

Combination Strategies to Improve Targeted Radionuclide Therapy

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Noteworthy

- Targeted radionuclide therapy is growing in popularity for cancer treatment.
- Efforts to improve targeted radionuclide therapy have led to increasing numbers of combination strategies being attempted.
- Increasing our understanding of the radiobiology of targeted radionuclide therapy will help inform the successful implementation of combination strategies.

Running title: Improving Targeted Radionuclide Therapy

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Abstract

In recent years, targeted radionuclide therapy (TRT) has emerged as a promising strategy for cancer treatment. In contrast to conventional radiotherapy, TRT delivers ionising radiation to tumours in a targeted manner, reducing the dose that healthy tissues are exposed to. Existing TRT strategies include the use of ^{177}Lu -DOTATATE, ^{131}I -MIBG, Bexxar[®] and Zevalin[®], clinically approved agents for the treatment of neuroendocrine tumours, neuroblastoma and non-Hodgkin's lymphoma respectively. Although promising results have been obtained with these agents, clinical evidence acquired to date suggests that only a small percentage of patients achieve complete response. Consequently, there have been attempts to improve TRT outcomes through combinations with other therapeutic agents; such strategies include administering concurrent TRT and chemotherapy, and the use of TRT with known or putative radiosensitisers such as PARP and mTOR inhibitors. In addition to potentially achieving greater therapeutic effects than the respective monotherapies, these strategies may lead to lower dosages or number of cycles required and, in turn, reduce unwanted toxicities. As of now, several clinical trials have been conducted to assess the benefits of TRT-based combination therapies, sometimes despite limited preclinical evidence being available in the public domain to support their use. Whilst some clinical trials have yielded promising results, others have shown no clear survival benefit of particular combination treatments. Here, we present a comprehensive review of combination strategies with TRT reported in the literature to date and evaluate their therapeutic potential.

Key Words: targeted radionuclide therapy; combination therapy; radiotherapy

Introduction

Targeted radionuclide therapy (TRT) involves the use of radiopharmaceuticals designed to specifically target cancer cells. These radiopharmaceuticals consist of beta, alpha or Auger electron-emitting radionuclides coupled to a tumour-targeting vector, such as a monoclonal antibody or peptide. In recent years, TRT has rapidly grown in popularity, with agents such as [¹⁷⁷Lu-DOTA⁰,Tyr³]octreotate (¹⁷⁷Lu-DOTATATE), ¹³¹I-metaiodobenzylguanidine (¹³¹I-MIBG), Bexxar[®] and Zevalin[®] now clinically approved for the treatment of low-grade neuroendocrine tumours (NETs), neuroblastoma and non-Hodgkin's lymphoma respectively. ¹⁷⁷Lu-PSMA, which targets prostate-specific membrane antigen (PSMA), is also emerging as an attractive strategy for metastatic castration-resistant prostate cancer, with large late-phase clinical trials underway. Due to its highly targeted approach, the application of TRT could result in fewer side-effects than conventional external beam radiotherapy (EBRT) and allow for more effective treatment of disseminated cancers (1). Promising results have been obtained with several TRTs in clinical trials, but there remains room for improvement; for instance, whilst the Phase III NETTER-1 trial showed that ¹⁷⁷Lu-DOTATATE can be a life-extending treatment, with significant improvement in the median progression-free survival being reported, objective response was observed in just 18% of patients (2). To further improve TRT outcomes, administering TRT at earlier disease stages, as is being investigated in the UpFrontPSMA trial (NCT04343885), and the use of combination therapies is being attempted.

Combination strategies investigated thus far include the use of agents to improve tumour perfusion to allow better distribution of the radiopharmaceutical (3), upregulation of the target receptor to increase cellular uptake (4), the combination of TRT with other DNA-damaging drugs (5–7), radiosensitisation through inhibiting essential processes such as DNA damage repair (8–10), and the use of immune checkpoint inhibitors (11–13). Several of these strategies have shown promise in preclinical studies and are currently in clinical trials, but there remains a lack of research into TRT radiobiology, making it difficult to predict which of these strategies will prove most effective. Attempts to enhance tumour perfusion and cellular uptake have shown promise, but we do not currently know the exact absorbed radiation dose

required for a complete response, making the optimisation of this strategy challenging (3,4). Efforts towards modelling the necessary dose required for tumour response after ^{177}Lu -PSMA therapy are underway, but this is difficult to achieve given the heterogeneity of PSMA expression (14). Additionally, although there are a wide variety of drugs being combined with TRT in an attempt to achieve effective radiosensitisation, the mechanisms by which these combination therapies work and their effects on downstream biological pathways are not necessarily fully understood. Many of these combination strategies make use of known radiosensitisers of EBRT, but due to differences in the dose rates, duration of radiation exposure, and radiobiological effects between EBRT and TRT, our understanding of EBRT radiobiology cannot be simply extrapolated to TRT (1,15–17). Radiosensitisers of EBRT may therefore not always be effective radiosensitisers of TRT, and vice versa. Here, we present a comprehensive review of TRT-based combination therapies tested to date, focusing primarily on those involving beta-emitting TRTs as several of these combinations are being evaluated clinically (**Figure 1; Supplemental Table 1-8**). We also discuss possible limitations of such studies and suggest further research that is needed if these combination strategies are to be effectively translated to patients.

