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# Peptide Receptor Radionuclide Therapy with $^{67}$Cu-CuSarTATE is Highly Efficacious Against a Somatostatin Positive Neuroendocrine Tumor Model

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**Disclosure**

C. M. Jeffery, E. M. van Dam and M. J. Harris were/are employed by Clarity Pharmaceuticals, the licensee of the intellectual property for SarTATE. C. M. Jeffery and P.S. Donnelly have potential financial interests in Clarity Pharmaceuticals. P. S. Donnelly is an inventor of intellectual property, in this area of research, which has been licensed from the University of Melbourne to Clarity Pharmaceuticals. Paul Donnelly serves on the Scientific Advisory Board of Clarity Pharmaceuticals. Unrelated to this project, Rodney Hicks has share options in Telix Radiopharmaceuticals that are held

on behalf of the Peter MacCallum Cancer Centre. No other potential conflict of interest relevant to this article was reported.

**Running Title:** $^{67}$Cu-CuSarTATE for Radionuclide Therapy

**ABSTRACT**

Peptide receptor radionuclide therapy using radiolabeled octreotate is an effective treatment for somatostatin receptor 2 expressing neuroendocrine tumors. The diagnostic and therapeutic potential of copper-64 and copper-67, respectively, offers the possibility of using a single somatostatin receptor targeted peptide conjugate as a theranostic agent. A sarcophagine cage amine ligand, MeCOSar, conjugated to ($Tyr^3$)-octreotate called, $^{64}$Cu-CuSarTATE, was demonstrated as an imaging agent and potential prospective dosimetry tool in ten patients with neuroendocrine tumors. This study aimed to explore the antitumor efficacy of $^{67}$Cu-CuSarTATE in a preclinical model of neuroendocrine tumors and compare it with the standard peptide receptor radionuclide therapy agent, $^{177}$Lu-LuDOTA-$Tyr^3$-octreotate ($^{177}$Lu-LuTATE).

**METHODS:** The antitumor efficacy of various doses of $^{67}$Cu-CuSarTATE in AR42J (rat pancreatic exocrine) tumor bearing mice was compared with $^{177}$Lu-LuTATE. **RESULTS:** Seven days after a single administration of $^{67}$Cu-CuSarTATE (5 MBq) tumor growth was inhibited by 75% compared to vehicle control. Administration of $^{177}$Lu-LuTATE (5 MBq) inhibited tumor growth by 89%. Survival, was extended from 12 days in the control group to 21 days following following treatment with both $^{67}$Cu-CuSarTATE and $^{177}$Lu-LuTATE. In a second study, the efficacy of fractionated delivery of PRRT was assessed, comparing the efficacy of 30 MBq $^{67}$Cu-CuSarTATE or $^{177}$Lu-LuTATE, either as a single intravenous injection or as two 15 MBq fractions two weeks apart. Treatment of tumors with two fractions significantly improved survival when compared to delivery as a single fraction ($^{67}$Cu-CuSarTATE: 47 vs 36 days; $P$= 0.036; $^{177}$Lu-LuTATE: 46 vs 29 days; $P$=0.040). **CONCLUSION:** This study demonstrates that $^{67}$Cu-CuSarTATE is well tolerated in Balb/c nude mice and highly efficacious against AR42J tumors *in vivo*. Administration of $^{67}$Cu-CuSarTATE and $^{177}$Lu-LuTATE divided into two fractions over two weeks was more efficacious than that of a single fraction. The antitumor activity of $^{67}$Cu-CuSarTATE in the AR42J tumor model demonstrating the suitability of this novel agent for clinical assessment in the treatment of somatostatin receptor 2 expressing neuroendocrine tumors.

