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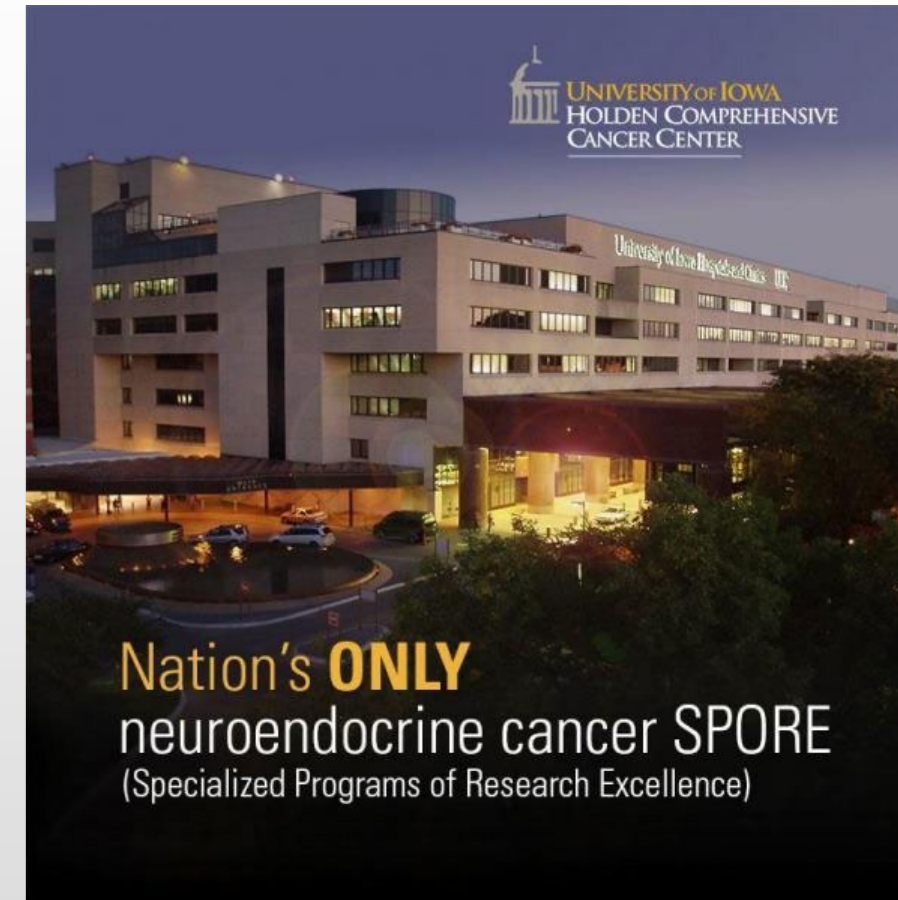


# Preclinical Evaluation of Chemokine Receptor 4 Antagonists for High Grade NETs and NECs Theranostics

Dijie Liu<sup>1</sup>, Mengshi Li<sup>2</sup>, Dongyool Lee<sup>2</sup>, Kimia Nourmahnad<sup>1</sup>, Nicholas J Baumhover<sup>3</sup>, Michael Schultz<sup>2,4</sup>, Andrew M Bellizzi<sup>5</sup>, M Sue O'Doriso<sup>1</sup>

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## Abstract

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**Background:** Treatment strategies targeting Somatostatin receptor subtype 2 (SSTR2) achieve partial response or stabilize disease and improve quality of life for patients with grade 1 and 2 neuroendocrine tumors (NETs), but have little effect for G3 NETs and neuroendocrine carcinomas (NECs), suggesting a critical need for new target. Preliminary data has validated positive chemokine receptor 4 (CXCR4) expression in patients' specimens and NET cell lines together with <sup>68</sup>Ga-Pentixafor (CXCR4 antagonist) PET/CT imaging in tumor-bearing mice. In this study, we determined CXCR7 expression which shares the same ligand as CXCR4 in NET cell lines, and further investigate known and newly designed CXCR4 antagonists for in vitro and in vivo evaluation to facilitate peptide-receptor radionuclide therapy in the future.

