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Combined Targeted Radiopharmaceutical Therapy and Immune Checkpoint Blockade: From Preclinical Advances to the Clinic

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Running Title: Progress in Combined TRT and ICI

ABSTRACT

Immune checkpoint inhibitors (ICI) have revolutionized cancer care, but many patients with poorly immunogenic tumors fail to benefit. Preclinical studies have shown that external beam radiotherapy (EBRT) can synergize with ICI to prompt remarkable tumor regression and even eradication. However, EBRT is poorly suited to widely disseminated disease. Targeted radiopharmaceutical therapy (TRT) selectively delivers radiation to both the primary tumor and metastatic sites, and promising results achieved with this approach have led to regulatory approval of certain agents (ex. ^{177}Lu-PSMA-617/Pluvicto™ for metastatic prostate cancer). To further improve therapeutic outcomes, combining TRT and ICI is a burgeoning research area, both preclinically and in clinical trials. Here we introduce basic TRT radiobiology and survey emerging and clinically translated TRT agents that have been combined with ICI.

INTRODUCTION

Blocking suppressive interactions that inhibit antitumor immune activation with antibody-based immune checkpoint inhibitors (ICI) has led to unprecedented and durable responses in patients with numerous cancer types (*1*). Most notable of these are antibodies to the PD-1/PD-L1 axis (programmed death receptor 1 and its ligand) and to CTLA-4 (cytotoxic T lymphocyte antigen 4), which may be used together given the non-redundant roles of these mediators in tumor immune evasion (*2*). However, poorly immunogenic tumors in particular may not respond to ICI, and for those that do eventual immune escape often occurs (*1,3*).

In rare cases, combining external beam radiation therapy (EBRT) and ICI has prompted regression of non-irradiated metastases (the abscopal effect) in patients (*4*). Further, preclinical studies demonstrate that EBRT can induce responses in tumors initially refractory to ICI and improve ICI effectiveness in responsive 'hot' tumors (*2,5*). EBRT causes accumulation of damaged DNA in the tumor cell cytosol, which prompts a type I interferon response via activation of the stimulator of interferon

genes (STING) adaptor protein (*6*). These signals, with concurrent upregulation or secretion of damage-associated molecular patterns (e.g. high mobility group box protein 1) due to tumor cell death (*7*), may stimulate dendritic cells (DCs) to cross-prime naïve $CD8^+$ T cells with released tumor antigens (*8*). The irradiated tumor and tumor draining lymph nodes become hubs for antigen presentation (*9*), leading to diversification and clonal expansion of the T-cell receptor (TCR) repertoire (*2*). Surviving tumor cells are sensitized to immune elimination via upregulation of immune susceptibility markers (e.g. MHC-I) and the display of tumor neoantigens (*10*) as well as altered expression of checkpoint molecules such as PD-L1 (*11*). Together, these tumor microenvironment (TME) modifications increase ICI efficacy when combined with radiotherapy.

Although low dose EBRT (2-3 Gy) can be administered safely to large fields or the whole body, it induces systemic lymphocyte depletion that may confound effective antitumor immunity (*12*). Also, delivering higher targeted EBRT doses to multiple small tumors or micrometastatic disease may not be feasible. Given these drawbacks, targeted radiopharmaceutical therapy (TRT), which systemically delivers radiation via a peptide, antibody, or other ligand carrier targeted to a tumor receptor or antigen, is more suitable. The radionuclide coupled to these carriers mainly decays via α or β particles, with or without low energy (e.g. Auger) electrons. Radionuclide selection is largely guided by matching the decay half-life to the biological half-life of the carrier molecule (*13*). As in EBRT, linear energy transfer (LET), the energy deposited per unit distance, dictates the extent of tissue and tumor penetration for TRT emissions. Alpha (α) particles have a LET of 50-230 keV/μm with tissue penetration depth of 50-100 μm, whereas β emissions have a LET of 0.2 keV/μm with a maximum penetration depth ≈ 12 μm, and Auger electrons have a LET of 4-25 keV/μm and a tissue penetration depth maximum < 1 μm (*13*). Radionuclides decaying by α particles and Auger electrons may be more apt to induce cell death and phenotypic modulation in individual tumor cells if internalized (*14*). Yet these radionuclides may be less suited towards targeting larger tumors or may fail to modify the TME immune milieu as

