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Advances in Precision Oncology: Targeted Thorium-227 Conjugates As a New Modality in Targeted Alpha Therapy

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Abstract

Targeted α therapy (TAT) offers the potential for the targeted delivery of potent α-particle-emitting radionuclides that emit high linear energy transfer radiation. This leads to a densely ionizing radiation track over a short path. Localized radiation induces cytotoxic, difficult-to-repair, clustered DNA double-strand breaks (DSBs). To date, radium-223 (^{223}Ra) is the only TAT approved for the treatment of patients with metastatic castration-resistant prostate cancer. Thorium-227 (^{227}Th), the progenitor nuclide of ^{223}Ra, offers promise as a wider-ranging alternative due to the availability of efficient chelators, such as octadentate 3,2-hydroxypyridinone (3,2-HOPO). The 3,2-HOPO chelator can be readily conjugated to a range of targeting moieties, enabling the generation of new targeted thorium-227 conjugates (TTCs). This review provides a comprehensive overview of the advances in the preclinical development of TTCs for hematological cancers, including CD22-positive B cell cancers and CD33-positive leukemia, as well as for solid tumors overexpressing renal cell cancer antigen CD70, membrane-anchored glycoprotein mesothelin in mesothelioma, prostate-specific membrane antigen in prostate cancer, and fibroblast growth factor receptor 2. As the mechanism of action for TTCs is linked to the formation of DSBs, the authors also report data supporting combinations of TTCs with inhibitors of the DNA damage response pathways, including those of the ataxia telangiectasia and Rad3-related protein, and poly-ADP ribose polymerase. Finally, emerging evidence suggests that TTCs induce immunogenic cell death through the release of danger-associated molecular patterns. Based on encouraging preclinical data, clinical studies have been initiated to investigate the safety and tolerability of TTCs in patients with various cancers.

Keywords: conjugate, precision oncology, targeted alpha therapy, ^{227}Th

Introduction

Recent progress in the field of oncology, particularly the clinical development of novel treatment modalities, has transformed the clinical outcomes for patients with specific cancers.[1] However, despite these advances, an unmet clinical need for novel therapeutic options to further improve clinical outcomes still exists,[2] particularly for patients who need more efficacious treatments for early stage disease or for those who become chemotherapy refractory or develop resistance to small-molecule inhibitors or targeted biological therapies.

Targeted α therapy (TAT) has emerged as a promising modality in cancer therapy. Following systemic administration, TAT specifically accumulates and delivers high linear energy transfer (LET) α particles directly to the tumor and its microenvironment, and the short-path length of 20–100 μm (2–10 cell diameters) minimizes damage to the surrounding

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healthy tissue.[3,4] High LET α particles (50–230 keV/μM) are highly cytotoxic due to the induction of difficult-to-repair clustered DNA double-strand breaks (DSBs). In contrast, radiation from β-particle emitters have a longer path length in tissue (1–10 mm), but a lower LET (0.2 keV/μM) leading to the induction of less complex damage, which is more readily repaired by the cell.[3,5]

The high potency of α particles is further highlighted by the fact that only 1–4 nuclear traversals are necessary to sterilize a bacterial cell, whereas around 2000 β particles are needed to induce an equivalent effect.[6,7] In addition, the longer path length of β^--particle emitters may induce more damage to tumor-adjacent normal tissue.[3,5]

Over the past two decades, a number of α-particle-emitting radionuclides, including bismuth-213 (^{213}Bi, half-life of 45.6 min), actinium-225 (^{225}Ac, half-life of 9.9 d), astatine-211 (half-life of 7.2 h), radium-223 (^{223}Ra, half-life of 11.4 d), and thorium-227 (^{227}Th, half-life of 18.7 d) have been investigated preclinically as TATs.[8] Some have progressed to clinical development,[8] however, to date only ^{223}Ra has been approved by the United States Food and Drug Administration[9] and the European Medicines Agency.[10] Based on the pivotal ALSYMPCA study,[11] ^{223}Ra is indicated for the treatment of patients with castration-resistant prostate cancer with symptomatic bone metastases and no known visceral metastatic disease.[9,10]

^{223}Ra is a calcium mimetic which accumulates at sites of increased bone turnover, such as metastatic lesions, through its active incorporation by osteoblasts and passive binding to hydroxyapatite.[4,8–12] Bone metastases originating from prostate cancer are characterized by an osteoblastic growth pattern driven by positive bidirectional paracrine feedback loops between cancer cells and osteoblasts, which lead to tumor cell proliferation and intralesional new bone formation.

Locally deposited ^{223}Ra induces clustered DNA DSBs in cancer cells, osteoblasts, and osteoclasts located within a range of 2–10 cell diameters. The subsequent reduction in the levels of secreted paracrine growth factors may have an impact on the viability of more distantly located cancer cells.[4,12] Moreover, in *in vitro* models, ^{223}Ra stimulated the lysis of cancer cells through CD8+ cytotoxic T lymphocytes through a mechanism involving an induction of the endoplasmic reticulum stress response and stimulator of interferon genes (STING) pathways.[12]

The clinical efficacy of ^{223}Ra has been reported in the phase 3 ALSYMPCA study in patients with metastatic castrate-resistant prostate cancer (mCRPC) who showed a median overall survival of 14.9 compared with 11.3 months in the control group (hazard ratio [HR] 0.70, 95% confidence interval [CI] 0.58–0.83; $p < 0.001$) and a longer time to first symptomatic skeletal event (HR 0.66; 95% CI 0.52–0.83; $p < 0.001$) without major safety concerns and low myelosuppression incidence.[11,13] Moreover, ^{223}Ra significantly improved the quality of life in patients with mCRPC.[14] Thus, the ^{223}Ra efficacy and safety data provide proof of concept for further clinical development of α emitters, including TATs, as anticancer therapeutics. Due to the inherent bone-seeking characteristics of ^{223}Ra, its clinical applicability has been limited to patients with metastatic bone disease.[15]

The paucity of efficient chelator systems for the conjugation of ^{223}Ra to targeting moieties has prevented the development of this radionuclide as a ligand-based targeted radioimmunotherapy.[16]

The clinical potential of radionuclide-labeled antibodies has been demonstrated in preclinical and clinical studies of the anti-human epidermal growth factor receptor 2 (HER-2) monoclonal antibody (trastuzumab) conjugated to the β-emitter lead-212 (^{212}Pb, half-life of 10.64 h) that deposits 93.4% of its energy to the cell through its daughter α-emitters ^{212}Bi (half-life of 61 min) and polonium-212 (half-life of 0.3 μs).[17] ^{212}Pb-labeled trastuzumab showed potent antitumor activity as monotherapy or in combination with chemotherapeutic regimens in preclinical models of disseminated peritoneal ovarian and gastric cancer, and in the orthotopic model of prostate cancer.[18–21] Phase I studies highlighted a favorable toxicity profile following intraperitoneal administration of ^{212}Pb-labeled trastuzumab in patients with HER2-positive peritoneal carcinomas.[22–24]

