Development of AlphaMedix (^{212}Pb-DOTAMTATE)

Phase 1 clinical trial results

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Targeted Alpha-Emitter Therapy With ^{212}Pb-DOTAMTATE for the Treatment of Metastatic SSTR-Expressing Neuroendocrine Tumors: First-in-Human, Dose-Escalation Clinical Trial

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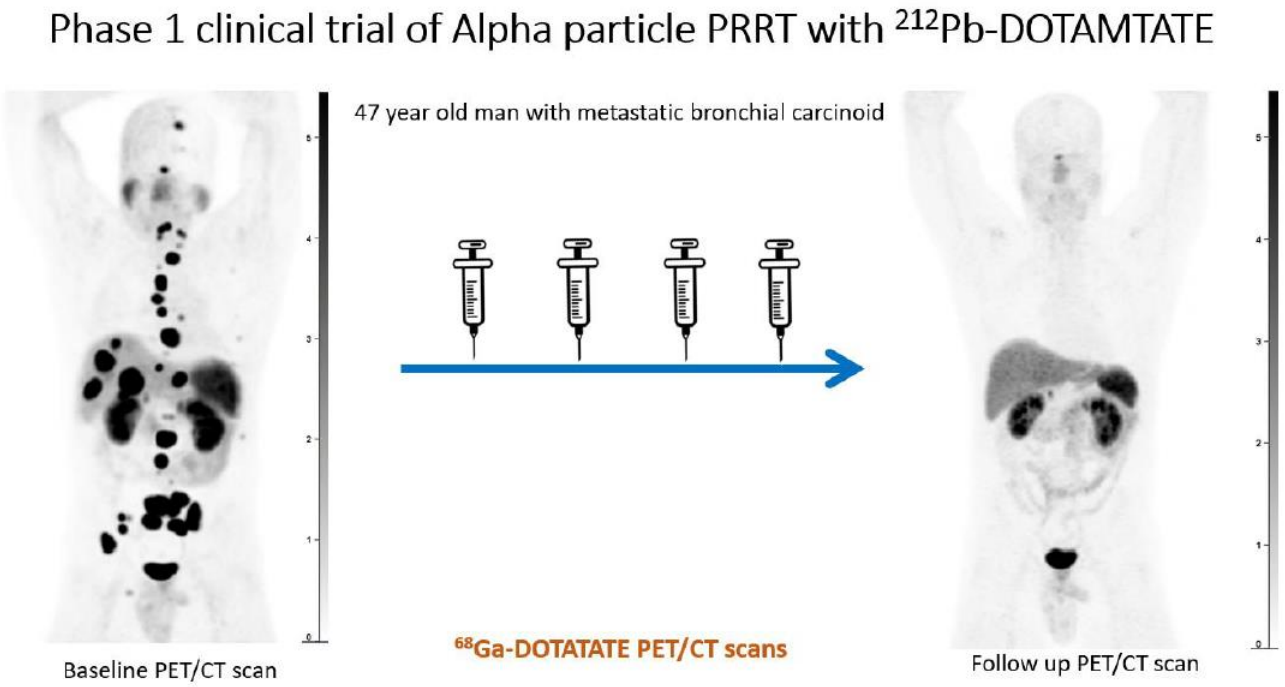

Patient with widespread metastatic neuroendocrine tumors which resolved after treatment with AlphaMedix (phase I clinical trial)

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Targeted Alpha-Emitter Therapy With ^{212}Pb-DOTAMTATE for the Treatment of Metastatic SSTR-Expressing Neuroendocrine Tumors: First-in-Human, Dose-Escalation Clinical Trial

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Short running title (31 characters; limit, 40 characters): TAT of NET with ^{212}Pb-DOTAMTATE. NEN

ABSTRACT (326 words; limit, 350)

Peptide receptor radiotherapy (PRRT) with somatostatin analogs has been successfully utilized as a treatment for somatostatin overexpressing tumors for years. Treatment of neuroendocrine tumors (NETs) with the beta particle emitter ^{177}Lu-DOTATATE is currently considered the standard of care for subjects with gastroenteropancreatic NETs (GEP-NETs). Despite the success of ^{177}Lu-DOTATATE, there remains significant room for improvement in terms of both safety and efficacy. Targeted alpha-emitter therapy with isotopes such as lead-212 (^{212}Pb) has the potential to improve both. Herein, we present the preliminary results of the phase 1 first-in-human dose-escalation trial evaluating ^{212}Pb-DOTAMTATE in patients with somatostatin receptor positive NETs.

Methods: A total of 20 subjects with histologically confirmed NETs, prior positive somatostatin analogue scans, and no prior history of ^{177}Lu/^{90}Y/^{111}In PRRT, with different primary sites of the disease, were enrolled. Treatment began with single ascending doses of ^{212}Pb-DOTAMTATE, with subsequent cohorts receiving an incremental 30% dose increase, which was continued until a tumor response or a dose-limiting toxicity was observed. This was followed by a multiple ascending dose regimen. The recommended phase 2 dose (RP2D) regimen consisted of 4 cycles of 2.50 MBq/kg (67.6 μCi/kg) of ^{212}Pb-DOTAMTATE administrated at 8-week intervals, intravenously.

Results: Ten subjects received the highest dose of 2.50 MBq/kg/cycle (67.6 μCi/kg/cycle). Treatment was well tolerated, with the most common treatment-emergent adverse events (TEAEs) being nausea, fatigue, and alopecia. No serious TEAEs were related to the study drug, and no subjects required treatment delay or a dose reduction. An objective radiological response (ORR) of 80% was observed for the first 10 subjects treated at the RP2D.

Conclusion: Targeted alpha therapy with ^{212}Pb-DOTAMTATE has been shown to be well-tolerated. Preliminary efficacy results are highly promising. If these results are confirmed in a larger, multicenter clinical trial, it would provide a substantial benefit over currently FDA approved therapies for patients with metastatic or inoperable SSTR-expressing NETs regardless of the grade and location of the primary tumor.

Key Words: TAT, PRRT**,** ^{212}Pb-DOTAMTATE, NET, NEN, phase 1

INTRODUCTION

Neuroendocrine tumors (NETs) are a heterogeneous group of rare neoplasms that originate from neuroendocrine cells. These neoplasms occur mostly in the gastrointestinal tract and pancreas but can also occur in other tissues including thymus and lung, as well as uncommon sites such as ovaries, heart, and prostate. Regardless of their primary site, NETs share histological, immunohistochemical, and ultrastructural features. NETs retain multi-potent differentiation capacities including the ability to produce and secrete a variety of metabolically active substances such as amines, peptides, and prostaglandins [1].

Most NETs strongly express somatostatin receptors (SSTRs), predominantly of the somatostatin 2 subtype [2], providing the basis of antisecretory and antiproliferative therapy with somatostatin analogs (short- and long-acting octreotide and long-acting lanreotide). These drugs are highly effective in controlling symptoms associated with carcinoid syndrome and have been shown to improve progression-free survival (PFS) in the metastatic setting in gastroenteropancreatic (GEP) NETs [3]. Although PFS can be prolonged, a high percentage of patients will progress and require additional therapy. Current guidelines recommend that patients with locoregional advanced disease and/or distant metastases for NETS of the gastrointestinal tract be treated with systemic therapy such as everolimus, sunitinib, or peptide receptor radionuclide therapy (PRRT) with the beta-emitter ^{177}Lu-DOTATATE [4]. The latter is currently the only PRRT approved by the US Food and Drug Administration for patients with SSTR-expressing GEP NETs [5]. The NETTER-1 study demonstrated a clinically meaningful and statistically significant increase in PFS and objective radiological response (ORR) in subjects with advanced GEP NETs treated with ^{177}Lu-DOTATATE and long-acting octreotide (30 mg) compared with those treated with high dose long-acting octreotide. At the data-cutoff date for the primary analysis, the estimated PFS at Month 20 was 65.2% in the ^{177}Lu-DOTATATE group and 10.8% in the control group [6]. Although the NETTER-1 trial demonstrated a tremendous benefit in PFS and overall survival (OS), the ORR was only 13% in the ^{177}Lu-DOTATATE group versus 3% in the octreotide group, with only 1 complete response (CR) and 14 (12%) partial responses (PR) in the ^{177}Lu-DOTATATE group [7]. It stands to reason that a radiopharmaceutical that provides a superior ORR will likely also improve PFS and OS.

^{212}Pb-DOTAMTATE is the first ^{212}Pb-labeled octreotate analogue to treat SSTR-expressing NETs and targets SSTR-expressing malignancies regardless of their primary organ of origin and their proliferative index. The

drug consists of 3 linked components: the ^{212}Pb isotope, a bifunctional metal chelator (DOTAM), and the SSTR-targeting peptide (TATE).

The physical half-life of ^{212}Pb is 10.6 hours, and it is an *in vivo* generator of alpha-emitting particles. ^{212}Pb itself is not an alpha-emitter, but its decay scheme includes 2 alpha-particles (one per branch) with potent cytotoxicity to cell nuclei [8, 9].

Compared with currently used beta-emitters, such as ^{177}Lu-DOTATATE [10, 11], ^{212}Pb-DOTAMTATE provides a significantly higher linear energy transfer delivered in a shorter path length. In theory, a higher linear energy transfer should induce more double-stranded DNA damage to the tumor cells, ultimately resulting in irreparable tumor cell injury, apoptosis, and cell death. Additionally, due to the shorter path length, there are fewer side effects for subjects receiving targeted alpha therapy (TAT) [12]. Accordingly, to address an unmet need of TAT in the field of PRRT for NET, we are undertaking a phase 1 study with the main objective of determining the safety and dose-limiting toxicity (DLT) of ascending doses of ^{212}Pb-DOTAMTATE used for TAT in subjects with SSTR-expressing NETs. A secondary objective was to determine the pharmacokinetic properties as well as the preliminary effectiveness of ascending doses of ^{212}Pb-DOTAMTATE.

MATERIALS AND METHODS

Study Design

This open-label, nonrandomized, dose-escalation and expansion, phase 1 trial (NCT03466216) was conducted at a single center in the US (Excel Diagnostics Nuclear Oncology Center, Houston, Texas). This prospective study was performed in accordance with the Helsinki Declaration and followed the International Conference on Harmonization Good Clinical Practice guidelines. The study was approved by the Biomedical Research Alliance of New York Institutional Review Board; all subjects gave written informed consent prior to enrollment. The study was conducted in full compliance with the US Health Insurance Portability and Accountability Act. Eligible patients included males and females aged ≥18 years with an ECOG performance status of 0–2 and a life expectancy of at least 12 weeks with a histologically confirmed diagnosis of NET, either unresectable or metastatic progressive disease, with at least 1 site of measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. All patients were required to have SSTR imaging within 4 weeks of the first dose. Patients who had been treated with prior whole-body radiotherapy or PRRT using ^{177}Lu/^{90}Y/^{111}In-DOTATATE/DOTATOC or TAT were excluded. Therapeutic use of any

somatostatin analogue, including long-acting octreotide acetate (within 28 days) and octreotide acetate (within 1 day), prior to administration of study drug was exclusionary.

The study was designed as a single ascending dose (SAD)/multiple ascending dose (MAD) trial utilizing a 3+3 dose-escalation scheme with an 8-week DLT period. Dose escalation proceeded as per Table 1. The initial dose to be examined was 1.13 MBq/kg (30.7 µCi/kg), and subsequent cohorts received an incremental 30% dose increase until a tumor response or a DLT was observed. The maximum total dose per subject in the SAD cohort regimen was 296 MBq (8 mCi). The maximum total dose per subject in the MAD cohort was 888 MBq (24 mCi). All these limits were assigned by human dosimetry calculations performed on subjects having received the ^{203}Pb-AlphaMedix™ surrogate under IND 130,960. The activity of each cycle was not to exceed 203.5 MBq +/- 10%. (5.5 mCi +/- 10%), regardless of the subject's weight, not to exceed the cumulative dose of 888 MBq (24 mCi). The data safety monitoring board (DSMB) was responsible for determining both dose escalation in the SAD cohorts and dose at which expansion into the MAD cohort(s) would occur. The DSMB made the recommendation to transition to the MAD cohort due to the clinical efficacy and lack of any dose limiting toxicities.

Non-hematologic DLTs were defined as all Grade 3 toxicities (except alkaline phosphatase) not responsive within 72 hours of supportive care and any Grade 4 toxicities. Hematologic DLTs were defined as any toxicity that did not recover to $\leq$ Grade 2 within 8 weeks after administration of the study drug. A dose-modifying toxicity was defined as any Grade 3 or 4 hematological toxicity (except lymphopenia) that did not resolve within 8 weeks from the prior administration or a Grade 2 or higher serum creatinine level that did not resolve within 8 weeks from the prior administration.

The MAD treatment regimen began at the 1.92 MBq/kg (52.0 µCi/kg) dose level and was escalated to the fourth cohort (MAD4) at a dose of 2.50 MBq/kg (67.6 µCi/kg/cycle). The cohort was then expanded to include 7 more patients for a total of 10. Thirty minutes prior to each dose, an amino acid solution of lysine and arginine was administered at 250 mL/h over 4 hours for kidney protection against the effects of radiation. Before each injection cycle subjects had a physical exam, filled out a quality-of-life questionnaire (EORTC QLQ-C30), and had routine blood testing including complete blood count, comprehensive metabolic panel with estimated glomerular filtration rate (eGFR), tumor markers, electrocardiogram, and medical imaging. Baseline and follow up imaging included, contrast enhanced MRI or CT scan for RECIST 1.1. evaluation ^{99m}Tc-DTPA renal scan, ^{68}Ga-DOTATATE PET/CT

were also performed. ^{18}F-FDG PET/CT, and bone scan were performed in selected patients, at the P.I.'s discretion, ^{18}F-FDG PET/CT scan was repeated, if positive at the baseline evaluation.

For all subjects, safety follow-up visits were scheduled at 2, 5, 8, and 12 months after the single injection in the SAD cohorts and after the fourth injection in the MAD cohorts. The 12-month safety follow-up visit was also the end of study visit. From months 13 to 36, a structured, semi-annual phone call follow-up was performed to collect late toxicity, any hospitalization, recent imaging results, and new treatment. Efficacy assessments per RECIST 1.1 were collected after each cycle as was functional imaging. Objective radiographic response (ORR) was assessed according to RECIST 1.1. .Following our own pre-established criteria PET/CT imaging response was defined as complete when all SSTR- positive lesions were resolved or PR, when there was a reduction of more than 50% of the "visually estimated" tumor burden. Visual estimation of the overall tumor burden for each patient by 68Ga-DOTATATE PET/CT, was a subjective assessment done by an experienced (more than 25 years) board certified Nuclear Medicine physician estimating the reduction in the patients tumor burden, considering that the baseline 68Ga-DOTATATE PET/CT scan reflected 100% of the tumor burden.Duration of response was defined as the time that measurement criteria were first met for CR/PR by RECIST 1.1. criteria until the date that recurrent or progressive disease was objectively documented [13] or last clinical contact (June 2021). Time to response was defined as the time between the first administration of study drug and the time when RECIST measurement criteria were first met for CR/PR.

The primary endpoint was assessment of the safety and DLTs of ascending doses of ^{212}Pb-DOTAMTATE used for targeted alpha-emitter therapy of subjects with SSTR-expressing NETs. Secondary endpoints included pharmacokinetics, dosimetry, and determination of preliminary effectiveness of ^{212}Pb-DOTAMTATE. Adverse events collected were coded using the National Cancer Institute Common Terminology Criteria for Adverse Events v4.03. Estimated glomerular filtration rate was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation. Pharmacokinetics were evaluated through the collection of several blood samples at multiple time points and urine collection before and after the intravenous administration of ^{212}Pb-DOTAMTATE.

Dosimetry data were obtained for 6 subjects in the MAD4 cohort and will be reported in a separate manuscript.

Students t test was used to compare the means and derive p values using JMP® Clinical Version 8.0. (SAS Institute Inc., Cary, NC),

RESULTS

A total of 20 PRRT naïve subjects (10 male, 10 female) have been treated to date (median age, 62 years; range, 27–80), with 10/10 subjects (100%) receiving 4 cycles of ^{212}Pb-DOTAMTATE at the highest dose level of 2.50 MBq/kg/cycle (67.6 µCi/kg/cycle) (Table 2). The mean cumulative dose administered over 4 cycles based on a dose of 2.50 MBq/kg (67.6 µCi/kg) was 791 MBq (21.4 mCi), with a range of 681–873 MBq (18.4–23.6 mCi). All patients had received or declined all FDA approved medications for their disease except for PRRT including somatostatin analogues and progressed prior to enrollment. The time span between the histophatologic diagnosis and the first cycle of treatment with ^{212}Pb-DOTAMTATE, varied considerably between patient to patient, ranging from 0.3 to 10.7 years with a mean of 5.36 years.

Radiographic Results

No objective radiological response by RECIST 1.1 criteria was seen in cohorts SAD1 and SAD2 or the first MAD cohort (MAD3). In the MAD4 cohort, the ORR by RECIST was 80% (1 CR, 7 PR, 2 stable disease). One subject (10%) in the MAD4 cohort (MAD4-06) demonstrated an objective response 8 weeks after the first injection, 6/7 subjects [86%]) after the third cycle of therapy, with 1 subject (MAD4-07) achieving a partial response after completion of all 4 cycles. The only CR was in subject MAD4-03, who obtained a CR by RECIST after the 10-month visit (Fig. 1). Four patients (40%) had ≥ 50% decrease of sum of the diameters (SOD) of the target lesions. The largest percentage decrease in SOD that was not a CR was seen in subject MAD4-02, with an 85% decrease (Fig. 2 and Fig. 3). The median decrease in SODs for all patients was 41%.