**Methods:** The expression of SSTR2, CXCR4 and CXCR7 on cells, mice tumor xenografts and patient tissue microarray, is determined by immunohistochemistry and/or flow cytometry. CXCR4 antagonists were radiolabeled with <sup>68</sup>GaCl<sub>3</sub> for PET/CT imaging in mice followed by biodistribution. <sup>90</sup>Y-, <sup>177</sup>Lu- or <sup>212</sup>Pb-antagonists were tested for in vitro binding. Effect of antagonists on chemotaxis signal is determined by cell migration and invasiveness assay (ongoing).

**Results:** Bon, GQP-1 and H727 cell lines express weak SSTR2 but moderate CXCR4 and CXCR7 by flow cytometry. H727 mouse xenograft expresses moderate CXCR4 while IMR32 the neuroblastoma xenograft expresses high CXCR4 by IHC and PET imaging. 85% of poorly-differentiated NECs patients demonstrated high CXCR4 expression. <sup>68</sup>Ga-CXCR4 antagonists showed high radiochemical purity as radiotracer for series of PET/CT imaging in tumor-bearing mice. Biodistribution displayed specifically tumor targeting, main kidney excretion and relatively high abdomen and blood retention.

**Conclusion:** <sup>68</sup>Ga-CXCR4 antagonists are specific PET/CT radiotracers for high-grade NETs and NECs diagnosis. Radio-therapeutic antagonists need further investigation of their safety and efficacy in G3 NETs and NECs.



