clinical studies. Finally, two case reports documented clear clinical benefits (including systemic disease control) for TRT combined with ICIs in one patient with recurrent pituitary carcinoma [90], and one patient with metastatic Merkel cell carcinoma [91]. Based on these promising findings, a number of clinical trials are now open to test TRT in combination with ICIs in patients with a variety of neoplasms (Table 1)

Taken together, these preclinical and clinical observations suggest that TRT may represent a generally safe approach to extend the clinical benefits of ICIs in at least some oncological indications.

Concluding remarks

The ability of ICIs to elicit clinically meaningful tumor-targeting immune responses is currently restricted to a fraction of patients with specific cancer types [1,2]. In this setting, TRT may constitute a viable approach to extend the clinical benefits of ICIs to an increased number of patients and/or additional oncological indications, as amply discussed herein. As in the case of EBRT [8], however, the design of successful TRT/ICI combinatorial regimens

appears to require attentive consideration of multiple factors that may ultimately dictate safety and efficacy. Such factors include not only the implementation of stringent dosimetric protocols (Box 2), but also (1) the identification of biomarkers reliably defining patients that are likely to respond to TRT/ICI combinations [92], (2) the translational potential of currently available mouse models for immuno-oncology [93], and (3) treatment-intrinsic parameters that are insufficiently investigated at preclinical levels, notably radionuclide activity and sequencing of administration [8] (see Outstanding questions).

Indeed, while at least one biomarker of sensitivity to ICIs has been identified and is routinely employed in the clinic (*i.e.*, PD-L1 expression levels for ICIs targeting the PD-1 axis) [92], and the vector target must obviously be expressed by malignant cells for TRT to function [94], whether these and/or other parameters can reliably predict the likelihood of individual patients to obtain clinical benefits from TRT/ICI combinations remains to be investigated [95]. Moreover, the widespread use of transplantable mouse cancer cell lines for the establishment of tumors in immunocompetent syngeneic hosts not only fails to recapitulate the heterogeneity of human malignancies, but also harnesses a system that has been robustly immunoedited as it evaded immunosurveillance in the original host from which these cells have been initially established [8]. In this context, genetically engineered and/or carcinogen-driven models may be better suited to recapitulate the initial dialogues between forming tumors and the host immune system, and hence be superior in terms of translational potential (at least in the setting of immuno-oncology) [96]. As these models are few and often suffer from other limitations [93], it will be important to foster development in this space. While the impact of TRT activity (and hence absorbed dose) on the immunological alterations caused by radiopharmaceuticals has been investigated only in a few studies [44,62], it seems clear that using high *vs* low activities results in different immunological events in the TME. Intriguingly, such immunological differences may at least hypothetically involve the activation of different cell death mechanisms by TRT employed at high *vs* low activity [97], a possibility that remains to be formally addressed. Thus, the design of successful TRT/ICI combinations appear to require further preclinical work aimed at determining which specific TRT activity is best suited for combination with ICIs in a specific oncological indication. Similar considerations can be made for sequencing of administration [80], a parameter that is often underestimated during the design of novel combinatorial regimens. Indeed, while the concomitant administration of two is expected to be superior to the administration of either when the intent is purely cytotoxic, tumor-targeting immunity is a step-wise reaction, implying that concomitant design may ultimately be suboptimal [8]. Systematic preclinical evaluation of the best treatment sequencing hence stands out as a *sine qua non* for the development of successful TRT/ICI combinations.

In summary, TRT stands out as a promising combinatorial partner for ICI, at least in some oncological indications. It is tempting to speculate, but remains to be formally investigated, that (similar to EBRT) conventional TRT approaches may need to be revisited to enable improved interactions with ICIs [8]. Additional preclinical and clinical work formally validating this possibility is urgently awaited.

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Box 1.