Increasing DNA Damage with Traditional Chemotherapies

As TRT primarily acts by inducing DNA damage, the efficacy of TRT can be increased by creating additional DNA damage through combining TRT with conventional chemotherapy or radiotherapy. Prominent examples of this include the combination of ^{177}Lu -DOTATATE with the anti-metabolite capecitabine, alkylating agent temozolomide, or both (CAPTEM) for the treatment of NETs (**Supplemental Table 1**). These are all standard chemotherapy options for advanced NETs, making their combination with ^{177}Lu -DOTATATE easily translatable if effective. To date, promising tumour response rates have been observed with ^{177}Lu -DOTATATE + capecitabine and/or temozolomide (6,18,19), and Phase II clinical trials are currently underway (NCT02736500; NCT02358356). Initial results from the Phase I/II CAPTEM trial suggest that whilst the objective tumour response rate in the cohort treated with ^{177}Lu -DOTATATE + CAPTEM was higher than in patients treated with ^{177}Lu -DOTATATE alone, more

treatment-related adverse effects were observed (20). Though rare, both ^{177}Lu -DOTATATE and CAPTEM have been associated with haematological toxicities when used as monotherapies (2,21); thus, the increase in adverse events observed in the combination group may be due to overlapping toxicities. The final outcomes of the Phase I/II CAPTEM trial should provide further insight into this.

To our knowledge, there exists just one preclinical study attempting to optimise ^{177}Lu -DOTATATE + capecitabine +/- temozolomide chemotherapy (3). In human small cell lung cancer H69 tumour-bearing mice, Bison *et al.* noted that whilst temozolomide outperformed ^{177}Lu -DOTATATE as a monotherapy, the combination of ^{177}Lu -DOTATATE + temozolomide led to additive effects (**Figure 2**) (3). Moreover, administering temozolomide 14 days before ^{177}Lu -DOTATATE treatment was found to be significantly more effective than vice versa, which was attributed to enhanced tumour perfusion, radiosensitivity and tumour oxygenation (3). In clinical studies involving ^{177}Lu -DOTATATE + capecitabine +/- temozolomide therapy, chemotherapy is typically initiated before or concomitantly with ^{177}Lu -DOTATATE (5,7,19). Further studies are necessary to optimise the dosing schedules to produce the greatest survival benefits, particularly for CAPTEM protocols where the relative timings of capecitabine and temozolomide administration are already known to affect synergism (22).

Aside from ^{177}Lu -DOTATATE-based combination therapies, there are several examples of other TRTs being combined with chemotherapy, but there is currently insufficient evidence to support the use of these combinations over their respective monotherapies (**Supplemental Table 1**). Recent reports have, however, alluded to significantly longer overall survival times in chemotherapy-naïve patients treated with TRT compared to patients with a history of chemotherapy (23–25). Until the precise reason behind these observations are understood, TRT + chemotherapy combinations should be carefully monitored if implemented.

Radiosensitisation – Inhibiting DNA Repair

Inhibiting key proteins involved in the DNA damage response (DDR) could lead to radiosensitisation of TRT. To date, there are several examples of this strategy being used: the majority

of these involve poly(ADP-ribose) polymerase (PARP) inhibitors, but inhibitors of other DDR proteins, heat shock protein 90 (HSP90) and checkpoint kinase 1 (CHK1) have also been used (**Supplemental Table 2**). Inhibition of DNA topoisomerases I and II (TOP1 and TOP2) has also been suggested to induce radiosensitisation by disrupting DNA repair (15,16). It should be noted, however, that many DDR-targeting agents are associated with bone marrow and gastrointestinal toxicities, which could lead to increased adverse side-effects when combined with TRT (2,26,27). Careful monitoring of normal tissue toxicity is therefore warranted for these combinations.

DDR Inhibitors. PARP enzymes constitute a family of proteins that are vital for the maintenance of cellular homeostasis and form an essential part of DDR. PARP proteins – in particular, PARP-1 – have been implicated in the repair of both single-stranded (SSBs) and double-stranded breaks (DSBs) (28). As the main mechanism by which ionising radiation causes cell death is through the induction of DNA damage, reducing the capacity for DNA repair with PARP inhibitors (PARPi) is a potential radiosensitisation strategy (29,30). A number of preclinical studies investigated whether PARPi can also potentiate TRT with ¹⁷⁷Lu-DOTATATE: in vitro with the human cancer cell lines osteosarcoma U2OS+SSTR, gastroenteropancreatic BON-1, and bronchopulmonary NCI-H727, and in vivo in AR42J tumour-bearing mice (8–10). The combination of PARPi with other TRTs, such as ¹³¹I-MIP-1095, ¹⁷⁷Lu-RM2 and ²²⁷Th-HER2, have also been tested with promising results (Supplemental Table 2).

While few studies have aimed to elucidate the mechanism behind PARPi-induced radiosensitisation of TRT, ¹⁷⁷Lu-DOTATATE + PARPi treatment has been associated with an increased number and persistence of DSBs, increased levels of DNA damage markers, such as γ H2AX, phosphorylated p53, and 53BP1, and increased cell cycle arrest (**Figure 3**) (8–10). Because of this, it has been proposed that PARP inhibition leads to an inability to repair the SSBs caused by ¹⁷⁷Lu-DOTATATE, resulting in the formation of additional DSBs upon cellular replication and, ultimately, cell death (8–10). Several clinical trials assessing the safety and efficacy of TRT + PARPi are underway

(NCT04086485; NCT04375267; NCT0387484) and the field is looking forward to their outcome. In the future, it would be of interest to compare the effects of TRT + PARPi in patients with deficient HR (e.g. with BRCA1/2 mutations) to those with functioning HR as mutations in HR repair proteins are known to induce PARPi sensitivity (31).