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Abstract

Introduction

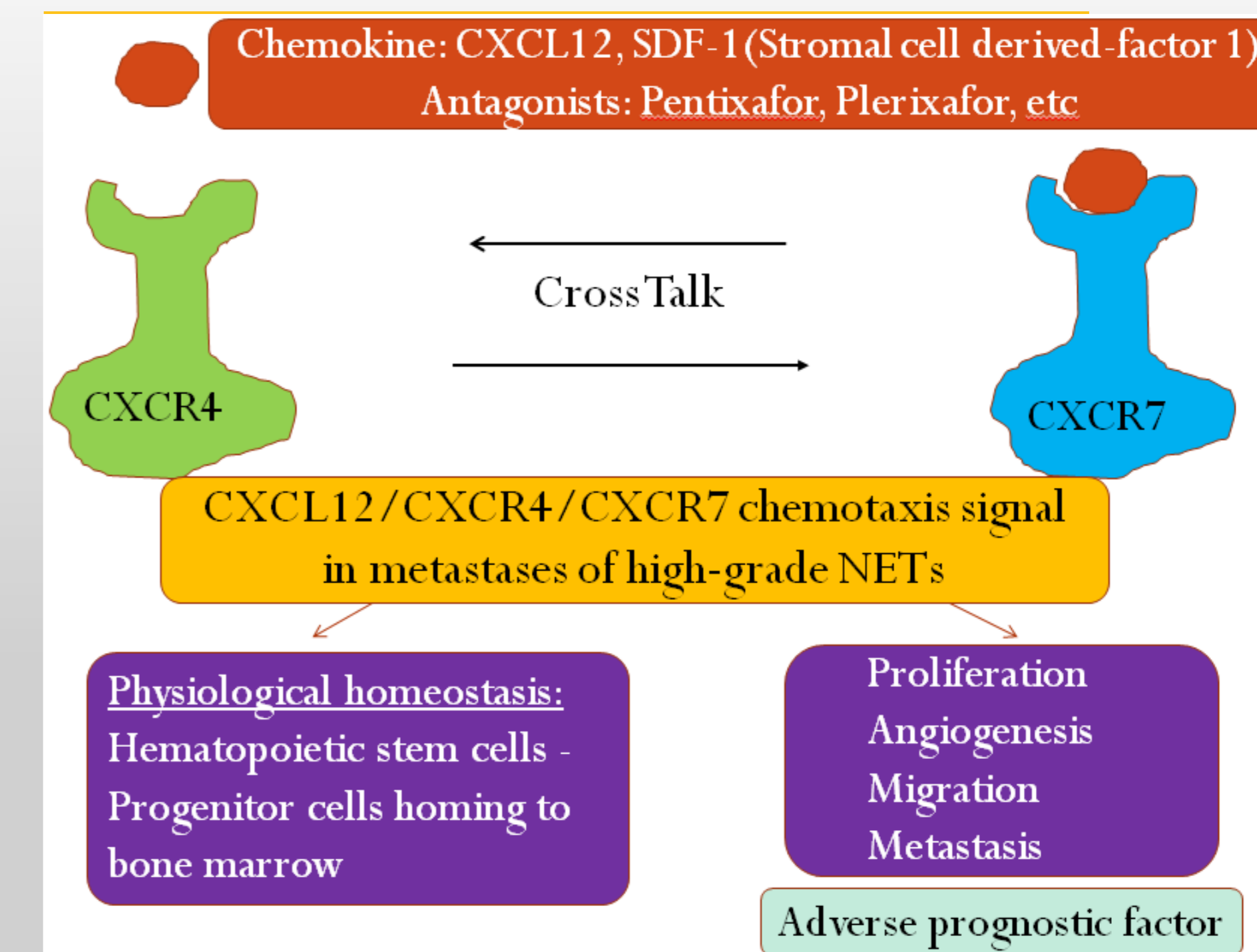