EBRT as a combinatorial partner for ICIs

External beam radiation therapy (EBRT) has attracted consistent attention as a clinically viable strategy to extend the benefit of immune checkpoint inhibitors (ICIs) to a larger fraction of patients with cancer [8]. This not only reflects the widespread availability of linear accelerators for EBRT and its well-recognized and hence predicable safety profile [8,98], but also the notion that (at least when delivered focally and according to specific doses and fractionation schedule) EBRT can mediate robust immunostimulatory effects [99,100]. However, not all prospective clinical trials testing EBRT in combination with ICIs reported to date have documented a clinical benefit for the combinatorial regiments over EBRT or ICIs employed alone [8]. Thus, while on the one hand a positive interaction between EBRT and ICIs has been observed in cohorts of patients with non-small cell lung carcinoma (NSCLC) [101–103], and esophageal or gastroesophageal junction cancer who received neoadjuvant chemoradiotherapy [104], the same did not hold true in cohorts of patients with glioblastoma [105,106], and head and neck squamous cell carcinoma [107]. While multiple reasons can be invoked to explain these contradicting results, including tumor type and/or anatomical location, the insufficient (preclinical and early clinical) investigation of factors including EBRT dose and fractionation, EBRT target volume, and sequencing of EBRT vs ICI administration is likely to contribute a non-negligible detrimental role in this context [8]. Emerging evidence suggests indeed that conventional EBRT protocols might need to be partially revisited to enable superior cooperativity with ICIs. It is likely, but remains to be formally determined, that similar considerations also apply to TRT.

Box 2.

Principles of TRT dosimetry

The biological effects of external bear radiation therapy (EBRT), including tumor control probability (TCP) as well as normal tissue complication probability (NTCP) are directly proportional to absorbed dose (Gy) [108]. Along similar lines, external beam radiation therapy (EBRT) dose (and fractionation schedule) has a major impact on its ability to enable tumor-targeting immune responses [8,109–112]. However, while essential in tailoring targeted radionuclide therapy (TRT), dose assessment is not routinely performed in TRT-treated patients, and treatment schedules are still based on injected activities (as measured in Bq), which cannot be directly correlated with TCP or NTCP [113]. TRT dosimetry as based on the medical internal radiation dose (MIRD) formalism is indeed a more complex and time-consuming task with several constraints for the patients then conventional EBRT dose assessment [10]. Total dose and dose rate depend indeed on pharmacodynamic features of the radiopharmaceutical (e.g., uptake and retention in the tumor, as dictated for instance by vascularization and vector target expression), as well as on (1) physical properties of the radionuclide (e.g., particle nature and energy, half-life, etc..), and (2) anatomical aspects (e.g., tumor burden, distance between the tumors and other organs, etc..) [10]. Thus, for the same TRT, absorbed doses are highly variable not only across different patients, but also across different neoplastic lesions from the same individual, ranging from <10 Gy to > 100 Gy [114,115]. Importantly, such discrepancies in absorbed dose considerably complicate the establishment of dose-response curves. However, it seems that beyond a specific dose threshold, TRT efficiently curtails tumor progression [116]. A similar threshold can be established for TRT toxicity on normal tissues including the kidney and bone marrow [116]. These data are essential as they demonstrate that a minimal dose of 80–100 Gy is required for efficient tumor control by TRT, but that further escalating therapy may not be beneficial. Moreover, these observations highlight the complexity to achieve therapeutically active doses in all neoplastic lesions from the same individual, which – at least in some cases – may not be possible despite repeated treatment cycles (which also increase the risk for normal tissue toxicity) [117]. Notably, TRT dose rates are generally very low (0.1 Gy h^{-1}) as compared to standard EBRT dose rates (2 Gy min^{-1}), which may have advantages from an immunological perspective (see also main text).

Outstanding questions

Can standardized dosimetric protocols be used in clinical TRT routine?

Can robust predictive biomarkers of responsiveness to TRT/ICI combinations be identified?

Can novel mouse models be developed that faithfully mimic oncogenesis, tumor progression, and treatment responses in the context of natural immunosurveillance?