limited dose reaches the tumor stroma. Those with longer-range emission (e.g. β particles) target tumor cells via 'crossfire' radiation - emissions from TRT bound to adjacent cells (*15*). As such, β particles are less likely to effectively target small tumor cell clusters or circulating tumor cells. Two peptide TRT ligands most under study in current/published clinical trials and recently approved by the United States Food and Drug Administration (FDA) and/or European Medicines Agency (EMA) utilize the β-emitter lutetium-177 - ^{177}Lu-DOTATATE (Lutathera®) to treat neuroendocrine tumors (NETs) and ^{177}Lu-PSMA-617 (Pluvicto™) for metastatic castration-resistant prostate cancer (mCRPC). Importantly, it has been demonstrated *ex vivo* that dose-equivalent β TRT can achieve comparable STING activation to EBRT (*16*), which is crucial to its translational potential in combination with ICI. However, TRT-induced alterations to antitumor immunity have only begun to be elucidated (*17,18*). From our understanding of EBRT-mediated effects and preliminary studies with TRT, a putative mechanism for TRT and ICI cooperation is shown in Figure 1.

At present, most studies involving combined TRT and ICI have been conducted preclinically, with minimal Phase I and case report data available, although numerous clinical trials are ongoing. In this review, we will discuss the recent progress of TRT + ICI therapy and future considerations to optimize clinical efficacy.

PRECLINICAL STUDIES SUGGEST SYNERGY BETWEEN TRT AND ICI

Peptide TRT + ICI

Peptide-based TRT agents have been widely investigated due to their greater solid tumor penetration and lower capacity for immunogenicity relative to antibodies or antibody fragments (*19*). Lutathera® targeting somatostatin receptor subtype 2 (SSTr2) was the first peptide TRT agent to be FDA approved in 2018. Other cell surface proteins overexpressed in malignancy and that facilitate angiogenesis (integrin $\alpha_v\beta_3$) and/or metastatic spread (integrin $\alpha_4\beta_1$/VLA-4) have received increased

interest (*20,21*). Recently, pioneering peptide TRT studies directed to these targets improved therapeutic outcomes in combination with ICI in B16F10 melanoma (*22,23*) and MC38 colorectal cancer (*24*). Choi *et al* demonstrated in B16F10 melanoma that lutetium-177 labeled LLP2A, a peptidomimetic selective to VLA-4, with dual ICI (anti-CTLA-4 and anti-PD-1 or anti-PD-L1), significantly improved survival relative to either TRT or dual ICI (*22*). Combining a modified RGD peptide to bind integrin $\alpha_v\beta_3$ labeled with lutetium-177 paired with anti-PD-L1, Chen *et al* showed that concurrent administration significantly reduced tumor volume and extended survival versus a sequential approach in MC38 colorectal cancer (*24*). A significant drawback of peptide TRT is relatively rapid clearance from the blood, limiting tumor accumulation and response duration (*25*). To extend circulation lifetime, carrier PEGylation (*26*) and incorporation of albumin-binding moieties (*24,25*) have been explored.

Antibody and Antibody Fragment TRT + ICI

As antibodies bind with high affinity and selectivity to their epitope, in addition to their commercial availability, they have been extensively implemented for TRT ('radioimmunotherapy', RIT) (*27*). Maximum tumor accumulation and blood clearance is typically not achieved until 5-10 d post-injection (*28*). As such, long-lived radionuclides (^{177}Lu, $t_{1/2}$: 6.7 d, ^{225}Ac, $t_{1/2}$: 9.9 d) may be optimal to deliver a therapeutic dose. Due to the long circulation time of full-length antibodies (serum half-life of 1-3 weeks (*27*)), non-target tissues may receive substantial radiation doses. Alternatively, radiolabeled engineered antibody fragments (e.g. minibodies, single domain antibodies) may be utilized. Antibody fragments also exhibit increased tumor penetration, albeit at the expense of lower tumor uptake due to more rapid blood clearance. However, antibody fragments of MW<60 kDa clear primarily through the kidney, which can result in renal toxicity (*27*).

