Joining Forces: Prospects for Targeted Radionuclide Therapy in Combined-Modality Regimens

[1]Martin R. Gill PhD, [1]Nadia Falzone PhD, [2]Yong Du MBBS, [1]Katherine A. Vallis MBBS

[1]CR-UK/MRC Oxford Institute for Radiation Oncology, Department of Oncology, University of Oxford, Old Road Campus Research Building, Off Roosevelt Drive, Oxford OX3 7DQ, UK

[2]The Royal Marsden Hospital NHS Foundation Trust, Sutton, Surrey, SM2 5PT, UK

Corresponding author:
Professor K.A. Vallis,
CR-UK/MRC Oxford Institute for Radiation Oncology,
University of Oxford, Old Road Campus Research Building,
Off Roosevelt Drive,
Oxford, OX3 7DQ,
Tel: +44 (0)1865 255209,
Fax: +44 (0)1865 857533,
Email: katherine.vallis@oncology.ox.ac.uk

Summary

Targeted radionuclide therapy (TRT) is a branch of cancer medicine concerned with the use of radioisotopes, radiolabelled molecules, nanoparticles or microparticles that either naturally accumulate in or are designed to home to tumours. They combine the specificity of molecular and, sometimes, physical targeting with the potent cytotoxicity of ionising radiation. Targeting vectors for TRT include antibodies, antibody fragments, proteins, peptides, and small molecules. The diversity of available carrier molecules together with the large panel of suitable radioisotopes with unique physicochemical properties allows vector-radionuclide pairings to be matched to the molecular, pathological and physical characteristics of a tumour. Some agents are designed for dual therapeutic and diagnostic ("theranostic") applications. TRT use has increased with the introduction of agents that are indicated for common oncological conditions, including ^{90}Y-microspheres for the treatment of hepatic tumours and $^{223}RaCl_2$ for bone metastases. This raises the question of how best to integrate TRT into multi-modality protocols. Achievements in this area and future prospects are reviewed here.

Introduction

The central goal of cancer medicine is to target and destroy malignant cells without damaging healthy tissue. Cytotoxic chemotherapy and external beam radiotherapy (EBRT) remain the mainstay of non-surgical treatment. However, the lack of specificity of these treatments often results in toxicity. It is therefore not surprising that the advent of targeted therapy, bringing with it the promise of excellent cancer-specificity, was greeted with great excitement by clinicians. This enthusiasm has been dampened, however, by the realisation that acquired resistance to targeted agents is common and that many are cytostatic, halting tumour growth rather than killing cells, making conversion of early responses into durable remissions or cure an on-going challenge.[1] To overcome the emergence of resistance, investigators are increasingly turning to combination treatments to yield a clinical benefit.[2] Another approach is to add a toxic payload to a targeted therapeutic to achieve tumour cell killing. Within this category, a particularly attractive strategy is targeted radionuclide therapy (TRT), in which a carrier molecule that seeks functional or molecular targets is labelled with an isotope that emits charged particles.[3] Further flexibility is built into the system since TRT may be applied to the treatment of localised or metastatic tumours through loco-regional or systemic administration.[4] There are three types of particulate radiation pertinent to targeted radionuclide therapy: β-particles, α-particles and Auger electrons, which can irradiate tissue volumes with

multicellular, cellular and subcellular dimensions respectively. In some cases, isotopes that also emit γ-rays or annihilation photons are used for theranostic applications, as they can be used for both imaging and therapy. The key considerations for radionuclide selection are the energy released by the emitted particles over unit distance (linear energy transfer; LET), their range in tissue and physical half-life. The LET of α-, β-particles and Auger electrons are 50-230 keV/µm, 0·2 keV/µm and 4-26 keV/µm with ranges in tissue of 20-100 µm, 0·05-12·0 mm, and <20 µm, respectively. β-emitters are considered ideal for targeting large tumours as their long track length means that even neighbouring cells that lack expression of the molecular target will be exposed to radiation, the "cross-fire" effect, thus overcoming heterogeneous target expression within a cancer.[3] Short-range, high-energy α-emitters are viewed as superior for treating micro-metastasis as well as blood or bone-marrow malignancies while Auger electron-emitters are suited to target single cells.[5]

Targeting vectors that have been tested for TRT include antibodies, a variety of antibody fragments and platforms, proteins, peptides, and small molecules. This rich choice of carrier, together with the availability of a range of isotopes with unique physicochemical properties, allows vector-radioisotope pairings to be tailored precisely to specific clinical scenarios. With the availability of generators for in-house production of therapeutic radiopharmaceuticals,[6] the use of radionuclide therapy is increasing. In the UK there was a 38% increase in the number of treatments during the 5-year period 2007 to 2012, and this increase is likely to continue with the adoption into practise of ^{90}Y-labelled microspheres for the treatment of hepatic tumours, $^{223}RaCl_2$ for metastatic castrate-resistant prostate cancer (mCRPC) and ^{177}Lu-Dotatate (DOTA-TATE or DOTA-octreotate) for neuroendocrine tumours.[7, 8] Currently, TRT is most often used as a monotherapy, typically in patients with end-stage refractory disease. TRT has been used with notable success in the treatment of lymphoma, a highly radiosensitive cancer, but has been less successfully deployed as a curative treatment of common solid tumours.[9]

Barriers to the success of TRT include the heterogeneous expression of the molecular marker that the radiotherapeutic is designed to target, and poor vasculature of the tumour preventing the delivery of the agent. The biological response to TRT of individual cells within a tumour or organ may vary, depending on cell-specific radiosensitivity and distribution of the radiotherapeutic agent at the macroscopic, multicellular and subcellular levels.[10] Other variables that are important in TRT include the relative biological effectiveness of the emitted particles, dose rate and induction of the bystander responses.[11] These properties of TRT may

be exploitable in rationally designed combination protocols, aiming to compliment the strengths of TRT with other therapeutics, taking into consideration optimal timing and sequencing. The aim of this review is to survey recent progress towards the integration of TRT agents in combination with other modalities with the goal of improving outcome, combating resistance and minimizing off-target effects. Illustrative clinical studies and trials that cover the main classes of targeting vector are discussed as are emerging preclinical candidates and combinations.

TRT in combination with chemotherapy: underlying principles

Combined-modality chemotherapy and EBRT is used routinely in the radical treatment of many common cancers including lung, head and neck and cervical cancer. The radiosensitising effect of cytotoxic drugs such as the platinum-based drugs, etoposide and 5-fluorouracil among others underlies the success of this approach. As is the case for EBRT, TRT agents cause DNA damage and are therefore also likely to be enhanced through combination with chemotherapeutic radiosensitisers. However, the interaction of the different classes of radionuclides with radiosensitisers may vary. For example, radionuclides with low LET emissions may be potentiated by DNA damage repair inhibitors or cell-cycle control disruptors. High LET radionuclides, such as alpha emitters, are well-suited to the treatment of hypoxic tumours and may be optimally combined with radiosensitisers that do not depend on the generation of reactive oxygen species. Published examples of clinically approved TRT agents used in combination with a range of chemotherapy drugs are summarised in Table 1.