What are the best administration schedules for combining TRT and ICIs in patients with cancer?

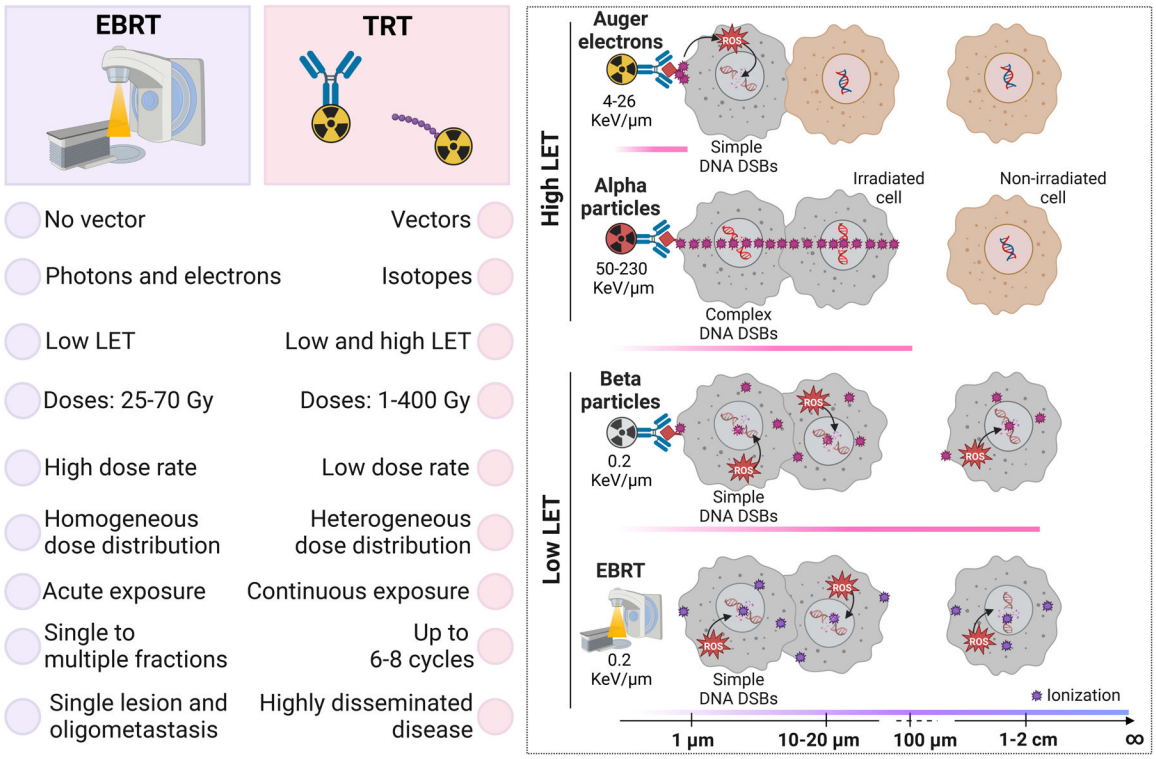

Figure 1. Biophysical principles of TRT vs EBRT.

While both targeted radionuclide therapy (TRT) and conventional (photon- or electron-based) external beam radiation therapy (EBRT) rely on the exposure of malignant cells to ionizing radiation, resulting in direct and reactive oxygen species (ROS)-driven damage to DNA and other macromolecules, these treatment modalities are fundamentally different with respect to a number of parameters, which render them suitable for the management of different oncological scenarios. These parameters include not only the type of radioactive emitters employed, which directly determine linear energy transfer (LET) and range, but also total dose, dose-rate, dose distribution, type of exposure and administration schedule. DSB, double strand break.