Jiao et al reported notable tumor growth delay and improved survival for melanoma-bearing mice receiving an anti-melanin antibody (h8C3) labeled with the α-emitter ^{213}Bi + anti-PD-1 relative to

anti-PD-1 alone (*29*). In a follow-up study with longer-lived isotopes (^{177}Lu,^{225}Ac) and to deduce the mechanisms involved, ^{225}Ac-h8C3 provided no improvement ± anti-PD-1 (*30*). Although low-dose ^{177}Lu-h8C3 + anti-PD-1 significantly slowed tumor growth and improved survival, no difference was observed in tumor-infiltrating T cells (TILs) versus untreated controls. A fully-human anti-mesothelin antibody labeled with the α-emitter ^{227}Th (^{227}Th-TTC) spurred multiple immunostimulatory pathways in murine colorectal cancer expressing human mesothelin that increased $CD8^+$ T cell infiltration while reducing $CD4^+$ T cells, the effects of which were augmented by anti-PD-L1 (*31*). Depletion of suppressive cells in the TME by β RIT (^{177}Lu-anti-CD11b) increased dual ICI (anti-CTLA-4 and anti-PD-1) efficacy in a glioma model, without other significant alterations to the TME immune cell composition (*32*). Others have utilized ICIs themselves as radioimmunotherapy agents, particularly anti-PD-L1 given its demonstrated clinical prognostic value in determining responsiveness to PD-1/L1 therapy (*33*). PD-L1 monoclonal antibodies have been labeled with both α (^{213}Bi (*34*))- and β (^{177}Lu (*35*))-emitters to simultaneously invigorate an antitumor TME milieu and deplete tumor cells. Enhanced therapeutic efficacy versus the isotype or unlabeled control was evidenced against human melanoma xenografts (*34*) and mouse colorectal cancer (*35*).

Small Molecule TRT + ICI

Much of the current work with small molecule TRT involves optimizing PSMA-targeted ligands to maximize tumor uptake while diminishing toxicity. PSMA is a hallmark antigen expressed by most prostate cancers, and its upregulation is associated with castration resistance and metastasis (mCRPC) (*36*). PSMA-617 is the lead TRT candidate under study preclinically and was FDA-approved on March 23, 2022. Although Phase III clinical results of ^{177}Lu-PSMA-617 in mCRPC were impressive (*37*), from a meta-analysis, 30% of patients are refractory to β therapy (no decline in serum prostate specific antigen, PSA) (*38*). The effectiveness of targeted α therapy (^{225}Ac-PSMA-617) can vary among these patients, given the disease state (early vs. late mCRPC), the extent of pretreatment, and metastatic profile (*39,40*). PSA

reduction with ^{225}Ac-PSMA-617 in TRT-naïve tumors can be more substantial than those reported for ^{177}Lu-PSMA-617, as expected given the greater LET of ^{225}Ac (*40*). In a murine prostate cancer model, Czernin *et al* aimed to exploit potentially increased tumor immunogenicity spurred by ^{225}Ac-PSMA-617 by adding anti-PD-1 (*41*). The combination synergized to improve survival and delay time to progression, but the immune correlates were not reported.

Directing α-therapy to the tumor cell nucleus prompts extensive DNA double strand breaks, inducing antitumor T cell activation that can be invigorated by ICI. Dabagian *et a*l utilized an astatine-211 (^{211}At)-labeled small molecule inhibitor of PARP, a class of nuclear enzymes that facilitate double strand break repair (*42*). With anti-PD-1 in a mouse glioblastoma model, the combination nearly doubled the mean progression free duration of ICI (65d vs. 36d) and led to complete response in all mice, compared to 60% of mice receiving ICI alone. Interestingly, TRT increased macrophage recruitment while depleting circulating T cells. The authors postulate that the improved therapeutic effect of the combination is due to activated macrophage proinflammatory signaling maintained by blocking PD-1.