In addition to PARPi, inhibitors of ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3-related (ATR), and DNA-dependent protein kinase (DNA-PK), key effector proteins involved in DDR, have also been investigated as radiosensitisers. These combination studies are mainly with EBRT (32–34), but there are a few examples showing their ability to also radiosensitise TRT (35,36); however, it remains to be seen how these TRT combinations perform clinically. Combining TRT with inhibitors of epidermal growth factor receptor (EGFR), which modulates DNA repair through activation of DNA-PK, has also been attempted in a small number of preclinical studies (37,38).

HSP90 Inhibitors. ¹⁷⁷Lu-DOTATATE has been combined with the HSP90 inhibitors (HSP90i) onalespib and gantespib in vivo, with promising results and a favourable toxicity profile reported in NET xenografts (39,40). HSP90 is a molecular chaperone, which participates in key processes such as protein degradation, folding, and intracellular transport. Clients for HSP90 include kinases involved in cell growth, such as EGFR and protein kinase B (AKT), and DDR proteins, such as ATR and CHK1 (41,42). HSP90i have been reported to induce radiosensitisation when combined with EBRT, but due to the multiple clients associated with HSP90, the exact mechanism of HSP90i-induced radiosensitisation is unknown. However, it is known to negatively affect DNA repair signalling and the activation of cell cycle checkpoints, potentiating radiation-induced damage (43,44). To date, no study has sought to elucidate the mechanism of HSP90i-induced radiosensitisation in the context of TRT. Moreover, although several HSP90i have been tested in clinical trials as potential monotherapies, none have been clinically approved due to a lack of efficacy and unacceptable toxicity, making this strategy challenging to translate to patients (45,46).

Topoisomerase Inhibitors. TOP1 and TOP2 are nuclear enzymes that are essential for maintaining the correct topological state of DNA, which is crucial for RNA transcription, chromatin remodelling, and DNA replication. Through the formation of a TOP-DNA covalent intermediate, the TOP cleavable complex (TOPcc), the main role of TOP1 and TOP2 is to catalyse the relaxation of positive or negative DNA supercoiling by nicking DNA strands to allow controlled rotation, followed by re-ligation (47). Many inhibitors of TOP1 and TOP2 (TOPi) work by stabilising TOPcc, leading to the accumulation of SSBs and DSBs respectively (47). In addition to creating DNA damage, numerous studies have shown that TOPi can also act as radiosensitisers of EBRT (48–50); however, the precise radiosensitisation mechanism remains unelucidated. Shih *et al.* suggested that TOPi-mediated cytotoxicity and radiosensitisation occur via different pathways as the former is Ku80-independent, whilst the latter is Ku80-dependent (51). Ku80 is required for non-homologous end joining (NHEJ), suggesting that TOPi can potentiate radiation-induced DNA damage through interfering with DDR. The schedule of TOPi administration is also thought to be crucial for effective radiosensitisation of EBRT, with varying degrees of radiosensitisation observed depending on the dosing frequency (49,50).

Several studies investigated the use of TOP1i with TRT, with the most frequently tested combination being ¹³¹I-MIBG + topotecan for the treatment of advanced neuroblastoma. In vitro and in vivo studies using the neuroblastoma SK-N-BE(2c) and glioma U251/NAT models have shown that ¹³¹I-MIBG + topotecan treatment leads to superadditive DNA damage and reduced efficiency of DNA repair (52,53). As with EBRT + TOPi combinations, the benefits of TRT + TOPi treatment appears to depend on the timing of TOPi administration: McCluskey *et al.* reported greater tumour growth delay in SK-N-BE(2C) and U251/NAT tumour-bearing mice when topotecan was administered simultaneously with ¹³¹I-MIBG compared to administration 24 hrs before or after (52,53). This observation has been linked to differences in cell cycle distribution induced by the different schedules; however, further studies are needed to confirm this (53). Several clinical studies have been conducted with ¹³¹I-MIBG + topotecan in neuroblastoma patients, but it remains unclear whether the addition of topotecan leads to any significant benefit over ¹³¹I-MIBG monotherapy (Supplemental Table 2).

Radiosensitisation – Inhibiting PI3K/AKT/mTOR Signalling

Mammalian target of rapamycin (mTOR) is a serine/threonine kinase that forms two complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), which differ in their subcellular location, structure and function: mTORC1 regulates cell growth and metabolism, and mTORC2 regulates cell proliferation and survival (54). mTOR activity is primarily regulated by the phosphoinositide-3-kinase/AKT/mTOR (PI3K/AKT/mTOR) signalling pathway. In many human cancers, mTOR signalling is hyperactivated, leading to increased tumorigenesis, tumour progression, and decreased survival (55,56). mTOR is also known to influence DDR; for example, mTOR can positively and negatively regulate ATM (57).

Activation of the PI3K/AKT/mTOR pathway has been linked to the development of radioresistance, and multiple studies have investigated mTOR inhibitors (mTORi) for their potential radiosensitising effects with EBRT (58–60). Multiple preclinical studies have demonstrated that EBRT + mTORi leads to increased radiosensitivity through decreased expression of NHEJ and homologous recombination (HR) repair pathway proteins, and increased expression of apoptosis pathway proteins (58–60). However, the use of mTORi as radiosensitisers for TRT remains controversial as preclinical studies investigating the efficacy of ¹⁷⁷Lu-DOTATATE + mTORi have reported contrasting results: Johnbeck *et al.* observed greater anti-tumour effects with ¹⁷⁷Lu-DOTATATE + everolimus treatment compared to ¹⁷⁷Lu-DOTATATE alone in the mouse H727 non-small cell lung carcinoma model, Zellmer *et al.* found that the combination treatment was equally effective as ¹⁷⁷Lu-DOTATATE alone in the athymic mouse AR42J pancreatic model, and Pool *et al.* reported reduced anti-tumour effects with the combination treatment in the immunocompetent rat CA20948 pancreatic tumour model (61–64). Pool *et al.* also noted the development of distant metastases in > 70% of the CA20948 rats in the everolimus-treated groups, whilst no metastases were observed in the control or ¹⁷⁷Lu-DOTATATE only groups (61,62). This increased propensity for metastasis formation was not observed in athymic AR42J tumour-bearing mice or in the Phase I NETTLE trial evaluating the maximum tolerated dose of everolimus when administered with ¹⁷⁷Lu-DOTATATE (64,65); thus, it is probable that the increased metastases observed