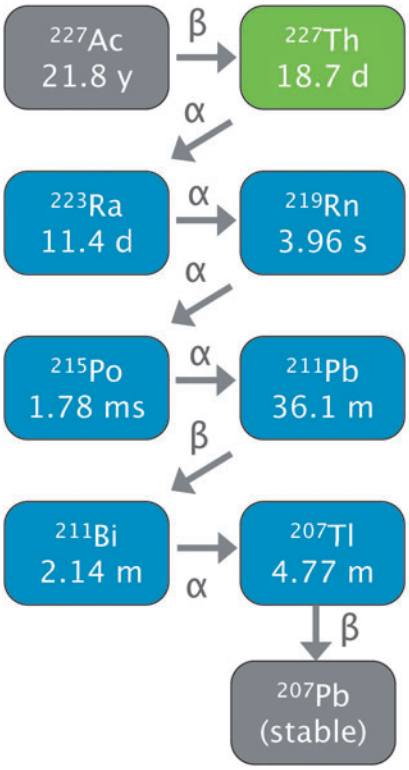
Thorium-227 (^{227}Th) can be efficiently complexed with octadentate 3,2-hydroxypyridinone (3,2-HOPO) chelators that are conjugated to antibodies or other targeting moieties,[25] resulting in highly stable targeted thorium-227 conjugates (TTCs). TTCs, therefore, represent a new promising class of TAT for cancer therapy capable of delivering a high-energy α-particle radiation to tumors by targeting antigens specifically expressed or overexpressed on cancer versus healthy tissue.[3,4]

Resistance to therapy, which develops to some cancer drugs, is unlikely to occur to α-particle-emitting radionuclides,[26] although a high prevalence of mutations in the DNA damage response (DDR) genes was reported in a small group of patients who had not responded to ^{225}Ac-prostate-specific membrane antigen (PSMA)-617 treatment.[27] Moreover, the high energy of α particles, delivered within a small area in the tumor, damage cancer cells independently of local oxygen levels, thus overcoming hypoxia-induced resistance to therapy.[26,28] Notably, due to their short path length, potent radiation, and normal tissue sparing, TTCs may be a particularly attractive therapeutic choice for controlling the growth of micrometastatic cancer deposits in patients with disseminated cancer.[29] It remains to be determined if the penetration range and activity of TTCs can be optimized to benefit patients with macroscopic tumor burden.[29]

The Building Blocks of TTCs

TTCs consist of three main building blocks. Following the β^--particle decay of actinium-227 (half-life 21.8 years), the α-particle-emitting radionuclide ^{227}Th is purified by ion exchange chromatography (Fig. 1). ^{227}Th is produced from the same supply chain as ^{223}Ra, and is available in quantities that fully support the drug development and commercialization programs by Bayer. ^{227}Th decays by α-particle emission with an energy of 5.9 MeV and a half-life of 18.7 d to ^{223}Ra with further decay releasing four α particles, with a mean energy of 6.6 MeV and two β particles, ending the cascade with the formation of stable ^{207}Pb (Fig. 1).[30]

The second building block is a siderophore-derived chelator containing HOPO groups bearing four 3-hydroxy-*N*-methyl-2-pyridinone moieties on a symmetrical polyamine scaffold functionalized with a carboxylic acid linker for bioconjugation. Conjugation to targeting moieties can be achieved through the amide bond formation with the ε-amino groups of lysine residues (Fig. 2). These octadentate 3,2-HOPO chelators can be very efficiently labeled with



Decay	Energy [MeV]
$^{227}Th \rightarrow {}^{223}Ra$	5.9
$^{223}Ra \rightarrow {}^{219}Rn$	5.7
$^{219}Rn \rightarrow {}^{215}Po$	6.8
$^{215}Po \rightarrow {}^{211}Pb$	7.4
$^{211}Pb \rightarrow {}^{211}Bi$	0.4
$^{211}Bi \rightarrow {}^{207}Tl$	6.6
$^{207}Tl \rightarrow {}^{207}Pb$	0.5

FIG. 1. Decay cascade of ^{227}Th purified from an ^{227}Ac source. ^{227}Th decay scheme. ^{227}Th is purified from an ^{227}Ac-generator and decays through its α- and β-particle-emitting daughters, ^{223}Ra, ^{219}Rn, ^{215}Po, ^{211}Pb, ^{211}Bi, and ^{207}Tl, to form a stable nonradioactive ^{207}Pb. ^{227}Ac, actinium-227; ^{211}Bi, bismuth-211; d, days; m, minutes; ms, milliseconds; ^{207}Pb, lead-207; ^{211}Pb, lead-211; ^{215}Po, polonium-215; ^{223}Ra, radium-223; ^{219}Rn, radon-219; s, seconds; ^{227}Th, thorium-227; ^{207}Tl, thallium-227; y, years.

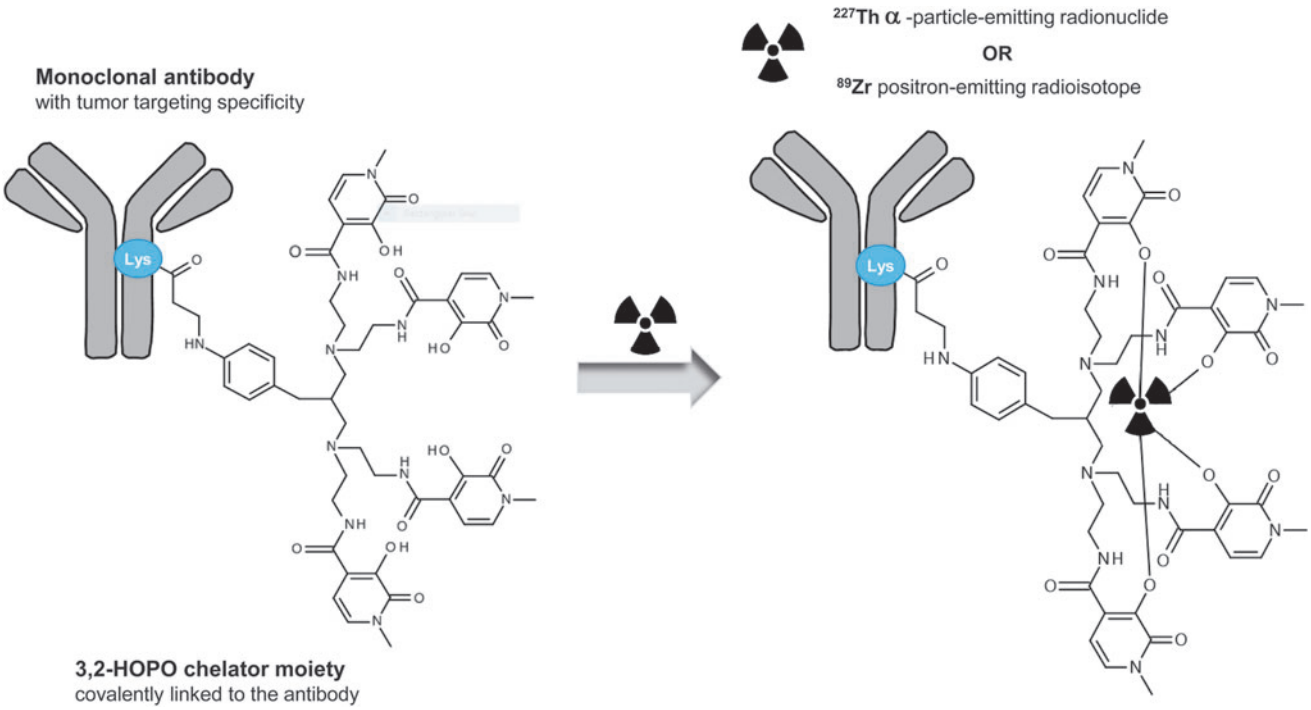


FIG. 2. Generation of TTCs. Schematic representation of the generation of TTCs. Monoclonal antibodies with tumor-targeting specificity are covalently linked to octadentate 3,2-HOPO chelator through the ε-amino groups of lysine residues to generate the antibody-3,2-HOPO chelator conjugate. The binding of a radionuclide (^{227}Th or ^{89}Zr) to the chelator involves the formation of several bonds, resulting in a stable radionuclide-labeled antibody-3,2-HOPO chelator complex. 3,2-HOPO, 3,2-hydroxypyridinone; ^{227}Th, thorium-227; TTCs, targeted thorium-227 conjugates, ^{89}Zr, zirconium.