TRT in combination with chemotherapy: clinical studies

Small molecule-based TRTs

Small molecules such as hormones, steroids, and neurotransmitters that are internalised by specific receptors have been tested as vehicles for TRT. A clinically important example is metaiodobenzylguanidine (MIBG), a structural analogue of the neurotransmitter norepinephrine that incorporates either the β-emitter, ^{131}I, or, when used primarily for SPECT imaging, the Auger electron and gamma-emitter, ^{123}I. MIBG is used for treatment of patients with relapsed or refractory neuroblastoma, neuroendocrine tumours (NET) and medullary thyroid cancers.[12] Although effective as a single agent, strategies to improve ^{131}I-MIBG therapy by combining it with radiosensitisers have been investigated. The long half-life of ^{131}I, 8 days, provides the opportunity for extensive overlap between ^{131}I-MIBG and radiosensitiser exposure to maximise efficiency. ^{131}I-MIBG has been explored in combination with topoisomerase I

inhibitors, a selection based on the antitumour activity of these chemotherapeutics against neuroblastoma and their radiosensitizing effects.[13] Phase I/II trials in patients with advanced neuroblastoma established the combination of ^{131}I-MIBG with vincristine and irinotecan is tolerable and active (NCT02035137)[14] while the combination with topotecan has been tested in a European multi-centre phase II clinical trial in patients with refractory or relapsed neuroblastoma (NCT00960739). ^{131}I-MIBG plus chemotherapy is also being investigated as part of an induction regimen prior to stem cell transplant in newly diagnosed high-risk neuroblastoma patients (NCT01175356).

Peptide-based TRT

The design and synthesis of peptide-drug conjugates is an emerging area of research. Their well-defined conjugation chemistry, efficient penetration in solid tumours, and lower production costs render them attractive alternatives to antibodies as carriers of a toxic payload. Peptide-radiometal conjugates that bind cell-surface receptors have been used clinically for many years, an approach known as peptide receptor radionuclide therapy (PRRT). Somatostatin receptor (SSTR)-binding radiopeptides are capable of delivering a tumouricidal radiation dose to inoperable or metastasized neuroendocrine tumours (NET) through β-decay. The ^{90}Y and ^{177}Lu conjugates of analogues of somatostatin, DOTA-TATE and DOTA-TOC (DOTA, Tyr3-octreotide), are two of the most widely used radiopharmaceuticals in this field.[15, 16] The use of ^{111}In- or ^{68}Ga-labelled analogues compatible with SPECT or PET imaging respectively provides a theranostic radionuclide pair. Trials designed to explore the combination of ^{177}Lu-DOTA-TATE with 5-FU chemotherapy, have shown that patients with progressive NET or uncontrolled symptoms experienced increased overall survival without significant bone marrow or renal toxicity compared to those receiving monotherapy.[17-19] More recently, a phase II study evaluated the combination of ^{177}Lu-DOTA-TATE with capecitabine, a 5-FU pro-drug, as salvage therapy for patients with advanced stage NET.[20] Tumour control and stabilization of disease was achieved in 94% of a cohort of 33 patients. Together, these encouraging results suggest that an advantage exists for combined treatment when compared to series in which ^{177}Lu-DOTA-TATE was used alone[21] and furthermore exemplifies the possibility of tailoring the choice of chemotherapy to agents that demonstrate independent efficacy against the cancer in question.[22] With this in mind, the combination of ^{177}Lu-DOTA-TATE with capecitabine plus the alkylating agent temozolomide has been tested, aiming to exploit the synergy and radiosensitizing effects of both cytotoxics. In a group of 30 patients with advanced pancreatic NET, the overall response rate was 80% (95% CI 66-93) with no

grade 4 adverse events.[23] Further preclinical and preliminary clinical trials include the use of carboplatin and etoposide (small cell carcinoma)[24] or the mTOR inhibitor everolimus (NET)[25] to accompany PPRT. These studies also demonstrate the effective use of ^{68}Ga-octreotate and PET imaging to assess the metabolic and objective responses of primary tumours and hepatic metastases to treatment.

The use of two therapeutic radionuclides in combination is an emerging option. This takes advantage of differences in the energy and range of particles emitted from each radionuclide. Using a pre-therapy biodistribution theranostic approach and accompanying dosimetry, more precise matching of the target lesion size to the optimal radionuclide combination required for its eradication without exceeding normal organ dose limits may be achieved. For example, ^{177}Lu-DOTA-TATE (β-particle range in tissue, 2 mm) has been tested in combination with ^{90}Y-DOTA-TOC (β-particle range, 11 mm) in patients with refractory NET in an effort to deliver an effective absorbed radiation dose while avoiding increased toxicity.[26] Administration of ^{177}Lu-DOTA-TATE (5·55 GBq) and ^{90}Y-DOTA-TATE in tandem (2·6 GBq) resulted in objective responses in 43% of patients and was well tolerated with no significant renal toxicity. Combination TRT has also been explored for small molecule/PRRT. The addition of ^{131}I-MIBG to ^{90}Y DOTA-Phe1-Tyr3-octreotide (^{90}Y-DOTATOC) in patients with advanced stage midgut NETs confirmed the feasibility of this strategy.[27] Of particular interest is how these findings will translate to new PRRT agents that possess higher target affinities than traditional SSTR agonists. In a small pilot study a SST2-receptor antagonist, ^{177}Lu-DOTA-JR11, was shown to possess superior intratumoural residence time and higher tumour uptake over ^{177}Lu-DOTA-TATE; properties that make it well-suited to a radiosensitisation strategy.[28]

The success of the somatostatin analogues in the treatment of SSTR-positive tumours has stimulated a concerted research effort to identify other targets for PRRT. Radiopeptides that target the gastrin-releasing peptide receptor, chemokine receptor-4 or glucagon-like peptide-1 receptor, have been designed for both imaging and therapeutic applications[29] and an appraisal of their role in combination with other anti-cancer agents is likely to follow. More recently, ^{177}Lu-labelled prostate-specific membrane antigen (PSMA) ligand has been tested in mCRPC with promising preliminary results.[30] Although the majority of PRRT agents are β-emitters, there is growing interest in the use of α-emitters such as ^{213}Bi, ^{225}Ac and ^{211}As.[31] A targeted α-emitter can selectively kill individual cancer cells with a single atomic decay, achieving higher

specificity than β-emission. ^{213}Bi-DOTA-TOC has been shown to induce remission in NET patients refractory to non-radioactive octreotide and to ^{90}Y/^{177}Lu-DOTATOC,[32] while ^{213}Bi-labelled bombesin analogues are currently in pre-clinical and phase I development for the treatment of prostate cancer.[33] A recent report described two patients with metastatic prostate cancer who experienced a complete response to ^{225}Ac-PSMA.[34] Auger electrons have LET values intermediate between those of α- and β-emitters and Auger electron emitters such as ^{111}In have been tested in the context of PRRT. For example, ^{111}In-DTPA-EGF, a radiopharmaceutical designed to bind the epidermal growth factor receptor (EGFR) was found to inhibit the growth of EGFR over-expressing breast cancer xenografts and has been evaluated in a first-in-human trial, where SPECT imaging provided evidence of accumulation of ^{111}In-DTPA-EGF in at least one known site of disease in 47% of patients.[35, 36] The combination of Auger electron emitters plus chemotherapy has been tested in only a small number of trials although in one, in which ^{111}In-octreotide was administered with 5FU to patients with NET, a high rate of symptomatic response was noted suggesting that this approach merits further study.[18] It is important to note that the decay of some medically important β-emitting radionuclides, including ^{177}Lu, is accompanied by the emission of Auger electrons, and they may contribute to the anticancer effects of these isotopes.[37]

Antibody-based TRTs

Antibody-drug conjugates (ADCs) combine the specificity of antibody affinity for cell-surface proteins with a cytotoxic payload. The same principle underlies the design of radioimmunotherapeutic agents (RIT) where a radionuclide acts as the cytotoxic warhead.[38] For non-radionuclide ADCs, it is important that drug cleavage only occurs once uptake into a cancer cell has taken place and incorporation of an effective cleavable linker is a crucial and challenging aspect of design and synthesis. A major advantage of RIT agents is that internalisation is not required due to the range of α- and β-particles. Here we highlight notable examples of RIT agents that have been tested in combination regimens.