are specific to the genotype of the CA20948 model. Alternatively, there is evidence that mTOR inhibition can influence endothelial-mesenchymal transition (EndMT), with both the promotion and suppression of EndMT being observed when using mTORi under different conditions (66,67). EndMT is known to contribute towards tumour progression and metastasis, and thus, the precise role of mTORi on EndMT when combined with TRT merits close investigation (68). Understanding the source of these increased metastases is crucial, particularly as a Phase I/II trial aimed at assessing ¹⁷⁷Lu-DOTATATE + everolimus treatment in NET patients is underway (NCT03629847).

In addition to its role in tumorigenesis, mTOR plays an essential part in immune system regulation, which should be considered when designing TRT + mTORi therapies. Everolimus is clinically used as an immunosuppressant because of its ability to promote the expansion of regulatory T cells (69). A study testing ¹⁷⁷Lu-DOTATATE + everolimus in non-tumour-bearing rats saw no significant increases in renal or haematological toxicities compared to ¹⁷⁷Lu-DOTATATE treatment, but decreases in the white blood cell count were noted, in line with the immunosuppressive effects of everolimus (70). Although immunosuppression is typically undesirable in cancer treatment as it decreases immune surveillance, there is little evidence suggesting that using mTORi as a monotherapy could promote tumour growth (71). Nevertheless, in LEW/SsNHsd Lewis rats – a sub-strain which shows an enhanced autoimmune response – bearing CA20948 tumours, Bison *et al.* observed complete tumour regression in 50% of the rats in the control group, compared to 12.5% in the group treated with everolimus alone (62). No cases of complete tumour regression were seen in control LEW/HanHsd rats, which possess a less active immune response (62). Both findings indicate immune system involvement and suggest that any immunosuppressive effects should be considered when developing treatment combinations with TRT.

Radiosensitisation – Inhibiting Hedgehog Signalling

The Hedgehog signalling pathway is involved in embryonic development and is abnormally activated in many cancers. Inhibitors of the transmembrane protein Smoothed (SMOi), a key

component of the Hedgehog pathway, have been investigated as potential anti-cancer drugs, with several SMOi now approved by the United States Food and Drug Administration. Spetz *et al.* showed that combining ¹⁷⁷Lu-DOTATATE with the SMO antagonist sonidegib in GOT1 tumour-bearing mice led to a greater time to progression compared to the respective monotherapies (72). Moreover, pathway analysis predicted that this combination therapy impacted cancer-related pathways, such as Wnt/ β -catenin, Notch and NF- κ B, differently than either sonidegib or ¹⁷⁷Lu-DOTATATE alone (72). Although experimental validation is required for these predictions, it suggests that radiosensitisation of ¹⁷⁷Lu-DOTATATE by sonidegib could involve multiple biological pathways and may therefore be challenging to optimise.

Radiosensitisation – Inhibiting p53-MDM2 Interactions

The tumour suppressor protein p53 regulates cell cycle progression, DNA repair and apoptosis, and is negatively regulated by murine double minute 2 (MDM2). MDM2 is overexpressed in many human tumours, leading to decreased p53 activity; thus, disruption of the p53-MDM2 interaction is a promising therapeutic strategy. Several studies have shown that ionising radiation induces p53-dependent MDM2 expression, and p53/MDM2 inhibitors may therefore act as radiosensitisers by promoting p53-dependent apoptosis (73,74). Despite p53-MDM2 inhibitors being investigated as radiosensitisers for EBRT in multiple studies, there are few examples of them being used with TRT to date and further studies are needed to validate this approach (**Supplemental Table 5**). It should be noted, however, that this strategy depends on the presence of wild type p53 and it may therefore not be as effective in cancers in which *TP53* mutations are common, such as metastatic castration-resistant prostate cancer (75,76).

Radiosensitisation – Disrupting Cell Cycle

Microtubules, composed of heterodimers of α - and β -tubulin, are essential for cell signalling, division, mitosis, and maintaining cellular structure (77). Taxane drugs stabilise microtubules by binding to β -tubulin, promoting microtubule polymerisation and, ultimately, G2/M cell cycle arrest and apoptosis

(77). The cell cycle plays an important role in radiosensitivity, with cells being most sensitive in the G2/M phases and most resistant in the S phase (78). Due to their effect on the cell cycle, there are many examples of the use of taxanes as radiosensitisers for EBRT, with encouraging outcomes observed (79). However, there exist only a few examples of TRT + taxane combination therapies being investigated; while promising preclinical results have been reported with ^{177}Lu - and ^{188}Re -based TRTs, Kessel *et al.* reported that the benefits of ^{177}Lu -PSMA was decreased in patients with a history of second line cabazitaxel therapy, suggesting that further investigation into the mechanism of TRT + taxane combinations is warranted (**Supplemental Table 6**) (80). In particular, the sequencing of the therapies is likely to be important as ionising radiation itself influences the cell cycle (81); Liebmann *et al.* noted that irradiating human breast MCF-7 and lung A549 adenocarcinoma cells with EBRT before or concurrently with paclitaxel antagonised paclitaxel cytotoxicity (82). Optimising this sequencing with TRT is likely to be challenging as, in contrast to EBRT, TRT delivers radiation heterogeneously over an extended period with less temporal control over the absorbed radiation dose (1).