**KEY WORDS**: radiopharmaceuticals; copper-64; copper-67; peptide receptor radionuclide therapy; theranostics

## INTRODUCTION

Overexpression of the somatostatin subtype 2a receptor on certain types of neuroendocrine tumors, as well as neuroblastoma, phaeochromocytoma/paraganglioma, Merkel cell carcinoma and meningioma, leads to this receptor being a valid target for diagnostic imaging and peptide receptor radionuclide therapy. Diagnostic positron emission tomography (PET) imaging with the gallium(III)-68 complex ($t_{1/2}$ = 68 min) complex of DOTATATE, where the macrocycle DOTA is conjugated to $Tyr^3$-octreotate, an 8-amino acid peptide analogue of somatostatin, has emerged as a valuable tool to identify patients suitable for peptide receptor radionuclide therapy with the $\beta^-$-emitting lutetium complex $^{177}$Lu-LuDOTA-$Tyr^3$-octreotate ($^{177}$Lu-LuTATE)(*1-5*). The safety and efficacy of peptide receptor radionuclide therapy relies on selectively delivering the highest possible dose of radiation to the tumor whilst sparing organs from radiation toxicity, particularly the kidneys, which is a critical target organ for radionuclide therapy. Accurate prospective dosimetry would allow prescription of an administered activity that maximizes therapeutic efficacy within the tolerance of organs, such as the kidney. The use of a short-lived radionuclide (gallium-68, $t_{1/2}$ = 68 minutes) to predict dosimetry for subsequent therapy with long-lived radionuclide lutetium-177 ($t_{1/2}$ = 6.65 d, $\beta^-$ = 100%, $E_{\beta^-}$(mean) = 134 keV) introduces limitations in modelling dosimetry. Furthermore, the use of two different chemical elements (gallium and lutetium) with different chemistries can lead to inconsistent tissue biodistribution as it is likely that peptide-metal complex assemblies prepared with different metal ions do not have the same binding and internalization interactions and altered excretory pathways(*6-8*). Furthermore, the use of the same element for both imaging and therapy would represent an important advance for radionuclide therapy, particularly if the half-life of the diagnostic agent is sufficient to evaluate clearance kinetics from critical target organs. There are two copper radionuclides, copper-64 and copper-67, that offer a matched theranostic pair. Positron-emitting copper-64 ($t_{1/2}$ = 12.7 h, $\beta^+$ = 17.4%, $E_{\beta^+}$(mean) = 278 keV) can be used as a companion diagnostic agent to plan use for PRRT using the $\beta^-$-emitting copper-67 ($t_{1/2}$ = 61.9 h, $\beta^-$ = 100%, $E_{\beta^-}$(mean) = 141 keV) (*9-11*). The $\beta^-$-emissions of copper-67 have a mean range of 0.2 mm and are appropriate for the treatment of small tumors down to 5 mm in diameter and disseminated metastatic disease(*12-14*). Copper-67 has a higher fraction of γ-emission than lutetium-177 but the mean energy of their relevant peaks are similar (copper-67: 185 keV 49% and 93 keV 16%; lutetium-177: 208 keV 11% 113 keV 6%),(*15*) making it suitable for post-treatment whole-body scintigraphy for calculation of percentage retention of administered dose, planar or quantitative single photon emission computed tomography (SPECT) dosimetry.

Pioneering studies that identified the therapeutic potential of copper-67 used derivatives of the tetrazamacrocyles, such as cyclam, to coordinate the copper(II) radionuclide(*16*). In one example, a

monofunctionalised cyclam derivative, 4-[(l,4,8,11-tetraazacyclotetradec-l-yl)methyl]benzoic acid, (CPTA) was conjugated to an antibody against anti-carcinoembryonic antigen (AB35) and the conjugate radiolabelled with copper-67. Evaluation of this conjugate in a mouse LoVo tumor model demonstrated high tumor uptake (15 ± 3 % injected activity (IA)/g after 24 h and 32 ± 7 % IA/g after 96 h)(*17*). This work was extended to a comparison of the biodistribution of copper-67-labelled AB35 with iodine-125-labelled AB35 in six patients with primary colorectal cancer. The copper-67 conjugate had higher tumor uptake than the radioiodinated antibody but also high non-specific liver and bowel uptake(*18*).

The therapeutic efficacy of both copper-64 and copper-67-labelled mouse anti-human colorectal cancer monoclonal antibody (called 1A3) was investigated in a GW39 human colon carcinoma carried in hamsters. Both the copper-64 and copper-67-labelled antibodies were able to cause complete remission in small tumors but to account for the different physical decay and radioactive half-lives of copper-64 and copper-67 five times more copper-64 agent was administered(*9*). In another demonstration of therapeutic potential of copper-67, a monoclonal antibody (Lym-1) that preferentially targets malignant tissue was functionalised with a derivative of the tetraazamacrocycle 1,4,7,11-tetraazacyclotetradecane-N,N',N'',N''-tetraacetic acid (TETA) to permit radiolabelling with copper-67. An evaluation of this conjugate in eleven lymphoma patients resulted in tumor regressions despite patients only receiving imaging doses (126 – 477 MBq)(*19,20*). The potential for peptide receptor radionuclide therapy with a copper-64 labelled somatostatin targeting complex, $^{64}$Cu-CuTETA-TATE has been demonstrated previously(*21*). Relatively high doses (555 MBq) were required to reduce tumor burden in CA20948 tumor bearing rats(*21*).