Response by ^{68}Ga-DOTATATE PET/CT scan in the MAD4 cohort demonstrated 3 CR (patients MAD4-02, -03, and -06), 4 PR, and 3 stable disease (Fig. 4). The mean decrease in SOD per RECIST in those patients that demonstrated a CR by ^{68}Ga-DOTATATE PET/CT was 84% (range, 70%–100%). Although patient MAD4-04 did not meet the definition of PR per RECIST, with only a 26% decrease in SOD of the target lesions, ^{68}Ga-DOTATATE PET/CT imaging demonstrated obvious improvement in tumor burden.

No progression of disease was noted for 9/10 subjects (90%) who completed treatment. One subject experienced disease progression approximately 10 months after completing all 4 cycles of treatment (16 months after treatment initiation). Interestingly, the new lesions were not seen on ^{68}Ga-DOTATATE PET/CT but were seen on ^{18}F-FDG PET/CT, suggestive of an undifferentiated NET or a non–SSTR-expressing malignancy.

At time of the last data collection, all MAD4 patients were alive, with median length of follow-up of 17.4 months (range, 9–26). The median duration of response was 14 months (range, 5–22), and the median time to

response was 5.2 months (range, 1.7–10.3).

Safety

No DLTs were noted during dose escalation or expansion, and no subject required delay in treatment or dose reduction or cancellation. A total of 170 adverse events were reported. Eighty-two (46%) were reported in the SAD cohort and 97 (54%) in the MAD cohort. For all reported adverse events, 49 (29%) were Grade 2, 7 (5%) were Grade 3, and zero (0%) was Grade 4. Thirty-two TEAEs were considered related to the study drug, with the most common being alopecia (25%) and nausea (31%).

Fifteen serious TEAEs, including 2 deaths, were reported (Table 3). The majority of serious TEAEs were reported in the SAD cohorts (9/15) and were reported by 4 patients. Six serious TEAEs were reported in the MAD cohort by only 2 patients. Preferred terms for the reported serious TEAEs, by patient, include SAD1-01: disease progression; SAD2-02: pain, malignancy associated pain, dehydration, low GFR (Grade 3), and disease progression; MAD3-01: acute renal failure and renal failure; MAD4-01: worsening achalasia; and MAD4-03: fatigue, acute cerebrovascular accident, hypoglycemia, dyspnoea, and chronic kidney disease. None of the reported serious TEAEs were considered related to the study drug.

Vital Signs

There were no clinically significant changes in systolic blood pressure, diastolic blood pressure, heart rate, or QT interval from baseline compared to the last cycle of treatment for all subjects.

Hematologic, Hepatic, and Renal Parameters

In the MAD4 cohort, there was no statistically significant difference from screening compared with 6 months after treatment in: platelets (median, 264 x10^9/L; range, 124–417 x10^9/L; p=.304), hemoglobin (median, 13.4 g/dL; range, 10.3–16.3 g/dL, p=0.1475), absolute neutrophil count (median, 3.52 x10^9/L; range, 2.08–5.1 x10^9/L, p=0.1833), or white blood cells (median, 5.7 x10^9/L; range, 3.27–9 x10^9/L; p=0.0868).

Comparison of the mean lymphocyte count of each cycle to baseline showed that there was no significant change from baseline screening to Cycle 1. There was a statistically significant difference when comparing Cycle 2: screening (P = 0.0043); Cycle 3: screening (P = 0.0003); and Cycle 4: screening (P = 0.0014); however, at 6 months after treatment, there was no statistically significant difference compared with screening (median, 1.09 x10^9/L 0.3–2.42 10^9/L. p= 0.0508).

There were no statistically significant differences 6 months after treatment in alanine transaminase (p=0.091984). There were statistically significant differences in aspartate aminotransferase (p=0.0454) when comparing screening to the 6-month timepoint.

The mean and median baseline eGFRs of all enrolled patients were 86.4 mL/min/1.72 m^2 and 92.7 mL/min/1.72 m^2, respectively. The mean eGFR for the MAD4 cohort at screening was 90.4 mL/min/1.72 m^2 and at the end of Cycle 4 it was 86.64 mL/min/1.72 m^2. There was no statistically significant difference in eGFR at the completion of 4 cycles compared with screening in the MAD4 cohort (p=0.06499). At 3 months after completion of Cycle 4, the mean eGFR was 78.34mL/min/1.72 m^2, and there was no statistically significant difference in the eGFR compared with baseline (p=0.8650). At the 6-month follow-up, the mean eGFR was 73.38 mL/min/1.72 m^2, which was statistically significant ($P < 0.001$) compared with screening, but with no clinical significance.

DISCUSSION

The current study in adults with progressive metastatic or inoperable SSTR-expressing NETs suggests that treatment with ^{212}Pb-DOTAMTATE provides a clear clinical benefit regardless of the location of the primary site or grade of the tumor. This is a paradigm change from conventional ideas that PRRT needs to be only done on G1-G2 GEP-NET tumors. We are treating these patients based on their molecular biology and receptor affinity. Of the 10 subjects who received all 4 cycles, 8 (80%) demonstrated an objective, long-lasting radiologic response by RECIST 1.1, which is highly encouraging. The pivotal multinational, randomized, double-blind, placebo-controlled phase 3 trial of sunitinib in patients with advanced, well-differentiated pancreatic NETs, sunitinib demonstrated ORR of 9.3 % compared with 0% in the placebo group [14]. In the more recent NETTER-1 study, which only enrolled patients with midgut NETs, initial ORR was found to be 18% in the ^{177}Lu-DOTATATE group compared with only 3% in the octreotide group [7]. These data were later updated to a 13% ORR in the ^{177}Lu-DOTATATE group and 4% in the control group [6]. Despite the relatively low ORR, substantial improvements were made in both PFS and OS. In the current study, although the number of patients is small, at the time of this data evaluation (median follow-up, 17.4 months), the duration of response (14 months) is extremely encouraging. Follow-up continues to determine the true duration of response. The phase II study is planned.

In terms of safety, ^{212}Pb-DOTAMTATE appears to be well tolerated, with mild and manageable side effects. We did not find clinically significant hematologic or hepatic toxicity, although the number of patients

treated at the highest dose was small and further follow-up is necessary. We did find a statistically significant change from baseline in AST, likely explained by 1 subject whose AST was approximately 1.5 times the upper limit of normal and likely of no clinical concern.

We did not observe any statistically significant changes in most hematologic parameters; however, we did observe an expected, statistically significant decrease in the absolute lymphocyte count during treatment that trended toward normal after completion of therapy. Although there was a decrease in the lymphocyte count, the absolute neutrophil count remained normal throughout the treatment period. No other hematological parameters measured had a statistically significant change in values at the 6-month follow-up compared with baseline.

Kidney reabsorption of radiolabeled peptides can lead to dose-limiting nephrotoxicity after PRRT. The timeframe for a kidney damage is unknown; however, data from external beam radiotherapy indicate that chronic kidney failure may occur in up to 5% of patients within 5 years of a dose higher than 23 Gy [15, 16]. This concept has not been proven to be accurate for radioligand therapy but at the moment is the only available principle for regulatory agencies. Nevertheless, in some centers strictly following the recommended kidney tolerance thresholds and not exceeding 4 × 7.4 GBq 177Lu-Dotatate, reported either no grade 3 or 4 subacute nephrotoxicity in 323 patients [17] or only 1.5% grade 3 or 4 nephrotoxicity in 807 patients [18] Results from a recently published retrospective review on a 5 year follow up of NET patients treated with ^{225}Ac-DOTATOC, show there was an average eGFR-loss of 8.4 ml/min per year, which was more pronounced in patients treated with higher dosages [19]. In the present study, 3 patients experienced serious TEAEs related to the kidney. Two patients in the SAD cohort had transient decreases in renal function due to dehydration that were determined to be unrelated to the investigational drug. Patient MAD4-03, who obtained a complete response from treatment, experienced acute kidney injury and resultant persistent chronic kidney disease. This 75-year-old patient had a number of confounding factors, including a long-standing history of obesity, hypertension, and poorly controlled diabetes mellitus type 2, and experienced a cerebrovascular accident prior to kidney insult. Baseline serum creatinine and eGFR were 0.84 mg/dL and 92.5 mL/min/1.72m^2, respectively. Both values remained relatively stable throughout treatment and began to change approximately 2 weeks after the last treatment with ^{212}Pb-DOTAMTATE. The serum creatinine continued to rise to a high of 1.97 mg/dL, and the eGFR decreased to 28.5 mL/min/1.72m^2, consistent with Stage 3 chronic kidney disease. Renal function data collection continues for all MAD4 cohort patients, and long-term follow-up should shed light on what impact, if any, ^{212}Pb-DOTAMTATE has on the kidneys. The most common

non-hematological, non-renal, or non-hepatic adverse event reported was nausea, followed by transient alopecia. Alopecia was moderate, and hair growth resumed quickly after treatment had been completed.

It is difficult to perform appropriate comparisons with the few published clinical trials of TAT in NET patients, since the radiopharmaceuticals used, and subject selection, among other factors, differ from ours. Nonetheless, data published by Kratochwill et al in 2016 showed preliminary good results using ^{213}Bi-DOTATOC TAT [20]. Prolonged responses were reported for the 7 patients in the study. Recently, data from Ballal et al using ^{225}Ac-DOTATATE TAT in advanced, progressive, and/or PRRT refractory GEP NET patients reported a PR in 15/24 (62.5%) evaluable patients (by conventional imaging) and stable disease in 9/24 (37.5%) [21]. In contrast with our study, all patients in the Ballal et al study had already been treated with ^{177}Lu-DOTATATE and more than half (56%) had progressive disease. Nevertheless, the results show that ^{225}Ac-DOTATATE TAT is a promising treatment option, even in patients previously treated with ^{177}Lu-DOTATATE PRRT.

To the best of our knowledge, our study is the first in-human study evaluating the safety and response of ^{212}Pb-DOTAMTATE in NET patients. Although the number of patients is small, the results are very promising (Fig. 4).

Strengths of this study include robust imaging data and inclusion of subjects with progressive metastatic NETs regardless of location of primary tumor and Ki-67 grade. Limitations of this study include a small number of patients recruited from only one clinical site, lack of central imaging, and limited follow-up.

CONCLUSION

^{212}Pb-DOTAMTATE is safe. Preliminary efficacy results are highly promising. If these results are confirmed in a larger, randomized, multicenter clinical trial, would provide a substantial benefit over currently FDA approved therapies for patients with metastatic or inoperable SSTR-expressing NETs regardless of the grade and location of the primary tumor.

DISCLOSURE

Research was sponsored by RadioMedix, Inc. Ebrahim S. Delpassand and Izabela Tworowska are employees and equity holders of RadioMedix. Julien Torgue and Jason Hurt are employees at Orano Med. No other potential

conflict of interest relevant to this article was reported. Authors Rouzbeh Esfandiari, Afshin Shafie, and Rodolfo Núñez had control of the clinical trial data.

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KEY POINTS:

QUESTION: Is ^{212}Pb-DOTAMTATE TAT a feasible and effective treatment modality for NET patients?

PERTINENT FINDINGS: The preliminary results in this first-in-human study of ^{212}Pb-DOTAMTATE TAT show that it is a well-tolerated treatment with an overall response rate of 80% in the first 10 subjects treated with the effective dose.

IMPLICATIONS FOR PATIENT CARE: TAT with ^{212}Pb-DOTAMTATE in NET patients has shown great potential, exceeding the standard of care treatments currently available, and, thus, a phase 2 study will start in the near future.

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FIGURE LEGENDS

FIGURE 1. Volume rendered images of the ^{18}F-FDG PET/CT scans from subject MAD4-03 before (left) and after (right) treatment with 4 cycles of ^{212}Pb-DOTAMTATE.

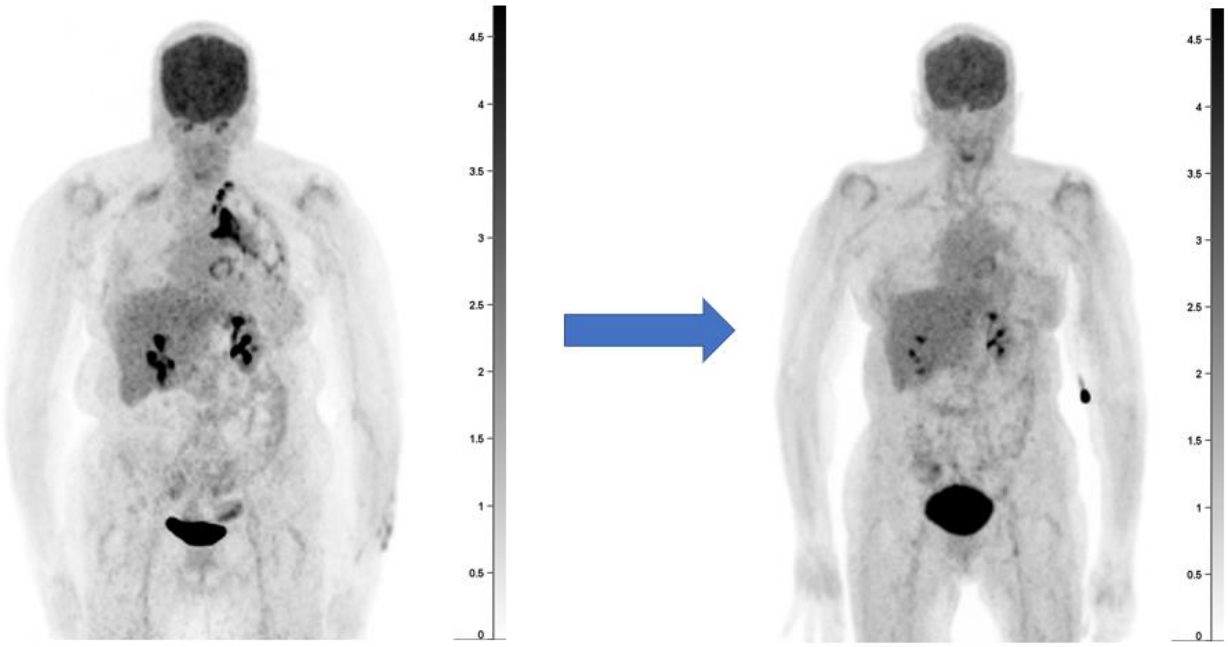


FIGURE 2. MRI of the liver before (A) and after (B) treatment with 4 cycles of ^{212}Pb-DOTAMTATE in MAD4-02 subject. Yellow arrows point to the liver metastases. Notice the near complete resolution of liver metastases on image B.