Radiosensitisation – Disrupting NAD⁺ Metabolism

Nicotinamide phosphoribosyl transferase (NAMPT) is an enzyme that is essential for nicotinamide adenine dinucleotide (NAD⁺) metabolism. NAD⁺ is required for many cellular processes, including the activation of PARP-1, and is regenerated via a salvage pathway involving NAMPT. NAMPT inhibitors (NAMPTi) have been proposed to work as radiosensitisers of TRT by preventing NAD⁺ regeneration; upon PARP-1 activation due to TRT-induced DNA damage, NAD⁺ is consumed and cannot be regenerated, leading it to drop to lethally low levels (83,84). To date, there is just one example of TRT + NAMPTi being investigated: Elf *et al.* showed that the combination of ^{177}Lu -DOTATATE and the experimental NAMPTi GMX1778 led to reduced tumour volumes and prolonged anti-tumour response in GOT1 tumour-bearing mice (83). However, more studies are required to assess the efficacy of this

strategy and determine the exact radiosensitisation mechanism. Moreover, since NAD⁺ is involved in many other processes, this approach could lead to off-target effects.

Radiosensitisation – Blocking Immune Checkpoints

Irradiation in the form of EBRT is known to have several immunomodulatory effects; for instance, EBRT can enhance tumour immunogenicity by inducing immunogenic cell death and promoting the release of tumour-associated antigens, while simultaneously reducing tumour immunogenicity by upregulating programme death ligand 1 (PDL1) expression (85). Combining EBRT with immune checkpoint blockade (ICB) antibodies targeting programmed death 1 (PD1), PDL1 and cytotoxic T-lymphocyte antigen 4 (CTLA-4) represents an attractive strategy to potentiate radiation-induced anti-tumour immunity and has produced encouraging results (86,87). While little is currently known about the effects of TRT on tumour immunogenicity, preclinical studies have shown that TRT also leads to upregulation of PDL1 expression and that combinations of TRT with ICB can lead to improved survival (11–13). Several clinical trials combining TRT + PD1 inhibitors are currently underway (e.g. NCT03325816; NCT03658447), though it is likely that further optimisation of when immunotherapy should be administered during TRT treatment will be required; as with EBRT, Chen *et al.* noted in their preclinical studies that the benefits of combining TRT + anti-PDL1 varies depending on whether the two treatments are given concurrently or sequentially (12,86).

Outlook

The rising interest in TRT has led to numerous combination strategies being evaluated, with promising results being reported and increasing numbers of clinical trials being conducted. In addition to the combination strategies discussed here, alternative strategies that have been proposed include those aimed at increasing the cellular uptake of radiopharmaceuticals and the rates of tumour perfusion (3,4). Multiple studies have also investigated the combination of TRT with EBRT, as well as with other beta- or

alpha-emitting TRTs (88–90). Growing numbers of triple combination therapies are also being evaluated, such as TRT + CAPTEM (NCT02358356) and TRT + vincristine + irinotecan (NCT01313936), though it remains to be seen whether these strategies are effective in increasing the therapeutic index.

Despite the growing number of studies being conducted in this field, there remain few key outcomes and increasing our understanding of TRT radiobiology is critical if we are to fully capitalise on the potential benefits of these combination therapies. For instance, the synergistic effects of taxanes and TOPi in combination with TRT are believed to be cell cycle-dependent, and the effects of both the drugs and TRT on the cell cycle must be understood before we can optimise the dosing schedule (53,81,82). Greater characterisation of the tested combinations over a range of concentrations is also necessary to identify combinations that are superadditive, as opposed to simply additive or, even, antagonistic. Moreover, many of the current combination strategies being employed for TRT involve known radiosensitisers for EBRT, despite its markedly different radiobiology (1,15,16). Whilst this may identify combinations that are effective with both forms of ionising radiation, it is likely that there exist effective radiosensitisers for TRT that are ineffective when combined with EBRT and vice versa. In addition, although this review focused on beta-emitting TRTs, there is growing interest in the use of alpha and Auger emitters (91–93). However, due to differences in radiobiology and therefore biologically effective dose, effective radiosensitisers of beta-emitting TRTs may not be effective radiosensitisers of alpha and auger-emitting TRTs. Differences may also exist between different beta-emitters due to differences in dose rates and dose deposition profiles.

Furthermore, many of the commonly used preclinical models thus far are not entirely clinically relevant; for example, despite U2OS+SSTR and BON-1 cells being used to evaluate ¹⁷⁷Lu-DOTATATE combination therapies, U2OS+SSTR is a non-NET cell line and BON-1 cells express much lower levels of SSTR than are found in human NET tumours (4,8). BON-1 cells also show mutations of key DNA damage response genes such as *TP53*, which are rarely seen in G1 and G2 NETs (94,95). Preclinical studies in a greater variety of models are necessary, as well as identification of more clinically relevant model systems. Most preclinical studies to date have been conducted in immune-compromised mice and

possible influences of the immune system on combination therapies and vice versa have not been explored in detail.