Methods

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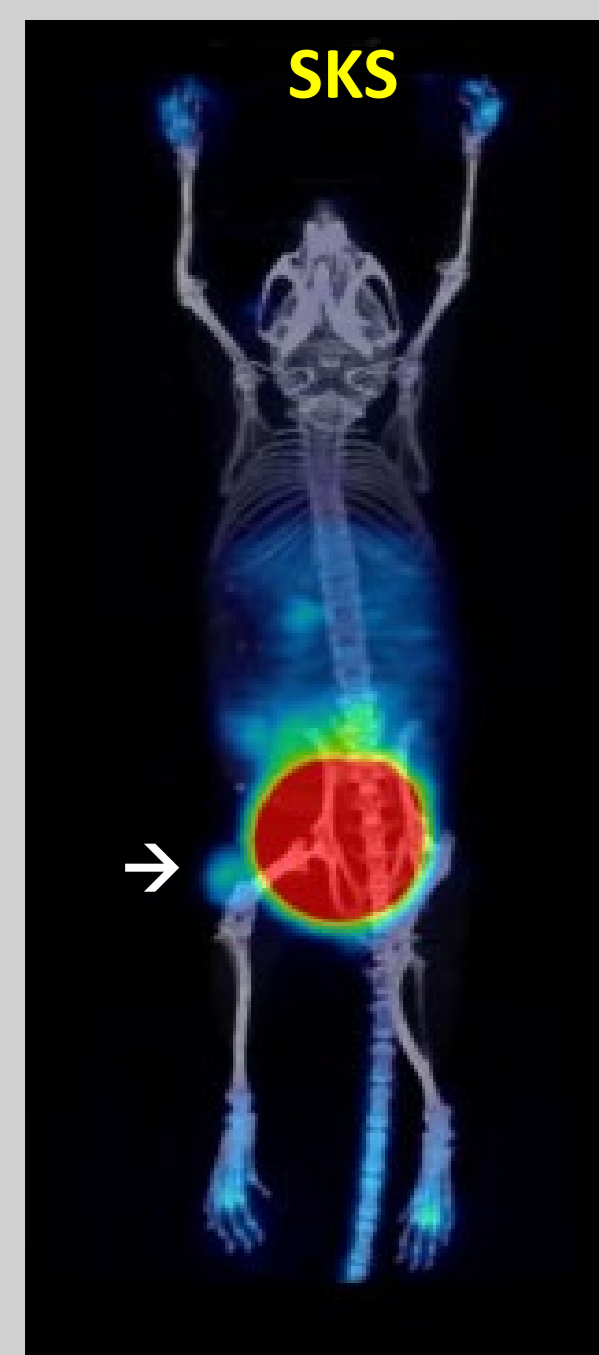
Conclusion