A prominent example of antibody-based TRT is ^{90}Y-ibritumomab tiuxetan (^{90}Y-IT; Zevalin®) which is used in the treatment of lymphoma, a radiosensitive malignancy.[38] ^{90}Y, a pure β-emitter, is conjugated to a monoclonal antibody that recognises the CD20 antigen, a protein found on the plasma membrane of B-lymphocytes in 95% of B-cell lymphomas. Other CD20-targeting RITs include ^{131}I-Tositumomab (Bexxar®) and ^{131}I-rituximab. Although Bexxar was withdrawn in 2014 due to infrequent use, ^{131}I-rituximab is employed in first-line treatment of

non-Hodgkin lymphomas (NHL) in conjunction with rituximab[39] and has been shown to achieves complete remission in half of patients suffering from relapsed or refractory indolent NHL.[40, 41] ^{90}Y-IT is currently indicated for the treatment of recurrent or relapsed CD20-positive NHL and as consolidation therapy following response to first-line chemotherapy. Numerous studies have been performed to define the role of ^{90}Y-IT in combination with chemotherapy in both indolent and aggressive NHL. The therapeutic gain offered by ^{90}Y-IT consolidation in advanced-stage follicular lymphoma was demonstrated in a randomized, phase III trial (NCT00185393). In a group of 409 patients, ^{90}Y-IT was added after completion of numerous chemotherapy regimens (CVP/CHOP, CHOP, fludarabine, chlorambucil, and rituximab combinations). Compared to the control arm, addition of ^{90}Y-IT resulted in a complete response (CR) rate of 87% versus 53% and an estimated 8-year progression free survival (PFS) rate of 41% versus 22%.[42] ^{90}Y-IT has also been combined with high-dose chemotherapy in autologous stem cell transplant (ASCT) procedures. A randomized study of 43 patients compared ^{90}Y-IT plus high-dose BEAM chemotherapy (carmustine, etoposide, cytarabine and melphalan) versus BEAM alone before ASCT. Compared to chemotherapy alone the ^{90}Y-IT-containing arm was associated with significantly improved overall survival (OS) at 2 years; 91% versus 62% (NCT00491491).[43] Based on these findings, the role of ^{90}Y-IT in the management of NHL in combination with other immunotherapy and chemotherapy agents is the subject of continuing clinical research.[44] The potential of α-particle RIT has been explored in leukaemia using ^{213}Bi-lintuzumab, composed of a humanized anti-CD33 antibody conjugated to the heavy metal α-emitter, ^{213}Bi. Early results indicate that, in a cohort of 18 patients with relapsed and refractory acute myelogenous leukemia (AML) or chronic myelomonocytic leukemia, 78% experienced a reduction in the percentage of blasts in the bone marrow.[45] ^{213}Bi-lintuzumab has also been administered sequentially with cytarabine, an anti-metabolite which inhibits DNA synthesis, in a phase I/II trial of patients with AML.[46] The same group is currently investigating the combination of ^{225}Ac-lintuzumab with cytoreductive chemotherapy (NCT02575963), and the substantially longer half-life of ^{225}Ac ($t_{1/2}$, 10 days) compared to ^{213}Bi ($t_{1/2}$, 45·6 mins) is expected to provide a better indication of whether this combination can control AML.

There is good preclinical evidence for the efficacy of radioimmunotherapeutics in the treatment of non-haematological malignancies. Promising candidate RIT agents are being tested and successes in combination regimens have been reported. ^{177}Lu-J591 is a RIT agent that targets PSMA, a transmembrane glutamate carboxypeptidase II protein. A series of prostate cancer trials demonstrated that ^{177}Lu-J591 targets known sites of metastatic disease in 90% of cases

and, when co-administered with a standard course of docetaxel, caused a significant fall in prostate-specific antigen (PSA) in 80% of participants.[47] No dose limiting toxicity (DLT) was observed leading to the conclusion that this combination is clinically acceptable.[47] Several RIT agents that target the anti-carcinoembryonic antigen (CEA), which is overexpressed in lung, breast, and pancreatic cancers among others, have been tested clinically. This includes ^{131}I-A5B7, which has been explored in combination with the vascular disrupting agent combretastatin-A4-phosphate.[48] This provides a striking example of how to achieve complementary drug selection, where two agents that target different biophysical compartments of the tumour may be combined safely. Trastuzumab, a monoclonal antibody directed against the HER2 receptor, is a widely used breast cancer treatment. Preclinical studies of radiolabelled trastuzumab conjugates showed modest therapeutic benefit even when conjugated to α-emitters such as ^{213}Bi and there are surprisingly few studies employing β-emitting radionuclides conjugated to trastuzumab for therapy.[49] ^{111}In-labelled trastuzumab was originally designed as an imaging probe to predict therapy response and visualise metastases.[50] However, attachment of a nuclear localisation sequence (NLS) had the effect of increasing the radiation-absorbed dose to DNA with a concomitant increase in *in vitro* cytotoxicity.[51] ^{111}In-trastuzumab-NLS proved effective against trastuzumab-resistant breast cancer cells, and cytotoxicity was enhanced by addition of the radiosensitizing cytotoxic, methotrexate.[52]

Genetically engineered antibody fragments including monomeric scFv fragments, diabodies, minibodies and other formats retain the targeting specificity of the whole antibody and offer improved tumour uptake and more rapid clearance.[38] Many studies exploring a range of radiolabelled antibody fragments for imaging and diagnosis have been reported but less progress has been made in defining their therapeutic applications. One example is the ^{131}I-labelled minibody, ^{131}I-F16SIP (Tenarad), which targets tenascin C, an extracellular matrix component on newly formed blood vessels, and is currently being explored in phase I/II trials for recurrent Hodgkin's lymphoma.[53] It is not known yet whether the use of antibody fragments will provide an advantage over intact antibodies within a combination strategy. However, a shorter circulation time and more rapid tumour uptake may make it possible to precisely time the administration of other systemically delivered drugs to coincide with the peak in tumour accumulation of the RIT agent.

Although most RIT agents in clinical use are directed against cell-surface antigens, there is also interest in the development of internalising antibodies. This can be achieved by exploiting receptor trafficking or by the addition of a cell-penetrating peptide (CPP) to the antibody. This

approach was used in the design of ^{111}In-anti-γH2AX-TAT, an experimental RIT agent that incorporates the CPP, TAT (trans-activator of transcription), and targets γH2AX, a DNA damage response protein.[54] ^{111}In-anti-γH2AX-TAT amplified pre-existing DNA damage through intranuclear decay of ^{111}In.[55] This agent would therefore be well-suited as an adjunct to EBRT or to genotoxic chemotherapy, treatments that induce DNA double-strand breaks (DSB) and γH2AX expression.