^{227}Th, with high yield, purity, and stability at ambient conditions (Fig. 2).[25,31,32] Compared with the tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) chelator, which often requires heating, the HOPO chelators are superior due to efficient radiolabeling at ambient temperatures and high stability of formed complexes.[33,34]

Mode of Action of TTCs

α-Particle emitters exert their effects independently of cell proliferative state and cell cycle phase. Both slow proliferating as well as actively dividing cells can be targeted by TTCs as long as the target antigen is expressed on the cell surface.[5–7]

TTCs induce clustered DNA DSBs and cell cycle arrest in the G2/M phase resulting in cell death through apoptotic and necrotic pathways.[3,5–7,35,36] *In vitro* exposure of cancer cells to TTCs leads to a release of markers of danger-associated molecular patterns (DAMPs), which are upregulated by dying cells to alert the immune system and to initiate immunogenic cell death.[36,37]

Due to an inherent cross-fire effect of α-particle emitters and their ability to penetrate 2–10 cell layers in tissue,[3] the biological activity of TTCs, in contrast to antibody-drug conjugates, does not strictly depend on antigen internalization and is broadly independent of homogeneity of antigen expression.[3,5–7] A critical parameter for their optimal efficacy, however, is the efficient delivery, accumulation, and retention in the tumor tissue to ensure treatment efficacy and a minimal damage to the surrounding healthy tissue.

^{227}Th decay releases ^{223}Ra from the chelator due to recoil energy (Fig. 1), with a proportion of ^{223}Ra being retained at the site of ^{227}Th decay, possibly through the internalization of TTCs into the target cell, and a small proportion being redistributed to the bone.[4,15] Remaining ^{223}Ra is excreted through the small intestine before further decay.[38] ^{223}Ra is well tolerated,[39] and as the majority of daughters decay in the gut, minimal toxicity is expected.

TTCs for Hematological Cancers

CD22-TTC

CD22, a member of the transmembrane sialic acid-binding immunoglobulin-type lectins (siglec) glycoprotein family (siglec-2), is expressed on mature B cells, but not on non-B lymphoid cells, myeloid cells, and hematopoietic stem cells. CD22 is believed to be implicated in the regulation of B cell function and survival.[40,41] CD22 is detected on malignant B cells in more than 90% of patients with non-Hodgkin's lymphoma (NHL), including diffuse large B cell lymphoma and follicular lymphoma,[42,43] and in all patients with relapsed and chemotherapy-refractory B-precursor acute lymphoblastic leukemia (ALL). Therefore, CD22 seems to be an ideal target for the treatment of NHL and ALL.[44]

Epratuzumab is a humanized monoclonal antibody with high-affinity *in vitro* binding to the recombinant extracellular portion of CD22.[45] In a phase I/II clinical study in patients with aggressive NHL, epratuzumab has demonstrated a favorable safety profile and antitumor activity.[46] The recombinant anti-CD22 immunotoxin, moxetumomab pasudotox, was recently approved for the treatment of patients with hairy-cell leukemia,[47] based on results of a pivotal phase III study,[48] validating, therefore, the CD22-targeting approach in CD22-positive cancers.

CD22-TTC reduced cell viability in a panel of hematological cancer cell lines (Table 1), with cytotoxicity correlating with levels of expression of cell-surface CD22. Furthermore, cytotoxicity was independent of the cellular subtype, including activated B cell and germinal center B cell, as defined by gene expression profiles.

The *in vivo* antitumor activity of CD22-TTC was evaluated in xenograft models in athymic nude mice bearing subcutaneous Ramos (Burkitt's lymphoma) tumors. A single dose of 300 kBq/kg resulted in a tumor growth inhibition and increased overall survival compared with the vehicle or radiolabeled isotype control groups (Table 1). In a disseminated xenograft model established from the human B cell Daudi cell line, animals injected with a single dose of 100 kBq/kg gained significant survival extension compared with the group treated with vehicle or a nonradiolabeled CD22 antibody–chelator conjugate (Table 1). A significant and equivalent increase in survival was also demonstrated when CD22-TTC was administered as either a fractionated dose of 3×100 kBq/kg or a single dose of 300 kBq/kg (Table 1), the former potentially offering an improvement of the toxicity profile and a therapeutic window.[34]

CD33-TTC

CD33 is a member of the siglec glycoprotein family (siglec-3) and an inhibitory sialoadhesin receptor expressed on hematopoietic cells.[49] High expression of CD33 has been reported on leukemic myeloblasts in 85%–90% of patients with acute myeloid leukemia (AML),[50] whereas hematopoietic stem cells are devoid of CD33, thus rendering CD33 an excellent target for AML therapy.[50]

Using the high-affinity anti-CD33 monoclonal antibody lintuzumab, a CD33 chelator conjugate was prepared for radiolabeling with ^{227}Th. CD33-TTC was subsequently evaluated *in vitro* and *in vivo* in CD33-positive human leukemia cell line-derived models (Table 1).[51] Exposure of human leukemia cells to CD33-TTC resulted in the specific reduction of cell viability correlating with the level of expression of cell-surface CD33 (Table 1). Importantly, exposure of a CD33-negative cell line to CD33-TTC did not decrease cell viability.[51] Analysis of the mode of action (MoA) of CD33-TTC showed an increase in DNA DSBs and cell cycle arrest in the G2/M phase.[51]

Evaluation of biodistribution using the human promyelocytic leukemia cell line HL-60 subcutaneously grown in mice demonstrated specific tumor uptake 7 d after administration of CD33-TTC.[51] *In vivo* efficacy studies using a subcutaneous HL-60 tumor model showed that administration of a single dose of 700 kBq/kg resulted in the complete regression of already established tumors (Table 1).[51] In a HL-60 disseminated model, a statistically significant, dose-dependent improvement in median survival time (MST) was observed in CD33-TTC-treated animals at doses as low as 50 kBq/kg compared with animals treated with a radiolabeled isotype control or vehicle alone (Table 1).[51]

TTCs for Solid Tumors

Mesothelin-TTC

Mesothelin (MSLN) is a cell-surface glycoprotein that mediates cell–cell adhesion and promotes metastatic spread, cell proliferation, and resistance to apoptosis.[52] MSLN expression

Table 1. Preclinical Characterization of Targeted Thorium-227 Conjugates and Key Experiments