## Introduction

- Somatostatin receptor subtype 2 (SSTR2) targeting is a mainstay of diagnosis and therapy for low grade neuroendocrine tumors (NETs), and SSTR2 directed peptide-receptor therapy (PRRT) is effective in patients with G1 and G2 NETs.
- However, the approach is relatively ineffective for neuroendocrine carcinomas (NECs) and G3 NETs because SSTR2 expression is low in these tumor types. Though newly approved by FDA, Lutether (177Lu-DOTATATE) has demonstrated an 18% objective response.
- Thus, there is a critical need for new theranostic targets that are expressed in high-grade NETs and NECs.
- Chemokine receptor 4 (CXCR4): G-protein coupled receptors(GPCR) family, partial function CXCR7
- CXCR4 ligands: <sup>68</sup>Ga-Pentixafor/<sup>90</sup>Y-, <sup>177</sup>Lu-Pentixather potential for radio-theranostics



PET/CT imaging of <sup>68</sup>Ga-Pentixafor

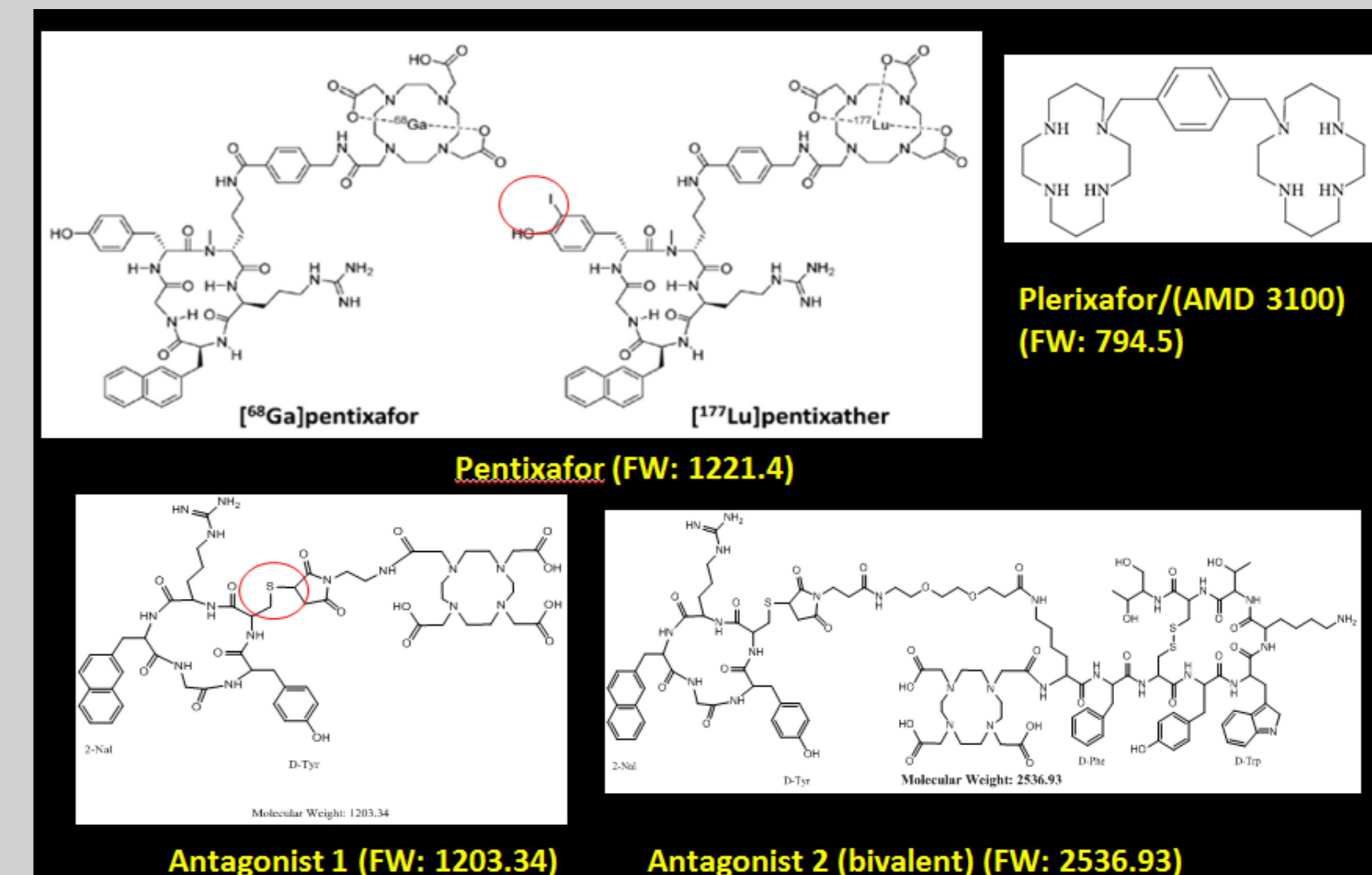


## Objective

In this study:

- We evaluate the expression of CXCR4 expression in NET cell lines, mice tumor xenografts and patient specimens;
- We evaluate the CXCR4 antagonists as imaging and therapeutic agents for theranostics in preclinical mice model bearing human NET or NEC tumor.

## Structure of CXCR4 ligands





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## Materials & Methods

### Tissue Microarray (TMA)



- **Cell lines.** SKS cells were obtained from the JCRB, Bon, QGP-1, SKNSH, SHSY-5Y and IMR-32 cells from ATCC and maintained according to recommended medium.
- **RNA isolation and GPCR array expression.** Total RNA was extracted using TriZOL, and cDNA was synthesized using High Capacity cDNA Reverse Transcription Kit and evaluated using Applied Biosystems TLDA GPCR array.
- **Tissue Microarray (TMA).** Specimens were collected from patients of NETs and NECs by the department of pathology and prepared for immunohistochemistry (IHC) staining for SSTR2 and CXCR4.
- **Flow cytometry.**  $1 \times 10^6$  cells were incubated with BV421-CXCR7, APC-CXCR4, PE-SSTR2 antibody. Cells were analyzed using a Beckmann Dickinson LSR-Violet Flow Cytometer. Hoechst channel was used for viability monitoring.