Emerging TRT-chemotherapy combinations

Building on the clinical success of the β-emitters, ^{89}Sr and ^{153}Sm, to alleviate pain caused by bone metastases, the α-emitter, $^{223}RaCl_2$ (Xofigo®), has been shown to confer a significant survival benefit in patients with CRPC and bone metastases.[56] Several studies have established that $^{223}RaCl_2$ can be administered safely to patients following docetaxel.[57] The results of a phase I/II trial in which concurrent $^{223}RaCl_2$ plus docetaxel is compared to docetaxel alone are awaited (NCT01106352). Phase I trials of the β-emitting agents ^{186}Re-HEDP (HEDP = 1,1-hydroxyethylidene diphosphonate) and ^{188}Re-HEDP in mCRPC, have established that ^{186}Re-HEDP may be safely combined with docetaxel[58], and ^{188}Re-HEDP with capecitabine.[59]

PSMA has recently emerged as an important target for TRT in prostate cancer. A ^{177}Lu-conjugated small molecule ligand of PSMA (^{177}Lu-PSMA) was tested in a cohort of 56 patients, 80% of whom experienced a decrease in PSA level. Twenty-five patients were evaluated for response using ^{68}Ga-PSMA as a companion diagnostic PET tracer, and partial response was noted in 14.[60] The immediate priority for this and other radiolabelled PSMA ligands is to evaluate them in clinical trials including randomised assessments, and then to define their role in combination with other treatments, such as $^{223}RaCl_2$. A new application of radionuclide therapy that has emerged over the last decade is radioembolisation of hepatic tumours by the intra-arterial delivery of ^{90}Y-labelled resin or glass microspheres. This treatment, selective internal radiation therapy (SIRT), has been tested in combination with 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX) chemotherapy with or without bevacizumab in patients with previously untreated metastatic colorectal cancer. The SIRFLOX phase III trial (530 patients) showed that while the SIRT/FOLFOX combination did not increase progression free survival (PFS), disease progression in the liver was significantly delayed.[61] The results of a second phase III trial, FOXFIRE, of similar design are awaited.[62] ^{131}I-lipiodol or ^{188}Re-lipiodol, also delivered via the hepatic artery, has emerged as an effective and affordable treatment for

hepatocellular carcinoma in South-East Asia, Sub–Saharan Africa, and Japan: regions where this cancer has a high incidence.[63]

TRT agents in combination with molecularly targeted therapeutics: emerging concepts and pre-clinical studies

In addition to non-specific chemotherapy, TRT has been investigated in combination with several classes of molecular targeted drugs, aiming to use the ability of a cancer-specific pathway agonist, antagonist, or inhibitor to complement the effects of TRT. This approach places greater focus on elucidation of the molecular mechanism of action and cellular response to the specific TRT. Successful co-targeting of the relevant pathway with the molecular targeted therapeutic may potentiate cytotoxicity and act to combat resistance, or a complementary molecular targeted therapeutic could be employed to combat tumour heterogeneity. Recent progress in this area is described, highlighting several promising research themes under development (Table 2).

DNA damage response inhibitors

An important mechanism by which to attain synergy with TRT is through concurrent inhibition of DNA repair. As cancer cells possess aberrations in their ability to elicit the correct cellular responses to DNA damage, they are particularly susceptible to this strategy. To exploit this vulnerability, numerous trials combining DNA damage response (DDR) inhibitors with chemotherapy or EBRT have been reported[64] and this strategy is now gaining traction for TRT. Pre-clinical examples include enhanced cytotoxicity for a combination of gemcitabine, the Chk1 inhibitor PF-477736, and ^{177}Lu-cetuximab, an anti-EGFR antibody, in a pancreatic ductal adenocarcinoma cell line and patient-derived xenograft models.[65] Employing the PARP inhibitor olaparib achieved similar results.[66] While encouraging, these examples each contain four separate components: a DDR inhibitor, gemcitabine, ^{177}Lu, and the anti-EGFR antibody cetuximab that, in itself, may possess inherent anti-proliferative activity towards EGFR-positive cells. Thus, elucidation of the relationship between components is particularly challenging and identifying synergistic relationships for further advancement is difficult. In a recent study, PARP inhibition using olaparib was shown to enhance the cytotoxic effect of ^{177}Lu-DOTA-TATE by increasing double-strand breaks (DSBs) in somatostatin receptor-expressing human osteosarcoma cells.[67]

Other inhibitors

Although it is generally accepted that the cellular DNA is the main target for TRT, studies by Pouget *et al.* on Auger electron-mediated damage have challenged this premise.[68] The possibility that TRT activates alternative cell signalling pathways opens up the possibility of interaction with other classes of inhibitor, including those that block oxidative stress responses or cell proliferation pathways. For example, EGFR inhibition has long been viewed as an attractive target for cancer therapy and EGFR inhibitors are applied in combination with EBRT and chemotherapy.[69] In preclinical experiments EGFR inhibition by gefitinib potentiated the cytotoxicity of ^{111}In-DTPA-hEGF by increasing the level of nuclear uptake and resultant DNA damage.[70] Furthermore, the EGFR tyrosine kinase inhibitor AG1478 plus ^{177}Lu-hu3S193, an anti-Lewis Y antibody, were shown to have an additive effect on DU145 (prostate cancer) xenografts and to prolong survival compared to ^{177}Lu-hu3S193 treatment alone.[71]

Hormone therapy and immunotherapy

The concurrent use of $^{223}RaCl_2$ and hormone therapy has been tested in a large, multinational single arm trial (839 patients) which established that addition of abiraterone and/or enzalutamide to $^{223}RaCl_2$ was safe and resulted in an increase in median survival.[72] Analogous trials of hormone therapy in combination with PSMA-targeted TRT will be of interest. An emerging challenge is how best to combine TRT with immunotherapy. This question has been explored for EBRT, as plentiful evidence that ionising radiation boosts the tumour-targeting immune response has accumulated. Several on-going clinical trials are designed to exploit this phenomenon.[73] The combination of $^{223}RaCl_2$ with the immunotherapeutic sipuleucel-T, which is used for asymptomatic or minimally symptomatic bone metastases in CRPC, is being investigated and may provide the first clues as to whether this approach holds promise (NCT02463799).

TRT in combination with EBRT: underlying principles and clinical studies

Considering that EBRT is a central component of treatment in up to 50% of cancer patients, it is surprising that there are few reported examples of TRT plus EBRT combinations.[74] The arguments for supporting this approach are many. The dose limiting organs of the two modalities differ, thus allowing escalation of the combined radiation absorbed dose to tumour without exceeding the maximum tolerated dose of the limiting organs.[74] The organs at risk in the case of EBRT are those that lie close to the tumour or are in the path of incident beams, whereas myelosuppression has been the main dose limiting toxicity of systemically

administered TRT. Another exploitable interaction between the two modalities is the effect of EBRT on blood flow and vessel permeability[75] where enhanced intratumoural accumulation of targeted radionuclides as a result of vasculature perturbation following EBRT has been reported.[76] This implies prior treatment with EBRT before the administration of TRT could also ameliorate the problems associated with non-uniform distribution of radioactivity in tumours which is compounded by heterogeneous expression of the antigen to which the TRT agent binds.[3] Furthermore, prior treatment with EBRT could be used to de-bulk the tumour which would allow for more effective treatment with TRT which is often limited by the range of the emissions from a specific radionuclide. A combination of EBRT and TRT could exploit the radiobiological differences observed in dose and dose-rate effects. While hypoxic cells are resistant to low LET photon irradiation, which is used for conventional EBRT, hypoxia is less of a barrier to efficacy in the case of intermediate LET intranuclear incorporated Auger electron emitters and high-LET α-emitters. It may also be possible to capitalise on dose-rate differences between EBRT and TRT. EBRT is delivered at high dose-rates, typically about 50-60 Gy/h, in short, repeated daily fractions of approximately 2 Gy. With TRT, however, the absorbed dose is delivered continuously but slowly with a dose-rate of 0·01-0·1 Gy/h that diminishes over time as the radionuclide decays. The difference in dose-rate of the two forms of radiotherapy results in profoundly different radiobiological effects. For example, an "inverse dose-rate effect" has been described whereby a given radiation dose delivered at a low dose-rate is more cytotoxic than when delivered more rapidly.[77] Also there is evidence to suggest that protracted low dose-rate, low dose radiation may sensitize cancer cells to a subsequent high dose-rate exposure.[78] Interestingly, differing dose-rates have been shown to cause differences in gene expression, mode of cell death and the type of DNA damage that is induced.[78] It is plausible that if these effects were better understood they could be exploited through smart scheduling and dosing of EBRT and TRT. It is also true that the phenomenon of "spatial co-operation", originally used to describe the interplay between chemotherapy and EBRT, is also likely to exist for the combination of TRT and EBRT.[79] Synergy arises because EBRT acts locally on the primary tumour, while TRT also targets metastases beyond the EBRT field. Highlighted clinical examples include the combination of EBRT and a radiolabelled anti-EGFR antibody (^{125}I-mAb 425) with or without temozolomide in a phase II study of 192 patients with glioblastoma multiforme (GBM).[80] The inclusion of temozolomide was associated with an increase in median survival (20·2 versus 14·5 months). The RIT agent, ^{131}I-L19SIP (Radretumab), which binds to fibronectin extra domain-B for vascular targeting, is being tested