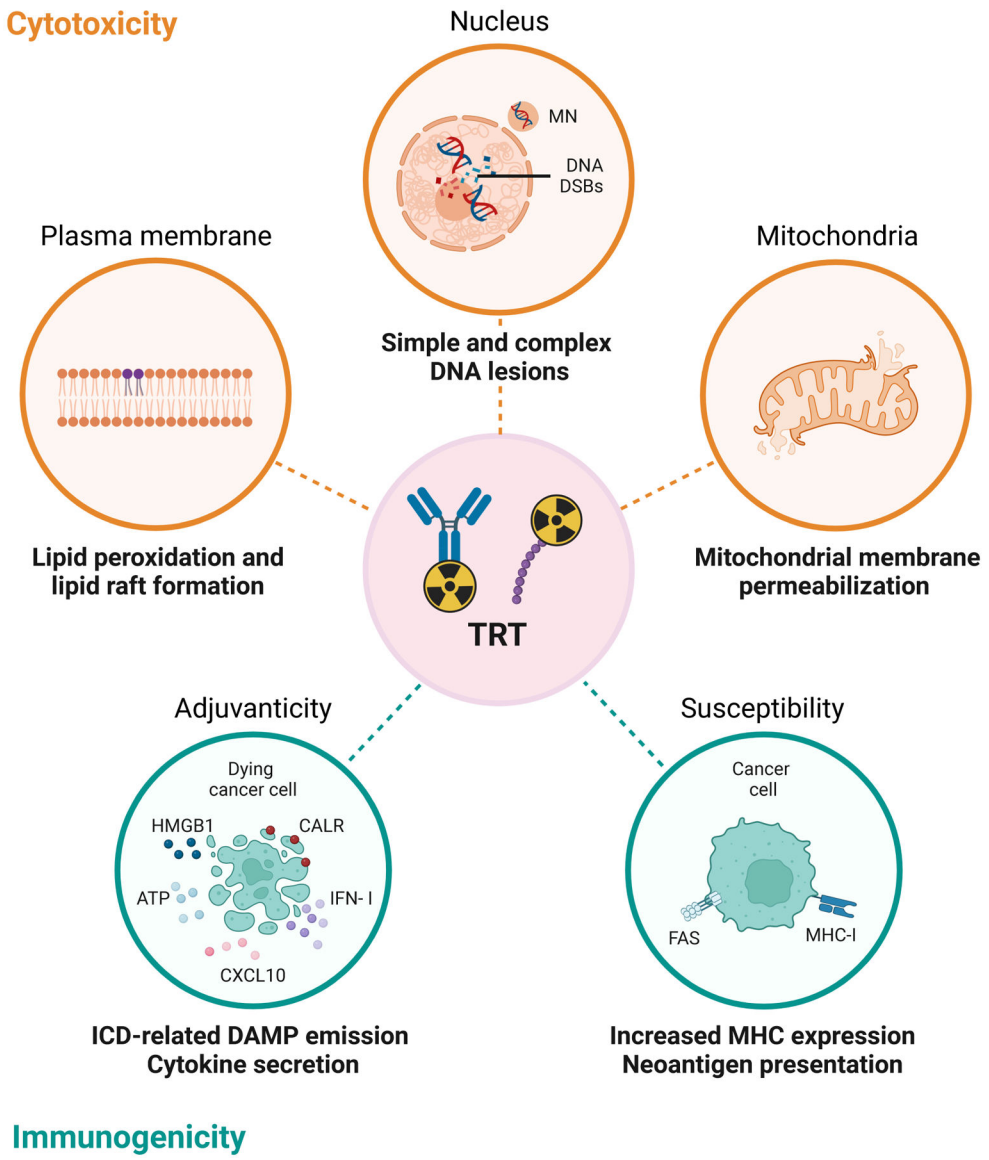

Figure 2. Molecular mechanisms of TRT-driven cytotoxicity and immunogenicity.