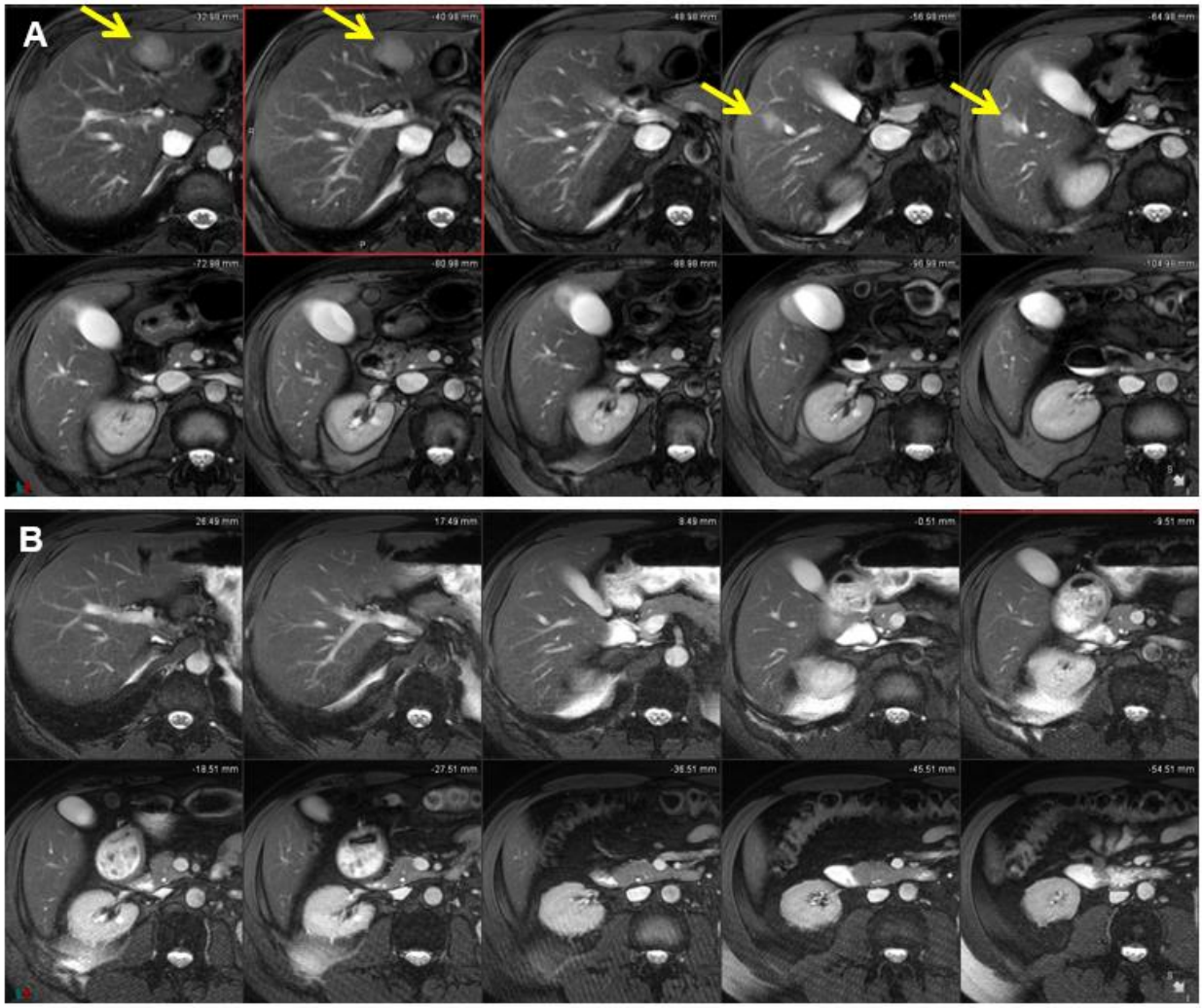
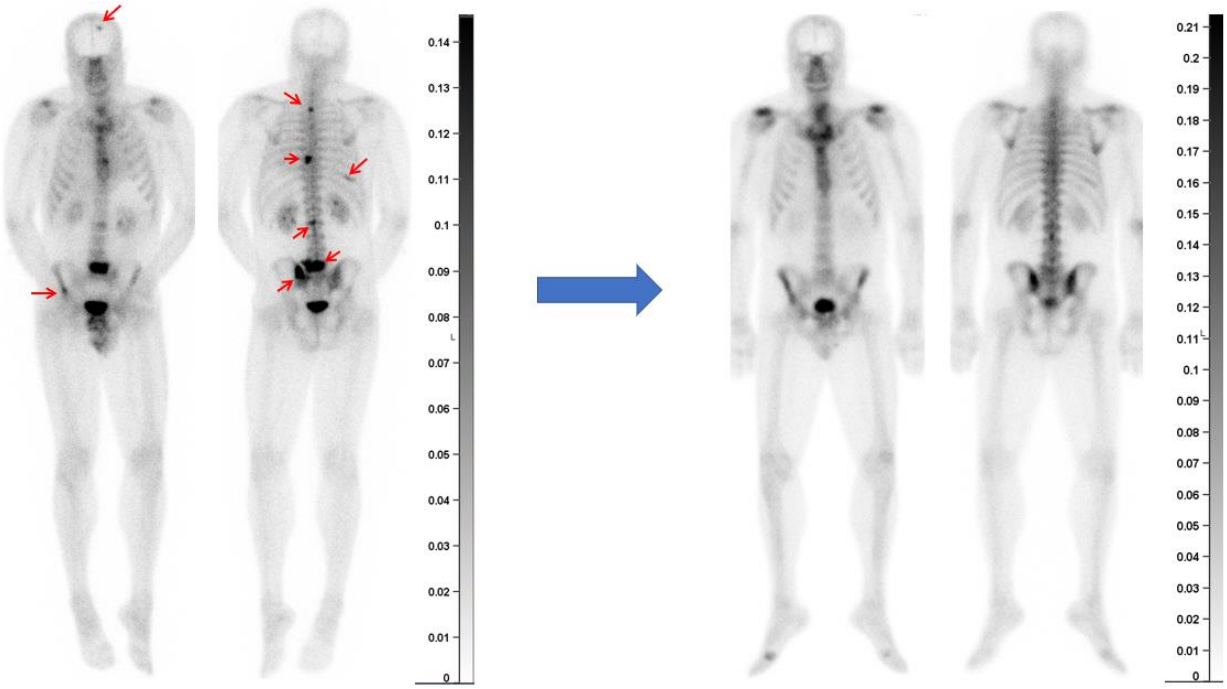


FIGURE 3. Bone scans of subject MAD4-02 before (left) and after (right) treatment with 4 cycles of ^{212}Pb-DOTAMTATE. Most of the lesions seen on the initial baseline bone scan (red arrows on left image) are completely healed in the bone scan after treatment (right).



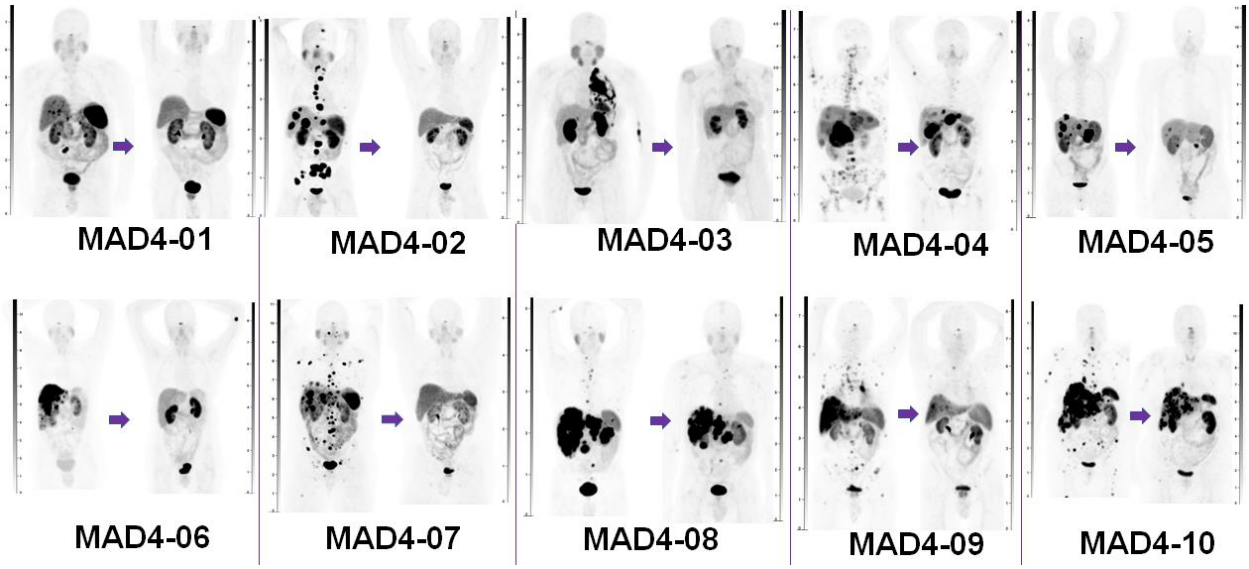


FIGURE 4. Volume rendered images of ^{68}Ga-DOTATATE PET/CT scans from the first 10 subjects enrolled in cohort 4 (MAD4), before (image on the left) and after treatment (image on the right) with 4 cycles of ^{212}Pb-DOTAMTATE at a dose of 2.50 MBq/kg (67.6 μCi/kg), for each cycle.

TABLES

TABLE 1 SAD and MAD Cohorts Dose Escalation per Cycle

Cohort	Dose per cycle, MBq/kg* (µCi/kg)
1	1.13 (30.7)
2	1.48 (40.0)
3	1.92 (52.0)
4	2.50 (67.6)

*+/-10%.

MAD = multiple ascending dose; SAD = single ascending dose.

TABLE 2 Patient Characteristics Including Relevant Clinical Trial Data

Patient*	Age	Sex	Type of NET	Grade	Ki-67	Stage	Time Gap (years)	No. of cycles	Total dose MBq (mCi)	RECIST 1.1 response†	^{68}Ga PET response‡	Duration of response (m)§
SAD1-01	75	M	Small bowel	G2	4	IV	6.4	1	81 (2.2)	SD	N/A	NP
SAD1-02	76	F	PNET	G2	N/A	IV	8.8	1	85 (2.3)	SD	N/A	NP
SAD1-03	77	M	PNET	G3	27	IV	4.5	1	85 (2.3)	SD	N/A	NP
SAD2-01	56	M	Rectal	G2	N/A	IV	5.2	1	122 (3.3)	SD	N/A	NP
SAD2-02	27	F	Small bowel	G1	N/A	IV	4.7	1	100 (2.7)	SD	N/A	NP
SAD2-03	72	F	Small bowel	G1	2	IV	6.1	1	115 (3.2)	SD	N/A	NP
MAD3-01	61	F	Small bowel	G2	6	IV	10.7	3	574 (15.5)	SD	NSC	0
MAD3-02	62	F	PNET	G2	3	IV	7.2	2‖	329 (8.9)	SD	NSC	0
MAD3-03	68	F	Small bowel	N/A	N/A	IV	10.7	3	266 (7.2)	SD	NSC	0
MAD3-04	51	M	PNET	N/A	N/A	IV	5.6	3	455 (12.3)	SD	(-40%)	0
MAD4-01	62	M	Small bowel	G3	22	IV	2.2	4	814 (22.0)	PR	(-95%)	22
MAD4-02	45	M	Bronchial carcinoid	G1	>20	IV	6.2	4	796 (21.5)	PR	(-100%)	22
MAD4-03	71	F	Bronchial carcinoid	G2	15	III	4.8	4	707 (19.8)	CR	(-100%)	20
MAD4-04	39	F	Rectal	G3	30	IV	5.1	4	807 (21.8)	SD	(-40%)	0
MAD4-05	62	M	PNET	G1	2	IV	7.3	4	873 (23.6)	PR	(-80%)	8
MAD4-06	49	F	PNET	G2	19	IV	2.9	4	681 (18.4)	PR	(-100%)	14
MAD4-07	45	M	Rectal	G2	12	IV	5.7	4	858 (23.2)	PR	(-95%)	5
MAD4-08	60	M	Small bowel	G2	5	IV	0.3	4	692(18.7)	SD	(-15%)	0
MAD4-09	80	M	Bronchial carcinoid	G2	10	IV	1.1	4	836 (22.6)	PR	(-60%)	1
MAD4-10	59	F	Bronchial carcinoid	G2	5	IV	1.8	4	847 (22.9)	PR	(-30%)	5

*The MAD3 cohort started as a SAD3 cohort, with a single injection with 1.92 MBq/kg (52.0 μCi/kg). When QOL improvements were reported after the first cycle, approval was obtained from the disease safety monitoring board and these subjects were transitioned to a MAD cohort with 2 additional cycles at the same dose administered at 8-week intervals.

†For SAD cohort subjects, response stablished 2 months after the injection, before starting other therapies.

‡The percentage decreased in overall tumor burden detected on the ^{68}Ga-DOTATATE PET/CT is “visually” estimated.

[§]As of June 2021.

[‖]Subject dropped out of the study after the second cycle.

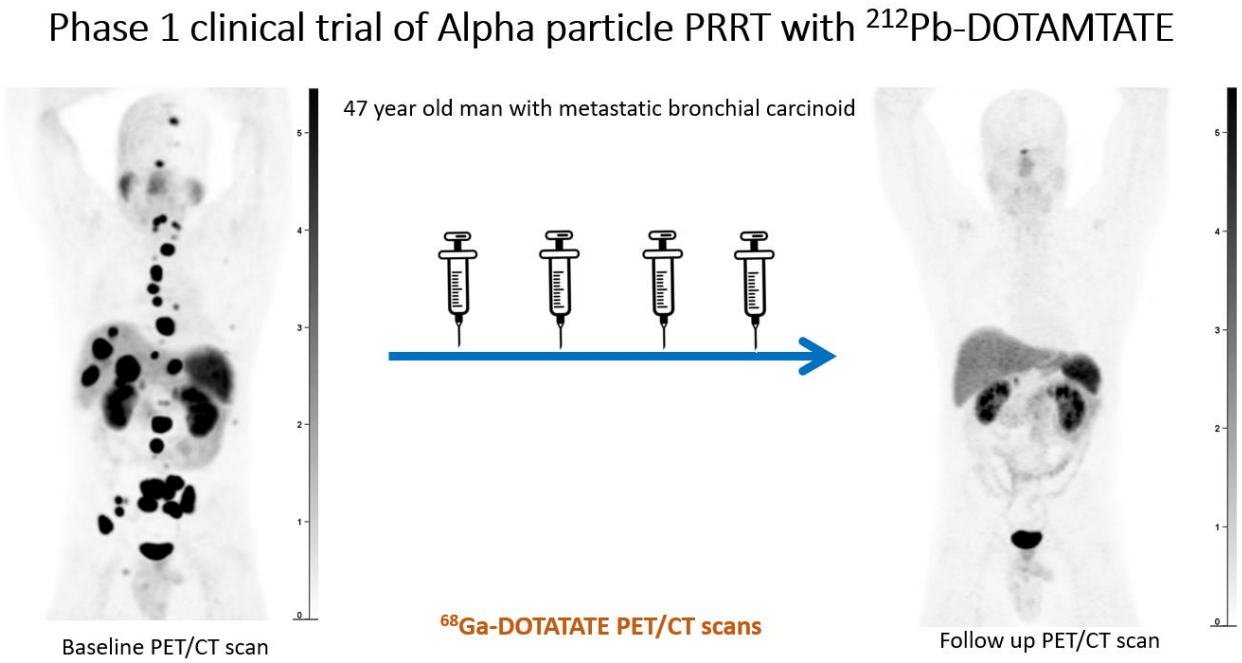
CR = complete response; F = female; G = grade (as per 2017 WHO Classification: G1=<3; G2=3-20, G3=>20 Ki-67 Index); LG = low grade; M = male; MAD = multiple ascending dose; N/A = not available; NET = neuroendocrine tumor; NP = not applicable; NSC = no significant change; PNET = pancreatic neuroendocrine tumor; PR = partial response; QOL = quality of life; RECIST 1.1 = Response Evaluation Criteria in Solid Tumors 1.1; SAD = single ascending dose; SD = stable disease; Time Gap = Time difference in years between the original histophatologic diagnosis and the first treatment cycle with ^{212}Pb-DOTAMTATE.

TABLE 3 All Serious Adverse Events

Subject	Event preferred term	Causality	Grade	Outcome
SAD1-01	Disease progression	Not related	G5	Fatal
SAD2-02	Pain	Not related	G2	Recovered
SAD2-02	Cancer pain	Not related	G2	Recovered
SAD2-02	Dehydration	Not related	G2	Recovered
SAD2-02	Disease progression	Not related	G5	Fatal
SAD2-02	Low GFR	Not related	G3	Recovered
MAD3-01	Acute kidney injury	Unlikely	G2	Recovered
MAD3-01	Renal failure	Unlikely	G3	Not recovered
MAD3-04	Abdominal pain	Unlikely	G3	Recovering
MAD4-01	Worsening achalasia	Not related	G3	Recovered
MAD4-03	Dyspnea	Unlikely	G3	Recovered
MAD4-03	Fatigue	Unlikely	G2	Resolved
MAD4-03	Hypoglycemia	Unlikely	G2	Resolved
MAD4-03	Cerebrovascular accident	Unlikely	G2	Resolved
MAD4-03	Chronic kidney disease	Unlikely	G2	Not recovered

G = grade; GFR =glomerular filtration rate; MAD = multiple ascending dose; SAD = single ascending dose.

GRAPHICAL ABSTRACT



Poster presented at ASCO 2022

Results on subjects who Progressed

Following Prior ^{177}Lu/^{90}Y-PRRT

Targeted Alpha-Emitter Therapy with ^{212}Pb-DOTAMTATE in Neuroendocrine Tumor Subjects who Progressed Following Prior ^{177}Lu/^{90}Y-PRRT (Abstract 382731)

Ebrahim Delpassand, MD[1]; Izabela Tworowska, PhD[1]; Rouzbeh Esfandiari, MD[2], Julien Torgue, PhD[3]; Jason Hurt, MD[3]; Rodolfo Nuñez, MD[2]
[1]RadioMedix, [2]Excel Diagnostics and Nuclear Oncology, [3]OranoMed, LLC

Background

- Targeted Alpha Therapy (TAT) with ^{212}Pb-DOTAMTATE has been shown to be safe and effective in subjects with neuroendocrine tumors (NET) who have not received previous PRRT[1], however, data is lacking for the use of TAT once progression occurs.

Study Design and Methods

- Subjects with biopsy-proven unresectable or metastatic SSTR expressing NETs from different primary sites with at least one measurable lesion who progressed after receiving prior PRRT received up to four 8-week cycles of ^{212}Pb-DOTAMTATE at 67.6 µCi/kg/cycle.
- Response to treatment was measured per RECIST 1.1 and ^{68}Ga/^{64}Cu-DOTATATE PET/CT.
- Safety parameters were also obtained.

