^{89}Zr-DFO-TOC for PET Imaging of Somatostatin Receptor Positive Neuroendocrine Tumors

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Introduction

Neuroendocrine tumors (NETs) are clinically diverse types of tumors originating from neuroendocrine cells that can arise anywhere in the body. An estimated 12,000 people are diagnosed with NETs in the U.S. each year, with 1- and 5-year mortality rates reaching 27.2% and 60.4%, respectively, emphasizing the need for early diagnosis. Previous studies have shown that somatostatin receptors (SSTRs) are overexpressed on NET cell membranes relative to healthy tissue, allowing for tumor targeting through radiolabeled somatostatin analogues (SSAs). The FDA has already approved three positron-emitting radiopharmaceuticals for PET imaging of somatostatin receptor positive (SSTR+) NETs, making PET the leading diagnostic tool for NETs.

This work aims to develop a novel ^{89}Zr-labeled tracer incorporating the SSA, octreotide (TOC), for PET imaging of SSTR+ NETs, leveraging the excellent nuclear ($t_{1/2}$ = 3.27 days, $\beta+$ = 22.3%, βavg= 395.5 keV) and chemical characteristics (+4 oxidation state, preferential coordination number of 7/8, favorable aqueous chemistry) of ^{89}Zr. In combination with ^{89}Zr, the known in vivo stability of the chelator deferoxamine (DFO) gives reason to believe this radiopharmaceutical will be successful to study the suitability of detecting SSTR+ NETs [1-4].

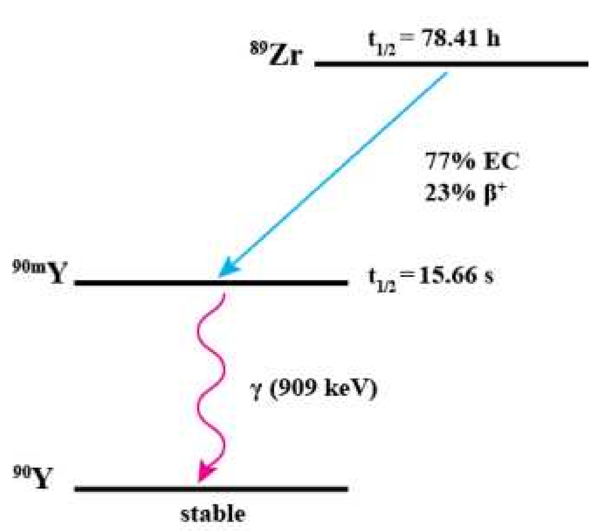

Figure 1: ^{89}Zr decay scheme.

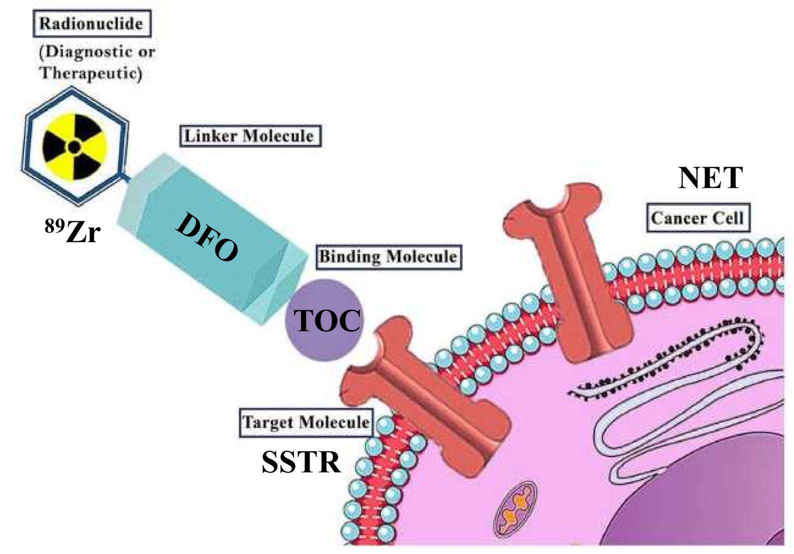

Figure 2: Model of ^{89}Zr-DFO-TOC targeting SSTR.