TTC		*Preclinical findings*
CD22-TTC[a]	*In vitro*	Specific reduction in cell viability in a dose- and receptor-dependent manner in a panel of different cell lines (Daudi, Raji, DOHH-2, Ramos, HC-1, Granta-519, REH, and five DBLCL cell lines) Upregulation of DAMPs
	In vivo	Specific tumor accumulation in athymic mice bearing Burkitt lymphoma Ramos cell line xenograft Increased MST (>60 d) at a single-dose administration of 300 kBq/kg Specific increase in MST in a disseminated human B cell Daudi mouse model at a single dose of 300 kBq/kg (39 d) as well as following dose fractionation at 3×100 kBq/kg (39 d)
CD33-TTC[51]	*In vitro*	Dose-dependent reduction in cell viability of CD33-positive HL-60 and KG-1 cell lines and induction of DNA DSBs followed by cell cycle arrest in the G2/M phase
	In vivo	Specific tumor accumulation and retention in the CD33-positive HL-60 tumor xenograft model 7 d after dose administration Specific tumor growth inhibition at a single dose administration of 700 kBq/kg: 10/10 animals had CR and 15/18 were without palpable tumors at the end of the study Specific increase in survival in a disseminated model established using HL-60 cells at doses as low as 50 kBq/kg
MSLN-TTC[36]	*In vitro*	Specific dose- and receptor-dependent reduction in cell viability in a panel of 12 cell lines Induction of DNA DSBs and cell cycle arrest in the G2/M phase resulting in apoptosis, as determined by caspase-3 activity and cytochrome c release in the cytoplasm; detection of necrosis Upregulation of DAMPs
	In vivo	Biodistribution demonstrates receptor-dependent retention in tumor models (cell line-derived colorectal, pancreatic, and ovarian and patient-derived ovarian and breast cancer xenograft models) for up to 4 weeks. Potent *in vivo* tumor growth inhibition in CDX and PDX models Fractionated dosing of 2×250 and 4×125 kBq/kg vs. single 500 kBq/kg dose resulted in equivalent antitumor activity (ST2185B, Capan-2 and ST103 xenograft models) Detection of DNA damage (γH2AX) and apoptotic (cleaved caspase-3) markers by IHC in isolated tumors Reversible myelosuppression observed in several models and no measurable body weight loss >10%
PSMA-TTC[65]	*In vitro*	Specific reduction of cell viability in a panel of six PSMA-positive cell lines Induction of DNA DSBs resulting in cell cycle arrest in the G2/M phase and apoptosis (measured by caspase 3/7 activity)
	In vivo	Biodistribution in CDX and PDX models demonstrated specific accumulation and retention in tumors for up to 21 d Potent tumor growth inhibition in LNCaP, MDA-PCa-2b, 22Rv1, C4-2, and LNCaP-luc CDX and LuCaP 86.2, KUCaP-1, and ST1273 PDX models; fractionated dosing demonstrated equal potency compared with a single-dose administration Strong activity in an orthotopic/intratibial mouse model using the luciferase-labeled cell line LNCaP-luc Detection of DNA damage (γH2AX) and apoptotic (cleaved caspase-3) markers by IHC in isolated tumors Reversible myelosuppression of white blood cells and no signs of significant weight loss
CD70-TTC[72]	*In vitro/ in vivo*	Strong *in vitro* and *in vivo* potency in the RCC cell line 786-O with high and specific tumor accumulation 7 d after dose administration Strong and specific tumor growth inhibition at doses as low as 50 kBq/kg Reversible myelosuppression of white blood cells
FGFR2-TTC[77]	*In vitro*	Specific receptor-dependent reduction in viability of gastric, colorectal and esophageal cancer cell lines and TNBC (Kato III, SUM-52PE, MFM-223, NCI-H716, SNU-16, RERF-GC-1B, and OACM5.1C) Induction of DNA DSBs resulting in cell cycle arrest in the G2/M phase (tested in SUM-52PE cells)
	In vivo	Biodistribution demonstrates specific accumulation in a colorectal cancer xenograft model Tumor growth inhibition at a single dose of 500 kBq/kg in NCI-H716 (colorectal), SNU-16 (gastric), and MFM-223 (TNBC) xenograft models No significant body weight loss

[a]Manuscript in preparation.

CDX, cell line-derived xenograft; CR, complete response; DAMPs, danger-associate molecular patterns; DLBCL, diffuse large B cell lymphoma; DSBs, double-strand breaks; FGFR2, fibroblast growth factor receptor 2; γH2AX, γ-H2A histone family member X; IHC, immunohistochemistry; kBq, kilobecquerel; MSLN, mesothelin; MST, median survival times; PDX, patient-derived xenograft; PSMA, prostate-specific membrane antigen; RCC, renal cell carcinoma; TNBC, triple-negative breast cancer; TTCs, targeted thorium-227 conjugates.

in normal human tissues is limited to the mesothelial cells lining the pleura, peritoneum, and pericardium.[53] The expression of MSLN in cancers varies with the highest expression rate reported in mesotheliomas (80%–95%), pancreatic cancer (80%–85%), and ovarian cancers (60%–65%).[54,55] A number of different MSLN-targeted therapies, including MSLN-targeting antibodies, antibody–drug conjugates, immunotoxins, and chimeric antigen receptor T cells, have been either clinically tested or are currently in early clinical development.[56]

Using the anti-MSLN antibody anetumab (BAY 86-1903), a MSLN-targeted ^{227}Th conjugate (MSLN-TTC) was developed.[36] MSLN-TTC showed strong activity in several *in vitro* and *in vivo* preclinical models.[36] Indeed, incubation of multiple MSLN-expressing cancer cell lines with MSLN-TTC resulted in a specific decrease in cell viability, which correlated with MSLN expression. In-depth analysis of the MoA of MSLN-TTC in OVCAR-3 and HT29-MSLN cell lines has shown an accumulation of DNA DSBs and subsequent cell cycle arrest in the G2/M phase (Table 1).[36]

Interestingly, MSLN-TTC induced cell death even when the activity of the apoptotic enzyme caspase-3 was abolished by a pan-caspase inhibitor z-VAD-FMK.[36] Also, blockade of the necrotic pathway with necrostatin-1 did not rescue cells from death.[36] Exposure of MSLN-positive cells to MSLN-TTC resulted in a release of reactive oxygen species, which are often observed during apoptosis (Table 1).[36] In summary, the α-particle radiation emitted from ^{227}Th results in cell death induced through multiple pathways.

In biodistribution studies, MSLN-TTC rapidly cleared from blood with a significant accumulation and retention for up to 4 weeks in mice bearing subcutaneously implanted tumors of multiple tissue origins (Table 1), which was dependent on the levels of MSLN expression.[36]

The observed antigen-dependent retention of MSLN-TTC in tumors resulted in a potent antitumor activity in colorectal, pancreatic, ovarian, and breast cancer murine models (Table 1). Tumor regression was observed in mice bearing HT29-MSLN, a colorectal adenocarcinoma cell line engineered to overexpress high levels of MSLN, xenografts, and OVCAR-3, an ovarian cancer cell line considered to be a medium MSLN expressor, xenografts (Table 1).[36] Similarly, potent *in vivo* efficacy was observed in ovarian and breast cancer patient-derived xenograft models (Table 1).

To test the efficacy of MSLN-TTC in tumor models with heterogeneous MSLN expression, a xenograft model was developed, whereby the proportion of MSLN-expressing cells was varied between 0% and 100% in growing tumors. MSLN-TTC exerted significant growth inhibition in tumors harboring as few as 20% of MSLN-positive cells. These data suggest that the crossfire effect of MSLN-TTC may potentially overcome target expression heterogeneity.