Targeted radionuclide therapy (TRT) kills malignant cells by interacting with multiple subcellular compartments, including: (1) the plasma membrane, resulting in lipid peroxidation and the formation of lipid rafts; (2) the nucleus, culminating with the accumulation of DNA double stand breaks (DSBs) and micronuclei (MN), and (3) mitochondria, resulting in mitochondrial membrane permeabilization in support of apoptotic cell death. In addition, TRT causes (1) the immunogenic cell death (ICD)-associated emission of damage-associated molecular patterns (DAMPs) and immunostimulatory cytokines from dying cancer cells, hence increasing their adjuvanticity, and (2) the upregulation of MHC Class I (MHC-I) and death receptors (DRs) on cancer cells resisting TRT cytotoxicity, which improves their susceptibility to recognition and lysis by immune effector cells. CALR, calreticulin, CXCL10, C-X-C motif chemokine ligand 10; FAS, Fas cell surface death receptor; HMGB1, high mobility group box 1; IFN-I, type I interferon.

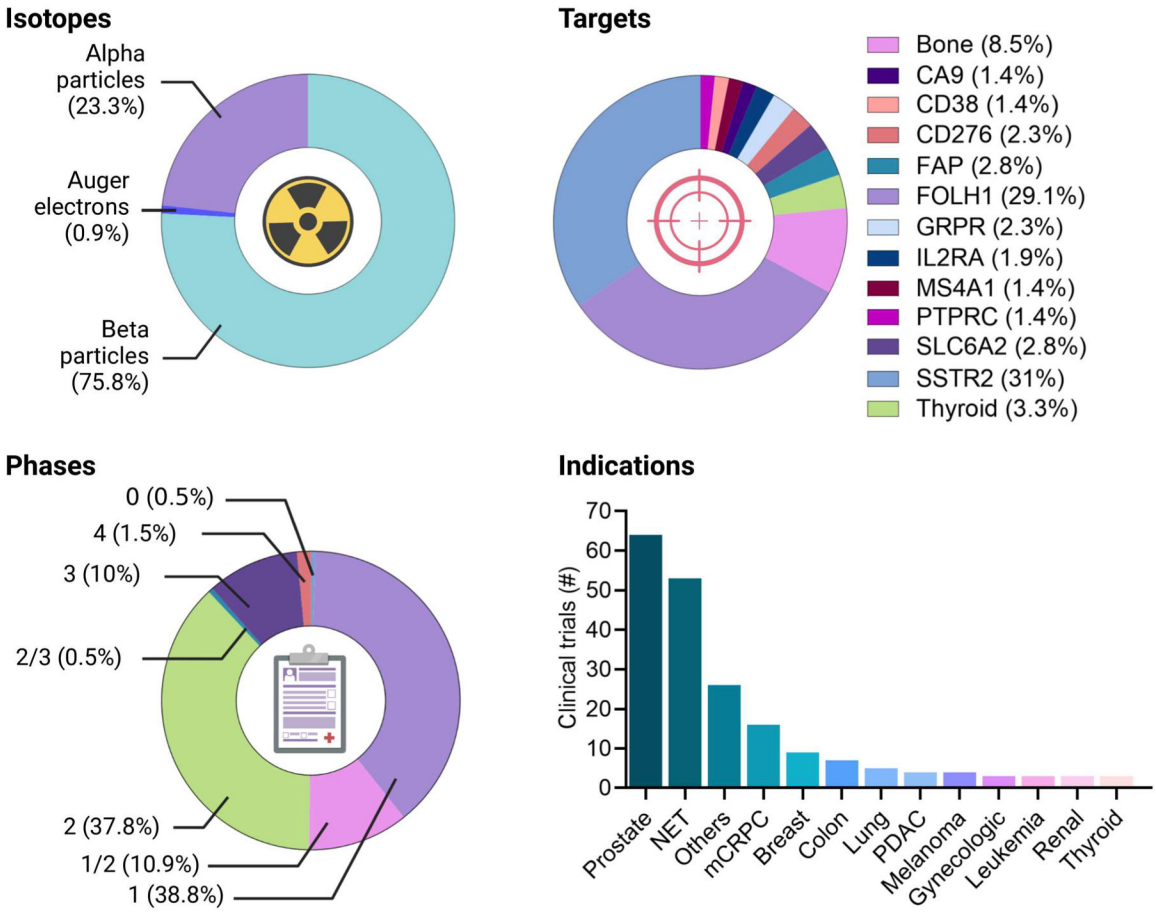

Figure 3. Clinical studies currently investigating TRT in patients with cancer.