Table 1: Subject Demographics

Subject	Age	Sex	Tumor Type	Grade	Previous RTx	Previous* ChemoTx	Total Activity† (mCi)
01	79	M	Sm. Bowel	G1	^{177}Lu	Som/Ever	20.0
02	65	M	Thymus	n/a	^{177}Lu	Som/Carbo/ CapTem/ Lomustine	23.3
03	70	M	Pulmonary	G3	^{177}Lu	Som/Ever	22.6
04	64	F	Pancreatic	G2	^{177}Lu	Som	22.7
05	56	F	Sm. Bowel	G2	^{177}Lu	Som	22.4
06	70	F	Pancreatic	G3	^{177}Lu	Som/CapTem/Ever Sut/5-Fu/Cabo/ FOLFOX	15.4
07	69	M	Sm. Bowel	n/a	^{177}Lu/^{90}Y	Som/Ever	23.1
08	61	M	Sm. Bowel	G2	^{177}Lu	Som/Ever/CapTem	5.8
09	53	M	Sm. Bowel	G1	^{177}Lu	Som	17.3
10	65	M	Pancreatic	n/a	^{177}Lu/^{90}Y	Som/Ever	15.8
11	35	M	Pancreatic	n/a	^{177}Lu	Som	23.2

*Som-somatostatin analogue; Ever-everolimus; CapTem- Capecitabine/Temolzolomide; Cabo-cabozantinib; Sut-sunitinib; Carbo-carboplatin; 5Fu-5-fluorouracil; FOLFOX: Leucovorin, 5FU, Oxaliplatin

† Total activity of ^{212}Pb-DOTAMTATE administered

Results

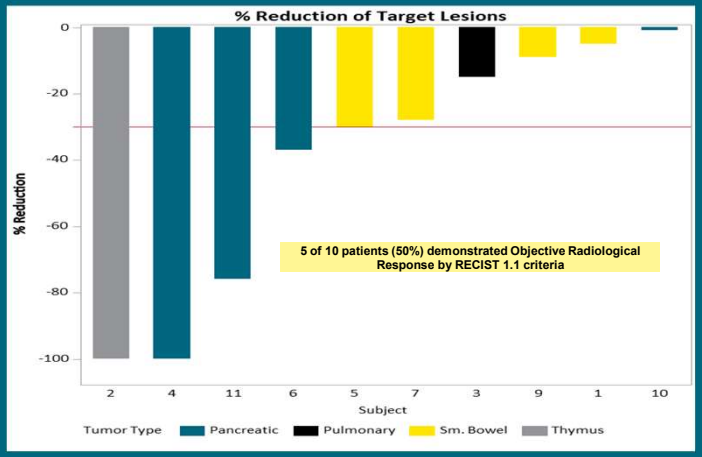


Figure 1: % reduction in the SOM of target lesions.

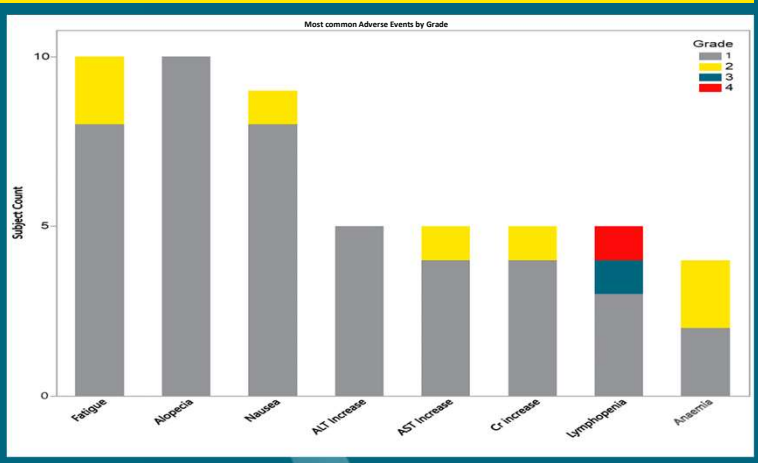


Figure 2: Most common adverse events reported by > 4 subjects

Figure 3: PET/CT Imaging After 4 Cycles of ^{212}Pb-DOTAMTATE (before and after treatment)

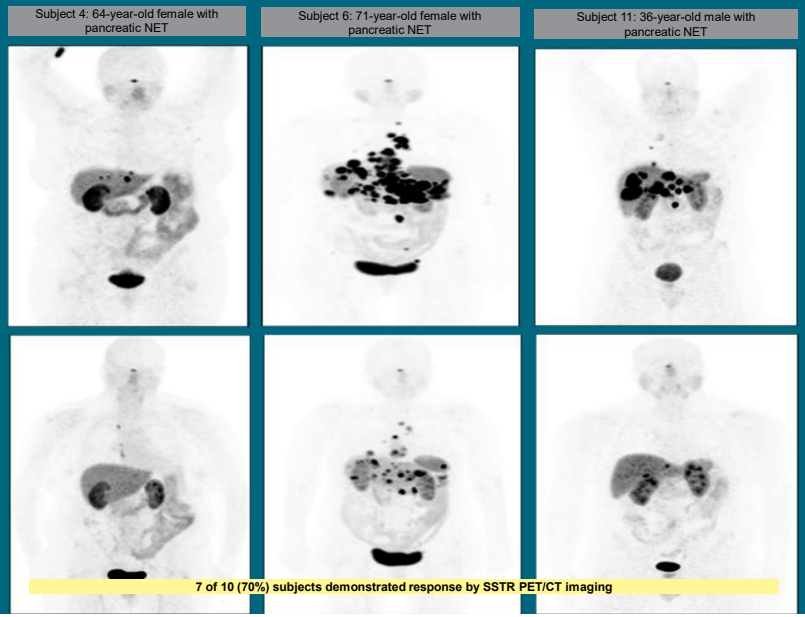
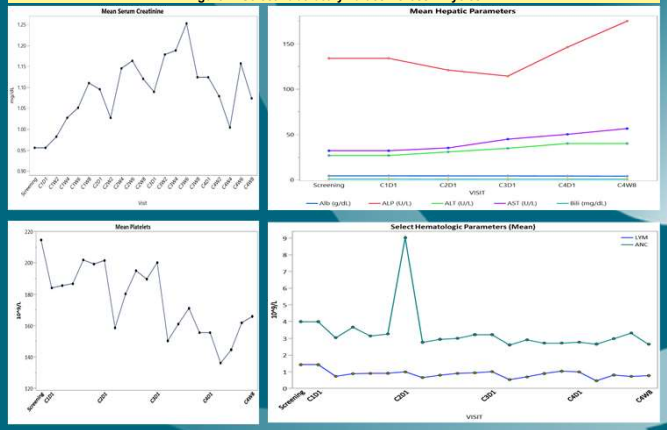


Figure 4: Select Laboratory Values Across 4 Cycles



Conclusions

- This is the first clinical trial of TAT with ^{212}Pb-DOTAMTATE in subjects with NETs who progressed following prior PRRT.
- The use of ^{212}Pb-DOTAMTATE in this setting is highly effective with manageable toxicity and warrants further investigation

Contact information: edelpassand@radiomedix.com

4128 **Poster Session**

Targeted alpha-emitter therapy with ^{212}Pb-DOTAMTATE in neuroendocrine tumor subjects who progressed following prior ^{177}Lu/^{90}Y-PRRT.

Ebrahim Delpassand, Rouzbeh Esfandiari, Izabela Tworowska, Julien Torgue, Jason Daniel Hurt, Rodolfo Nunez; Excel Diagnostics and Nuclear Oncology Center, Houston, TX; Radiomedix Inc, Houston; Orano Med, Plano, TX

Background: Targeted Alpha-Emitter Therapy (TAT) with ^{212}Pb-DOTAMTATE has been shown to be safe and effective in subjects with neuroendocrine tumors (NET) who have not received previous PRRT, however, data is lacking for the use of TAT once progression occurs. Herein, we present the safety and efficacy of ^{212}Pb-DOTAMTATE in subjects with recurrent NETs following prior ^{177}Lu/^{90}Y-PRRT. **Methods:** Subjects with biopsy-proven unresectable or metastatic SSTR expressing NETs from different primary sites with at least one measurable lesion who had received progressed after receiving prior PRRT were enrolled and received up to four 8-week cycles of ^{212}Pb-DOTAMTATE at 67.6 μCi/kg/cycle. Response to treatment was measured per RECIST 1.1 criteria and by ^{68}Ga/^{64}Cu-DOTATATE PET/CT. Safety parameters were also obtained. **Results:** A total of 11 PRRT subjects were enrolled regardless of primary tumor location (pancreas (4), small bowel (3), midgut (1), ileum (1), thymus (1), and lung (1)). 8/11 subjects (73%) completed all four cycles. The mean cumulative dose was 20.9 mCi. As of January 2022, an objective radiological response (ORR) was demonstrated in 30% of evaluable subjects (1CR, 2PR, and 7 SD). In addition, 70% (7/10) evaluable subjects demonstrated a response per ^{68}Ga/^{64}Cu-DOTATATE PET/CT SSTR imaging. One hundred forty-five AEs were reported, with Grade 1 (79%). There were 23 (16%) Grade 2 AEs and 5 (3%) Grade 3/ 4 AEs. Of the AEs reported, 84 (58%) were considered possibly related, and 61 (42%) were considered not related or unlikely related. The most frequent AEs (reported in $\geq$ 4 subjects) include: alopecia (100%), fatigue (100%), nausea (91%), anemia (36%), alanine aminotransferase increased (36%), aspartate aminotransferase increased (36%) and lymphopenia (46%). Three SAEs were reported (achalasia, asthma exacerbation, and septic shock) one of which resulted in the death of the subject (septic shock). No SAEs were considered related to study drug. **Conclusions:** This is the first clinical trial of ^{212}Pb-targeted alpha-emitter therapy in subjects with NETs who progressed following prior PRRT. The use of ^{212}Pb-DOTAMTATE in the recurrent setting is highly effective with manageable toxicity and warrants further investigation. Clinical trial information: NCT03466216. Research Sponsor: NCI SBIR direct to Phase II 1R44CA265421, Pharmaceutical/Biotech Company.

End of enrolment in phase II clinical trial

Press release

Press Release

RadioMedix and Orano Med Complete Patient Enrolment in Phase II Trial of Targeted Alpha-Emitter AlphaMedix in Neuroendocrine Cancers

Trial's Objective Response Rate Endpoint Already Achieved

Houston, TX, USA and Paris, France – May 16, 2023 – RadioMedix and Orano Med, two clinical stage radiopharmaceutical companies, today announced that the last patient has been dosed in the Phase II trial of the targeted alpha emitter therapy, ^{212}Pb-DOTAMTATE (AlphaMedix™). This trial is being conducted to evaluate the safety and effectiveness of AlphaMedix™ in peptide receptor radionuclide therapy (PRRT) of naive patients with somatostatin receptor-expressing neuroendocrine tumors (NET), regardless of the location of the primary tumor. Top-line data from the trial is expected in mid-2024. Remarkably, based on data already collected, the objective response rate (ORR) endpoint has already been achieved and is more than twice as high as the current standard of care.

"The completion of the Phase II trial enrolment is a significant milestone in the clinical development of our innovative targeted alpha-emitter radiotherapy, AlphaMedix™, and brings us one step closer to having this drug available to patients," said Ebrahim Delpassand, MD, Chairman and Chief Executive Officer of RadioMedix. "Previous studies have shown targeted alpha therapy (TAT) with AlphaMedix™ is well-tolerated. The preliminary efficacy data seen to date are very promising, particularly achieving the planned ORR endpoint. As the trial progresses, we believe the ORR could improve further. We look forward to reporting data on the study in 2024, which we believe will show that AlphaMedix™ will provide substantial benefit over currently FDA approved therapies for patients with metastatic or inoperable SSTR-expressing NETs."

This Phase II trial is a multi-center, single arm, non-randomized, open-label basket trial. Forty-one patients with histologically confirmed NETs and positive somatostatin analogue imaging who have not received prior PRRT have been enrolled across four sites in the United States. Treatment consists of four cycles of AlphaMedix™ at 8-week intervals. The primary endpoint of the trial is safety and effectiveness of AlphaMedix™. Efficacy endpoints include objective response rate (ORR) using RECIST v1.1 criteria, progression-free survival (PFS), and overall survival (OS). Additional information about the trial can be found on clinicaltrials.gov: NCT 05153772.

Julien Dodet, President, and Chief Executive Officer of Orano Med, noted: "Completing this Phase II trial enrolment on schedule is a great achievement everyone involved and confirms the strong interest of the medical community for targeted alphatherapies with lead-212. We are convinced that targeted alphatherapies, such as AlphaMedix™, are the future of radiopharmaceutical therapies, providing an increased cytotoxic potential against cancer cells with limited toxicity to surrounding healthy cells. This reinforces Orano Med's commitment to make innovative lead-212-based therapies available to the medical community and patients worldwide."

About Targeted Alpha Therapy

Targeted alpha therapy (TAT) relies on a simple concept: combining the ability of biological molecules to target cancer cells with the short-range cell-killing capabilities of alpha-emitting radioisotopes. Alpha decay consists of the emission of a helium nucleus (alpha particle) together with very high linear energy transfer and a range emission of only few cell layers, resulting in irreparable double strand DNA breaks in cells adjacent only to area of alpha emission. This approach results in an increased cytotoxic potential toward cancer cells while limiting toxicity to nearby healthy cells. As a result, alpha emitters are considered as the most powerful payloads to be found for targeted therapies.

About AlphaMedix™

AlphaMedix™ is a radiolabeled SSTR-targeting therapeutic investigational drug for the treatment of NETs patients. The product consists of SSTR-targeting peptide complex radiolabeled with ^{212}Pb that serves as an in vivo generator of alpha-emitting particles. ^{212}Pb isotope is particularly suitable for SSTR therapy applications based upon its half-life, energy, and decay properties.

About neuroendocrine tumors

Neuroendocrine tumors (NETs) are a heterogeneous group of rare neoplasms that originate from neuroendocrine cells. These neoplasms occur mostly in the gastrointestinal tract and pancreas but can also occur in other tissues including the thymus, lung, and other uncommon sites such as ovaries, heart, and prostate. Most NETs strongly express somatostatin receptors (SSTRs). In the United States, around 12,000 patients are expected to be diagnosed with neuroendocrine tumors, with an average 5-year survival rate of 60% at a metastatic stage.

About RadioMedix

RadioMedix, Inc. is a clinical-stage biotechnology company, and sponsor of the AlphaMedix™ trial, based in Houston and Humble, Texas. The company is focused on innovative targeted radiopharmaceuticals for diagnosis, monitoring, and therapy of cancer. RadioMedix is developing radiopharmaceuticals for PET imaging and therapy (alpha- and beta-labeled agents). The company established contract service facilities for academic and industrial partners including a drug discovery center for the early probe development, a pre-clinical core facility for in vitro and in vivo evaluation of radiopharmaceuticals, and cGMP and analytical suite for Phase I-III clinical trials, and the large-scale post-approval commercial manufacturing facility, the Spica Center.

More information about RadioMedix, visit: www.radiomedix.com.

About Orano Med

Orano Med is a clinical-stage biotechnology company which develops a new generation of targeted therapies against cancer using the unique properties of lead-212 (^{212}Pb), a rare alpha-emitting radioisotope and one of the more potent therapeutic payloads against cancer cells known as Targeted Alpha-Emitter Therapy (TAT). The company develops several treatments using ^{212}Pb combined with various targeting agents. Orano Med has ^{212}Pb manufacturing facilities, laboratories, and R&D centers in France and in the US and is currently investing to further expand its GMP-manufacturing capacities for ^{212}Pb radiolabeled pharmaceuticals in North America and Europe.

More information about Orano Med, visit: www.oranomed.com.

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Dosimetry and biodistribution – Clinical trial results

"Dosimetry and biodistribution of ^{203}Pb-AR-RMX in patients with somatostatin expressing neuroendocrine tumors"

E. S. Delpassand, T. A. Stallons, A. Hamidi, L. Bolek, M. Ali, A.Shafie, G. Vahdati, A. Saidi, F. Rojas-Quijano, P. Jurek, G. Kiefer, B. He, M. Ghaly, E. Frey, G. Sgouros, J. Torgue, I. Tworowska

10th International Symposium on Targeted Alpha Therapy 2017

RadioMedix INNOVATING THERANOSTICS

AREVA Med

Macrocyclics™ An AREVA Med Company

Targeted α-emitter therapy of neuroendocrine tumors (NETs)

Target disease SSTR-(+) neuroendocrine tumors

Current treatment option available No approved agents available for PRRT of NETs

Lu^{177}-DOTATATE Luthera (AAA)- expected approval 2017

Lu^{177-} OPS201 (SSTR-antagonist, OctreoPharm, Ipsen) clinical studies

β-emitter PRRT of NETs

- Multiple clinical studies (RadioMedix/Excel Diagnostic Clinic)
- mPFS of 18.2 and 33.0 months for ^{90}Y-DOTATATE and ^{177}Lu-DOTATATE; NETTER-1 Phase III clinical: the response rate was 18% in the ^{177}Lu- DOTATATE group versus 3% in the control group
- Hematologic/renal toxicity of PRRT reduced by dose fractionation.
- Complete response to beta-emitter PRRT rare.
- Reasons: heterogeneity of neuroendocrine tumors; advanced stage of disease at the time of diagnosis; resistance to nonradioactive octreotide and ^{90}Y/^{177}Lu PRRT during PRRT

α-emitter PRRT of NETs

- No approved agent available
- ^{213}Bi-DOTATOC; ^{225}Ac-DOTATOC - clinical studies
- Increased of FSR of NETs patient to 36 months (compared to 24 months during beta-emitter PRRT)
- TAT : potential to overcome patient resistance to β−PRRT (targets double stranded DNA)

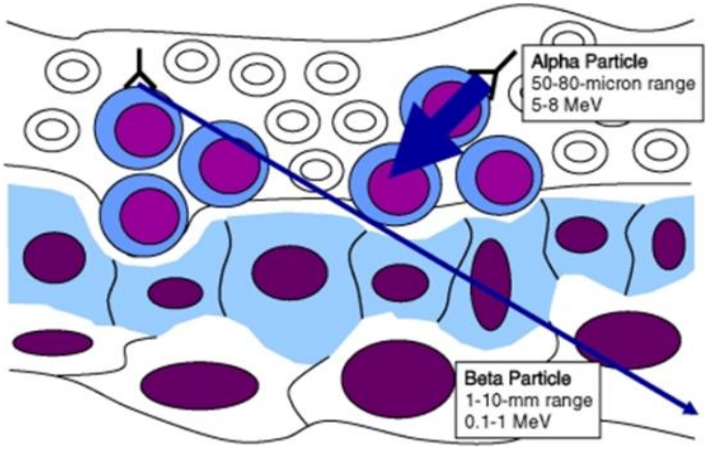
Physicochemical characteristics of β-emitters and α-emitters

β-emitters

- Intermediate energy (0.50–2.30 MeV) ; long range in tissues (1–12 mm of tissue penetration).
- β-particles range: target clusters of cells (from 10 to 1,000 cells)

α-emitters

- High-LET radiation (60–230 keV/μm)
- Short to intermediate path length (^{212}Pb: 50–80 μm) in tissues
- Path length: target several cells (2–10 cells)
- Irreversible damage of double stranded DNA



Nuclide	$T_{1/2}$	Emission	Mean path length
I-125	60.0d	auger	10nm
At-211	7.2h	alpha	65nm
Pb-212	10.6h	alpha	50-80 um (0.05-0.08mm)
Lu-177	6.7d	beta/gamma	0.7mm
Cu-67	2.58d	beta/gamma	0.7mm
I-131	8.04d	beta/gamma	0.9mm
Sm-153	1.95d	beta/gamma	1.2mm
Re-186	3.8d	beta/gamma	1.8mm
P-32	14.3d	beta	2.9mm
Re-188	17h	beta/gamma	3.5mm
In-114m	50d	beta/gamma	3.6mm
Y-90	2.67d	beta	3.9mm

Pb^{203}/Pb^{212} matched pair of isotopes

Pb^{203}/Pb^{212} for image guided targeted therapy

Selection of patients – Dose optimization

AREVA Med

Matched Pair Isotopes

Imaging

$^{203}Pb - t_{1/2} = 51$ h

SPECT

Therapy

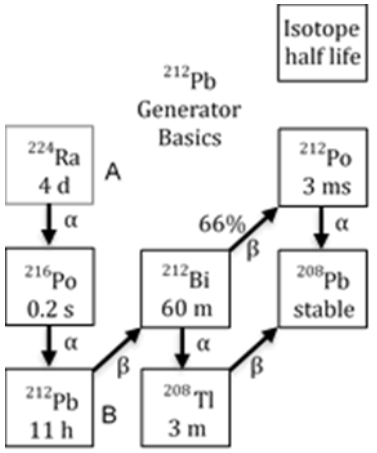
$^{212}Pb - t_{1/2} = 10$ h

Alpha Particle Therapy

^{212}Pb and ^{203}Pb behave precisely the same biochemically.