Finally, in an orthotopic bone/lung metastatic xenograft model established using luciferase-transfected NCI-H226 cells, a single dose of either 250 or 500 kBq/kg of MSLN-TTC resulted in a statistically significant reduction in tumor burden in the lungs and tibia (Fig. 3; Table 1).[57] In all preclinical models tested, MSLN-TTC appeared to be well tolerated, as evidenced by unaltered body weight and reversible myelosuppression, an observation typical for this class of compounds.[36]

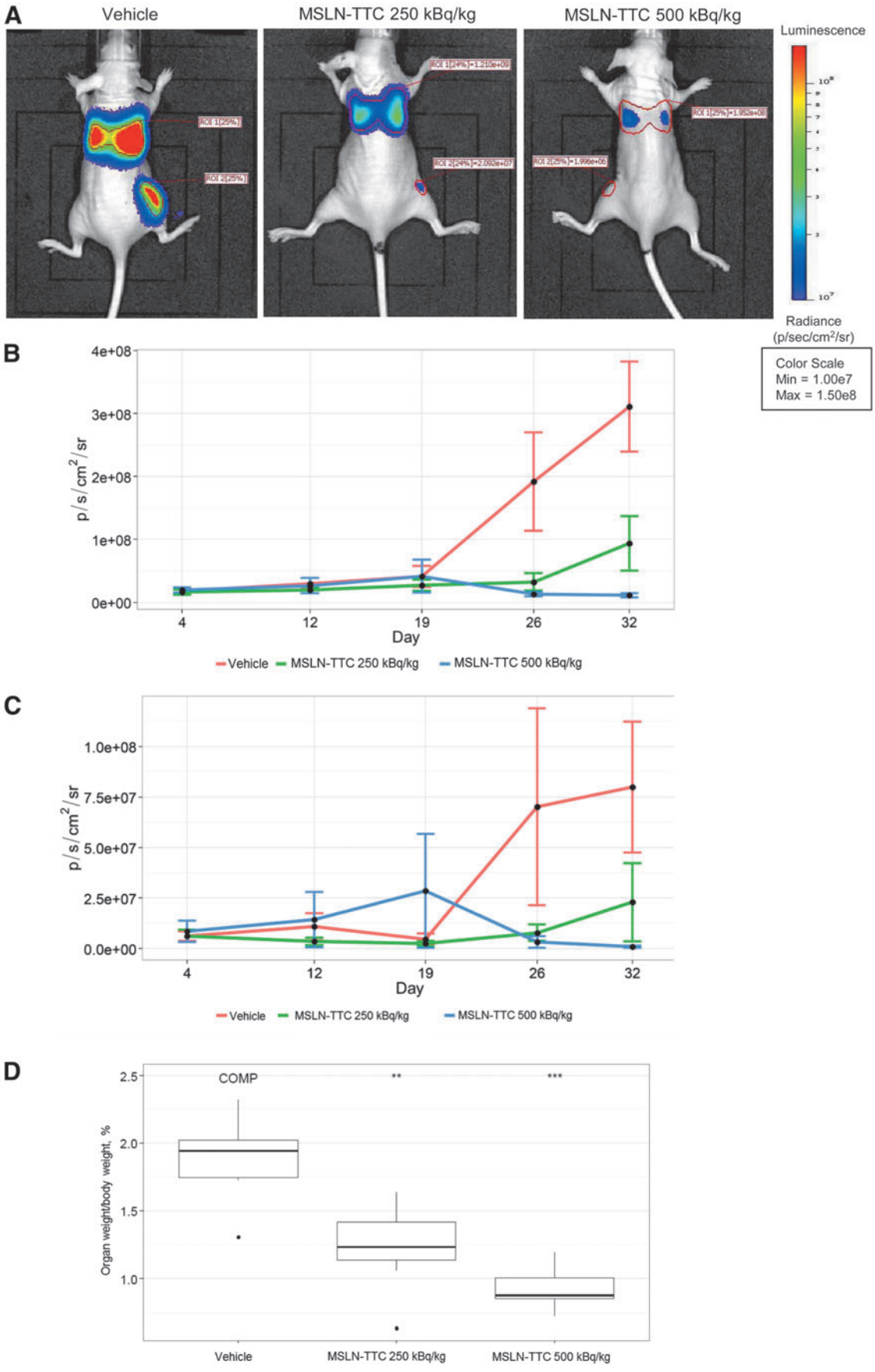
A pharmacokinetic/pharmacodynamic modeling approach was developed, which fitted the respective *in vivo* models with high accuracy. Furthermore, the potency parameter k2 of ^{227}Th with a value of 2×10^{-6} mL/Bq/h was independent of receptor density and tissue origin.[36]

PSMA-TTC

PSMA, a non-shed type II transmembrane glycoprotein, is highly expressed in nearly all prostate cancers, including castration-resistant disease, and in >80% of prostate cancer metastases. In normal tissues, the low-level expression is confined to the prostate, kidneys, small intestine, salivary glands, and central nervous system.[58,59] PSMA is downregulated by androgens and expression can be reversed by treatment with antiandrogen therapy in prostate carcinomas.[58,59]

PSMA-targeted radiotherapy using antibody- and peptide-based approaches has recently attracted growing interest. Promising prostate-specific antigen (PSA) responses have been reported in men with mCRPC who had received the small-molecule PSMA-617 radiolabeled with the β^--emitter lutetium-177 (^{177}Lu-PSMA-617),[60] which is now being evaluated in a pivotal trial in patients with mCRPC (NCT03511664).[61] Furthermore, in patients who became refractory to ^{177}Lu-PSMA-617, exchanging the radionuclide with the α-emitter ^{225}Ac (^{225}Ac-PSMA-617) has shown remarkable clinical responses in some patients.[62] While in a pilot study treatment with ^{225}Ac-PSMA-617 resulted in a more than 50% decline in lesions detected by positron emission tomography/computed tomography (PET/CT) in 88% of patients with mCRPC, and 65% of patients experienced the complete resolution of metastatic lesions seen on PET/CT scans.[63]

FIG. 3. Evaluation of MSLN-TTC in a lung/bone orthotopic model using the luciferase transfected human lung mesothelioma cell line NCI-H226. **(A)** BLI of athymic mice bearing orthotopic bone/lung metastatic xenograft tumors generated by inoculation of luciferase-transfected lung cancer NCI-H226 cells. Animals were treated with either vehicle ($n=7$), MSLN-TTC 250 kBq/kg ($n=7$) or MSLN-TTC 500 kBq/kg ($n=7$). Representative images of 1 animal from each of the treatment group at the end of the study (day 32) are shown. **(B)** Total tumor burden observed by BLI is shown as a sum of average radiance of tibia and lungs (photons/s/cm^2/steradian). Higher dose of MSLN-TTC decreased total tumor burden on the study day 32. Each treatment group consisted of 7 animals. **(C)** Tumor burden in the tibia was evaluated by BLI. Animals were treated with either vehicle ($n=7$), MSLN-TTC 250 kBq/kg ($n=7$), or MSLN-TTC 500 kBq/kg ($n=7$). Group means are shown. Higher dose of MSLN-TTC decreased tibia tumor burden on the study day 32. **(D)** Relative weight of the lungs (median ± IQR25% ± min/max). MSLN-TTC decreased relative lung weight in a dose-dependent manner compared with the vehicle group indicating antitumor effect in the lungs. Outliers are marked with *floating dots*, but they were not removed in the statistical analysis. Statistical analysis was performed using analysis of variance. As statistical differences were observed ($p=2.3115e-05$), the pairwise comparison was performed using Tukey's Honest Significant Difference test. ***p*-value <0.01; ****p*-value <0.001. BLI, bioluminescence imaging; COMP, comparison group; MSLN-TTC, mesothelin-targeted thorium-227 conjugate.