Moving forward, an increasing mechanistic understanding of TRT-based combination therapies is crucial if these strategies are to be effectively – and safely – translated to patients, particularly given the outcome of the Phase III ERA223 trial, where abiraterone acetate + prednisone/prednisolone + ^{223}Ra resulted in no improvements in survival compared to abiraterone acetate + prednisone/prednisolone alone, but was associated with an increased frequency of skeletal fractures (96). Similarly, initial results from the Phase I/II CAPTEM trial (NCT02358356) show that whilst ^{177}Lu -DOTATATE + CAPTEM treatment led to higher objective tumour response rates than ^{177}Lu -DOTATATE alone, it was associated with more treatment-related adverse effects (20). Recent evidence has also alluded to better survival in chemotherapy-naïve patients treated with TRT compared to those with a prior history of chemotherapy, possibly due to the acquisition of resistance mechanisms as has been suggested for second and subsequent lines of chemotherapy (25,97). The use of TRT combination therapies as first-line treatments may therefore produce greater survival benefits and should be explored further. In addition to potentially increasing therapeutic efficacy and minimising the occurrence of toxicities, greater understanding of the radiobiology behind these combination strategies may also allow us to stratify patients and tailor combination therapies to the grade and mutational landscape of each patient's cancer.

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Figures

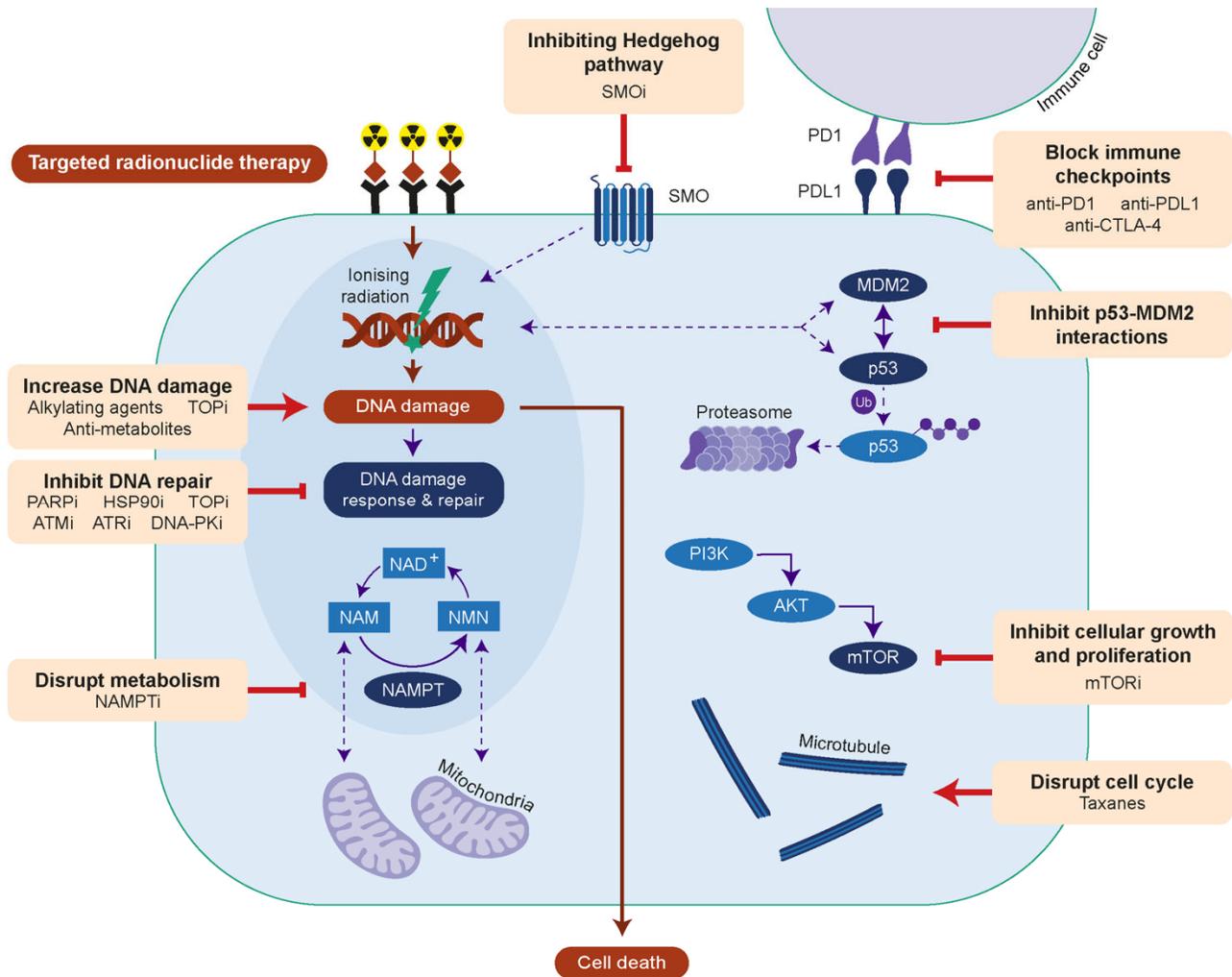


FIGURE 1. The efficacy of TRT can be improved by increasing DNA damage, inhibiting DNA repair, disrupting metabolism or the cell cycle, inhibiting Hedgehog, PI3K/AKT/mTOR or p53-MDM2 signalling, or blocking immune checkpoints.