^{203}Pb - very simple decay scheme ending at a ground state of stable ^{203}Tl

Isotope	^{203}Pb
Mass	202.97
Half-life*	51.88 hour
Binding Energy	1,602.27 MeV
γ Energy*	279keV (80.6%), 401keV (3.47%), 681keV (0.7%),
Decay mode	Electron capture



^{203}Pb-AR-RMX (Poster- Session I)

"Preclinical studies-^{203}Pb-AR-RMX conjugates for image guided TAT of neuroendocrine tumors (NETs)"

Phase I Exploratory study of ^{203}Pb-AR-RMX (IND # 130960)

"Dosimetry and Bio-distribution of ^{203}Pb –AR-RMX in Patients with Somatostatin Expressing Neuroendocrine Tumors. A Phase I Exploratory Study."

Sponsor: RadioMedix Inc.

Study site: Excel Diagnostics and Nuclear Oncology Center

Collaborator : AREVA Med LLC.

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Phase I Exploratory study of ^{203}Pb-AR-RMX (IND # 130960)

Open-label, single-dose, diagnostic study using ^{203}Pb-AR-RMX with intra-subject comparison of the biodistribution with ^{68}Ga-DOTATATE PET/CT or OctreoScan™.

Indication

Diagnosis of primary and metastatic somatostatin receptor-positive neuroendocrine tumors

Total number of patient:

6 subjects with histologically and/or clinically confirmed NET and prior somatostatin analogue scans (positive or negative)

Primary Objective:

To assess the dosimetry and biodistribution of ^{203}Pb-DOTAMTATE in patients with somatostatin expressing neuroendocrine cancers as a surrogate for ^{212}Pb-AR-RMX, an investigational radiopharmaceutical for TAT of NETs.

Secondary Objectives:

To compare distribution of currently available somatostatin receptor imaging agents such as Octreoscan or ^{68}Ga-SSTR PET/CT with distribution of ^{203}Pb-AR-RMX.

Safety assessment:

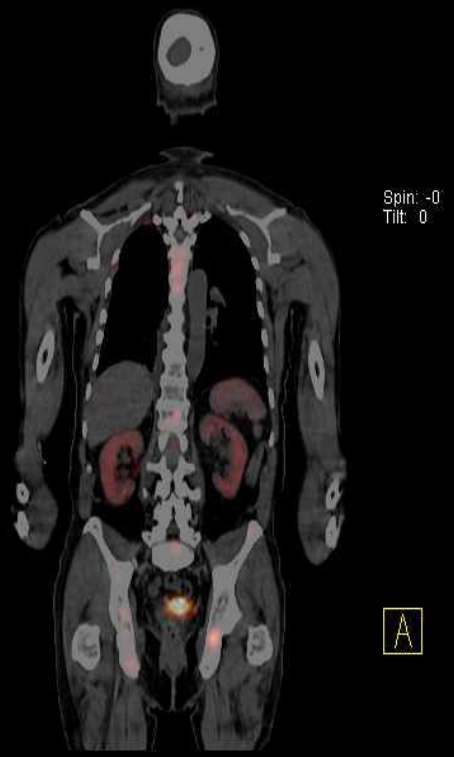
Assessment of dosimetry, clinical chemistries and haematology.

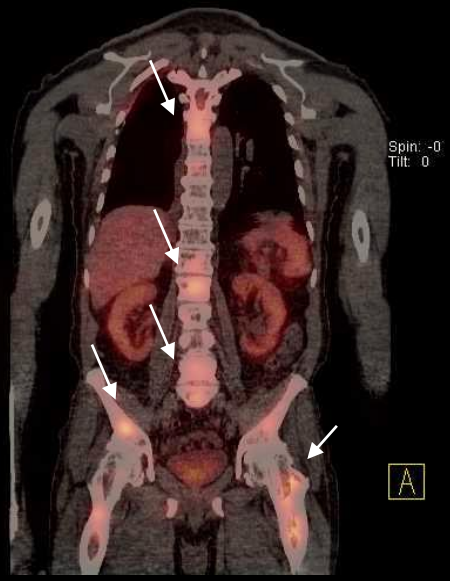
Measurement of vital signs at multiple time points after administration of ^{203}Pb-AR-RMX.

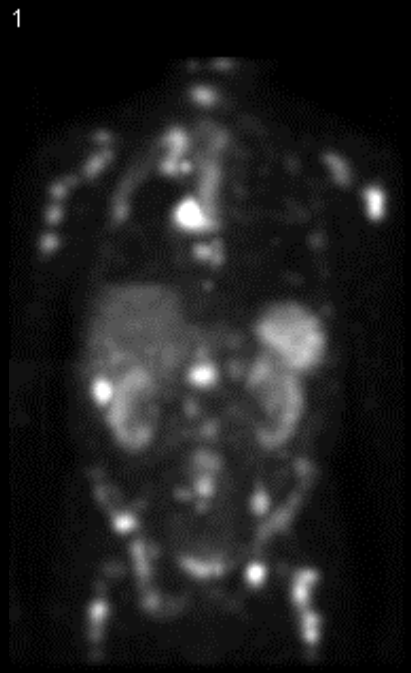
^{68}Ga-DOTATATE PET/CT
PT-005

^{203}Pb-AR-RMX SPECT/CT 4h (coronal)

^{203}Pb-AR-RMX SPECT







All patients (1 female and 5 male) received an average dose of 4.94 mCi of ^{203}Pb-AR-RMX SPECT-CT scans acquired at 1 h, 4 h, 24 h and 48 h post injection

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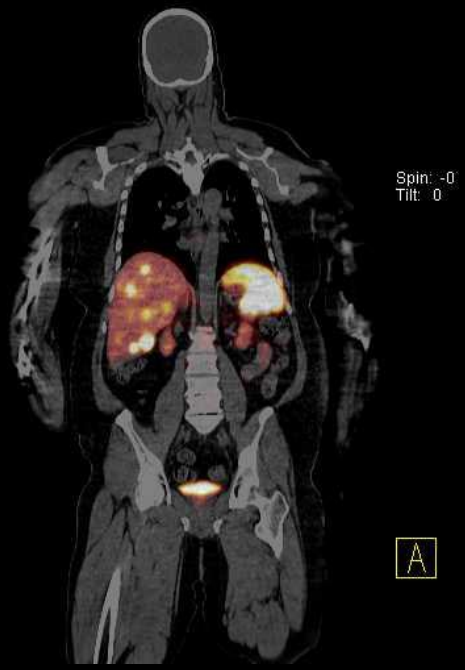
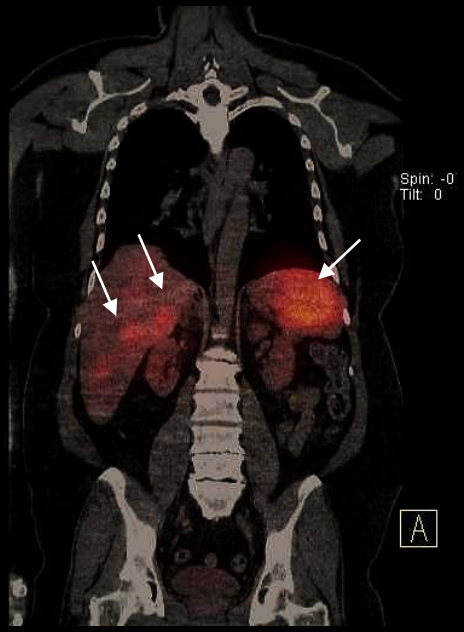
^{68}Ga-DOTATATE PET/CT
PT-006

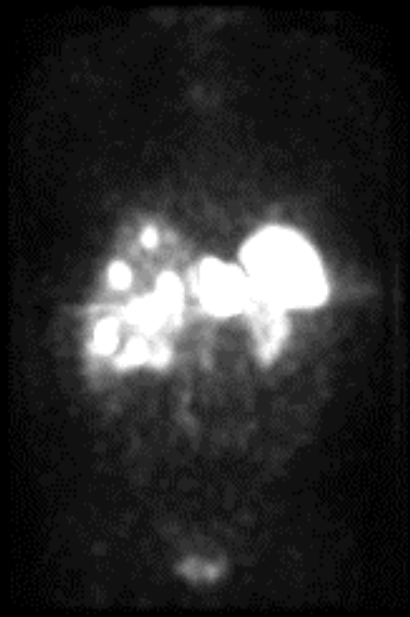
^{203}Pb-AR-RMX SPECT/CT 4h (coronal)

^{203}Pb-AR-RMX SPECT

1







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Safety evaluation

- All patients were evaluated and followed up for any evidence of renal, hepatic or hematologic toxicity using NCI common toxicities criteria Version 4.1 up to a month after the radiopharmaceutical injection.
- No significant acute toxicity was observed immediately following scan; no patients required supportive treatment during or after the scans.
- Clinically non-significant transient lymphocytopenia recorded in 5 of 6 patients 24 or 48 hr p.i.; no intervention was required; CBC returned to the baseline.

Comparison of ^{68}Ga -DOTATATE PET/ CT and ^{203}Pb- AR-RMX SPECT/CT scans

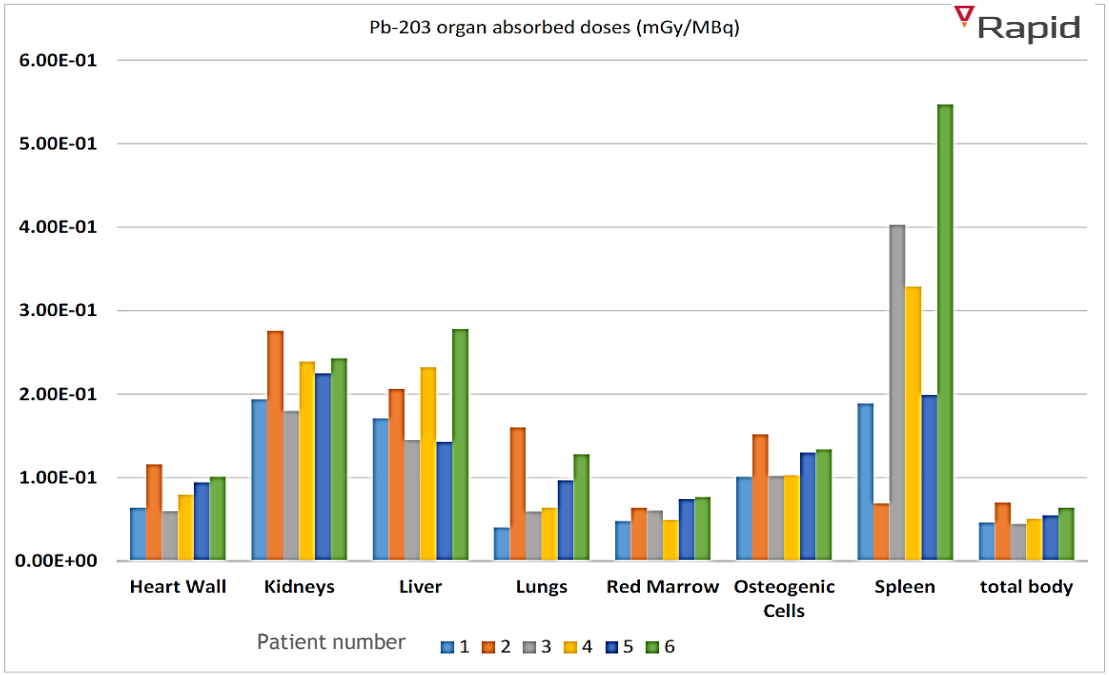
- Independent reads by two NP blinded to the results of the other study.
- ^{68}Ga-DOTATATE PET/CT scan: total number of 177 lesions detected in 6 patients.
- ^{203}Pb-Ar-RMX SPECT/CT : 109 lesions.
- Very close correlation (with correlation coefficient of 0.89) between lesions detected by these two modalities.

Comparison of ^{68}Ga –DOTATATE PET/ CT and ^{203}Pb- AR-RMX SPECT/CT scans

		^{68}Ga-DOTATATE	^{203}Pb-AR-RMX
Visceral			
	Head/Neck	0	0
	Chest (lungs, breast, heart)	8	5
	Gastrointestinal tract	0	0
	Liver	27	27
	Pancreas	2	2
	Other (kidney, spleen)	0	0
	Pelvis (below pelvic arch)	5	4
TOTAL/ORGAN		42	38
Nodal			
	Head/Neck	1	1
	Chest (including axilla)	9	9
	Abdomen and Pelvis	2	3
	Inguinal	0	0
TOTAL/ORGAN		12	13
SKELETON			
	Head/Neck	9	6
	Trunk (vertebrae, bony thorax, bony pelvis)	95	34
	Upper Extremities	7	7
	Lower Extremities	12	11
TOTAL/ORGAN		123	58
TOTAL /SCAN		177	109

^{68}Ga DOTATATE is more sensitive to detect bone lesions in the axial skeleton (total of 95) as compared to ^{203}Pb-AR-RMX (total of 34).

Dosimetry analysis of organ/tissues absorbed dose of ^{203}Pb-AR-RMX



The expected tissue absorbed of ^{212}Pb-AR-RMX

- The spleen - the highest absorbed dose (not dose-limiting organ);
- The kidneys and liver – the absorbed dose averaging 19 and 17 mGy/MBq;

EBR absorbed dose limits

- The whole kidney volume – limit 18-23 Gy absorbed dose - 5% risk of kidney injury in 5 years;
- The liver- limit 27-30 Gy absorbed dose (twice daily fractions, 1.5 Gy per fraction);
- The toxicity limit is not well established

SUMMARY

- ✓ No clinically significant acute toxicity observed after ^{203}Pb-AR-RMX; no patients required supportive treatment during or after the scans.
- ✓ No statistically significantly difference observed between the ^{68}Ga-DOTATATE PET/CT and ^{203}Pb-AR-RMX SPECT/CT
- ✓ ^{68}Ga DOTATATE PET/CT can be used to evaluate the eligibility of patients for ^{212}Pb-AR-RMX TAT.
- ✓ The clinical studies of ^{212}Pb-AR-RMX scheduled in Q4 of 2017.
- ✓ Intellectual Property: AR-RMX composition, their formulations and method of use for theranostics applications
- ✓ NIH/NCI SBIR Contract Grant Phase I (2016): PI: I. Tworowska (RadioMedix); J. Torgue (AREVA Med)

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Please visit our poster (Poster Session I):

^{203}Pb-AR-RMX conjugates for image guided TAT of neuroendocrine tumors (NETs)

I. Tworowska, T. A. Stallons, A. Saidi, N. Wagh, F. Rojas-Quijano, P. Jurek, G. E. Kiefer, J. Torgue, E. Delpassand

Thank you!