A
Vehicle
MSLN-TTC 250 kBq/kg
MSLN-TTC 500 kBq/kg
Luminescence
Radiance (p/sec/cm²/sr)
Color Scale
Min = 1.00e7
Max = 1.50e8
B
p/s/cm²/sr
Day
Vehicle
MSLN-TTC 250 kBq/kg
MSLN-TTC 500 kBq/kg
C
p/s/cm²/sr
Day
Vehicle
MSLN-TTC 250 kBq/kg
MSLN-TTC 500 kBq/kg
D
COMP
Organ weight/body weight, %
Vehicle
MSLN-TTC 250 kBq/kg
MSLN-TTC 500 kBq/kg

The clinical use of ^{225}Ac-PSMA-617, however, is limited by xerostomia, which becomes the dose-limiting toxicity due to the strong uptake of the PSMA-targeting ligands into the salivary glands.[62] In contrast, salivary gland uptake of PSMA-targeted antibodies has not been reported.[64]

Recently, promising preclinical antitumor activity has been shown for an antibody-based PSMA-targeted ^{227}Th conjugate (PSMA-TTC).[65] *In vitro*, a rapid accumulation of intracellular radioactivity was observed in PSMA-TTC-treated prostate cancer cells expressing high levels of PSMA but not in PSMA-negative cells, indicating fast internalization of PSMA-TTC in a target-dependent manner.[65] PSMA-TTC selectively reduced the viability of PSMA-expressing prostate cancer cells compared with isotype control-treated cells (Table 1), which was dependent on PSMA cell-surface expression levels.[65] PSMA-TTC induced DSBs in prostate cancer cells, cell cycle arrest in the G2/M phase, and apoptosis.[65]

Antitumor activity of PSMA-TTC was tested *in vivo* in PSMA-positive cell line- and patient-derived xenograft models that mimic different stages of prostate cancer, including models of resistance to androgen receptor inhibitors. PSMA-TTC showed specific accumulation and retention in xenograft tumors and significantly inhibited tumor growth at 75–500 kBq/kg administered either as a single dose or multiple fractionated doses (Table 1).[65] Analysis of xenograft tumor tissue by immunohistochemistry demonstrated the specific induction of DNA damage markers (γH2AX) and activation of the apoptotic marker cleaved caspase-3 in tumor cells.[65] Additionally, PSMA-TTC treatment reduced the level of serum PSA, while PSA levels increased in the control treatment groups.[65]

In the LNCaP-luc osseous prostate cancer model representing metastatic bone disease, tumor growth in the bone and tumor-induced changes in bone morphology were significantly reduced following a single administration of PSMA-TTC at 100 and 200 kBq/kg compared with the control groups.[65]

CD70-TTC

The membrane-bound protein ligand CD70 is a member of the tumor necrosis factor superfamily, and its expression is strictly regulated and transiently induced on activated T and B cells under physiological conditions.[66] High levels of CD70 expression have been reported in B cell chronic lymphocytic leukemia, follicle center B cell lymphomas, large B cell lymphomas, and mantle cell lymphomas,[67] as well as renal cell carcinomas (RCCs),[66,68,69] nasopharyngeal and ovarian carcinomas, glioblastomas, and melanomas.[66,70,71]

A CD70-targeted ^{227}Th conjugate (CD70-TTC) was developed, and its activity was tested on CD70-positive human RCC cells *in vitro* and *in vivo*.[72] *In vitro*, exposure to CD70-TTC reduced RCC 786-O cell viability in a dose-dependent manner (Table 1).[72] In a biodistribution study using 786-O tumor-bearing mice, specific uptake and retention of CD70-TTC at 7 d after administration was observed.[72] Dose-dependent and statistically significant inhibition of 786-O xenograft tumor growth in mice was achieved when CD70-TTC was administered as a single dose of 50, 100, 300, and 500 kBq/kg. The observed antitumor activity correlated with an increase in MST at the end of the experiment.[72] CD70-TTC was well tolerated, with only modest changes in hematological parameters detected in treated animals.[72]

Fibroblast growth factor receptor 2-TTC

Fibroblast growth factor receptor 2 (FGFR2), a transmembrane tyrosine kinase receptor, is activated through dimerization upon binding of its ligands, fibroblast growth factors.[73] FGFR2 expression levels in normal tissues are usually low.[73] However, genetic alterations in *FGFR2*, such as genomic amplifications, gene fusions, and mutations, have been reported in various malignancies, including gastric, colorectal, and triple-negative breast cancer (TNBC), resulting in overexpression or ligand-independent constitutive activation of FGFR2.[74] Higher levels of FGFR2 activity are usually associated with increased cancer cell survival and induction of resistance to targeted therapies.[75,76] Therefore, FGFR2 appears to be an attractive candidate for the development of an FGFR2-targeting ^{227}Th conjugate.

FGFR2-TTC reduced the viability of FGFR2-expressing cells, resulting in accumulation of DNA DSBs and cell cycle arrest in the G2/M phase (Table 1).[77] In a preclinical murine model of human gastric cancer, whole-body autoradiography visualized the FGFR2-TTC tumor uptake in mice bearing subcutaneous xenografts.[77] Furthermore, treatment with a single dose of 500 kBq/kg specifically reduced the tumor growth in human colorectal, gastric cancer, and TNBC xenograft mouse models (Table 1).[77] FGFR2-TTC was well tolerated, with no significant body weight loss observed in any of the xenograft models tested.[77]

Zirconium–HOPO–Antibody Conjugates and Their Use in Biodistribution Studies

Zirconium-89 (^{89}Zr), like ^{227}Th, exists as Zr^{4+} and forms very stable complexes with octadentate 3,2-HOPO chelators.[32,78,79] Zirconium-89 is a positron-emitting radioisotope and offers great potential for the development of diagnostic PET imaging tracers. Such tracers can be utilized in the preclinical and clinical development of TTCs by providing information on the pharmacokinetic and pharmacodynamic properties of the labeled conjugates (Fig. 2).

The PSMA antibody-3,2-HOPO chelator conjugate used in PSMA-TTC was radiolabeled with ^{89}Zr to form ^{89}Zr-HOPO-PSMA. *In vitro*, ^{89}Zr-HOPO-PSMA bound cell surface of PSMA-positive LNCaP cells in a PSMA-dependent manner, and its cellular internalization increased during the incubation time.[80]

^{89}Zr-HOPO-PSMA was evaluated further in a biodistribution study. A single dose of ^{89}Zr-HOPO-PSMA corresponding to 347 kBq/mouse at a total antibody dose of 0.072 mg/kg resulted in 40%–50% of injected dose per gram (ID/g) 24–240 h postinjection in PSMA-positive LNCaP xenograft tumors, as determined by radioactivity measurements of excised tumor tissue.[80] ^{89}Zr-HOPO-PSMA provided excellent image contrast of LNCaP xenografts in PET imaging for up to 7 d after administration of the tracer (4.3 MBq/mouse, 1.21 mg/kg total antibody), and the mean tumor accumulation was well in accordance with that from the biodistribution study (40%–72% ID/g 24–168 h postinjection; Fig. 4).[80] Importantly, the accumulated dose per gram was comparable with that seen in the PSMA-TTC biodistribution studies,[65,80–82] thus supporting the use of ^{89}Zr-HOPO-PSMA in guiding preclinical and clinical development of PSMA-TTC.