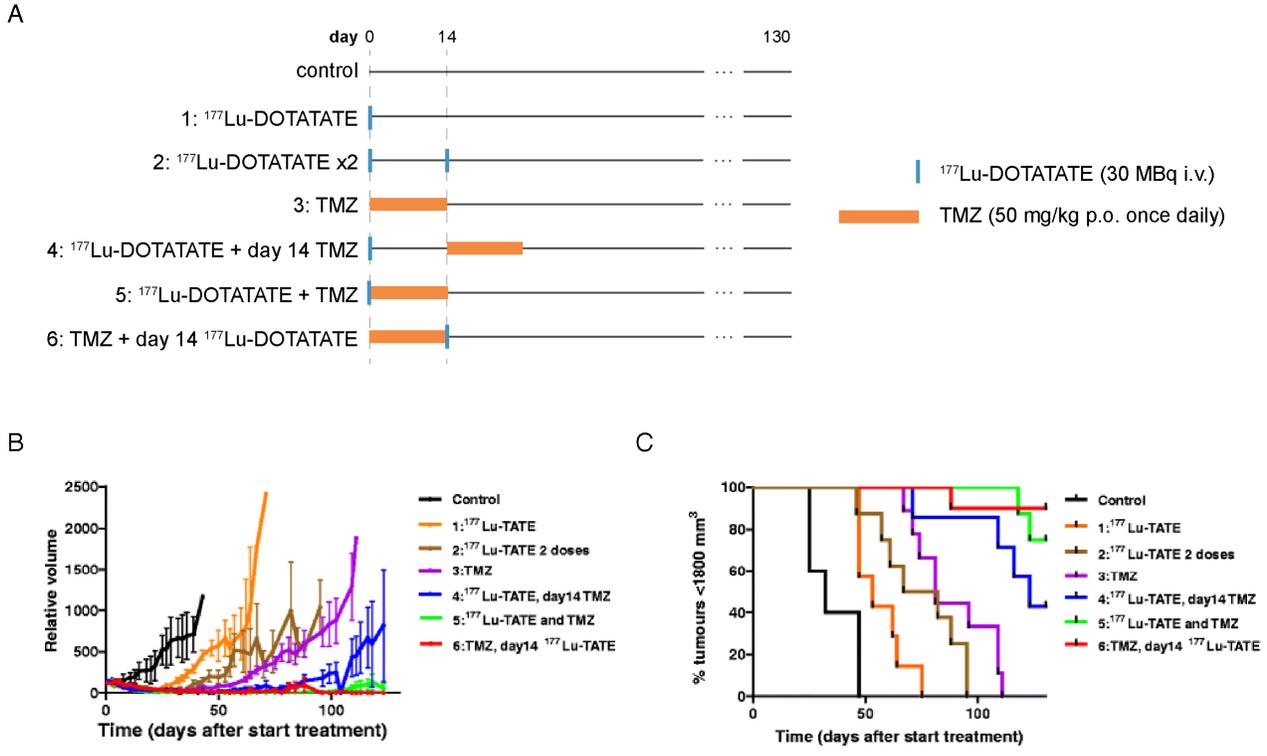


FIGURE 2. Evaluation of different ¹⁷⁷Lu-DOTATATE +/- temozolomide (TMZ) treatments in H69 tumour-bearing mice. **a)** Study timeline. **b,c)** Average tumour volume and percentage of mice with tumours < 1800 mm² (8-10 mice per group). CC license – Permission granted with citation (3).