TRT CAN SENSITIZE 'COLD' TUMORS TO ICI

The key promise of TRT + ICI is the capability to render immunologically 'cold' tumors (unresponsive to ICI alone) vulnerable to ICI via radiation-induced immune activation. Major cancer types resistant to ICI include colon, prostate, and breast cancer, although varied responses can occur even amongst tumors within the same patient (*1*). These tumors display minimal T cell infiltration and substantially impaired preexisting antitumor immunity. Radiation has been shown to elicit antitumor immune responses through induction of a cGAS-STING mediated type I interferon response, which is dose-dependent (*17*). From preclinical experiments, antitumor immunomodulation via EBRT occurs even at low doses (2-5 Gy) (*43*). This observation could be leveraged by rationally designed TRT to deliver low dose sufficient for immunostimulation while sparing radiosensitive lymphocytes systemically.

Patel *et al* recently employed this approach to evaluate the alkylphosphocholine analog NM600 labeled with the β-emitter ^{90}Y in combination with anti-CTLA-4 in multiple ICI-resistant tumor models (Fig. 2) (*17*). When low dose (2.5-5 Gy) ^{90}Y-NM600 was received by the tumor as determined from ^{86}Y-NM600 PET via a Monte Carlo dosimetry software, survival was significantly improved compared to ICI alone. Dramatic responses were observed, with up to two-thirds of mice receiving the combination experiencing complete response and tumor-specific T cell memory, compared to none in either single treatment group. No signs of toxicity were seen. The combined treatment increased T cell infiltration and mitigated exhaustion. Intriguingly, the authors showed that unlike a previous report utilizing moderate dose, single-tumor directed EBRT (*2*), low dose TRT did not expand TCR diversity despite the clonal expansion of TILs. By combining these modes of EBRT and TRT, their non-redundant effects better potentiated response to anti-CTLA-4, allowing for control of a secondary (received no EBRT) tumor and optimal survival relative to either TRT or EBRT + anti-CTLA-4.

CLINICAL TRIALS OF TRT + ICI

Although combination TRT + ICI clinical trials are ongoing, there are few recent reports of intentional TRT sensitization to ICI in the available clinical literature, enabled by compassionate use authorization. Two case reports demonstrate impressive therapeutic efficacy with TRT+ ICI in patients with metastatic Merkel cell carcinoma (MCC), an aggressive skin cancer, who progressed on first-(avelumab/anti-PD-L1) and/or second-line (ipilimumab/anti-CTLA-4 + nivolumab/anti-PD-1 + EBRT) therapies (*44,45*). Half of MCC patients may not respond or acquire resistance to ICI (*45*), yet MCC often expresses somatostatin receptors, allowing for targeting via ^{177}Lu-DOTATATE, a modified octreotide. A patient with heavy MCC metastatic burden who received ^{177}Lu-DOTATATE and resumed anti-PD-L1 demonstrated a response within days, with near complete response observed one month after initiation (Fig. 3) (*44*). In a separate report, a patient receiving the related ^{177}Lu-DOTATOC and resuming

ipilimumab + nivolumab experienced partial response that was maintained through the time of the manuscript submission (5 months) (*45*). Although the GoTHAM trial (NCT04261855) to evaluate ^{177}Lu-DOTATATE + avelumab for metastatic MCC has begun, survival data is unlikely to be available until 2024.

Despite the rapid pace of TRT development, most exploratory clinical trials combining TRT and ICI utilize established TRT agents (^{177}Lu-DOTATATE, ^{177}Lu-PSMA-617, $^{223}RaCl_2$). Those that are ongoing/have published results within the past four years are highlighted below.

^{177}Lu-DOTATATE (Lutathera®) + ICI

^{177}Lu-DOTATATE is the culmination of more than 20 years of somatostatin analog development for NET treatment, with wide clinical adoption following the phase III NETTER-1 trial (NCT01578239) (*46*). Somatostatin receptor expression has also been identified in a minority of small-cell lung cancer (SCLC) (*47*) . Due to its aggressiveness (5-year overall survival rate <10%), SCLC often presents once disseminated and is ultimately refractory to chemotherapy (*48*). As a subset of extensive-stage SCLC patients display durable responses to nivolumab, Kim *et al* conducted a phase I trial (NCT03325816) combining ^{177}Lu-DOTATATE and nivolumab at two TRT dose levels in patients with relapsed/refractory SCLC, SCLC remaining stable following first-line chemotherapy, or pulmonary NETs (*48*). Of the 7 patients with disease measurable by CT, one with extensive-stage SCLC showed partial response, and two others with atypical carcinoid displayed stable disease. The SCLC patient who experienced partial response showed avid tumor uptake of ^{68}Ga-DOTATATE. However, unlike observations mainly from extrapulmonary NETs (*46*), the extent of ^{68}Ga-DOTATATE uptake may not predict TRT efficacy in lung NETs/SCLC (*47*).