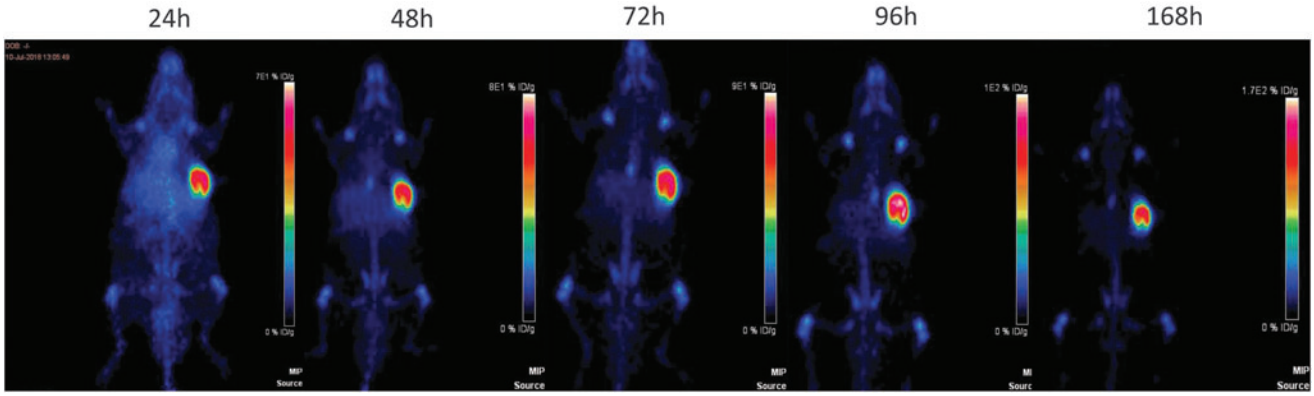


FIG. 4. PET imaging of LNCaP tumor-bearing mice using ^{89}Zr-HOPO-PSMA. LNCaP tumor-bearing mice were injected with ^{89}Zr-HOPO-PSMA and subjected to PET scans at 24–168 h postinjection. Maximal intensity projections of PET imaging at 24, 48, 72, 96, and 168 h after the ^{89}Zr-HOPO-PSMA injection are shown. HOPO, hydroxypyridinone; PET, positron emission tomography; PSMA, prostate-specific membrane antigen; ^{89}Zr, zirconium-89.

Rationale for Combining TTCs with Inhibitors of DDR

In response to DNA damage, the DDR pathways initiate cell cycle arrest to either promote repair of the DNA lesion, or, if the damage is irreparable, to initiate programmed cell death (apoptosis).[83] The ability of cancer cells to repair DNA lesions often reduces the therapeutic efficacy of DNA damage-inducing agents. However, cancer cells can become more vulnerable to apoptotic cell death in response to DNA-damaging agents, especially if they harbor inactivating genetic alterations in the DDR genes, such as *BRCA* genes.[83] Alternatively, agents inhibiting one of the DDR pathways may enhance sensitivity of cancer cells to the treatment with DNA-damaging TTCs.

As the key MoA of TAT is the induction of clustered and difficult-to-repair DNA DSBs, a combination of TTCs with inhibitors of specific DDR pathway is, therefore, expected to induce synthetic lethality in cancer cells. To test this hypothesis, several preclinical studies have been performed where TTCs were combined with DDR inhibitors.[84–86]

A humanized HER2-targeting IgG1 antibody derived from trastuzumab was conjugated to the 3,2-HOPO chelator and subsequently radiolabeled with ^{227}Th resulting in HER2-TTC.[25] Robust tumor growth inhibition was achieved when HER2-TTC was combined at a subefficacious monotherapy dose of 125 kBq/kg with the poly-ADP ribose polymerase 1/2 (PARP1/2) inhibitor olaparib in the DLD-1 BRCA2$^{-/-}$ xenograft model established from the DLD-1 colorectal cancer cell line with inactivated *BRCA2* gene, while no significant tumor inhibition occurred in the xenograft model established from the DLD-1 parental isogenic cell line (Table 2).[84]

The dosing regimen that combined 125 or 300 kBq/kg HER2-TTC and 50 mg/kg of olaparib resulted in a complete tumor growth inhibition in the DLD-1 *BRCA2*$^{-/-}$ xenograft model (Table 2). Importantly, the combination treatment appeared to be well tolerated.[84] These data support further investigation of the combinations of TTCs with specific DDR inhibitors in the *BRCA2*$^{-/-}$ setting.

In another study, concomitant treatment with FGFR2-TTC and ataxia telangiectasia and Rad3-related inhibitor (ATRi; BAY 1895344) led to an increased activity of both agents in FGFR2-expressing cell lines, including those of gastric cancer and TNBC, compared with the single-agent

Table 2. Efficacy of Targeted Thorium-227 Conjugates and DNA Damage Response Pathway Inhibitor Combinations in Preclinical *In Vivo* Models

Combination	*Preclinical findings*
HER2-TTC Olaparib[84]	Parallel assessment of the combination of HER2-TTC with olaparib in the human DLD-1 colorectal cancer xenograft model and the DLD-1 BRCA$^{-/-}$ xenograft model HER2-TTC in combination with olaparib demonstrated potent activity in both models. However, the potency of the combination was further enhanced in the DLD-1 BRCA$^{-/-}$ model due to DNA DSB repair deficiency
FGFR2-TTC ATRi[85]	FGFR2-TTC demonstrated increased potency in combination with ATRi when both agents were administered at subefficacious doses in mice bearing MFM-223 tumors (TNBC)
MSLN-TTC	Respective monotherapies did not show tumor growth inhibition at the same doses MSLN-TTC demonstrated synergistic activity when combined with ATRi at subefficacious doses in mice bearing OVCAR-3 tumors (ovarian cancer)
ATRi and olaparib[86]	Similarly, MSLN-TTC demonstrated additive activity when combined with olaparib at subefficacious doses in mice bearing OVCAR-3 tumors (ovarian cancer) Respective monotherapies did not show tumor growth inhibition at the same doses

ATRi, inhibitor of ataxia telangiectasia and Rad3-related; DSB, double-strand break; FGFR2, fibroblast growth factor receptor 2; HER2, human epidermal growth factor receptor 2; MSLN, mesothelin; TNBC, triple-negative breast cancer; TTC, targeted thorium-227 conjugate.