Recent mprovements in developing copper chelators and in the methods of linear accelerator based production of copper-67 have reinvigorated interest in this radionuclide(*22*). The use of copper radionuclides in radiopharmaceuticals is best achieved using chelators that form complexes with copper(II) that are stable *in vivo*. Hexamine cage ligands of the bicyclo[6.6.6]icosane type, given the trivial name of "sarcophagines", form complexes with copper(II) that are more stable *in vivo* than copper(II) complexes of DOTA(*23-28*). For example, incubation of $^{67}$Cu-[Cu(sar)]$^{2+}$ in blood plasma for 174 h revealed <2% of the copper(II) dissociated from the complex(*25*).

Preclinical evaluation of a sarcophagine ligand functionalised with Tyr$^3$-octreotate, $^{64}$Cu-CuMeCOSar-Tyr$^3$-octreotate, ($^{64}$Cu-CuSarTATE, Figure 1), in a murine xenograft model, revealed high the uptake of $^{64}$Cu-CuSarTATE in somatostatin receptor-expressing tumors at 2 hours post injection, 63.0 ± 15.0 % injected activity per gram (%IA/g) and remained high 24 hours after injection (105 ± 27 %IA/g)(*29*). This agent was subsequently evaluated in 10 patients with neuroendocrine neoplasia and

displayed high and late retention in tumor tissue suggesting it is a suitable for diagnostic for prospective dosimetry for $^{67}$Cu-CuSarTATE PRRT(*30*).

In this work, we confirm the $^{64}$Cu-CuSarTATE binds to somatostatin subtype 2a receptor positive tumors in a AR2J (rat pancreatic exocrine tumor) xenograft model using PET imaging and then compare the therapeutic efficacy of $^{67}$Cu-CuSarTATE to $^{177}$Lu-LuTATE, which has been proven to be effective in multiple studies(*31*).

< insert Figure 1 here>

## MATERIALS AND METHODS

### Materials

SarTATE was prepared by Auspep (Tullamarine, Melbourne, Victoria 3043, Australia) using a modified version of a method reported previously(*29*). $^{64}$Cu-$CuCl_2$ (700 MBq in 0.05 M HCl was provided by the Molecular Imaging and Therapy Research Unit, SAHMRI, Adelaide 5000, South Australia, Australia. $^{67}$Cu-$CuCl_2$ was provided by ISU Idaho Accelerator Center, Pocatello, ID, USA as a solution (up to 4 GBq) in 0.05-0.1 M HCl). $^{177}$Lu-LuTATE was prepared by the in-house Radiopharmacy, Peter MacCallum Cancer Centre following standard protocols(*32*).

### Preparation of $^{64}$Cu-CuSarTATE

$^{64}$Cu-CuSarTATE was prepared on the iPhase Multisyn synthesiser unit using a modified version of a previously described method.(*29,30*) Briefly: $^{64}$Cu-$CuCl_2$ (700 MBq in 0.05 M HCl (300 µL)) was added to SarTATE (20 µg, 13.7 nmol) in a solution of 10% ethanol in 0.1 M ammonium acetate (5 mL) containing gentisic acid, sodium salt (38 mg). The reaction mixture was incubated for 25 min at room temperature and then passed through a Strata X 33 µm Polymeric Reverse Phase cartridge (Phenomenex, Inc.). Cartridge-retained $^{64}$Cu-CuSarTATE was rinsed with saline for injection, then eluted with ethanol followed by saline for injection into a vial. The contents of this vial were filtered through a 0.22-µM filter. $^{64}$Cu-CuSarTATE was recovered in 60%–80% radiochemical yield with more than 95% radiochemical purity.

### Preparation of $^{67}$Cu-CuSarTate

Briefly, $^{67}$Cu-$CuCl_2$ (~ 4 GBq, 0.05-0.1 M HCl) was added to SarTATE (60 µg, 41.2 nmol) in a solution of 10% ethanol in 0.1 M ammonium acetate (5 mL) containing gentisic acid, sodium salt (38 mg). The

reaction mixture was incubated for 30 min at room temperature and then passed through a Strata-X 33 µm Polymeric Reverse Phase (Phenomenex, Inc.) cartridge (30 mg). Cartridge-retained $^{67}$Cu-CuSarTATE was rinsed with saline for injection before elution with ethanol into a vial containing saline for injection. The contents of this vial were filtered through a 0.22-mM filter to give $^{67}$Cu-CuSarTATE 60%–80% radiochemical yield with more than 95% radiochemical purity.