^{177}Lu-PSMA-617 (Pluvicto™) + ICI

Approximately one-third of patients do not respond to ^{177}Lu-PSMA-617 despite extensive PSMA expression evident from PET (*49*). In a recent phase II trial (NCT02787005), pembrolizumab (anti-PD-1)

demonstrated encouraging efficacy in pre-treated, bone-predominant mCRPC (*50*). Prasad *et al* observed 40% PSA decline in a 90-year-old patient with advanced mCRPC who initiated ^{177}Lu-PSMA-617 while receiving pembrolizumab for locally advanced squamous cell carcinoma (*49*). To interrogate potential synergy between ^{177}Lu-PSMA-617 and pembrolizumab, the phase Ib/II PRINCE trial (NCT03658447) was initiated. Although the study is ongoing, an interim report details a ≥ 50% PSA decline rate of near 75% among 37 patients (*51*). Seven of nine patients with measurable disease exhibited partial responses. Therapy with ^{225}Ac-PSMA-617 has shown remarkable efficacy (70% rate of PSA decline ≥ 50%, 29% complete response rate from ^{68}Ga-PSMA PET) in heavily-pretreated, TRT-naïve patients (*40*), but can be hampered by dose-limiting xerostomia (PSMA is expressed in the salivary glands) (*52*) which may be only partially resolvable (*39,40*). TRT via a PSMA-targeted antibody (J591) has circumvented this issue in patients (*53*), and a clinical trial to assess ^{225}Ac-J591 + pembrolizumab (NCT04946370) is now recruiting.

$^{223}RaCl_2$ (Xofigo®) + ICI

Xofigo® is non-chelated ^{223}Ra, an α-emitter with chemical similarity to calcium selectively trafficked to areas of increased bone stroma formation, as occurs within sclerotic or osteoblastic bone metastases (*54*). The vast majority (> 90%) of mCRPC patients display bone metastases radiographically, and a substantial fraction of mCRPC deaths result from these metastases and their complications. Due to the short range of α radiation, cytotoxicity is constrained to the target region, limiting myelotoxicity. From a landmark phase III clinical trial (NCT00699751), Xofigo® was demonstrated to significantly extend time to the first symptomatic skeletal event and overall survival (*54*). To investigate whether ^{223}Ra-mediated cell death potentiates pembrolizumab in intractable cancers, a phase II trial in mCRPC (NCT03093428) and a phase I/II trial in metastatic NSCLC (NCT03996473) patients with bone metastases are ongoing. Preliminary results from the mCRPC trial have not shown therapeutic benefit for the

combination (*55*). A phase Ib trial of Xofigo® + atezolizumab (anti-PD-L1) in mCRPC (NCT02814669) demonstrated increased toxicity without appreciable clinical benefit versus either alone (*56*).

OUTLOOK

TRT has been shown to enhance ICI in preclinical models, garnering increasing interest towards optimizing treatment strategies for clinical translation. Future preclinical work will likely involve elegant approaches to reduce off-target toxicity, such as pre-targeting for RIT (*28*), as well as triple combinations of TRT + ICI + other immunotherapies for 'cold' metastatic tumors resistant to ICI alone or with TRT. Given the distinct immunologic effects of TRT and EBRT, TRT + EBRT + ICI may be increasingly explored (*17*). In the clinic, α and β TRT may be used in tandem to improve efficacy due to complementary emission penetration or to mitigate toxicity/resistance, as demonstrated for ^{177}Lu/^{225}Ac-PSMA-617 (*57*). Therapeutic benefit could then be improved with ICI.