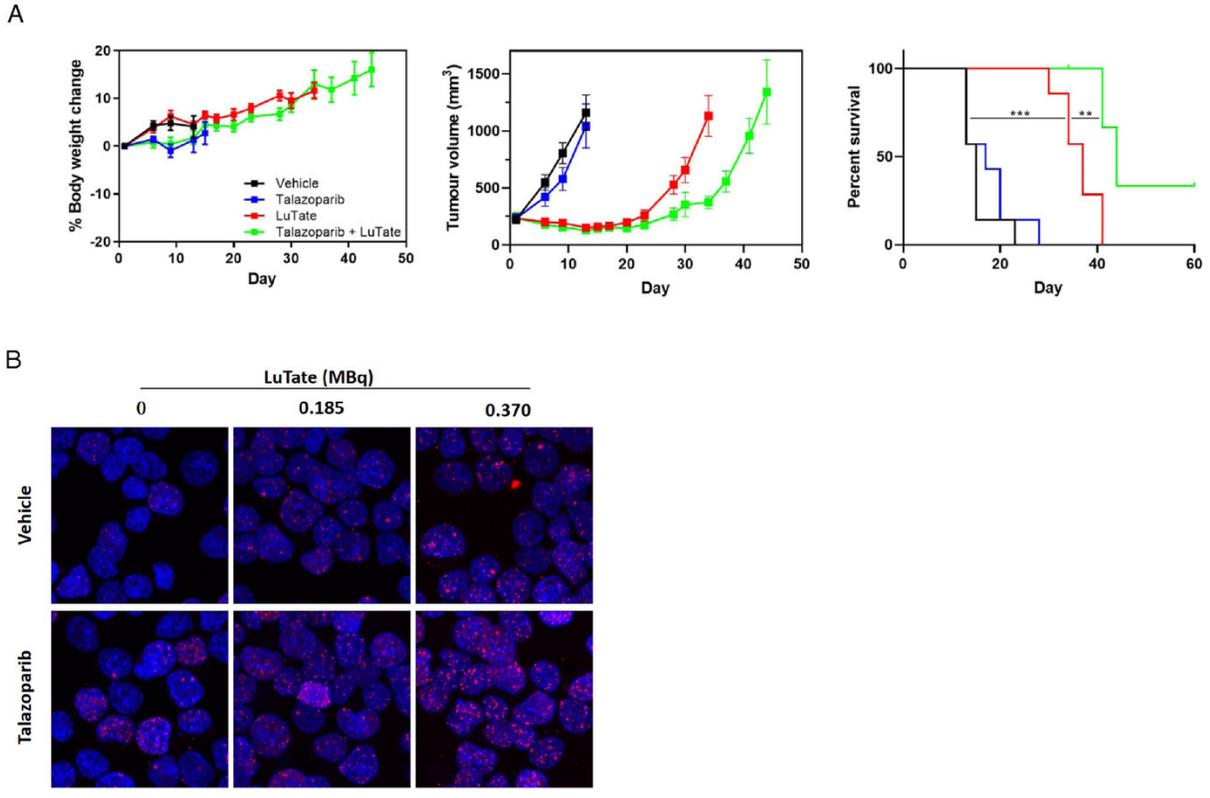


FIGURE 3. Evaluation of TRT + talazoparib treatment in exocrine pancreatic AR42J model. **a)** Body weight, tumour volume and percent survival of AR42J tumour-bearing mice treated with 30 MBq ¹⁷⁷Lu-DOTATATE (day 1) +/- 0.25 mg/kg talazoparib twice daily (day 1-5). **b)** γ H2AX staining of AR42J cells treated with ¹⁷⁷Lu-DOTATATE +/- talazoparib for 2 hrs. CC license – Permission granted with citation (10).

TABLE S1. Examples of combination strategies based on increasing DNA damage with traditional chemotherapies.

Class	TRT	Type	Stage	References
5-FU/CAP/TEM	¹³¹ I-huA33	Antibody	Clinical	(1,2)
			Preclinical	(3)
	¹⁷⁷ Lu-DOTATATE	Peptide	In trials	NCT02736500 NCT02358356
			Clinical	(4–12)
		Preclinical	(13)	

TABLE S2. Examples of combination strategies based on interfering with DNA repair.

Class	TRT	Type	Stage	References
ATM / ATR inhibitors	²²⁷ Th-MSLN	Antibody	Preclinical	(1)
CHK1 inhibitors	¹³¹ I-MIP-PSMA	Peptide	Preclinical	(14)
EGFR inhibitors	¹³¹ I-huA5B7	Antibody	Preclinical	(15)
	¹⁷⁷ Lu-hu3S193	Antibody	Preclinical	(16)
DNA-PK inhibitors	²¹³ Bi-cetuximab	Antibody	Preclinical	(17)
HSP90 inhibitors	¹⁷⁷ Lu-DOTATATE	Peptide	Preclinical	(18,19)
PARP inhibitors	¹³¹ I-MIP-1095	Peptide	Preclinical	(14)
	¹⁷⁷ Lu-DOTATATE	Peptide	In trials Preclinical	NCT04375267 NCT04086485 (20–22)
	¹⁷⁷ Lu-PSMA	Peptide	In trials	NCT03874884
	¹⁷⁷ Lu-RM2	Peptide	Preclinical	(23)
	²²⁷ Th-HER2	Antibody	Preclinical	(24)
TOP1/2 inhibitors	¹³¹ I-MIBG	Other	Clinical Preclinical	(25–27) (28,29)
	¹³¹ I-MIP-1095	Peptide	Preclinical	(14)

TABLE S3. Examples of combination strategies based on inhibiting PI3K/AKT/mTOR signalling.

Class	TRT	Type	Stage	References
mTOR inhibitors	¹⁷⁷ Lu-anti-L1CAM	Antibody	Preclinical	(30)
	¹⁷⁷ Lu-DOTATATE	Peptide	In trials	NCT03629847
			Clinical	(31)
			Preclinical	(32–36)
¹⁷⁷ Lu-PSMA	Peptide	Preclinical	(37)	
AKT inhibitors	¹⁷⁷ Lu-anti-L1CAM	Antibody	Preclinical	(30)

TABLE S4. Examples of combination strategies based on inhibiting Hedgehog pathway.

Class	TRT	Type	Stage	References
SMO inhibitors	¹⁷⁷ Lu-DOTATATE	Peptide	Preclinical	(38)

TABLE S5. Examples of combination strategies based on inhibiting p53-MDM2 interactions.

Class	TRT	Type	Stage	References
MDM2 inhibitors	¹³¹ I-MIP-1095	Peptide	Preclinical	(14)
	¹⁷⁷ Lu-DOTATATE	Peptide	Preclinical	(39)

TABLE S6. Examples of combination strategies based on disrupting cell cycle.

Class	TRT	Type	Stage	References
Mitotic inhibitors	¹⁷⁷ Lu-anti-L1CAM	Antibody	Preclinical	(30)
	¹⁷⁷ Lu-GRP	Peptide	Preclinical	(40)
	¹⁷⁷ Lu-hu3S193	Antibody	Preclinical	(16)
	¹⁸⁸ Re-HEDP	Other	Clinical	(41)
Preclinical			(42)	

TABLE S7. Examples of combination strategies based on disrupting NAD⁺ metabolism.

Class	TRT	Type	Stage	References
NAMPT inhibitors	¹⁷⁷ Lu-DOTATATE	Peptide	Preclinical	(43)

TABLE S8. Examples of combination strategies based on combining with immunotherapy.

Class	TRT	Type	Stage	References
CTLA-4 inhibitors	¹⁷⁷ Lu-LLP2A	Peptide	Preclinical	(44)
	⁹⁰ Y-NM600	Other	Preclinical	(45)
PD1 inhibitors	²²⁵ Ac-PSMA	Peptide	Preclinical	(46)
	¹⁷⁷ Lu-DOTATATE	Peptide	In trials	NCT03325816
				NCT03457948
				NCT04261855
¹⁷⁷ Lu-LLP2A	Peptide	Preclinical	(44)	
PDL1 inhibitors	¹⁷⁷ Lu-PSMA	Peptide	In trials	NCT03658447
				NCT03805594
				(47)
	¹⁷⁷ Lu-LLP2A	Peptide	Preclinical	(44)

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