**PET Imaging and Biodistribution Studies**

All *in vivo* studies were performed with the approval of the Peter MacCallum Cancer Centre Animal Experimentation Ethics Committee and in accordance with the Australian code for the care and use of animals for scientific purposes, 8$^{th}$ Edition, 2013. AR42J cells were obtained from the the American Type Culture Collection (ATCC). Cells (3 x 10$^6$) in 50% Matrigel in PBS were implanted subcutaneously into the right flank of 6-7 week old female Balb/c nude mice (Australian Bioresources, Australia). Once tumors reached a volume of 100-150 mm$^3$, mice were injected intravenously with 3 MBq (0.24 nmol) $^{64}$Cu-CuSarTATE. At 1 hr and 4 hr post injection the mice anaesthetised with 2% isoflurane in oxygen and placed into a G8 PET/CT scanner (Perkin Elmer). A CT image was then acquired over 1 min followed by a 10 min static PET image. The PET images were reconstructed using the on-board maximal likelihood and expectation maximization (ML-EM) algorithm and then analysed using VivoQuant (Invicro) software package. On completion of the 4 hr imaging, the mice were euthanised. Organs (blood, lungs, heart, liver, kidneys, muscle, spleen and tumor) were excised, weighed and the amount of radioactivity present in each organ was quantified using a Capintec (Captus 4000e) gamma counter. Data are presented as percent injected activity/gram tissue.

**In vivo Therapy Studies**

In the therapy experiments, Balb/c nude female mice with subcutaneous AR42J xenografts (100-150 mm$^3$) were either intravenously injected with vehicle (saline), 5 MBq (0.07 nmol) of $^{177}$Lu-LuTATE, or 5 MBq (0.37 nmol) of $^{67}$Cu-CuSarTATE) in a final volume of 100 µL (n = 7 for each group), or intravenously injected with vehicle (saline), with 30 MBq (0.4 nmol) of $^{177}$Lu-LuTATE or 30 MBq (2.2 nmol) of $^{67}$Cu-CuSarTATE) either as a single administration or as a fractionated dose 14 days apart (15 MBq x 2) (n=8 for each group). Mice were monitored twice weekly for tumor growth using calipers and general health. Mice were euthanised when tumor volume exceeded 1200 mm$^3$. Tumor volume (mm$^3$) was calculated as length (mm) x width (mm) x width (mm)/6.

**Data Analysis**

Percentage tumor growth inhibition (TGI) was calculated as 100 x (1- ΔT/ΔC) where ΔC and ΔT were determined by subtracting the mean tumor volume (in the vehicle control and treated groups, respectively) on day 1 of treatment, from the mean tumor volume on the last day all mice remained in the study. Statistical analysis was performed using Graph Pad Prism 8.0 (Graph Pad La Jolla, CA). An ANOVA analysis was performed followed by Dunnett's post hoc test to compare the tumor growth in the treated groups to the vehicle control. Survival curves were analysed using the Mantel Cox log rank test wheresurvival was defined as time for a tumor to reach a volume of ≥1200 $mm^3$.

**RESULTS**

**Synthesis of $^{64}$Cu-CuSarTATE and $^{67}$Cu-CuSarTATE**

Both $^{64}$Cu-CuSarTATE and $^{67}$Cu-CuSarTATE could be prepared in ammonium acetate buffer at room temperature in 30 minutes to give the complexes in 60-80 % radiochemical yield with >95% radiochemical purity.

**$^{64}$Cu-CuSarTATE has High Tumor Uptake in Somatostatin Receptor 2 Positive AR42J Xenograft Model**

PET images were acquired following administration of $^{64}$Cu-CuSarTATE (3 MBq, 0.24 nmol) *via* tail-vein injection to AR42J tumor bearing female Balb/c nude mice. PET/CT images at 1 h and 4 h post-injection revealed very high tumor uptake and low background (Figure 2). The tumors were clearly identified at 1 h after injection ($SUV_{max}$ 16.3 ± 1.7, 1 h post-injection) with excellent tumor to background ratios (66 ± 7 at 1 h post-injection). Clearance over 4 h leads to a further increase in the tumor to background ratios (76 ± 7; P = 0.027 versus 1 h). The high tumor uptake was confirmed with an *ex vivo* biodistribution analysis where the animals were euthanized after imaging at 4 h post-injection and the amount of radioactivity in the tumor and organs was quantified (Figure 2b). The tumor uptake 4 h post-injection was 61.8 ± 2.4 % injected activity per gram (%IA/g), kidney uptake was 16.0 ± 0.7 %IA/g and lung uptake was 12.2 ± 0.8 %IA/g.