in combination with whole brain radiotherapy for brain metastases in a phase I/II trial (NCT01125085).[81] Work in this area is not limited to RIT and the combination of fractionated EBRT with either PRRT or ^{131}I-MIBG have been explored in small feasibility studies.[82, 83] The timing of the therapies are crucial as a temporal separation in the delivery of EBRT and TRT could be deleterious compared to simultaneous or concurrent delivery.[84]

The design of treatment schedules that combine EBRT and TRT remains a challenge and it is likely that the optimal treatment schedule, dosimetry and radiobiology will differ for each vector-radionuclide pairing. A recent review highlighted that statistically significant dose-response relationships were found in 61% of studies of TRT.[85] This emphasises the requirement for accurate dosimetry to arrive at personalized administration for each TRT.

Future Directions and Conclusions

Multimodality approaches to cancer treatment are being widely adopted. In the case of TRT-containing regimens a deeper understanding of the radiobiological consequences of specific vector-radionuclide pairings is needed to exploit synergy between TRT and other modalities. Although TRT agents that incorporate β-emitting isotopes have been used successfully in combination with radiosensitizing chemotherapy, it remains to be seen how the emerging α-emitting radiotherapeutics should be combined with other treatments. The cytotoxic effects of high LET α-radiation are less dependent on cell-cycle phase and oxygen concentration and it follows that conventional radiosensitisers may not have the beneficial impact on α-particle therapy as demonstrated for β-emitting agents. Complementary TRT agents aimed specifically at different sub-populations of cancer cells and the strategy of enhancing TRT uptake by increased tumour vasculature permeabilisation, are interesting approaches that merit further investigation.

It is evident that dosing of TRT agents is often based on empirical observations and not subject to the precise, patient-specific treatment planning which is standard for EBRT. For the same amount of administered radioactivity, the tumour uptake and, therefore, the radiation-absorbed dose, may vary considerably from one patient to the next. Thus, there is a strong drive towards patient-specific planning in the TRT field, the aim being to use information about dose to the organ at risk, pharmacokinetics of the construct and tumour uptake derived from pre-treatment imaging to guide selection of the administered activity that will result in a pre-determined radiation absorbed dose to the tumour. This greater emphasis on patient-specific treatment

planning would allow escalation of tumour dose, avoidance of normal tissue toxicity and improve co-delivery of TRT to the required level for the precise combination with overall improved patient outcome.

Finally, a concentrated effort is required to shift practice from empirical observation to randomised controlled trials of combination treatments if the potential of TRT is to be fully exploited. The integration of TRT into first-line therapy, rather than as a treatment for end-stage disease as has been the norm to date, is likely to be beneficial.

References

1. Neal JW, Sledge GW. Decade in review-targeted therapy: successes, toxicities and challenges in solid tumours. *Nat Rev Clin Oncol* 2014; **11**: 627-8.
2. Lopez JS, Banerji U. Combine and conquer: challenges for targeted therapy combinations in early phase trials. *Nat Rev Clin Oncol* 2016: doi:10.1038/nrclinonc.2016.96.
3. Pouget J-P, Navarro-Teulon I, Bardies M, et al. Clinical radioimmunotherapy: the role of radiobiology. *Nat Rev Clin Oncol* 2011; **8**: 720-34.
4. Dumortier J, Decullier E, Hilleret MN, et al. Adjuvant intraarterial lipiodol or ^{131}I-lipiodol after curative treatment of hepatocellular carcinoma: a prospective randomized trial. *J Nucl Med* 2014; **55**: 877-83.
5. Chan HS, Konijnenberg MW, de Blois E, et al. Influence of tumour size on the efficacy of targeted alpha therapy with (213)Bi-[DOTA(0),Tyr(3)]-octreotate. *Eur J Nucl Med Mol Imaging Res* 2016; **6**: 6.
6. Edem PE, Fonslet J, Kjaer A, Herth M, Severin G. In vivo radionuclide generators for diagnostics and therapy. *Bioinorg Chem Appl* 2016; **2016**: 8.
7. Rojas B, Hooker C, McGowan DR, Guy MJ. Five years of molecular radiotherapy growth in the UK: survey results from 2007 to 2012. *Nucl Med Commun* 2015; **36**: 761-5.
8. Strosberg J, El-Haddad G, Wolin E, et al. Phase 3 trial of ^{177}Lu-dotatate for midgut neuroendocrine tumors. *N Engl J Med* 2017; **376**: 125-35.
9. Jackson MR, Falzone N, Vallis KA. Advances in anticancer radiopharmaceuticals. *Clin Oncol (R Coll Radiol)* 2013; **25**: 604-9.

10. Rajon D, Bolch WE, Howell RW. Survival of tumor and normal cells upon targeting with electron-emitting radionuclides. *Med Phys.* 2013; **40**:014101.
11. Brady D, O'Sullivan JM, Prise KM. What is the role of the bystander response in radionuclide therapies? *Front Oncol* 2013; **3**: 215.
12. Sze WCC, Grossman AB, Goddard I, et al. Sequelae and survivorship in patients treated with ^{131}I-MIBG therapy. *Br J Cancer* 2013; **109**: 565-72.
13. McCluskey AG, Boyd M, Ross SC, et al. [131I]meta-iodobenzylguanidine and topotecan combination treatment of tumors expressing the noradrenaline transporter. *Clin Cancer Res* 2005; **11**: 7929-37.
14. DuBois SG, Allen S, Bent M, et al. Phase I/II study of ^{131}I-MIBG with vincristine and 5 days of irinotecan for advanced neuroblastoma. *Br J Cancer* 2015; **112**: 644-9.
15. van Essen M, Krenning EP, Kam BLR, et al. Peptide-receptor radionuclide therapy for endocrine tumors. *Nat Rev Endocrinol* 2009; **5**: 382-93.
16. Kesavan M, Turner JH. Myelotoxicity of peptide receptor radionuclide therapy of neuroendocrine tumors: A decade of experience. *Cancer Biother Radiopharm* 2016; **31**: 189-98.
17. Hubble D, Kong G, Michael M, et al. ^{177}Lu-octreotate, alone or with radiosensitising chemotherapy, is safe in neuroendocrine tumour patients previously treated with high-activity ^{111}In-octreotide. *Eur J Nucl Med Mol Imaging* 2010; **37**: 1869-75.
18. Kong G, Johnston V, Ramdave S, et al. High-administered activity In-111 octreotide therapy with concomitant radiosensitizing 5FU chemotherapy for treatment of neuroendocrine tumors: preliminary experience. *Cancer Biother Radiopharm* 2009; **24**: 527-33.
19. Kong G, Thompson M, Collins M, et al. Assessment of predictors of response and long-term survival of patients with neuroendocrine tumour treated with peptide receptor chemoradionuclide therapy (PRCRT). *Eur J Nucl Med Mol Imaging* 2014; **41**: 1831-44.
20. Claringbold PG, Brayshaw PA, Price RA, Turner JH. Phase II study of radiopeptide ^{177}Lu-octreotate and capecitabine therapy of progressive disseminated neuroendocrine tumours. *Eur J Nucl Med Mol Imaging* 2011; **38**: 302-11.
21. Kwekkeboom DJ, de Herder WW, Kam BL, et al. Treatment with the radiolabeled somatostatin analog [177Lu-DOTA0,Tyr3]octreotate: Toxicity, efficacy, and survival. *J Clin Oncol* 2008; **26**: 2124-30.