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Preclinical results

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Preclinical investigation of ^{212}Pb-DOTAMTATE for peptide receptor radionuclide therapy in a neuroendocrine tumor model

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Abstract

Somatostatin analogs have been examined as a treatment for somatostatin receptor overexpressing tumors for years; specifically, octreotate (TATE) and octreotide (TOC). Several versions of these analogs coupled to beta or gamma nuclides are currently used as imaging agents, as treatments with peptide receptor radionuclide therapy (PRRT) for patients with neuroendocrine tumors or are being explored in preclinical and clinical settings. Our study describes the use of ^{212}Pb-DOTAMTATE, the octreotate analog, in combination with ^{212}Pb, the parent of an alpha emitter. Preclinical studies demonstrated tumor targeting of ^{212}Pb-DOTAMTATE of >20% ID/g up to 24hrs post drug injection. The addition of kidney protection agents, including L-lysine and L-arginine decreases drug accumulation in the kidneys and the addition of ascorbic acid to the chelation mixture reduces oxidation of the drug product. ^{212}Pb-DOTAMTATE displays a favorable toxicity profile with single dose injections of 20μCi showing 100% survival and with non-toxic cumulative doses up to 45μCi, when fractionated into three smaller doses of 15μCi. In an initial efficacy study, a single 10μCi of ^{212}Pb-DOTAMTATE extended the mean survival 2.4-fold. Efficacy was enhanced by giving three treatment cycles of ^{212}Pb-DOTAMTATE and reducing the time between injections to two weeks. Efficacy was optimized further by the addition of a chemo sensitizing agent, 5-fluorouracil, given in combination with three cycles of 10μCi ^{212}Pb-DOTAMTATE. These conditions led to 79% of the animals being tumor free at the end of the 31-week study suggesting that ^{212}Pb-DOTAMTATE alone or in combination with a chemotherapeutic may have positive clinical implications.

Keywords

^{212}Pb-DOTAMTATE; neuroendocrine tumor; cancer; PRRT; alpha-emitter therapy

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Conflict of Interest Disclosure:

Tania A Rozgaja Stallons, Amal Saidi and Julien J Torgue are Orano Med employees. Izabela Tworowska and Ebrahim S Delpassand are RadioMedix Inc. employees.

Introduction:

Although great strides have been taken to increase the success of cancer treatments, new and more specific strategies are urgently needed to increase cancer cytotoxicity while minimizing damage to healthy tissue. One such strategy is to link peptides targeting tumor-associated receptors to radioisotopes, to direct the killing power of these isotopes to tumor cells. For successful targeted radiation, crucial considerations must be addressed regarding emission type, energy/range of emission and half-life. ^{212}Pb provides a radiotherapeutic agent with short range cancer cell destruction (α-particles) and potential imaging (γ-ray) capabilities. The ^{212}Pb half-life of 10.6 hours provides clinical feasibility and allows for its production and world-wide distribution.

Peptide receptor radiotherapy (PRRT), specifically with somatostatin analogs has been examined as a treatment for somatostatin overexpressing tumors for years. The SSTR binding Tyr3-octreotate (TATE) peptide used in this study has been extensively evaluated in clinical studies in the USA and worldwide. Octreotate based compounds are routinely used in clinical studies for diagnosis of patients with SSTR positive neuroendocrine tumors (NETs) using gamma-emitting isotopes such as ^{68}Ga (US commercial name Netspot, Novartis) and ^{64}Cu as well as other radiolabeled analogs ^{111}ln-octreoscan and 99mTc-/EDDA/HYNIC-octreotide [1–4]. They have shown favorable results in therapy of NET patients using beta-emitting isotopes (^{177}Lu and ^{90}Y) [5, 6] and more recently with alpha particle emitting isotopes such as ^{225}Ac and ^{213}Bi [7]. The ^{177}Lu-DOTATATE Phase 3 study NETTER-1 trial demonstrated a statistically significant and clinically meaningful risk reduction of 79% in disease progression or death versus a treatment with a double dose of Octreotide LAR versus standard of care in patients with progressed midgut carcinoid tumors [8]. This study also demonstrated a favorable safety profile of ^{177}Lu-DOTATATE. The median progression-free-survival in the ^{177}Lu-DOTATATE arm (NETTER-1) at 30 months has not yet been reached, while the median progression free survival in the Octreotide LAR 60 mg arm was only 8.4 months. While beta-emitter peptide receptor radiotherapy (PRRT) showed very promising results and ^{177}Lu-DOTATATE (Lutathera) has recently been approved in US and Europe, it is known to be limited in some populations. Patients previously resistant to beta PRRT have responded favorably to alpha therapy [9]. Previous studies have demonstrated the low toxicity profile of alpha-emitter labeled SSTR targeting agents [7] but limited preclinical data is available. Further studies showed that PRRT could be combined with chemotherapeutics to enhance efficacy [10–14]. The studies presented here further support the use of alpha SSTR agents as treatment for NETs.

Extensive preclinical work, including relevant xenograft models of SSTR overexpressing tumors, has been accomplished showing tumor uptake > 20% ID/g at 1hr post injection and remaining for up to 24hrs, a reduction in kidney accumulation by the addition of positively charged amino acids and the reduction of drug oxidation by ascorbic acid added during the chelation step. Furthermore, ^{212}Pb-DOTAMTATE showed a favorable toxicity profile with a Highest Non-Severely Toxic Dose (HNSTD) dose of 20μCi and efficacy which can be improved by decreasing the timing between drug injections from three weeks to two weeks. Efficacy data showed a 2.4-fold increase in median survival in mice treated with a single 10μCi dose of ^{212}Pb-DOTAMTATE. This could be further enhanced by the addition of a

chemo-sensitizing agent, 5-fluorouracil, which when given in combination of ^{212}Pb-DOTAMTATE (at 2-week intervals) yielded 79% tumor free mice at the end of the 31-week study. These data suggest that there is therapeutic potential for ^{212}Pb-DOTAMTATE alone or in combination with a chemotherapy as a treatment of SSTR positive NET's and have supported the initiation of a phase I clinical study with ^{212}Pb-DOTAMTATE (NCT03466216).

Materials and Methods:

Cell line and Mice:

AR42J rat pancreatic cell line was purchased from ATCC. The cells were tested for mycoplasma by Hoechst DNA stain, Agar culture and PCR-based assay by ATCC and were not detected as per certificate of analysis. The cells were maintained in F12K media (Gibco) containing 20% fetal bovine serum (Gibco). Athymic nude mice were purchased from Charles River or Envigo and CD-1 mice were purchased from Envigo. All studies were conducted using female mice unless otherwise mentioned. All studies were conducted under the approval of the institutional IACUC committee.

Manufacturing and Radiolabeling

GMP DOTAMTATE ($C_{65}H_{93}N_{17}O_{16}S_2$, Figure 1) was manufactured by Macrocyclics using Fmoc solid phase peptide synthesis. DOTAMTATE was added to purified ^{212}Pb at a ratio of 2.4μCi/ng and incubated at 50°C for 10 minutes with shaking at 300rpm. For studies using the ascorbic acid enriched formulation, metal-free L-ascorbic acid (Honeywell) was diluted in Optima water (Fisher) and added prior to the drug chelation to a final injection concentration of 10mM.

iTLC was used to confirm chelation was greater than 95%. Samples were diluted to appropriate activity in PBS or saline prior to injection.

Cell Binding Assay

Peptide binding to somatostatin receptors 2 (SSTR2) and Kd was evaluated in SSTR2 expressing AR42J cells by growing 250,000 cells into the wells of a 24-well plate for 48hrs. Concentrations from 0.5nM to 64nM of ^{212}Pb-DOTAMTATE were incubated in the AR42J containing wells for 10 minutes at 37°C. Four replicates were performed for each concentration. Cells were then washed with PBS and cells from each well were counted for presence of radioactivity. Binding curves were then created and Kd calculated using GraphPad Prism software.

Cell killing Assay

30,000 AR42J cells were grown in a 96 well plate for 48hrs. Cells were then incubated for 4 hours with increasing ^{212}Pb-DOTAMTATE ranging from 0nCi/ml to 800nCi/ml. Eight well per group were treated. Cells were washed with PBS to remove the unbound peptide fraction and then fresh media was introduced. Cells were allowed to incubate for 4 days at 37°C. Cells were then rinsed and incubated with fluorescein diacetate for 30 minutes and read with

a fluorimeter at 485/535nm. Percentage of viable cells was calculated based on untreated cells as a control.

Tumor Models

For all tumor studies, two million (2×10^6) AR42J cells were implanted subcutaneously, in an equal volume mixture of GFR-Matrigel (Corning) and RPMI media (Gibco), into the right flank of each mouse and grown to a volume of ~200–300mm^3.

Preparation of Kidney Protection Agents

200μL of L-Lysine-L-Arginine (35mg/mL of each) diluted in saline or 10% dextrose, 200μL of L-lysine (35mg/mL or 70mg/mL) in saline or 200μL of L-arginine (70mg/mL) in saline were given via intravenous injection five minutes prior to drug injection.

Biodistribution studies

Female mice were grown until an approximate tumor volume of 300mm^3 was reached. 200μL of ^{212}Pb-DOTAMTATE (5μCi) was administered to the mice via the tail vein and mice were euthanized at predetermined timepoints. The background was automatically subtracted from the counts. A standard is also used for decay correction. %ID/g was calculated for each organ collected.

Alpha Imaging

E*x vivo* assessment of ^{212}Pb-DOTAMTATE localization and microdosimetry was performed on frozen section (10–12 μm) of AR42J xenograft tumors placed on a phosphor sheet (Eljen Technology) and imaged using a high-sensitivity QHYCCD camera (Andor). Images were analyzed with Micromanager software (Image J)

Radio HPLC Studies

^{212}Pb-DOTAMTATE was analyzed on an Agilent 1220 HPLC using a C18 reverse phase column (Restek) with an acetonitrile gradient. Fractions were collected off the column every 10 seconds for a total of 10 minutes and then analyzed for radiometric detection by auto gamma counter (Perkin Elmer).

Toxicity Studies

Female athymic nude mice received an injection of either 10μCi, 20μCi, 40μCi or 60μCi of ^{212}Pb-DOTAMTATE or control PBS intravenously. Animals were weighed three times per week and monitored daily for signs of termination criteria over a four-week period. For fractionated toxicity, studies animals (n=10 per group) received a single injection of 40μCi ^{212}Pb-DOTAMTATE, 2 × 20μCi of ^{212}Pb-DOTAMTATE or 3 × 15μCi of ^{212}Pb-DOTAMTATE. Repeat injections were given at three-week intervals. Control mice received PBS only. Blood was sampled via the retro-orbital plexus using potassium-EDTA capillaries and tubes (Greiner Bio-one) and complete cell blood count (CBC) was obtained using VETSCAN® HM5 hematology analyzer (Abaxis). Animals were euthanized when termination criteria were met.

Efficacy Study

Tumor bearing animals were injected with 100μL of 5μCi or 10μCi ^{212}Pb-DOTAMTATE or control (PBS or cold peptide). After three weeks mice who received the 5μCi dose, received a second dose of ^{212}Pb-DOTAMTATE. Animals were monitored daily and calipered three times per week to monitor tumor volume. Mice were sacrificed when termination criteria were met.

Combination Efficacy with 5-fluorouracil

All animals were grown with tumors as described above. Control groups were injected with saline alone or 15mg/kg 5-fluorouracil (Acros) once per week for nine weeks (5-fluorouracil alone). Radiotherapy only groups received 10μCi of ^{212}Pb-DOTAMTATE at two-week or three-week intervals. Combination therapy groups received a treatment of 5-fluorouracil (15mg/kg) followed 24hrs later by 10μCi of ^{212}Pb-DOTAMTATE. The 5-fluorouracil was continued weekly for a total of nine weeks for both treatment groups. 10μCi of ^{212}Pb-DOTAMTATE was given 24hrs after the first 5-fluorouracil injection and then at two or three-week intervals for a total of three injections. Animals were monitored daily for signs of termination criteria and calipered three times per week to monitor tumor volume. Animals were euthanized when termination criteria were met.

Termination Criteria

Mice were sacrificed when tumor volumes reached 3000mm^3 or other predetermined termination criteria were met (weight loss over 15% for two consecutive days or 20% weight loss from initial weight, serious bleeding, necrosis or ulceration of the tumor, scruffiness or lack of grooming over 5 days, lethargy over 3 days, weakness/balance issues over 5 days, hunchback appearance, diarrhea or hypothermia).

Statistical Analysis

Animals were randomly assigned to each group. An unpaired t-test was used for statistical analysis.

Patient Studies

The study was conducted in accordance with the Declaration of Helsinki ethical guidelines and upon signature of the IRB approved informed consent form. Studies were performed under FDA IND 130960.

Results:

In vitro Data

An *in vitro* binding study of ^{212}Pb-DOTAMTATE to SSTR2 expressing AR42J cells yielded a K_d of 12.9nM (Supplementary Figure S1A), which is in line with other studies that have examined the binding of octreotate peptides to somatostatin expressing cell lines [15]. In addition, a cytotoxicity assay showed a dose dependent cytotoxic effect of ^{212}Pb-DOTAMTATE for AR42J cells with complete death observed at 800nCi/mL and 50% viability observed between 12.5nCi/mL to 25nCi/mL (Supplementary Figure S1B). A ^{212}Pb-

chelate only negative control did not show a dose dependent cytotoxic effect with viability ranging from 47% to 156%.

Biodistribution Studies

All studies were conducted in female mice, as a biodistribution study showed that there was no significant difference in organ uptake between male and female mice (Supplementary Figure S2A) and the literature suggests that female mice may be more susceptible to toxicity and may provide a worst case scenario between the two sexes [16]. When animals were injected with a single dose of 5μCi ^{212}Pb-DOTAMTATE, the average tumor uptake exceeded 20% ID/g one hour after drug administration and remained constant through 4 and 24 hours post drug administration (Figure 1). The pancreas and kidneys were the two organs with the highest non-target uptake but these organs also showed significantly less accumulation by 24 hours post-injection. In further examining AR42J tumors for ^{212}Pb-DOTAMTATE distribution, no correlation between tumor volume and tumor uptake is visible in tumors up to 1500mm^3 (Supplementary Figure S3) and alpha imaging of tumors treated with ^{212}Pb-DOTAMTATE showed homogenous distribution of the drug at all tumor sizes up to 1500mm^3 (Supplementary Figure S4A). Three specific activities of 4.1ng, 22ng or 110ng per 10μCi were also examined via biodistribution study (Supplementary Figure S2B). 10μCi per 4.1ng (2.4μCi/ng) has been primarily used in ^{212}Pb-DOTAMTATE studies to date however a decrease in the specific activity does not appear to have a significant effect on tumor uptake. This suggests that receptor saturation is not occurring even at over 25-fold lower specific activity then what has been primarily used in these studies.

Reduction of Renal Retention of ^{212}Pb-DOTAMTATE

As kidney protection agents are often given with targeted radiotherapies to minimize nephrotoxicity, several kidney protection agents and diuretics were tested in combination with ^{212}Pb-DOTAMTATE through biodistribution studies for their ability to minimize drug accumulation in the kidneys (Figure 2). Of the five versions of amino acid combinations/concentrations given, all were able to significantly reduce ^{212}Pb-DOTAMTATE uptake in the kidneys ($p=<0.0001$) at 1hr post drug injection. Additional studies conducted with higher levels of L-lysine in tumor bearing mice at three timepoints showed that while kidney uptake is reduced no effect on drug accumulation in the tumor was observed (Supplementary Figure S4B).

Enhancing Stability of ^{212}Pb-DOTAMTATE binding with Ascorbic Acid

Oxidation of DOTATATE peptides, specifically on the indol ring of the tryptophan residue has been shown to occur when the peptide is labeled with radioisotopes and can be minimized by the addition of ascorbic acid [17]. The presence of an oxidized form of DOTAMTATE was also witnessed in our studies when the drug was prepared and not used immediately, however, it was not known if this oxidation influenced the drug binding to its SSTR targets. To test if the presence of oxidized DOTAMTATE influenced overall drug binding, a biodistribution study was conducted in AR42J tumor bearing mice. ^{212}Pb-DOTAMTATE prepared with and without ascorbic acid present during chelation was left overnight (to obtain a worst-case scenario) and oxidation confirmed the following day by Radio-HPLC before the biodistribution was conducted (Figure 3). Drug binding to the tumor

was significantly enhanced ($p>0.01$) in the presence of ascorbic acid during the chelation reaction at 1, 4 and 24hrs (33% ID/g 24hrs post drug injection) compared to the ascorbic acid-free formulation (10% ID/g 24hrs post drug injection) suggesting that oxidation was having a negative effect on the drug but could be minimized with the addition of the antioxidant.

^{212}Pb-DOTAMTATE toxicity studies

To assess the toxicity profile of ^{212}Pb-DOTAMTATE, a dose range finding study in female, athymic nude mice was first conducted as a basis for dose selection for subsequent efficacy studies and for a GLP toxicity study. A maximum tolerated dose was determined to be between 20μCi and 40μCi (Figure 4A).

A single dose GLP toxicity study was conducted in female CD-1 non-tumor bearing mice at doses of ^{212}Pb-DOTAMTATE ranging from 0μCi to 40μCi per mouse. Body weights, clinical chemistry and hematology parameters were examined throughout the nine-months duration of the study. The 2 and 10 μCi doses appeared to be reasonably well tolerated whereas administration of a single 40μCi intravenous dose of ^{212}Pb-DOTAMTATE was associated with adverse findings including mortality, decreased body weight gain, leukocyte, erythrocyte, serum albumin and organ weights as well as histopathologic findings of bone marrow depletion and gastrointestinal lesions. At 20 μCi, there were relatively mild and reversible effects on weight gain and leukocyte counts along with chronic glomerular nephritis which appears late in the study due to a combination of aging and dosing. Based on the study findings, the dose of 10 μCi was considered a no-observable effect (NOEL) dose and an HNSTD of 20μCi was determined (Supplementary Figure S5).