< Insert Figure 2 here >

**$^{67}$Cu-CuSarTATE is Highly Efficacious Against AR42J Tumors *In vivo***

Mice were inoculated with AR42J cells and once the tumors reached a volume of approximately 150 mm$^3$ the mice were randomized to receive saline, $^{177}$Lu-LuTATE or $^{67}$Cu-CuSarTATE *via* intravenous injection. The treatments were well tolerated causing only a transient reduction in animal body weight that did not exceed 5 % of that at baseline (Fig 3A). Seven days after a single administration of of $^{67}$Cu-CuSarTATE (5 MBq, 0.37 nmol) tumor growth was inhibited by 75% ($P$= 0.0001 vs control) compared to vehicle control (Figure 3B). Administration of $^{177}$Lu-LuTATE (5 MBq) inhibited tumor growth by 89% ($P$≤0.0004 vs control) (Figure 3B). Survival, defined as time to tumor volume greater than 1200 mm$^3$, was extended from 12 days in the control group to 21 days following following treatment with both $^{67}$Cu-CuSarTATE and $^{177}$Lu-LuTATE (Fig 3C).

< Insert Figure 3 here >

In a second study, the efficacy of fractionated delivery of PRRT was assessed. AR42J tumor bearing mice were treated with a total of 30 MBq $^{67}$Cu-CuSarTATE or $^{177}$Lu-LuTATE, either as a single intravenous injection or as two 15 MBq fractions two weeks apart. All treatments were well tolerated (Fig 4A) and induced tumor stasis with reinitiation of tumor growth seen earlier in the single fraction treated groups (Fig 4B). Futhermore, treatment of tumors with two fractions significantly improved survival when compared to delivery as a single fraction $^{67}$Cu-CuSarTATE: 47 vs 36 days; $P$= 0.036; $^{177}$Lu-LuTATE: 46 vs 29 days; $P$=0.00 (Fig 4C). Equivalent efficacy was observed between $^{67}$Cu-CuSarTATE and $^{177}$Lu-LuTATE following treatment on both the single and fractionated schedules (P = n.s.).

< insert Figure 4 here >

**DISCUSSION**

A sarcophagine cage amine ligand, MeCOSar, conjugated to (Tyr$^3$)-octreotate ($^{64}$Cu-CuSarTATE) was demonstrated to be an effective imaging agent and potential prospective dosimetry tool in ten patients with neuroendocrine tumors.(*30*) In this study, we aimed to investigate the therapeutic potential of $^{67}$Cu-CuSarTATE.

The previous preclinical evaluation of $^{64}$Cu-CuSarTATE for PET imaging somatostatin positive tumors(*29*) used a tumor model in which somatostatin receptor 2 is overexpressed through viral tranfection (A427-7)(*33*). In this work a different tumor cell line with endogenous expression of

somatostatin receptor 2 (AR42J) was used. This cell line is commonly used as a model of neuroendocrine tumor because of high somatostatin receptor 2 expression and reliable growth as a xenograft. This cell line was derived from a spontaneous pancreatic tumor in rats. The effective tumor targeting of $^{64}$Cu-CuSarTATE in the AR42J model was confirmed by PET imaging prior to proceeding with therapeutic evaluation of the ligand labelled with copper-67. High tumor uptake was evident in the PET images at 1 h post-injection and retention of this uptake led to images at 4 h post-injection with excellent tumor to background ratios (Figure 2). The high tumor uptake was confirmed by an ex-vivo biodistribution study (62 ± 2 %IA/g, 4 h post-injection).

Administration of either 5 MBq or 30 MBq, either as a single or fractionated dose, of $^{67}$Cu-CuSarTATE demonstrated similar efficacy as administration of the same activity of $^{177}$Lu-LuTATE. Both agents demonstrated significant reduction in tumor volume and increased life span (Figures 3 and 4). Fractionated dose protocols can lead to reductions in tumor burden with decreased toxicity by delivering a high cumulative dose of activity to the tumor whilst allowing non-target tissue to recover (*21*). It is also possible that cells in different phases of the cell cycle may be differentially sensitive to radiation and by fractionating doses, cells at different stages of the cell cycle may be more effectively targeted. Supporting the current clinical practice of performing several spaced cycles of PRRT, administration of a total of 30 MBq $^{67}$Cu-CuSarTATE or $^{177}$Lu-LuTATE, as two 15 MBq fractions two weeks apart, significantly improved survival when compared to delivery as a single fraction of 30 MBq.