To safely optimize TRT tumor dose delivery, individualized patient dosimetry will be required. Currently, TRT is given with a fixed dosing regimen regardless of the individual patient's tumor burden or tumor uptake of the companion pretherapy PET tracer, despite evidence that more tailored therapy may improve outcomes (*58*). *Current et al*. recently reported that intrasubject variability in lesion PSMA expression and PSMA$^{low/medium/high}$ cell abundance caused disparities in the therapeutic efficacy of PSMA-directed TRT in mouse prostate cancer models (*59*). TRT could treat low PSMA tumors but was most effective for extensive and homogenous PSMA expression. As such, a fixed dosing strategy could lead to undertreatment and the selection of TRT-resistant clones. Individualized dosimetry could account for this. Patients with homogenously high target expression could safely receive increased activity (*60*) and those with low and/or variable expression could be evaluated to predict therapeutic effect and the fraction of metastases that could be treated effectively. Individualized Monte Carlo-based dosimetry has demonstrated improved accuracy relative to standardized phantom-based methods in small patient

cohorts (*61*). Further, this pretherapy dosimetry could reliably predict tumor and/or at-risk organ doses for TRT (*62*).

Several outstanding mechanistic questions must be resolved, requiring an increased understanding of TRT radiobiology. For example, for a particular TRT use, it is often unknown whether a threshold, mean, or maximum dose absorbed by the tumor is optimal for antitumor efficacy, or even if this applies across tumor volumes (*13*). Little to no study of TRT dose, dose rate, and scheduling regarding radioresistance or immune checkpoint modulation has been performed. Towards combination with ICI, concomitant administration has demonstrated improved efficacy compared to staggered schedules (*24,31*). However, the mechanism remains elusive. Taken together, it can be anticipated that as our understanding of TRT radiobiology grows, more efficacious and patient-specific combinatorial regimens will emerge.

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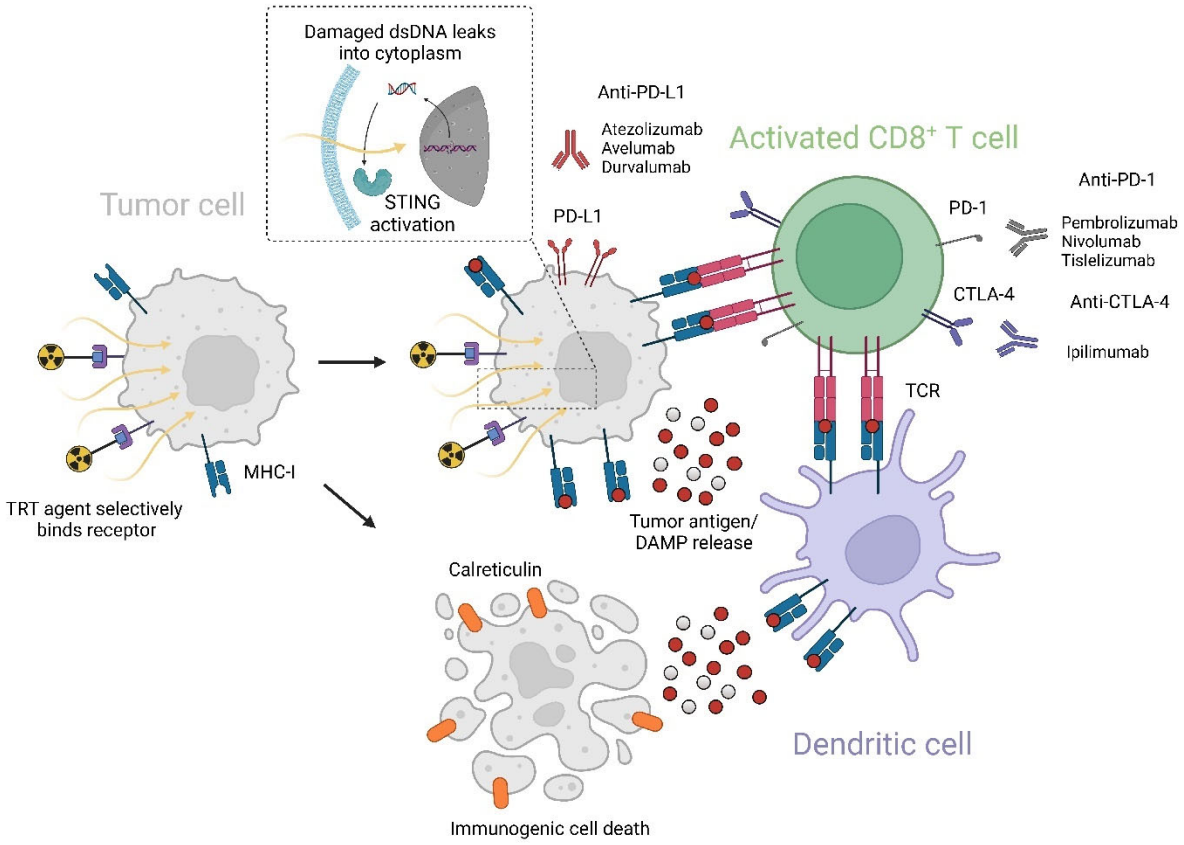