Methods

Radiochemical Tracer Assessment: ^{89}Zr-oxalate was obtained from the UAB Cyclotron Facility. 100 µCi of ^{89}Zr (pH 7.2) was incubated with varying concentrations of DFO-TOC in 0.5 M HEPES buffer for 30 minutes at 800 rpm and 37 °C. Radiochemical yield was determined via HPLC and iTLC. Stability on benchtop and in mouse serum were investigated at timepoints up to 9 days.

In Vitro Assays: Cell binding and competitive cell blocking assays were performed using 1 million cells/well of AR42J, PC-3, and PANC-1 cell lines, The cells were treated with 1 µCi ^{89}Zr-DFO-TOC (0.025 nmol binding, 100x excess blocking) at time points up to 9 days and assessed via gamma counting. The relative SSTR subtype expressions for each cell line are summarized in Table 1.

Table 1: SSTR subtype expression of cell lines [5-7].

Cell Line	Origin	SSTR Expression
AR42J	Epithelial-like cell isolated from the pancreas of a rat with a tumor[1]	SSTR1 (low) SSTR2 (high) SSTR3 (low SSTR5 (low)
PC-3	A cell line initiated from a bone metastasis of a grade IV prostatic adenocarcinoma from a 62-year-old, White, male[2]	SSTR2 (low) SSTR5 (moderate)
PANC-1	A cell line exhibiting epithelial morphology that was isolated from the pancreatic duct of a 56-year-old, White, male with epithelioid carcinoma	SSTR2 (low) SSTR5 (low)

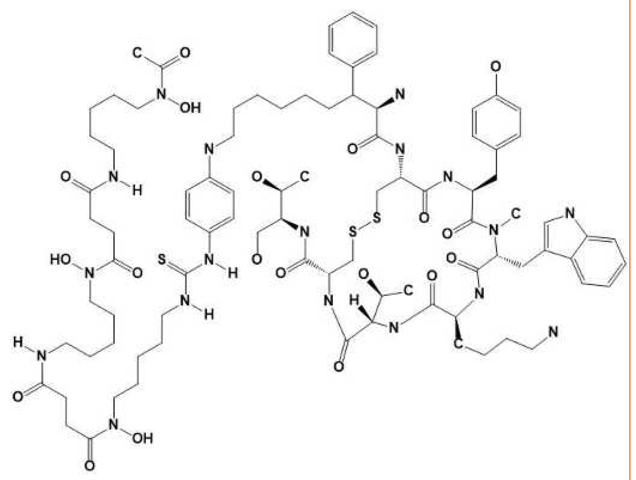

Figure 3: Structure of DFO-TOC.

Results

Radiochemical Tracer Assessment: Preliminary studies showed that amounts as little as 0.1 nmol DFO-TOC can be effectively radiolabeled with ^{89}Zr, as illustrated in Figure 4. This complex has been determined to be stable both on benchtop and in mouse serum at timepoints up to 9 days (Figure 5).

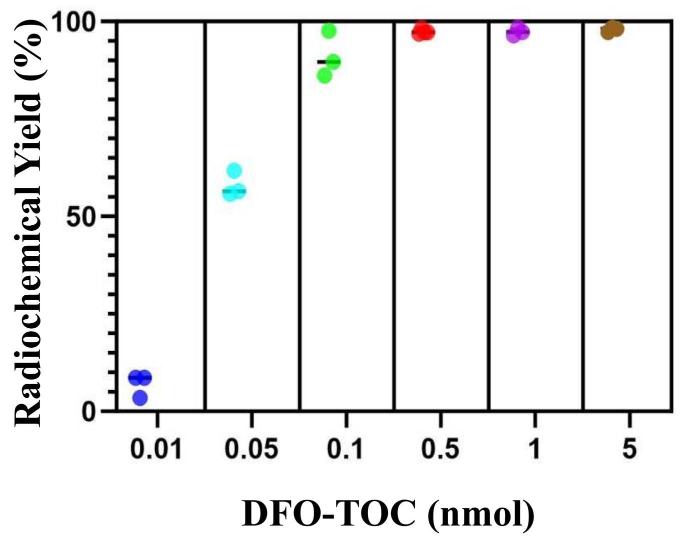

Figure 4: Radiochemical Yield of ^{89}Zr-DFO-TOC for varying DFO-TOC amounts.