22. Strosberg JR, Fine RL, Choi J, et al. First-line chemotherapy with capecitabine and temozolomide in patients with metastatic pancreatic endocrine carcinomas. *Cancer* 2011; **117**: 268-75.
23. Claringbold PG, Turner JH. Pancreatic neuroendocrine tumor control: durable objective response to combination ^{177}Lu-octreotate-capecitabine-temozolomide radiopeptide chemotherapy. *Neuroendocrinology* 2016; **103**: 432-9.
24. Lewin J, Cullinane C, Akhurst T, et al. Peptide receptor chemoradionuclide therapy in small cell carcinoma: from bench to bedside. *Eur J Nucl Med Mol Imaging* 2015; **42**: 25-32.
25. Claringbold PG, Turner JH. NeuroEndocrine tumor therapy with lutetium-177-octreotate and everolimus (NETTLE): A phase I study. *Cancer Biother Radiopharm* 2015; **30**: 261-9.
26. Seregni E, Maccauro M, Chiesa C, et al. Treatment with tandem [90Y]DOTA-TATE and [177Lu]DOTA-TATE of neuroendocrine tumours refractory to conventional therapy. *Eur J Nucl Med Mol Imaging* 2014; **41**: 223-30.
27. Bushnell DL, Madsen MT, O'cdorisio T, et al. Feasibility and advantage of adding (131)I-MIBG to (90)Y-DOTATOC for treatment of patients with advanced stage neuroendocrine tumors. *EJNMMI Res* 2014; **4**: 38.
28. Wild D, Fani M, Fischer R, et al. Comparison of somatostatin receptor agonist and antagonist for peptide receptor radionuclide therapy: A Pilot Study. *J Nucl Med* 2014; **55**: 1248-52.
29. Chatalic KL, Kwekkeboom DJ, de Jong M. Radiopeptides for imaging and therapy: a radiant future. *J Nucl Med* 2015; **56**: 1809-12.
30. Afshar-Oromieh A, Babich JW, Kratochwil C, et al. The rise of PSMA ligands for diagnosis and therapy of prostate cancer. *J Nucl Med* 2016; **57**: 79S-89S.
31. Sgouros G. Alpha-particles for targeted therapy. *Adv Drug Deliv Rev* 2008; **60**: 1402-6.
32. Kratochwil C, Giesel FL, Bruchertseifer F, et al. ^{213}Bi-DOTATOC receptor-targeted alpha-radionuclide therapy induces remission in neuroendocrine tumours refractory to beta radiation: a first-in-human experience. *Eur J Nucl Med Mol Imaging* 2014; **41**: 2106-19.
33. Wild D, Frischknecht M, Zhang H, et al. Alpha- versus beta-particle radiopeptide therapy in a human prostate cancer model (^{213}Bi-DOTA-PESIN and ^{213}Bi-AMBA versus ^{177}Lu-DOTA-PESIN). *Cancer Res* 2011; **71**: 1009-18.

34. Kratochwil C, Bruchertseifer F, Giesel FL, et al. ^{225Ac}-PSMA-617 for PSMA-targeted α-radiation therapy of metastatic castration-resistant prostate cancer. *J Nucl Med* 2016; **57**: 1941-4.
35. Chen P, Cameron R, Wang J, Vallis KA, Reilly RM. Antitumor effects and normal tissue toxicity of ^{111}In-labeled epidermal growth factor administered to athymic mice bearing epidermal growth factor receptor-positive human breast cancer xenografts. *J Nucl Med* 2003; **44**: 1469-78.
36. Vallis KA, Reilly RM, Scollard D, et al. Phase I trial to evaluate the tumor and normal tissue uptake, radiation dosimetry and safety of ^{111}In-DTPA-human epidermal growth factor in patients with metastatic EGFR-positive breast cancer. *Am J Nucl Med Mol Imaging* 2014; **4**: 181-92.
37. Hindie E, Zanotti-Fregonara P, Quinto MA, Morgat C, Champion C. Dose deposits from ^{90}Y, ^{177}Lu, ^{111}In, and ^{161}Tb in micrometastases of various sizes: implications for radiopharmaceutical therapy. *J Nucl Med* 2016; **57**: 759-64.
38. Steiner M, Neri D. Antibody-radionuclide conjugates for cancer therapy: Historical considerations and new trends. *Clin Cancer Res* 2011; **17**: 6406-16.
39. McQuillan AD, Macdonald WBG, Turner JH. Phase II study of first-line ^{131}I-rituximab radioimmunotherapy in follicular non-Hodgkin lymphoma and prognostic 18F-fluorodeoxyglucose positron emission tomography. *Leuk Lymphoma* 2015; **56**: 1271-7.
40. Leahy MF, Seymour JF, Hicks RJ, Turner JH. Multicenter phase II clinical study of iodine-131-rituximab radioimmunotherapy in relapsed or refractory indolent non-Hodgkin's lymphoma. *J Clin Oncol* 2006; **24**: 4418-25.
41. Leahy MF, Turner JH. Radioimmunotherapy of relapsed indolent non-Hodgkin lymphoma with ^{131}I-rituximab in routine clinical practice: 10-year single-institution experience of 142 consecutive patients. *Blood* 2011; **117**: 45.
42. Morschhauser F, Radford J, Van Hoof A, et al. ^{90}Yttrium-ibritumomab tiuxetan consolidation of first remission in advanced-stage follicular non-Hodgkin lymphoma: updated results after a median follow-up of 7.3 years from the international, randomized, phase III first-line indolent trial. *J Clin Oncol* 2013; **31**: 1977-83.
43. Shimoni A, Avivi I, Rowe JM, et al. A randomized study comparing yttrium-90 ibritumomab tiuxetan (Zevalin) and high-dose BEAM chemotherapy versus BEAM alone as the conditioning regimen before autologous stem cell transplantation in patients with aggressive lymphoma. *Cancer* 2012; **118**: 4706-14.