Overview of clinical trials based on targeted radionuclide therapy (TRT) registered at www.clinicaltrials.gov with a “Not yet recruiting”, “Recruiting”, “Enrolling by invitation” or “Active, not recruiting” status as per June, 1st 2023. CA9, carbonic anhydrase 9; FAP, fibroblast activation protein alpha; FOLH1, folate hydrolase 1; GRPR, gastrin releasing peptide receptor; IL2RA, interleukin 2 receptor subunit alpha; mCRPC, metastatic castration-resistant prostate cancer; MS4A1, membrane spanning 4-domains A1; PDAC, pancreatic ductal adenocarcinoma; PTPRC, protein tyrosine phosphatase receptor type C; SLC6A2, solute carrier family 6 member 2; SSTR2, somatostatin receptor 2.

Table 1.

Ongoing clinical trials combining TRT and ICIs[a,b]

Indication	TRT	ICI	Phase	Status	Note	Clinical trial number
Hepatic metastases NET	^{177}Lu-DOTA-TATE	Pembrolizumab	2	Recruiting	Comparison with 90^{Y} microsphere radioembolization	NCT03457948
mCRPC	^{177}Lu-PSMA-617	Pembrolizumab	1	Active, not recruiting	Dose- and schedule-finding study	NCT03805594
mCRPC	^{177}Lu-PSMA-617	Ipilimumab Nivolumab	2	Recruiting	Up to 6 cycles at 7.5GBq per cycle	NCT05150236
mCRPC	$^{223}RaCl_2$	Nivolumab	1/2	Recruiting	Assessment of ctDNA reduction after 6 weeks of treatment	NCT04109729
mCRPC	$^{223}RaCl_2$	Avelumab	1/2	Recruiting	Up to 6 cycles (activity N/A)	NCT04071236
mCRPC	$^{223}RaCl_2$	Pembrolizumab	2	Recruiting	At least 3 cycles (activity N/A)	NCT03093428
mCRPC	^{225}Ac-J591	Pembrolizumab	1/2	Recruiting	One dose at 65 or 90 KBq/Kg in optional further combination with an AI	NCT04946370
MCC	^{177}Lu-DOTA-TATE	Pembrolizumab	2	Not yet recruiting	Up to 4 cycles at 7.4GBq per cycle	NCT05583708
MCC	^{177}Lu-DOTA-TATE	Avelumab	1/2	Recruiting	Comparison with EBRT (2 fractions)	NCT04261855
NETs	^{177}Lu-DOTA-TATE	Nivolumab	2	Recruiting	Up to 4 cycles at 7.4GBq per cycle	NCT04525638
Renal cancer	^{177}Lu-Gerentuximab	Nivolumab	2	Recruiting	MTD assessment with 3.1 or 4.1GBq	NCT05239533
SCLC	^{177}Lu-DOTA-TATE	Tislelizumab	1	Recruiting	Safety study in further combination with carboplatin and etoposide	NCT05142696

[a] Abbreviations: AI, androgen inhibitor; ctDNA, circulating tumor DNA; EBRT, external beam radiation therapy; ICI, immune checkpoint inhibitor; MCC, Merkel cell carcinoma; mCRPC, metastatic castration-resistant prostate cancer; MTD, maximum tolerated dose; N/A, not available; NET, neuroendocrine tumor; SCLC, small cell lung cancer; TRT, targeted radionuclide therapy.

[b] Source www.clinicaltrials.gov, limited to studies with “Not yet recruiting”, “Recruiting”, “Enrolling by invitation” and “Active, not recruiting” status.