Table 3. Ongoing Clinical Development of Targeted Thorium-227 Conjugates

Treatment	*Phase*	*Status*	*Primary objectives*	*Secondary/exploratory objectives*	*Disease (estimated enrollment)*	*Estimated completion date (primary completion date)*	*Study ID*
MSLN-TTC	Phase I	Recruiting	Incidence of DLTs, TEAEs, drug-related AEs, and SAEs	RP2D and PK properties	Advanced recurrent epithelioid mesothelioma, serous ovarian cancer, metastatic or locally advanced pancreatic ductal adenocarcinoma (optional, dose expansion arm) ($n=228$)	February 2023 (June 2022)	NCT03507452
PSMA-TTC	Phase I	Recruiting	Incidence of DLTs and MTD	RP2D and PK properties	mCRPC ($n=111$)	November 2022 (September 2022)	NCT03724747
CD22-TTC	Phase I	Active, not recruiting	Safety, tolerability and MTD	Biodistribution, radiation dosimetry, PK/PD, immunogenicity, biomarkers, tumor response profile	Relapsed or refractory CD-22-positive non-Hodgkin's lymphoma ($n=21$)	January 2020 (April 2019)	NCT02581878
HER2-TTC	Phase I	Not recruiting yet	Incidence of TEAEs, TESAEs and DLTs, severity of TEAEs and TESAEs, and ORR by RECIST v1.1	Recommended dose for dose expansion cohorts, RP2D, and PK properties	HER2-expressing cancers ($n=176$)	May 2027 (April 2025)	NCT04147819

AEs, adverse events; DLTs, dose-limiting toxicities; HER2, human epidermal growth factor receptor 2; mCRPC, metastatic castrate-resistant prostate cancer; MSLN, mesothelin; MTD, maximum tolerated dose; ORR, objective response rate; PK, pharmacokinetic/s; PD, pharmacodynamics; PSMA, prostate-specific membrane antigen; RECIST v1.1, Response Evaluation Criteria in Solid Tumors version 1.1; RP2D, recommended phase 2 dose; SAEs, serious adverse events; TEAEs, treatment-emergent adverse events; TESAEs, treatment-emergent serious adverse events; TTC/s, targeted thorium-227 conjugate/s.

treatment.[85] *In vivo*, increased antitumor efficacy in the FGFR2-overexpressing human TNBC MFM-223 xenograft model was observed when the combination of FGFR2-TTC and ATRi were administered at subefficacious doses, while the respective monotherapy demonstrated only partial tumor growth inhibition (Table 2).[85]

Synergistic activity between MSLN-TTC and inhibitors of ataxia telangiectasia mutated, ATR (BAY 1895344), DNA-dependent protein kinase, and PARP1/2 has been investigated *in vitro* and *in vivo* using human ovarian cancer xenograft mouse models.[86] The treatment of mice bearing OVCAR-3 tumors with the combination of MSLN-TTC and either ATRi (BAY 1895344) or PARP1/2 inhibitor olaparib has shown the strongest increase in potency at doses which were nonefficacious when administered as monotherapy (Table 2).[86]

In summary, these data support further investigation of the combination of specific TTCs with the DDR pathway inhibitors to achieve synthetic lethal interactions and a higher tumor apoptotic index.

Immunostimulatory Effect of TTCs

Tumor-targeted delivery of ionizing radiation, specifically γ-rays or β^--particles, has been shown to induce an immunostimulatory response.[87] Indeed, ionizing radiation has been linked to immunogenic cancer cell death through radiation-induced release of DAMPs, which initiate and promote inflammatory responses mediated by activated dendritic cells presenting tumor-specific antigens to prime cytotoxic T cells.[88] External beam radiation doses above 12–18 Gy have been shown to activate the expression of DNA exonuclease Trex1, to degrade the DNA in the cytosol of irradiated cancer cells and to prevent activation of STING downstream of the type-I interferon pathway.[89]

Similar to the effects of γ- and β^--particles radiation, irradiation with the α-particle-emitting radionuclide ^{213}Bi complexed to bovine serum albumin induced a release of DAMPs when inoculated *in vitro* on murine adenocarcinoma MC38 cells, mimicking an *in situ* vaccination approach. This mixture of cells, when injected into immunocompetent mice, activated the adaptive immune system and provoked an antitumor response.[90,91]

Interestingly, the α-particle emitter ^{223}Ra significantly increased antitumor activity *in vitro* by enhancing the T cell-mediated lysis of cancer cells through CD8+ cytotoxic T lymphocytes. This resulted in concurrent increase in the expression of major histocompatibility complex class I and calreticulin, and the activation of the endoplasmic reticulum stress response pathway.[92] Furthermore, exposure of an ovarian cancer cell line to MSLN-TTC and B cell lymphoma cell lines to CD22-TTC elevated the expression of DAMPs calreticulin, heat shock protein (HSP) 70, HSP90, and high-mobility group box 1 protein.[36]

These data warrant future work to further investigate the immunostimulatory effects of TTCs and potentially synergistic therapeutic activity between TTCs and immune checkpoint inhibitors.

Clinical Development of TTCs

The TTC translational strategy is underpinned by the breadth of the preclinical data suggesting the therapeutic effectiveness of TTCs in patients with tumors harboring specific cell-surface molecules that can be targeted by the antibody component of TTCs to deliver high-energy α particles to the tumor site. Furthermore, TTCs show antitumor efficacy in various preclinical models resistant to standard of care, suggesting that TTCs may provide novel treatment options for patients who are or become resistant to customary treatment regimens. Therefore, the combination of TTCs with other targeted therapies, such as inhibitors of the DDR pathway, may induce synthetic lethality and lead to treatment alternatives to further improve clinical outcomes.

Currently, CD22-TTC, MSLN-TTC, and PSMA-TTC are undergoing clinical development in phase I trials. CD22-TTC is being investigated in an open-label study (NCT02581878) designed to evaluate the safety, tolerability, biodistribution, radiation dosimetry, and pharmacokinetics of CD22-TTC in patients with relapsed or refractory CD22-positive NHL (Table 3).[93]

MSLN-TTC is being investigated in patients with advanced malignant epithelioid mesothelioma or advanced recurrent serous ovarian cancer who have exhausted available therapeutic options, to evaluate the safety, tolerability, pharmacokinetics, and antitumor activity of MSLN-TTC (NCT03507452, Table 3).[94]

A first-in-human study (NCT03724747) has also been initiated to evaluate the safety, pharmacokinetics, and antitumor activity of PSMA-TTC in patients with mCRPC who are not responsive to standard therapy or are lacking appropriate treatment options (Table 3).[95]

A first-in-human study (NCT04147819) will be initiated shortly to evaluate the safety, pharmacokinetics, and antitumor activity of HER2-TTC in patients with breast or gastric cancer who are not responsive to standard therapy or are lacking appropriate treatment options.[96] These studies will establish the maximum tolerated doses and toxicity profiles of TTCs to guide further clinical development of TTCs in specific cancer patient subpopulations.

Summary and Future Perspectives

The TTC agents enable the highly localized and specific delivery of α-particle energy to cancer cells expressing target antigens and to the tumor microenvironment, thus minimizing damage to the neighboring healthy tissue. Studies using ^{223}Ra in patients with CRPC and metastatic bone disease have provided evidence and proof of concept for the clinical utility and benefits of TAT. The TTC agents have broad applicability across different tumor types, and preclinical studies have demonstrated their activity even in tumors with varying levels of expression of target molecules, indicating that the level of abundance of target cell-surface molecules may not be an activity-limiting step for TTC therapy. TTCs may be less susceptible to the development of acquired resistance compared with cytotoxic chemotherapy drugs or inhibitors of specific signaling molecules and, therefore, may be beneficial in early and late treatment lines.[26,97]

Future clinical studies designed to investigate the toxicity profiles of TTCs will be of paramount importance in determining the full potential of this class of cancer therapy. The MoA of TTCs offers the potential for new combinations with a range of current and emerging therapies, including inhibitors of the DDR pathway and potentially immune

checkpoint inhibitors for the effective treatment of patients with a variety of tumor types. Furthermore, the combination of TTCs with immune checkpoint inhibitors may show benefits in patients with less advanced cancer due to TTC-induced immunogenic effect and a lower likelihood of resistance to TTCs. Future studies should explore the genetic burden induced by TTCs in tumors and compare it with the high tumor mutational load, which has been previously linked to an enhanced preclinical efficacy of immune checkpoint inhibitors.[98]

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