44. Rizzieri D. Zevalin(®) (ibritumomab tiuxetan): After more than a decade of treatment experience, what have we learned? *Crit Rev Oncol Hematol* 2016; **105**: 5-17.
45. Jurcic JG, Larson SM, Sgouros G, et al. Targeted α particle immunotherapy for myeloid leukemia. *Blood* 2002; **100**: 1233-9.
46. Rosenblat TL, McDevitt MR, Mulford DA, et al. Sequential cytarabine and α-particle immunotherapy with bismuth-213-lintuzumab (HuM195) for acute myeloid leukemia. *Clin Cancer Res* 2010; **16**: 5303-11.
47. Vallabhajosula S, Nikolopoulou A, Jhanwar YS, et al. Radioimmunotherapy of metastatic prostate cancer with ^{177}Lu-DOTAhuJ591 anti prostate specific membrane antigen specific monoclonal antibody. *Curr Radiopharm* 2016; **9**: 44-53.
48. Meyer T, Gaya AM, Dancey G, et al. A phase I trial of radioimmunotherapy with ^{131}I-A5B7 anti-CEA antibody in combination with combretastatin-A4-phosphate in advanced gastrointestinal carcinomas. *Clin Cancer Res* 2009; **15**: 4484-92.
49. Milenic DE, Garmestani K, Brady ED, et al. Targeting of HER2 antigen for the treatment of disseminated peritoneal disease. *Clin Cancer Res* 2004; **10**: 7834-41.
50. Perik PJ, Lub-De Hooge MN, Gietema JA, et al. Indium-111-labeled trastuzumab scintigraphy in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer. *J Clin Oncol* 2006; **24**: 2276-82.
51. Costantini DL, Chan C, Cai Z, Vallis KA, Reilly RM. ^{111}In-labeled trastuzumab (herceptin) modified with nuclear localization sequences (NLS): an Auger electron-emitting radiotherapeutic agent for HER2/neu-amplified breast cancer. *J Nucl Med* 2007; **48**: 1357-68.
52. Costantini DL, Bateman K, McLarty K, Vallis KA, Reilly RM. Trastuzumab-resistant breast cancer cells remain sensitive to the Auger electron-emitting radiotherapeutic agent ^{111}In-NLS-trastuzumab and are radiosensitized by methotrexate. *J Nucl Med* 2008; **49**: 1498-505.
53. Aloj L, D'Ambrosio L, Aurilio M, et al. Radioimmunotherapy with Tenarad, a ^{131}I-labelled antibody fragment targeting the extra-domain A1 of tenascin-C, in patients with refractory Hodgkin's lymphoma. *Eur J Nucl Med Mol Imaging* 2014; **41**: 867-77.
54. Cornelissen B, Kersemans V, Darbar S, et al. Imaging DNA damage in vivo using γH2AX-targeted immunoconjugates. *Cancer Res* 2011; **71**: 4539-49.
55. Cornelissen B, Darbar S, Kersemans V, et al. Amplification of DNA damage by a γH2AX-targeted radiopharmaceutical. *Nucl Med Biol* 2012; **39**: 1142-51.

56. Nilsson S. Radionuclide therapies in prostate cancer: integrating radium-223 in the treatment of patients with metastatic castration-resistant prostate cancer. *Curr Oncol Rep* 2016; **18**: 14.
57. Hoskin P, Sartor O, O'Sullivan JM, et al. Efficacy and safety of radium-223 dichloride in patients with castration-resistant prostate cancer and symptomatic bone metastases, with or without previous docetaxel use: a prespecified subgroup analysis from the randomised, double-blind, phase 3 ALSYMPCA trial. *Lancet Oncol* 2014; **15**: 1397-406.
58. van Dodewaard-de Jong JM, de Klerk JMH, Bloemendal HJ, et al. A phase I study of combined docetaxel and repeated high activity ^{186}Re-HEDP in castration-resistant prostate cancer (CRPC) metastatic to bone (the TAXIUM trial). *Eur J Nucl Med Mol Imaging* 2011; **38**: 1990.
59. Lam MGEH, Bosma TB, van Rijk PP, Zonnenberg BA. (188)Re-HEDP combined with capecitabine in hormone-refractory prostate cancer patients with bone metastases: a phase I safety and toxicity study. *Eur J Nucl Med Mol Imaging* 2009; **36**: 1425-33.
60. Baum RP, Kulkarni HR, Schuchardt C, et al. ^{177}Lu-labeled prostate-specific membrane antigen radioligand therapy of metastatic castration-resistant prostate cancer: Safety and efficacy. *J Nucl Med* 2016; **57**: 1006-13.
61. van Hazel GA, Heinemann V, Sharma NK, et al. SIRFLOX: randomized phase III trial comparing first-line mFOLFOX6 (plus or minus bevacizumab) versus mFOLFOX6 (plus or minus bevacizumab) plus selective internal radiation therapy in patients with metastatic colorectal cancer. *J Clin Oncol* 2016; **34**: 1723-31.
62. Dutton SJ, Kenealy N, Love SB, Wasan HS, Sharma RA. FOXFIRE protocol: an open-label, randomised, phase III trial of 5-fluorouracil, oxaliplatin and folinic acid (OxMdG) with or without interventional Selective Internal Radiation Therapy (SIRT) as first-line treatment for patients with unresectable liver-only or liver-dominant metastatic colorectal cancer. *BMC Cancer* 2014; **14**: 497.
63. Lambert B, Bacher K, Defreyne L, et al. ^{188}Re-HDD/Lipiodol therapy for hepatocellular carcinoma: A phase I clinical trial. *J Nucl Med* 2005; **46**: 60-6.
64. O'Connor MJ. Targeting the DNA damage response in cancer. *Mol Cell* 2015; **60**: 547-60.
65. Al-Ejeh F, Pajic M, Shi W, et al. Gemcitabine and CHK1 inhibition potentiate EGFR-directed radioimmunotherapy against pancreatic ductal adenocarcinoma. *Clin Cancer Res* 2014; **20**: 3187-97.

66. Al-Ejeh F, Shi W, Miranda M, et al. Treatment of triple-negative breast cancer using anti-EGFR–directed radioimmunotherapy combined with radiosensitizing chemotherapy and PARP inhibitor. *J Nucl Med* 2013; **54**: 913-21.
67. Nonnekens J, van Kranenburg M, Beerens CEMT, et al. Potentiation of peptide receptor radionuclide therapy by the PARP inhibitor olaparib. *Theranostics* 2016; **6**: 1821-32.
68. Pouget JP, Santoro L, Raymond L, et al. Cell membrane is a more sensitive target than cytoplasm to dense ionization produced by auger electrons. *Radiat Res* 2008; **170**: 192-200.
69. Nyati MK, Morgan MA, Feng FY, Lawrence TS. Integration of EGFR inhibitors with radiochemotherapy. *Nat Rev Cancer* 2006; **6**: 876-85.
70. Bailey KE, Costantini DL, Cai Z, et al. Epidermal growth factor receptor inhibition modulates the nuclear localization and cytotoxicity of the Auger electron-emitting radiopharmaceutical ^{111}In-DTPA-human epidermal growth factor. *J Nucl Med* 2007; **48**: 1562-70.
71. Kelly MP, Lee ST, Lee FT, et al. Therapeutic efficacy of ^{177}Lu-CHX-A″-DTPA-hu3S193 radioimmunotherapy in prostate cancer is enhanced by EGFR inhibition or docetaxel chemotherapy. *Prostate* 2009; **69**: 92-104.
72. Saad F, Carles J, Gillessen S, et al. Radium-223 and concomitant therapies in patients with metastatic castration-resistant prostate cancer: an international, early access, open-label, single-arm phase 3b trial. *Lancet Oncol* 2016 **17**: 1306-16.
73. Bloy N, Pol J, Manic G, et al. Trial Watch: Radioimmunotherapy for oncological indications. *Oncoimmunology* 2014; **3**: e954929.
74. Dietrich A, Koi L, Zöphel K, et al. Improving external beam radiotherapy by combination with internal irradiation. *Br J Radiol* 2015; **88**: 20150042.
75. Ng QS, Goh V, Milner J, et al. Acute tumor vascular effects following fractionated radiotherapy in human lung cancer: In vivo whole tumor assessment using volumetric perfusion computed tomography. *Int J Radiat Oncol Biol Phys* 2007; **67**: 417-24.
76. Msirikale JS, Klein JL, Schroeder J, Order SE. Radiation enhancement of radiolabelled antibody deposition in tumors. *Int J Radiat Oncol Biol Phys* 1987; **13**: 1839-44.
77. Hall EJ, Brenner DJ. The dose-rate effect revisited: Radiobiological considerations of importance in radiotherapy. *Int J Radiat Biol Phys* 1991; **21**: 1403-14.