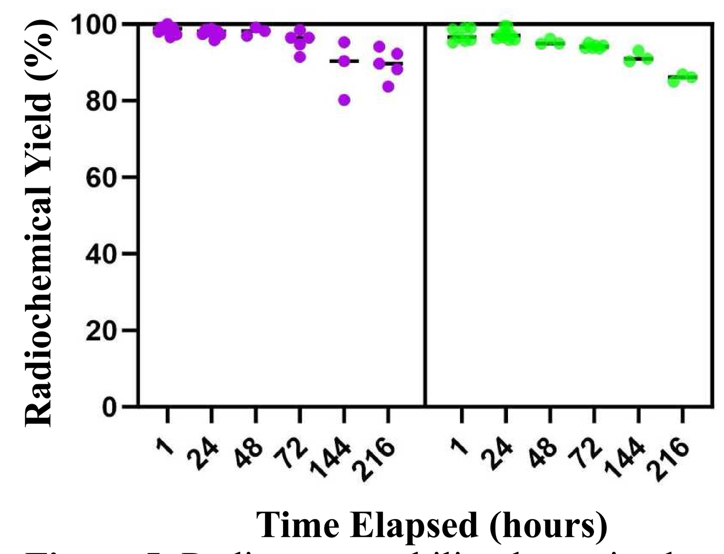

Figure 5: Radiotracer stability determined via HPLC on the benchtop (left) and in mouse serum (right).

In Vitro Assays: In vitro assays demonstrated a statistically significant difference in uptake between all cell lines (Figure 6). AR42J and PC-3 showed statistically significant ^{89}Zr-DFO-TOC uptake between total binding and blocking groups while PANC-1 showed no statistically significant difference.