The higher fraction of γ-emission of copper-67 when compared to lutetium-177 leads to 3.2 times higher γ-exposure per decay with a slightly less penetrating mean energy and will result in greater radiation cross dose to healthy tissues. This gamma cross exposure, however, has not been linked to adverse effects in lutetium-177 therapies where regions with primary uptake and exposure to beta electrons being most strongly implicated in tissue effects. The higher fraction of γ-emmision for copper-67 may be beneficial in terms of SPECT imaging potentially allowing dose verification following treatment by performance of SPECT with CT attenuation correction(*34*). By comparison, iodine-131 has approximately 12-fold higher γ-emission per decay compared to lutetium-177 and coupled with a greater mean gamma energy raises a number of radiation protection considerations. In the USA, the NUREG guidance indicate that up to 14 GBq of copper-67 can be administered on an outpatient basis if the external dose rate at 1 meter is less than 0.22 mSv/h(*35*). Extrapolation of measured patient dose rate data have suggested no issue with release of patients who have received up to 5 GBq of $^{67}$Cu-CuSarTATE (*36*).

Importantly, $^{67}$Cu-CuSarTATE displayed similar efficacy to $^{177}$Lu-LuTATE. The energy of the $\beta^-$-emissions from copper-67 are similar in energy to the the $\beta^-$-emissions from lutetium-177 but the significantly shorter half-life of copper-67 (2.58 d vs. 6.71 d) provides a higher dose-rate. The ability to perform prospective dosimetry is an advantage for agents that use the copper-64/67 theranotic pair, especially in patients with a large tumor burden (*37*) or impaired renal function and in the paediatric setting where PRRT is an emerging treatment for advanced neuroblastoma (*38*). It is acknowledged that diagnostic imaging and therapy often performed using different quantities of administered peptide and this needs to be considered as biodistribution can change depending on the amount of peptide injected. However, initial data on the biodistribution and radiation dosimetry of $^{64}$Cu-CuSarTATE and $^{67}$Cu-CuSarTATE in meningioma patients showed similar tumor clearance for the two agents and consistent organ dose estimations(*39,40*). In terms of the potential translation of the copper-64/67 theranostic pair to clinical studies it is pertinent that copper-64 is produced on a cyclotron and its half-lifec enables distribution to sites without on-site radiochemistry facilties and permits imaging at later times points than what is possible with gallium-68 based agents. In addition, copper-67 can be produced with e-accelerators in high specific activity (>150 Ci/mg) and radionuclide purity >99%, so its production is not reliant on nuclear reactors.

## CONCLUSION

As anticipated, $^{67}$Cu-CuSarTATE is well tolerated in Balb/c nude mice and highly efficacious against AR42J tumors *in vivo*. Administration of $^{67}$Cu-CuSarTATE and $^{177}$Lu-LuTATE divided into two fractions over two weeks was more efficacious than that of a single fraction. The antitumor activity of $^{67}$Cu-CuSarTATE in the AR42J tumor model suggests this novel agent warrants clinical assessment for the treatment of somatostain expressing neuroendocrine tumors.

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KEY POINTS

QUESTION: Is peptide receptor radionuclide therapy with $^{67}$Cu-CuSarTATE efficacious against a somatostatin positive xenograft model?

PERTINENT FINDINGS: $^{67}$Cu-CuSarTATE is well tolerated in Balb/c nude mice and is highly efficacious against AR42J tumors *in vivo*. The efficacy of $^{67}$Cu-CuSarTATE is similar to $^{177}$Lu-LuTATE.

IMPLICATIONS FOR PATIENT CARE: $^{64}$Cu-CuSarTATE offers the potential for diagnostic PET imaging to support prospective dosimetry for therapeutic treatment with $^{67}$Cu-CuSarTATE.

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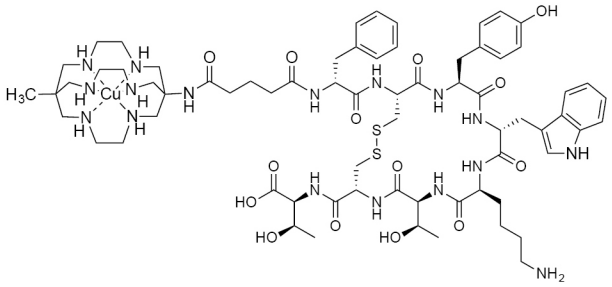