To further examine if the HNSTD determined in the single dose toxicity study could be overcome through fractionation of the ^{212}Pb-DOTAMTATE, a repeat dose toxicity study was conducted in non-tumor bearing CD-1 mice (Figure 4B). As hematological toxicity is routinely dose limiting for radiotherapeutics and is usually reversible with time at lower doses, fractionation was expected to overcome the lower HNSTD determined in the single dose study. Animals were given a single dose of 40μCi of ^{212}Pb-DOTAMTATE, two cycles of 20μCi or three cycles of 15μCi ^{212}Pb-DOTAMTATE every three weeks. Almost 40% of animals in the 1×40μCi group died nine days after injection but those that survived were able to survive through the remainder of the study. 50% of the animals in the 2 × 20μCi group died within four weeks of the study and one week after receiving the second dose. The animal group that received 3×15μCi of ^{212}Pb-DOTAMTATE were consistent with the control group. Hematological toxicity appeared to be the reason for death in the first two groups. This was evident by the significantly low white blood cell counts (WBC) and platelets (PLT) in the 1 × 40μCi and 2 × 20μCi groups after drug injections (Supplementary S6). Animals who received 3 × 15μCi doses of ^{212}Pb-DOTAMTATE also had a decrease in their WBC and PLT counts but were able to recover after each dose. This study suggests that a fractioned dose of drug is optimal as it allows the same cumulative dose but with recoverable hematological effects.

Efficacy Studies with ^{212}Pb-DOTAMTATE

An initial low-dose efficacy study of ^{212}Pb-DOTAMTATE was performed to examine the effectiveness of the drug in tumor bearing mice at 1/4 of the HNSTD. Animals were given one or two cycles of 5μCi ^{212}Pb-DOTAMTATE or 10μCi ^{212}Pb-DOTAMTATE. Control animals received cold-DOTAMTATE or PBS. Animals that were injected with cold-DOTAMTATE or PBS had similar median survival of 3.4 weeks and 3.5 weeks, respectively post injection. Mice that received one injection of 5μCi ^{212}Pb-DOTAMTATE had a median survival of 6.3 weeks while mice who received one injection of 10μCi ^{212}Pb-DOTAMTATE had a median survival of 8.5 weeks showing a dose dependent effect. Two injections of 5μCi ^{212}Pb-DOTAMTATE led to a median survival of 7.1 weeks (Figure 5). The median survival time was similar between animals that received 1×10μCi vs 2×5μCi of drug suggesting that at low doses a fractionated dose does not appear to be beneficial. Overall however, the ^{212}Pb-DOTAMTATE does show efficacy at these low doses but efficacy could likely be improved with higher treatment doses.

With this data, efficacy studies with ^{212}Pb-DOTAMTATE were further optimized with a combination therapy and treatment cycle study. The aim of this study was to optimize the timing of treatment cycles and to combine the radiotherapeutic with a subtherapeutic (~40mg/m^2 versus at least 400mg/m^2 in human) chemotherapy dose of 5-fluorouracil (5-FU) to maximize tumor devastation. Animals received saline only, 5-FU only, 3 × 10μCi of ^{212}Pb-DOTAMTATE at two-week or three-week intervals, or a combination of 5-FU and 3 × 10μCi ^{212}Pb-DOTAMTATE at two-week or three-week intervals (Figure 6). Animals that were injected with 5-fluorouracil alone had a median survival of 2.4 weeks while the saline alone group had a median survival of 3.1 weeks post cell injection. Mice that received three injections of ^{212}Pb-DOTAMTATE only at three-week intervals had a median survival rate of 9.4 weeks while combination therapy with 5-fluorouracil led to a longer median survival of 11.1 weeks with 20% of the mice alive and tumor free at the termination of the 31-week study. When ^{212}Pb-DOTAMTATE was given at two-week intervals the median survival was 11.9 weeks and this was further improved by the addition of 5-FU where 79% of the animals survived to the end of the 31-week study. This suggests that the timing of the drug treatment is critical in maximizing its effectiveness. Furthermore, optimal timing of the radiotherapeutic combined with a radiosensitizer can significantly enhance efficacy versus the drug alone and lead to a significant group of tumor-free animals.

Discussion:

Extensive preclinical work and optimization has been accomplished to demonstrate the feasibility, safety and therapeutic potential of ^{212}Pb-DOTAMTATE alone or in combination as a treatment for SSTR positive NETs. Specifically, in vitro assays have shown that the peptide binds to its SSTR receptor with an appropriate affinity for therapeutic use and has cytotoxic effects. Furthermore, in vivo tissue distribution studies in tumor bearing animals showed ^{212}Pb-DOTAMTATE has a high uptake in the tumor relative to other organs. Although some drug uptake and retention was observed in the kidneys and pancreas of animals, it decreased significantly by 24hrs post drug injection. This uptake is not unexpected as these organs have also shown high uptake in other nonclinical rodent studies

involving alpha emitters, which have not transliterated into adverse effects in human studies. [7, 18–21]. However, given the particularly high tumor uptake, the DOTAMTATE peptide has potential not only for therapeutic applications with ^{212}Pb but also for imaging applications using longer-lived and gamma-emitting lead isotope such as ^{203}Pb. Biodistribution studies conducted in our lab have shown that CD-1 mice given ^{203}Pb-DOTAMTATE did not show significantly different tissue uptake compared to mice treated with ^{212}Pb-DOTAMTATE in all critical organs (Supplementary Figure S7). This was confirmed by an exploratory IND (IND 102,590) conducted to examine the dosimetry and biodistribution of ^{203}Pb-DOTAMTATE in patients with SSTR expressing NET's as a surrogate for ^{212}Pb-DOTAMTATE. ^{203}Pb-DOTAMTATE showed similar PK properties to other commercially available octreotate drugs but with the advantage that the same metal could be used for imaging and therapeutic applications. This further confirms that the two isotopes have a similar physical property and pharmacokinetic profile and could therefore be used for theranostic purposes.

As with many PRRT treatments, the presence of radiolabeled somatostatin analogs in the kidneys is common due to their renal clearance and retention by megalin/cubulin receptors [20–22]. Kidney protection agents including L-lysine-L-arginine, mixtures of positively charged amino acids and amifostine are often given in combination with radiolabeled drugs [23–25]. With ^{212}Pb-DOTAMTATE specifically, multiple kidney protection agents were tested, and all were found to significantly reduce drug uptake in the kidneys 1 hr post drug injection. It should be noted, however, that these were given as a bolus injection 5 minutes prior to drug injection rather than an IV over the course of four hours which is done with patients, due to animal model constraints; therefore, the data may not directly translate into a clinical setting.

In addition to kidney uptake, another factor that must be considered with PRRT is the oxidation of peptides in the proximity of radionuclides. The presence of an oxidized form of DOTAMTATE was detected in our studies by radio-HPLC and was shown to have a negative impact on tumor binding through biodistribution studies. Free radicals have been shown to form in solutions containing high energy β-particles and tryptophan residues, specifically, can become oxidized [26–28]. The addition of the antioxidant, ascorbic acid, during the chelation reaction significantly enhanced (3X vs. a mostly oxidized peptide) tumor binding presumably by minimizing this tryptophan oxidation within the ^{212}Pb-DOTAMTATE peptide as confirmed by Radio-HPLC analysis.

To better characterize the safety profile of ^{212}Pb-DOTAMTATE a dose range finding study in female, athymic nude mice was conducted as a basis for dose selection for subsequent efficacy studies and a single dose GLP toxicity study with ^{212}Pb-DOTAMTATE. The dose range finding study led to a maximum tolerated dose between 20μCi and 40μCi and provided the preliminary information for a single dose GLP toxicity study, which included a 9-month follow-up to determine potential delayed toxicities in radiation-sensitive organs. Based on histopathology, body weights, hematology and clinical chemistry from this GLP toxicity study, an HNSTD of 20μCi was determined. An additional toxicity study showed that fractionating the dose was optimal and allowed for a cumulative dose that would be toxic if given as a single injection.

Efficacy studies showed that ^{212}Pb-DOTAMTATE has therapeutic potential as it was able to extend median life span 2.4-fold with a single treatment at low doses. Furthermore, it was able to cure approximately 50% of the animals when the timing of the drug was optimized. The time between cycles must be sufficient to allow for acute hematological toxicity recovery without being too long of a duration that the tumor growth rate renders the drug less effective. Furthermore, combination therapy with PRRT can be used to enhance the efficacy of the drugs beyond the additive efficacy of each. By targeting multiple mechanisms involved in tumor cell proliferation and resistance, combination therapies using two or more drugs achieve efficacy with lower doses or toxicity than individual treatments. Several radiosensitizers have shown additive or synergistic effects when combined with PRRT [10–14]. Fluorouracil acts as an inhibitor of thymidylate synthase (TS), which is a nucleoside required for DNA replication and DNA repair [29–31]. Fluorouracil's mechanism of action makes it an ideal candidate for combination therapy with PRRT as the main goal is to maximize irreversible DNA damage. ^{212}Pb-DOTAMTATE and fluorouracil combination therapy showed a significant improvement in tumor regression. A three-cycle ^{212}Pb-DOTAMTATE treatment combined with weekly subtherapeutic fluorouracil dosing was able to durably cure approximately 80% of the animals.

Overall, the non-clinical studies provide appropriate justification on the safety and efficacy of ^{212}Pb-DOTAMTATE in animals and have provided sufficient data to warrant a clinical trial study. The rodent models showed a promising safety index with a 3.2-fold increase in median survival and one third of the animals being tumor free. Somatostatin analogs have long been studied and used in preclinical and clinical settings for the treatment of SSTR expressing neuroendocrine tumors, but a successful TAT treatment remains elusive. The preclinical data presented supports the further progression of ^{212}Pb-DOTAMTATE into a clinical setting and was used to support the initiation of a Phase I study (NCT03466216, https://clinicaltrials.gov/ct2/show/NCT03466216?term=radiomedix&rank=2).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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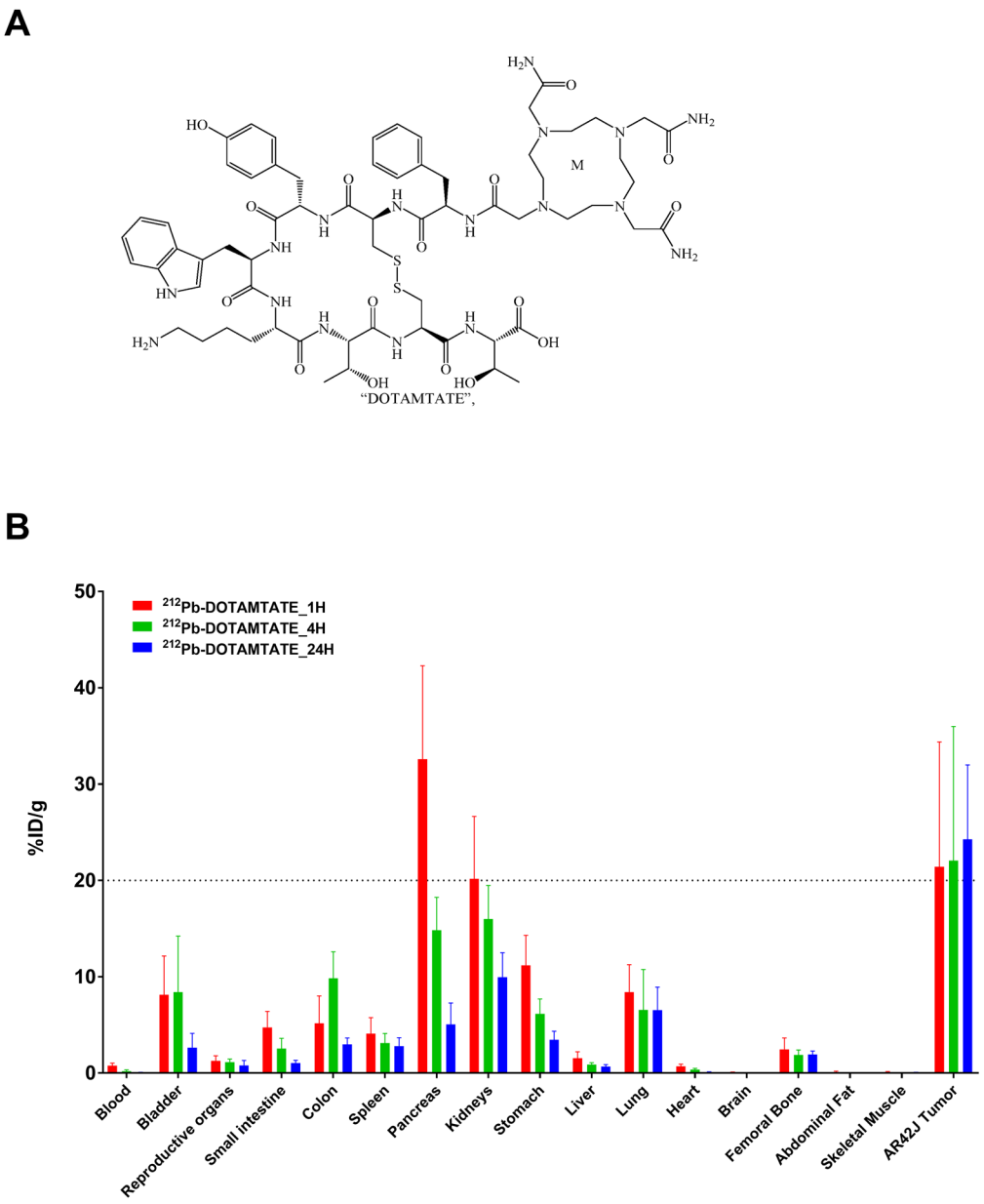

Figure 1. Drug Structure and Initial Biodistribution.

Figure 1 shows the chemical structure of DOTAMTATE and biodistribution of ^{212}Pb-DOTAMTATE in athymic nude tumor bearing mice.

A. Chemical structure of DOTAMTATE (Formula: C65H93N17O16S2) B. Drug was administered, and organs were collected from 5 mice per timepoint: 1 hour post (red), 4 hours (green) and 24 hours (blue) post injection.

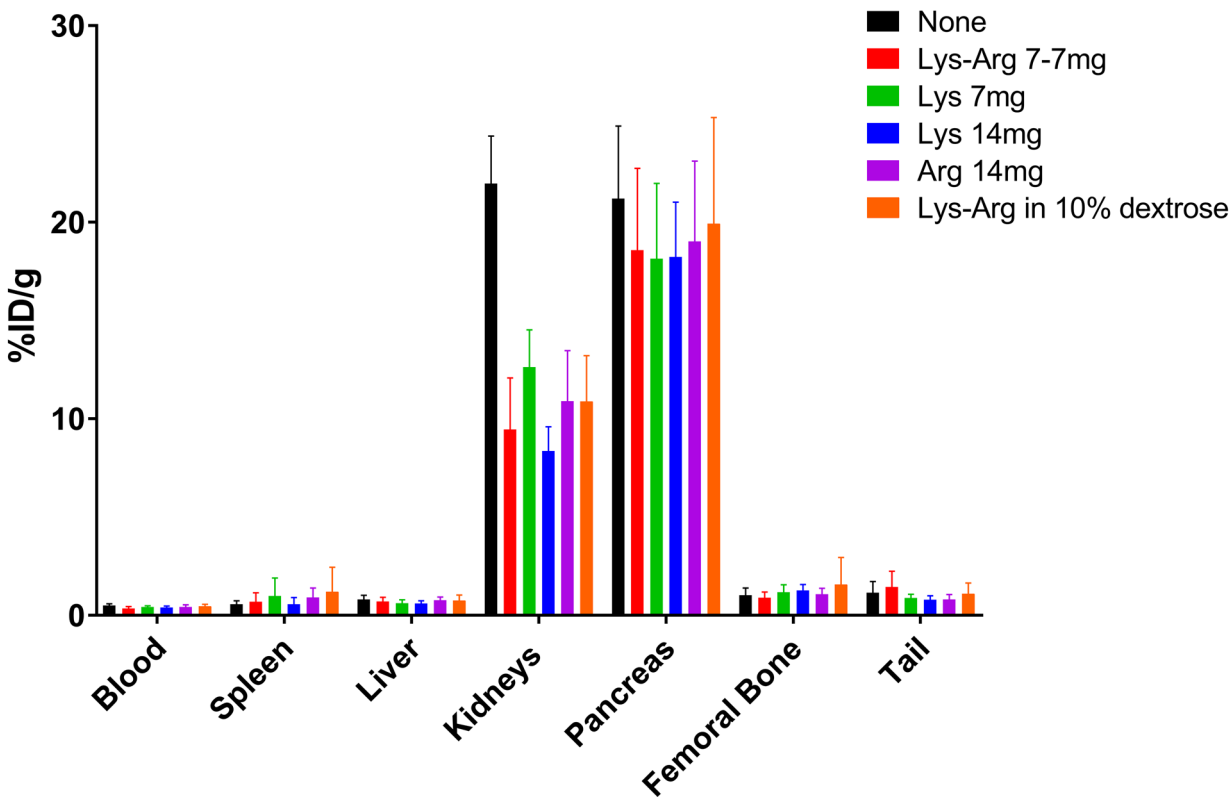

Figure 2. ^{212}Pb-DOTAMTATE in the Presence of Kidney Protection Agents.

Figure 2 shows a biodistribution of ^{212}Pb-DOTAMTATE in CD-1 mice at 1-hour post drug injection with kidney protection agents. 200μL of kidney protections agents 7mg Arg-7mg Lys in saline (red), 7mg Lys in saline (green), 14mg Lys in saline (blue), 14mg Arg in saline (purple) or 7mg Arg-7mg Lys in 10% dextrose (orange) were given IP five minutes before drug injection. No kidney protection agent control shown in black. 5 mice per group. Average of two studies displayed.