FIGURE 1.

TRT and ICI synergize via immune mechanisms. The TRT agent binds a tumor cell target receptor, and the emitted radiation induces the release of tumor-associated antigens and damage associated molecular patterns (DAMPs), causes DNA damage, and potentially prompts immunogenic cell death. Damaged cytoplasmic DNA stimulates STING, leading to a type I interferon response. Tumor MHC-I expression is increased as is neoantigen display, and the stimulated activation of dendritic cells (DCs) correspondingly increases antigen cross-presentation to T cells. The expression of immune checkpoint molecules is modulated, allowing for maintained immune activation with ICI. As a DAMP, calreticulin is newly expressed on the outer membrane of tumor cells undergoing immunogenic cell death (*18*), leading to phagocytosis by DCs that is central to their activation (*7*). Created with BioRender.com.

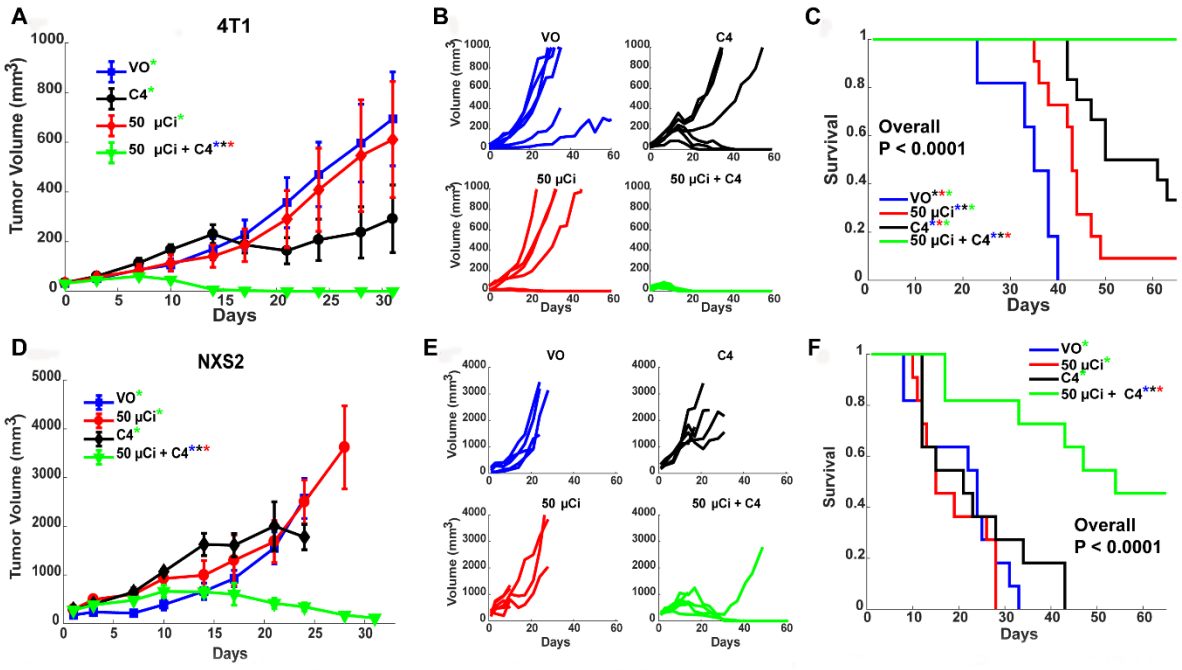

FIGURE 2.