**Figure 1**. The chemical structure of $^{64/67}$Cu-CuSarTATE.

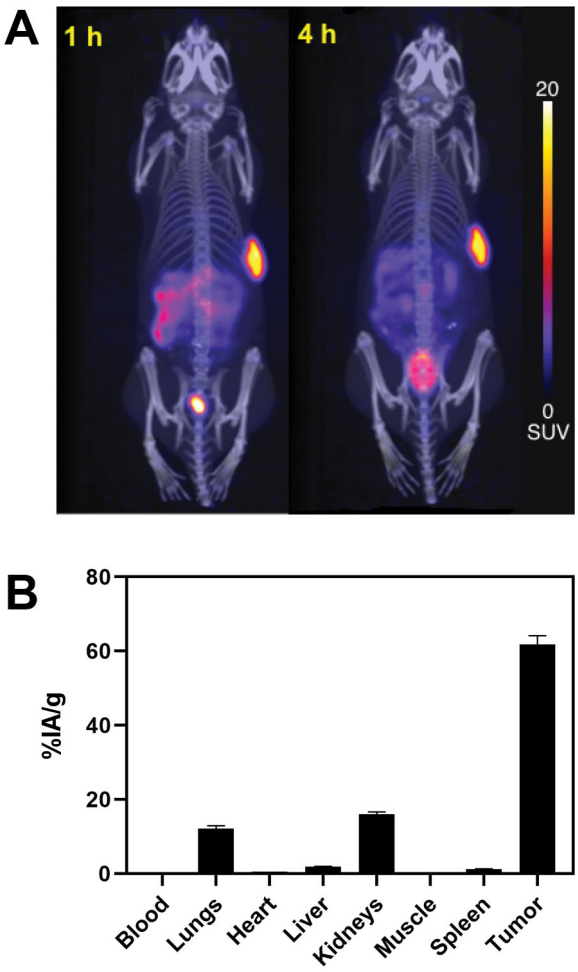


**Figure 2.** Representative maximum intensity projection PET/CT images of AR42J tumor bearing female Balb/c nude mice following injection of $^{64}$Cu-CuSarTATE (3 MBq, 0.24 nmol of peptide) at 1 and 4 hours post injection. b) *Ex vivo* biodistribution expressed as percent injected activity per gram tissue (%IA/g) (mean ± SEM, n = 3) was performed following imaging at 4 h after injection.

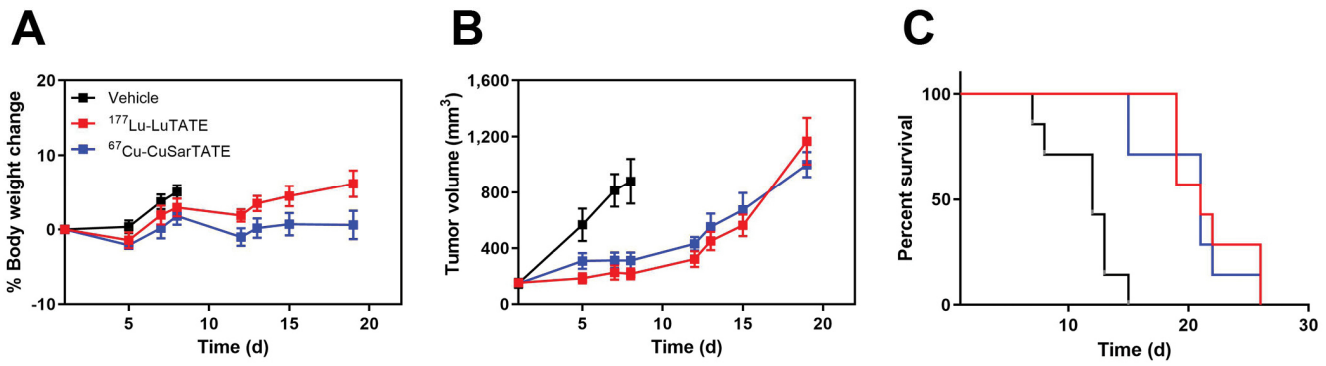


**Figure 3**. Inhibition of AR42J tumor growth by $^{67}$Cu-CuSarTATE. AR42J tumor bearing mice were treated with vehicle, 5 MBq $^{177}$Lu-LuTATE or 5 MBq $^{67}$Cu-CuSarTATE on Day 1 (vehicle and $^{177}$Lu-LuTATE) or Day 2 ($^{67}$Cu-CuSarTATE). Percent body weight change from baseline (a) and tumor volumes (b) were determined every 3-4 days. Data is shown until the first mouse from a group was removed from the study. Data is expressed as mean ±SEM; n=7 mice/group. Kaplan Meier survival analysis of data in (b) where survival was defined as time to tumor volume ≥1200 mm$^3$(c).