78. Williams JR, Zhang Y, Zhou H, et al. A quantitative overview of radiosensitivity of human tumor cells across histological type and TP53 status. *Int J Radiat Biol* 2008; **84**: 253-64.
79. Steel GG, Peckham MJ. Exploitable mechanisms in combined radiotherapy-chemotherapy: the concept of additivity. *Int J Radiat Oncol Biol Phys* 1979; **5**: 85-91.
80. Li L, Quang TS, Gracely EJ, et al. A Phase II study of anti-epidermal growth factor receptor radioimmunotherapy in the treatment of glioblastoma multiforme. *J Neurosurg* 2010; **113**: 192-8.
81. Poli GL, Bianchi C, Virotta G, et al. Radretumab radioimmunotherapy in patients with brain metastasis: a ^{124}I-L19SIP dosimetric PET study. *Cancer Immunol Res* 2013; **1**: 134-43.
82. Kreissl MC, Hänscheid H, Löhr M, et al. Combination of peptide receptor radionuclide therapy with fractionated external beam radiotherapy for treatment of advanced symptomatic meningioma. *Rad Oncol* 2012; **7**: 99.
83. Fishbein L, Bonner L, Torigian DA, et al. External beam radiation therapy (EBRT) for patients with malignant pheochromocytoma and non-head and -neck paraganglioma: Combination with ^{131}I-MIBG. *Horm Metab Res* 2012; **44**: 405-10.
84. Sun LQ, Vogel CA, Mirimanoff RO, et al. Timing effects of combined radioimmunotherapy and radiotherapy on a human solid tumor in nude mice. *Cancer Res* 1997; **57**: 1312-9.
85. Strigari L, Konijnenberg M, Chiesa C, et al. The evidence base for the use of internal dosimetry in the clinical practice of molecular radiotherapy. *Eur J Nucl Med Mol Imag* 2014; **41**: 1976-88.

Declaration of Interests

There are no conflicts of interest.

Author Contributions

MRG: Literature search, writing

NF: Writing

YD: Writing

KAV: Writing

Search Strategy and Selection Criteria

The information for this Review was compiled by searching PubMed and the US National Institutes of Health database for articles and clinical trials from Jan 1 2005 until February 1 2017. The search terms used included “radiopharmaceutical”, “radionuclide”, “radioimmunotherapy”, “molecular targeted radiotherapy”, or names of individual targeted radionuclide drugs, along with “combination”, “targeted therapy”, “external beam radiotherapy”, “chemotherapy”, or “clinical trial”. Full articles were viewed and references were checked while citations were examined using GoogleScholar for additional material, as appropriate. Any papers or clinical trials employing the radionuclide solely for SPECT/PET imaging were discounted. We apologise to authors whose papers are not cited due to space limits.

Sources of Funding

MG, NF and KAV are supported by Cancer Research-UK (grant C5255/A15935), the Medical Research Council (MC_PC_12004) and through a MRC Confidence in Concept Award (MG/KV). The funders had no role in writing the manuscript or in the decision to submit it for publication.

TRT agent	Target	Population	Combination	Details	Reference
^{131}I-MIBG	Norepinephrine transporter	Relapsed or refractory neuroblastoma	Irinotecan, vincristine	Phase I/II (n=32) Tolerable, optimised regimen of ^{131}I-MIBG with vincristine and shorter course, high-dose irinotecan	14 NCT02035137
^{177}Lu-DOTA-TATE	Somatostatin receptors	Neuroendocrine tumours	5-FU	Safe and well-tolerated with therapeutic benefit in patients with progressive disease or uncontrolled symptoms	17,18,19
^{177}Lu-DOTA-TATE	Somatostatin receptors	Advanced stage neuroendocrine tumours	Capecitabine -/+ temozolomide	Phase I/II studies (n=33 and n=35) Tumour control and stabilization of disease in 94% of patients	20, 23
^{177}Lu-DOTA-TATE	Somatostatin receptors	Metastatic neuroblastoma	^{90}Y-DOTA-TATE	Phase II (n=26) Objective responses in 43%	26
^{90}Y-IT	CD20	Stage III or IV follicular NHL	Consolidation after first-line CVP, CHOP, or CHOP-R	Phase III, randomized (n=414), estimated 8-year overall PFS increased from 22% to 41%	42 NCT00185393
^{90}Y-IT	CD20	Relapsed diffuse large B-cell lymphoma	RIT given prior to high-dose BEAM chemotherapy and ASCT	Phase III, randomized (n=43) Benefit of ^{90}Y-IT plus BEAM compared to BEAM. PFS: 59% vs 37% OS: 91% vs 62%	43 NCT00491491
^{223}Ra dichloride	Ca^{2+} analogue	CRPC with bone metastases	Abiraterone and/or enzalutamide	Phase III (n=839). Increase in median OS. Similar findings obtained with denosumab	72 NCT01618370

TRT=targeted radionuclide therapy. DOTA=1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid. TATE=octreotate. IT=ibritumomab tiuxetan. CHOP=cyclophosphamide, doxorubicin, vincristine, and prednisone. R=rituximab. CVP=cyclophosphamide, vincristine, prednisone. RIT=radioimmunotherapy. BEAM=carmustine, etoposide, cytarabine, melphalan. MIBG=metaiodobenzylguanidine. PFS=progression free survival. OS=overall survival. CRPC=castrate-resistant prostate cancer. ASCT=Autologous stem cell transplant.

Table 1: Clinically-approved targeted radionuclide therapeutics in combination protocols

TRT agent	Target	Population	Combination	Details	Reference
^{177}Lu-J591	PSMA	CRPC with bone metastases	Docetaxel/prednisone	Phase II (n=59) Targets sites of known metastates in 94% Evidence of anti-tumour efficacy	47
^{186}Re-HEDP	Bone	CRPC with bone metastases	Docetaxel	Phase I (n=14) Combined therapy well-tolerated and randomized Phase II underway	58
^{213}Bi-lintuzumab	CD33	Newly diagnosed or relapsed/refractory AML	Cytarabine	Phase I/II (n=31) Well-tolerated; some remissions. Trials with ^{225}Ac-lintuzumab underway	45, 46 NCT02575963
^{131}I-A5B7	CEA	Advanced gastrointestinal carcinomas	Combretastatin-A4-phosphate	Phase I (n=12) DLT, MTD, efficacy, and mechanism of combined treatment determined	48
^{125}I-mAb 425	EGFR	Glioblastoma multiforme	EBRT and temozolomide	Phase II (n=192) Median survival of RIT/EBRT improved by temozolomide inclusion	80
^{131}I-L19SIP (Radretumab)	Fibronectin	Brain metastases	Whole brain EBRT	Phase I/II (n=32), on-going	81 NCT01125085

TRT=targeted radionuclide therapy. PSMA=prostate-specific membrane antigen. CRPC=castrate-resistant prostate cancer. AML=acute myeloid leukaemia. HEDP=1,1-hydroxyethylidene diphosphonate. CEA=carcinoembryonic antigen. EGFR=epidermal growth factor receptor. RIT=radioimmunotherapy. EBRT=external beam radiation therapy. DLT=dose limiting toxicity. MTD=maximum tolerated dose.

Table 2: Investigational targeted radionuclide therapeutics in combination protocols