TRT sensitizes 'cold' murine tumor models to ICI. Tumor volume and survival in 4T1 breast cancer (A-C) and NXS2 neuroblastoma (D-F) in mice receiving 200 µg CTLA-4 (C4, 3x) with or without 50 µCi ^{90}Y-NM600 or saline control (vehicle only, VO) (n=5-6 each). Adapted with permission from (*17*).

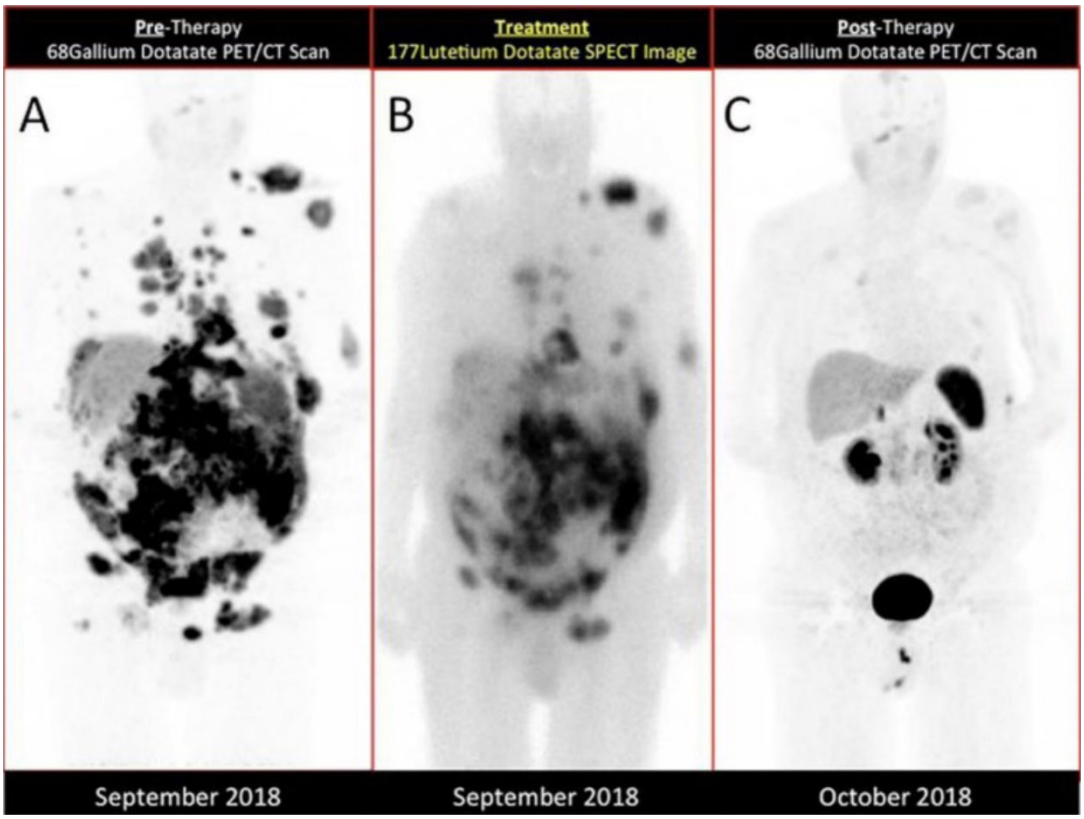

FIGURE 3.

Dramatic improvement in a patient refractory to anti-PD-L1 (avelumab) receiving a single off-label dose of ^{177}Lu-DOTATATE for heavily metastatic Merkel cell carcinoma and resuming avelumab. (A) Pre-treatment ^{68}Ga-DOTATATE PET/CT scan, (B) ^{177}Lu-DOTATATE SPECT/CT scan during TRT, and (C) ^{68}Ga-DOTATATE PET/CT scan 1 month post-treatment. Reprinted with permission from (*44*).