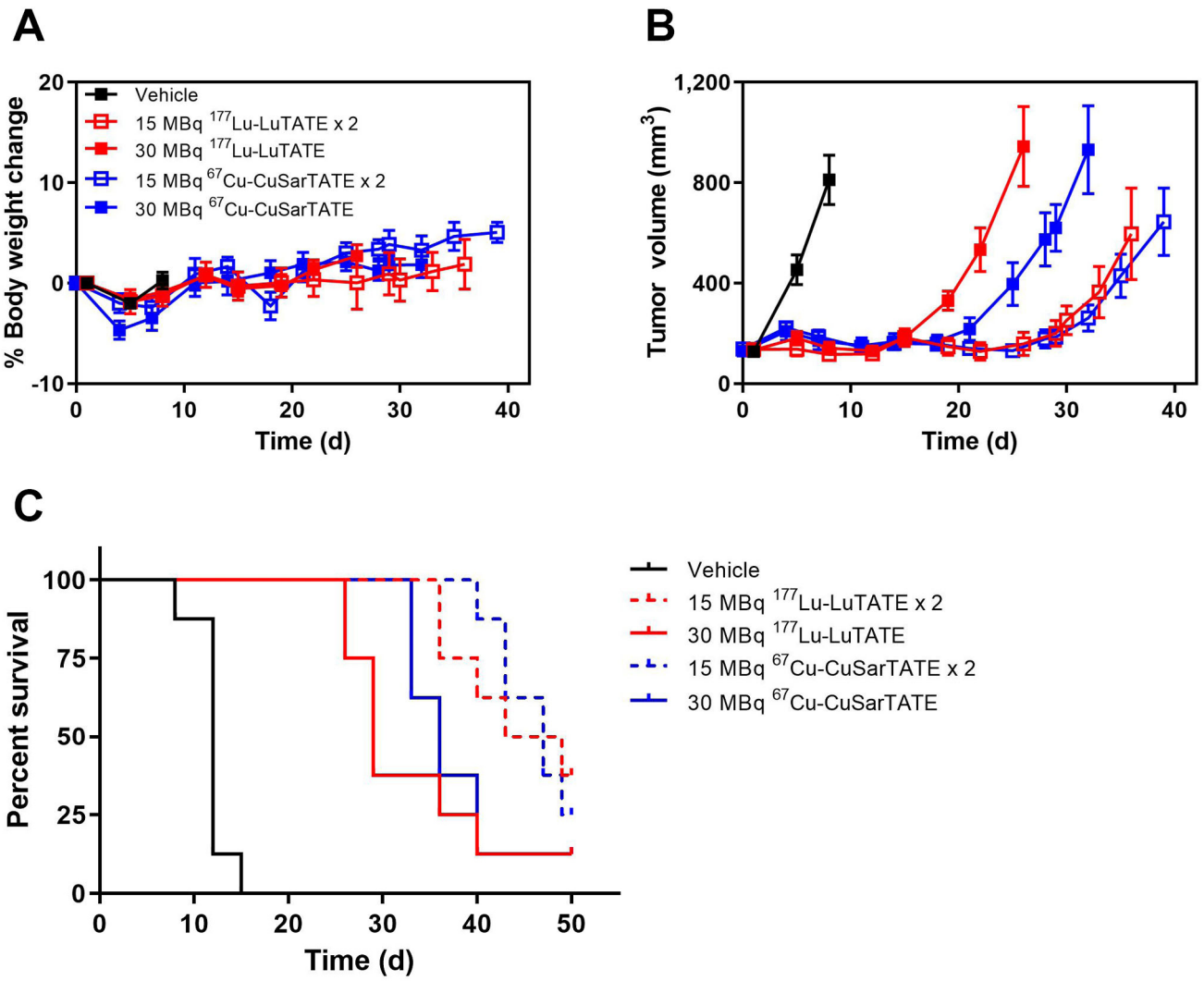


**Figure 4** Enhanced efficacy of fractionated $^{67}$Cu-CuSarTATE PRRT. AR42J tumor bearing mice were treated with vehicle on day 1, 30 MBq $^{177}$Lu-LuTATE on day 1, 30 MBq $^{67}$Cu-CuSarTATE on day 2, 15 MBq $^{177}$Lu-LuTATE on day 1 and 15 or 15 MBq $^{67}$Cu-CuSarTATE on days 2 and 16. Percent body weight change from baseline (a) and tumor volumes were determined every 3-4 days. Data is shown until the first mouse from a group was removed from the study. Data is expressed as mean ±SEM; n=8 mice/group. (c) Kaplan Meier survival analysis of data in (b) where survival was defined as time to tumor volume ≥1200 mm$^3$