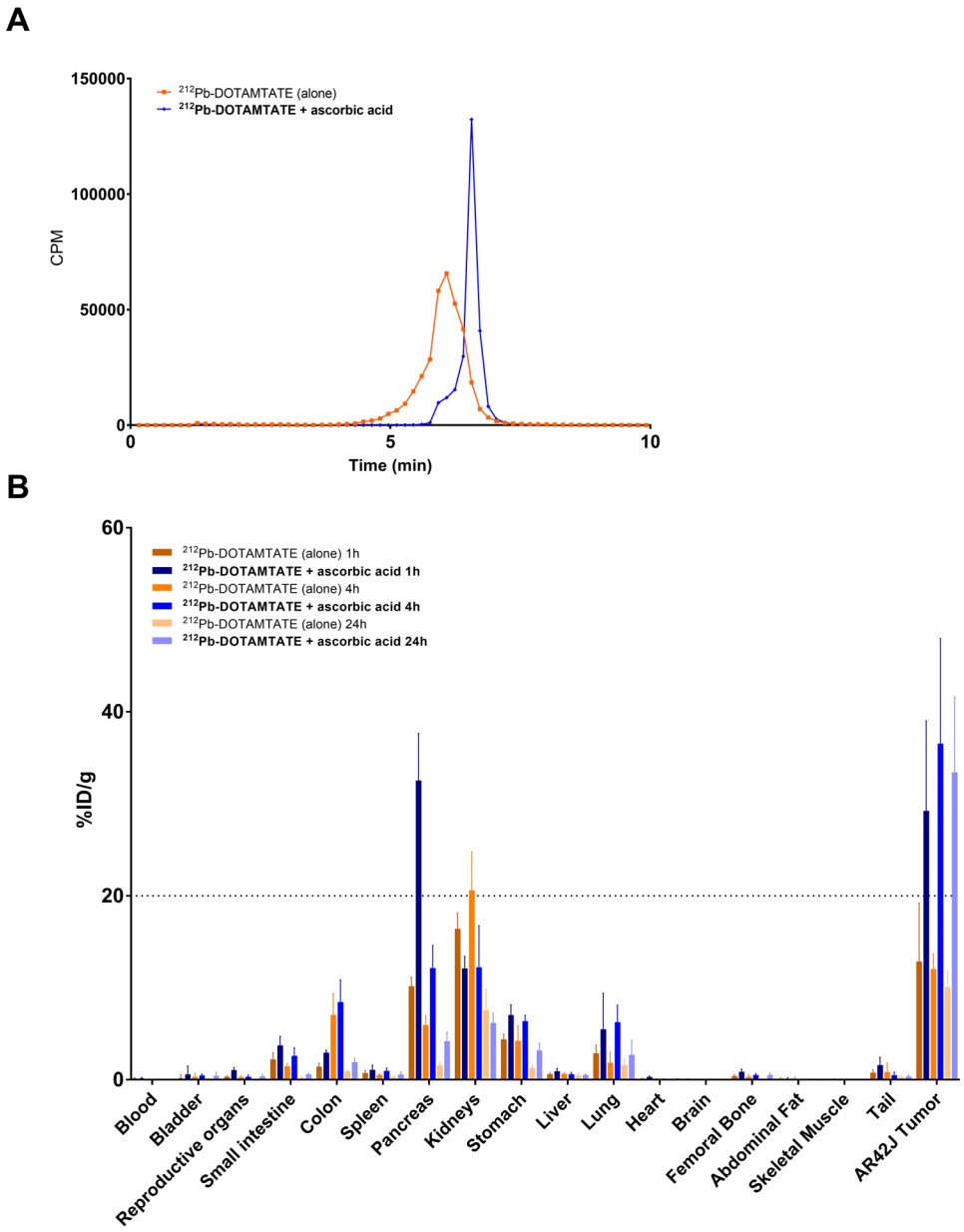

Figure 3. Addition of Ascorbic Acid to Drug Preparation.

Figure 3 shows radio HPLC and biodistribution studies of ^{212}Pb-DOTAMTATE with ascorbic acid. A. Radio-HPLC of ^{212}Pb-DOTAMTATE without ascorbic acid present during chelation (orange) or with 10mM final concentration of ascorbic acid (blue) prior to biodistribution. B. Biodistribution of ^{212}Pb-DOTAMTATE in AR42J tumor bearing mice (n=5 per group) at 1hr, 4hr and 24hrs post drug injection in the presence of 10mM ascorbic acid (blue) or none (orange).

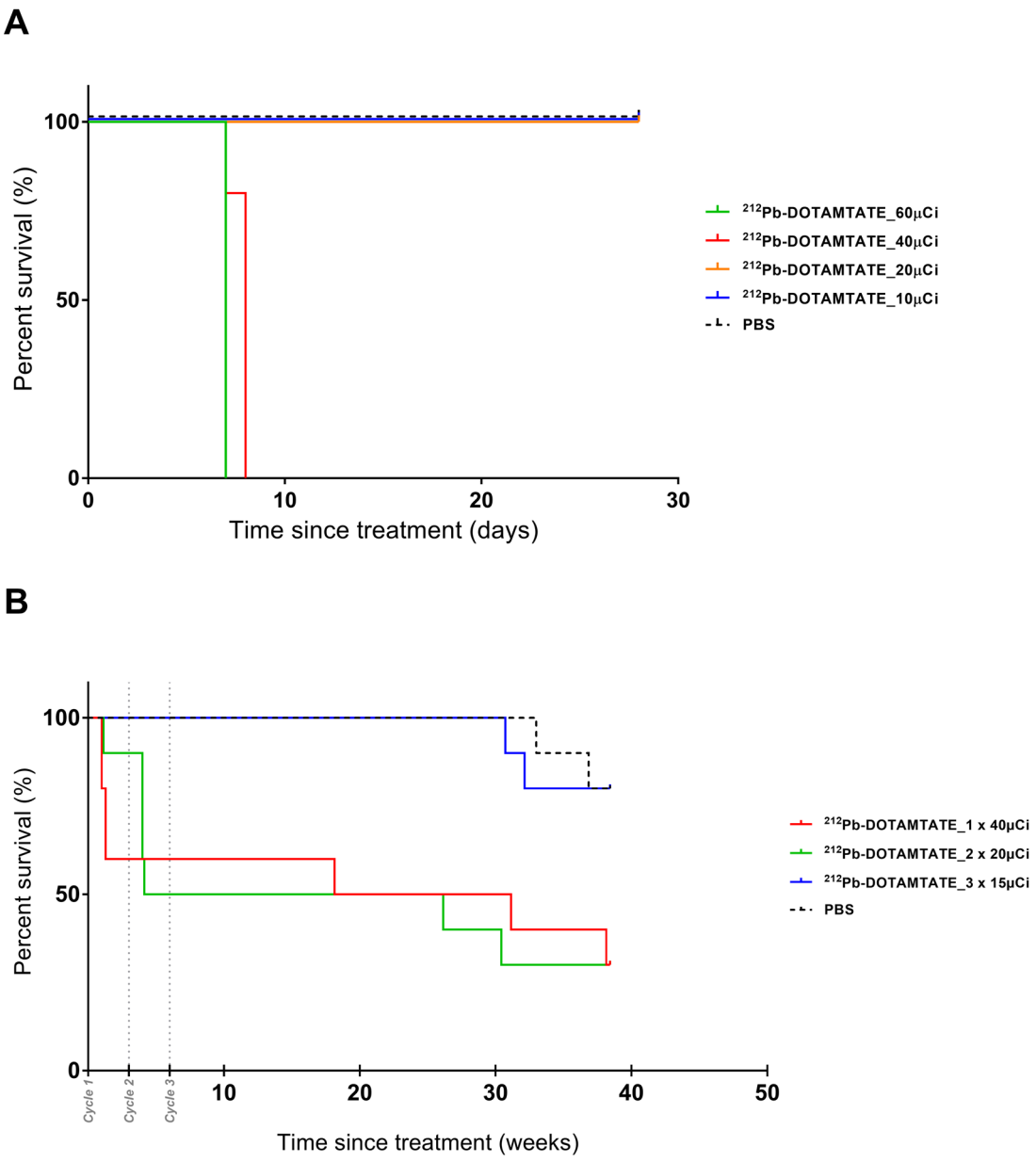

Figure 4. Dose Range Finding and Toxicity Studies.

Figure 4 shows initial dose range finding studies and fractionated dose toxicity studies with ^{212}Pb-DOTAMTATE in athymic nude mice. A. Kaplan-Meier survival curve of ^{212}Pb-DOTAMTATE treated athymic nude mice. Animals received a single dose of 10μCi (purple), 20μCi (blue), 40μCi (green), or 60μCi (red) of ^{212}Pb-DOTAMTATE or PBS control (black). n=5 mice per group. Survival of the animals are shown in days post injection during the 4-week study. B. Kaplan-Meier curve of ^{212}Pb-DOTAMTATE in CD-1 mice in a fractionated dose toxicity study. PBS alone, n=10 (black), 1 × 40μCi, n=10 (red), 2 × 20μCi, n=10 (green) and 3 ×15μCi, n=10 (blue) treatment groups. Drug cycles 1, 2 and 3 are shown with grey dots.

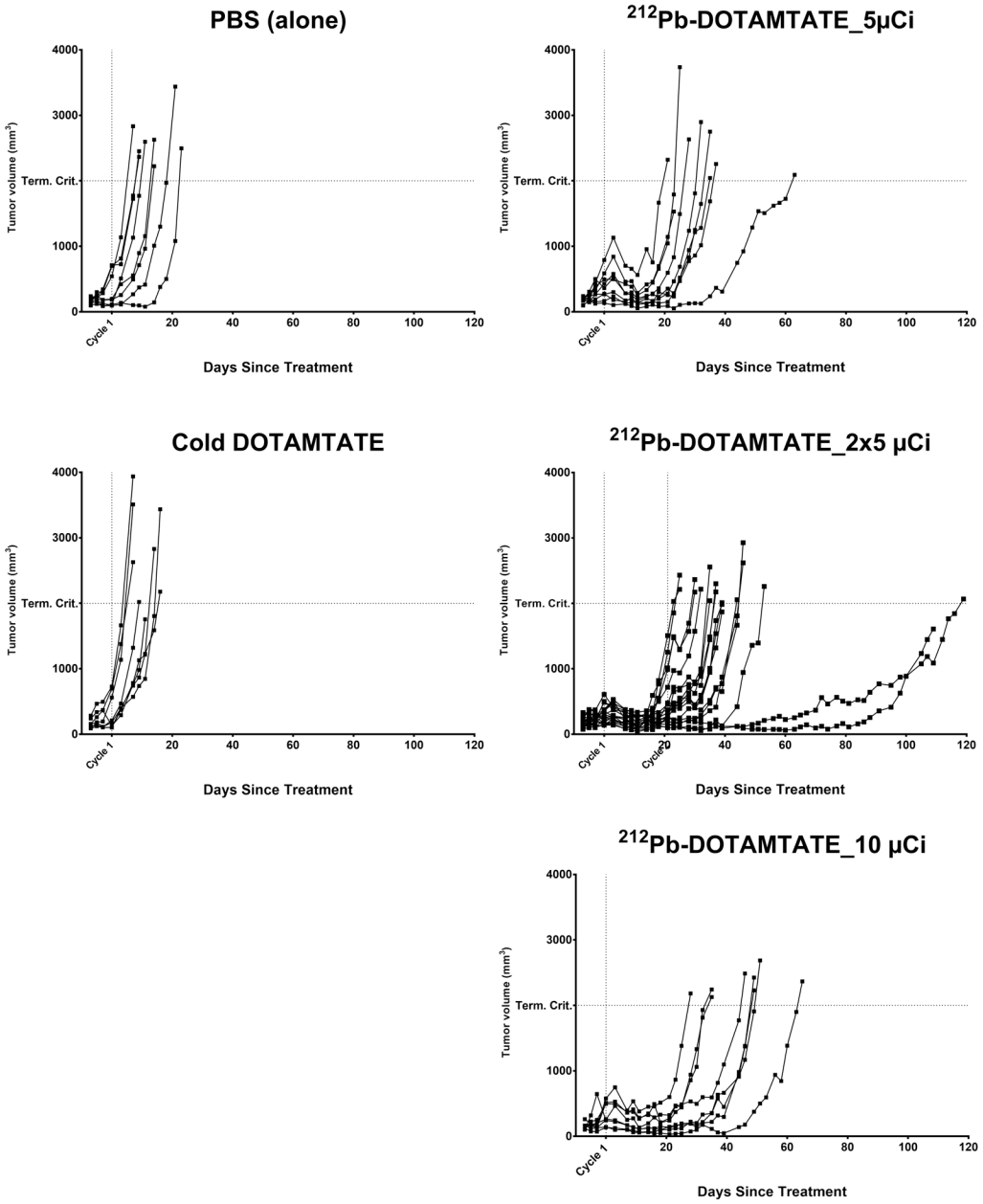

Figure 5. Single vs. multiple dose efficacy study.

Figure 5 shows the efficacy of mice treated with ^{212}Pb-DOTAMTATE in a single and multi-injection dose setting. Groups of animals were injected with cold DOTAMTATE (n=8), PBS (n=8), 5μCi ^{212}Pb-DOTAMTATE (n=9), 10μCi ^{212}Pb-DOTAMTATE (n=8), 2 × 5μCi ^{212}Pb-DOTAMTATE (n=18), Tumor volumes for individual mice per group are shown in mm3 over time.

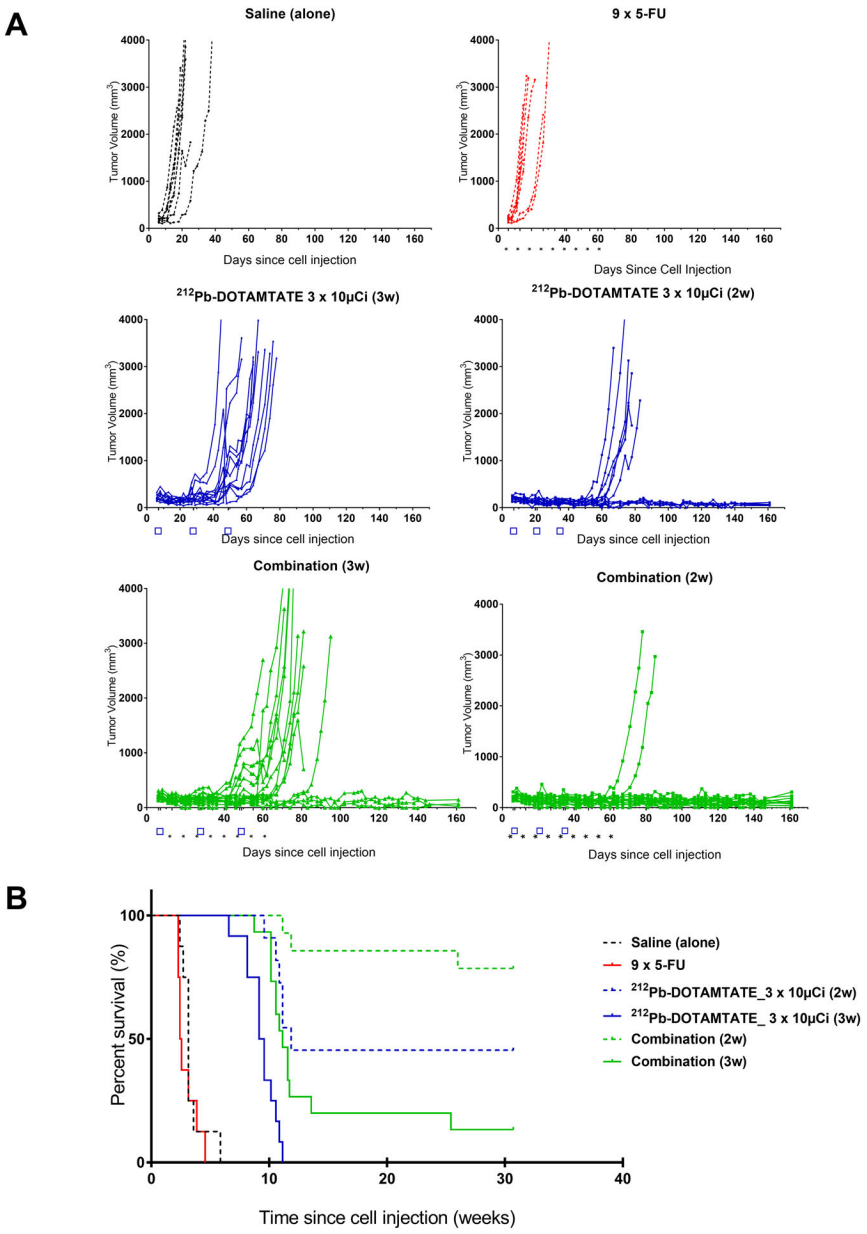

Figure 6. Combination Efficacy Study.

Figure 6 shows an efficacy study of AR42J tumor bearing mice treated with ^{212}Pb-DOTAMTATE in combination with 5-fluorouracil. A. Individual mouse efficacy data showing tumor volumes of mice injected with saline (black), 5-FU only (red), ^{212}Pb-DOTAMTATE only (blue) and in combination with 5-FU (green). B. Kaplan Meier of animals treated with saline (black), 5-FU only (red), ^{212}Pb-DOTAMTATE only at 2-week intervals (blue dashed), 3-week intervals (blue solid), in combination with 5-FU at 2-week intervals (green dashed) or three-week intervals (green solid).