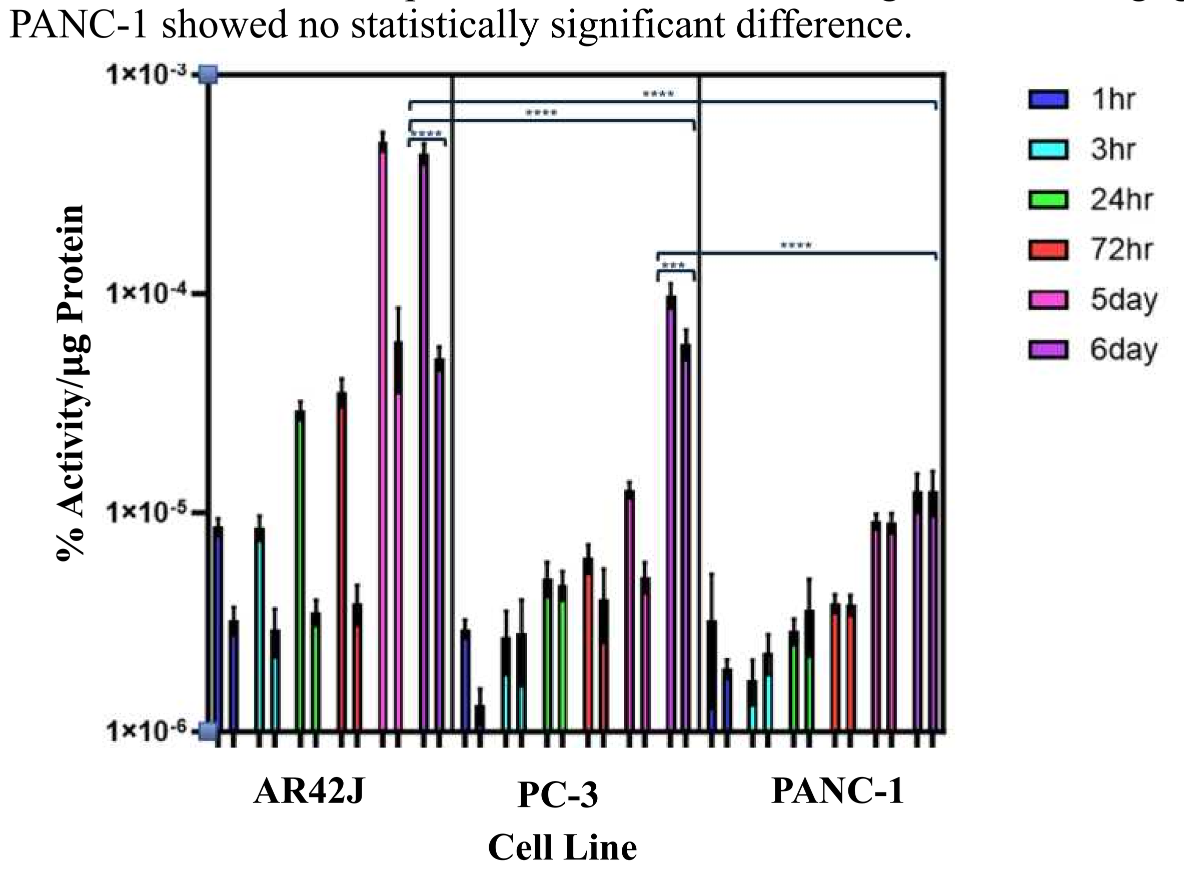

Figure 6: Cell binding assay comparing total binding and blocking of ^{89}Zr-DFO-TOC.

Discussion

This work demonstrated that high molar activity ^{89}Zr-DFO-TOC can be synthesized with high radiochemical purities under mild conditions necessary for in vivo studies. The stability of the tracer both on the benchtop and in mouse serum supports our hypothesis that the compound will potentially demonstrate enhanced stability in vivo.

The statistically significant difference between AR42J, PC-3, and PANC-1 cell uptake can be attributed to the relative SSTR expressions summarized in Table 1 and are consistent with the known binding affinities of TOC to SSTR2 and SSTR5. The statistically significant difference in uptake between binding and blocking groups for AR42J ($P < 0.0001$) and PC-3 ($P < 0.0005$) highlights the specificity of the radiotracer for the receptors. Although a statistically significant difference between binding and blocking groups was not observed for the PANC-1 cells, the results are consistent with the low SSTR subtype expressions.

Conclusion

In this work, ^{89}Zr-DFO-TOC was synthesized with radiochemical yields of up to 99% and was found to remain stable in vitro at extended timepoints. In vitro and in vivo studies are ongoing, and if successful, will demonstrate the ability to utilize the excellent nuclear and chemical properties of ^{89}Zr for sensitive detection of SSTR+ NETs, while providing information related to the dosimetry pertinent for future theranostic applications with long-lived therapeutic radionuclides, such as ^{177}Lu. This work has the potential to revolutionize patient care through earlier detection and improved NET diagnoses.

Future Work

- In vitro internalization assays using AR42J, PC-3, and PANC-1 cell lines
- In vivo PET imaging of murine models with SSTR+ xenograft tumors
- Therapy and dosimetry studies incorporating ^{177}Lu-DOTA-TOC
- Repeat studies using ^{89}Zr-DOTA-TOC to determine preferential binding

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Figure 7 (left): Alexis and Veronika Mocko performing experiments with radiolanthanides in the hot cell facility at LANL.

Figure 8 (right): Avery Pilot (TAMU), Alexis Sanwick, and Christine Lawrence (TAMU) posing in front of large hot cell 13.

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