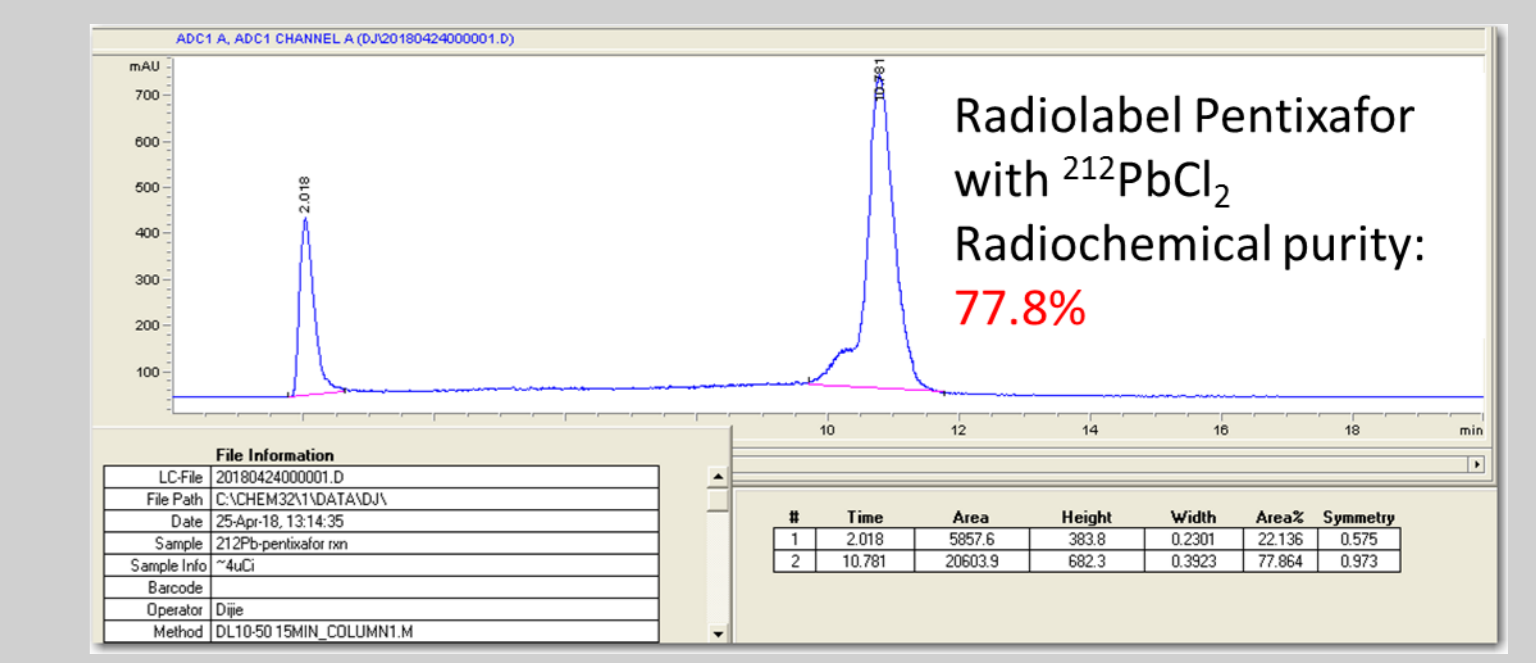
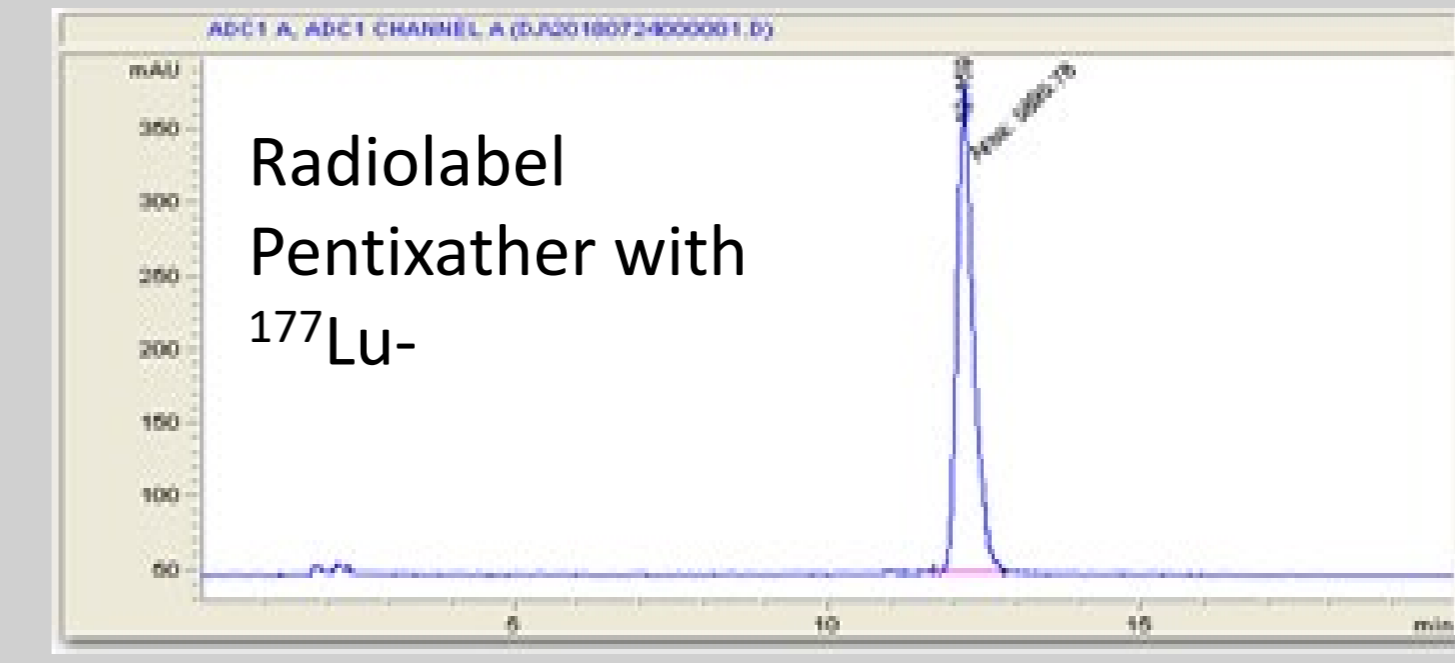
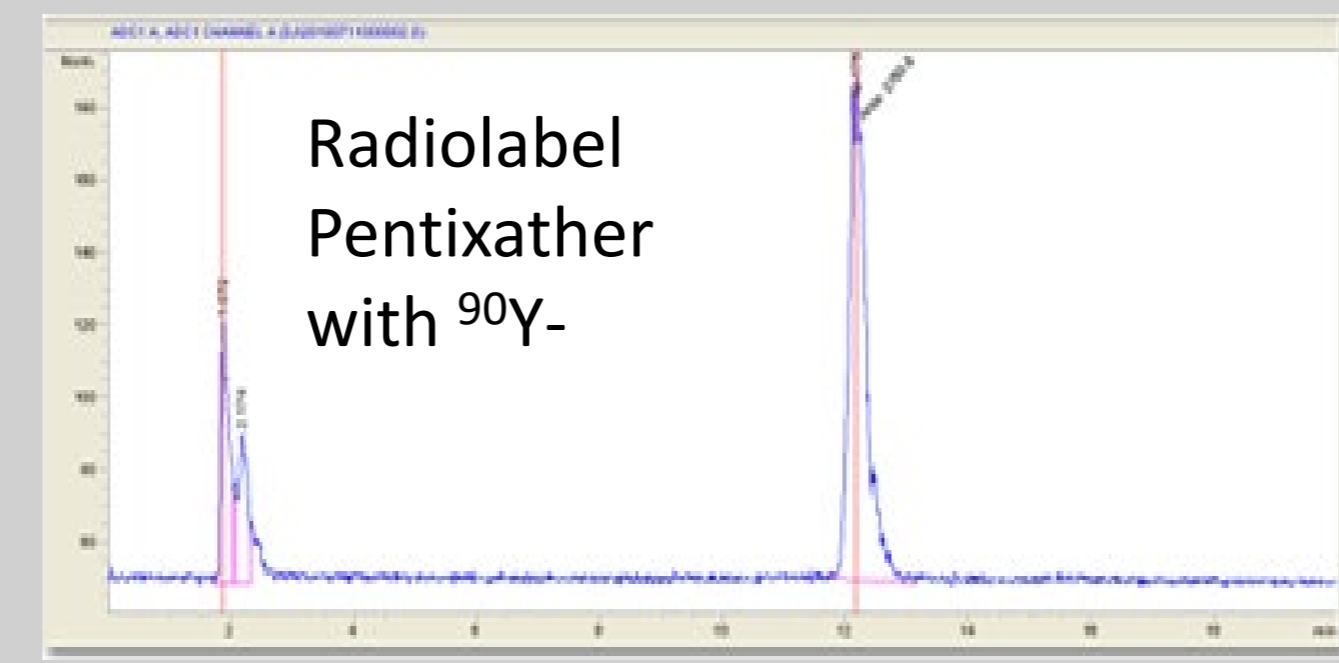
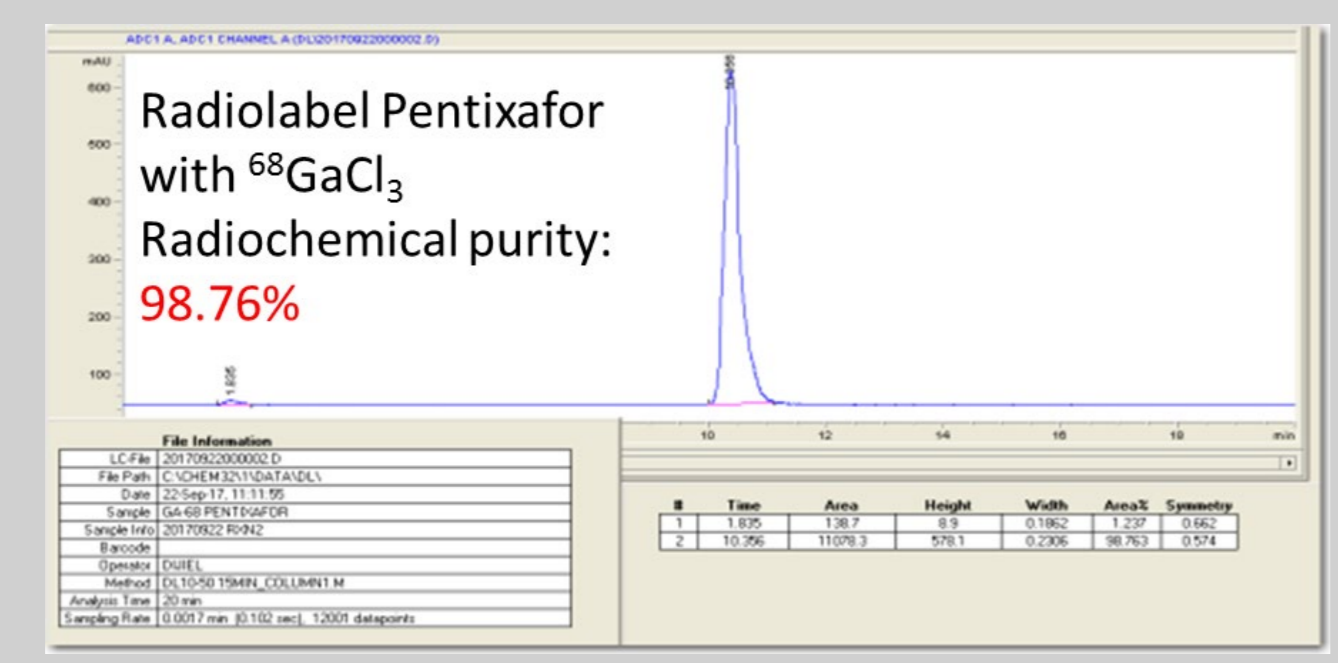
**Radiochemistry.** CXCR4 antagonists were labeled with in the acetate buffer at pH 5.3-5.5 with <sup>68</sup>Ga-, <sup>90</sup>Y-, <sup>177</sup>Lu-, or <sup>212</sup>Pb-, at 95°C for 15 min, purified by C18 exchange column in 50% ethanol solution.

In vitro binding. Radiolabeled ligands were incubated with cells for 45 min at RT. Cells were rinsed with binding buffer and lysed in 1 N NaOH for gamma counting or scintillation assay.

**In Vivo PET/CT imaging and biodistribution.** Tumor bearing mice were administered approximately 200 μCi of <sup>68</sup>Ga-CXCR4 antagonists via tail vein. PET/CT imaging was acquired by Siemens Inveon instrument and data was analyzed by Inveon Research Workshop. Blocking mice were co-injected 50 μg of Plerixafor in addition to radiotracer. Mice were euthanized at the end of imaging for ex-vivo biodistribution analysis. Biodistribution study was carried out in tumor bearing mice by injecting ~2 μCi of <sup>67</sup>Ga-Pentixafor. At different time points mice were euthanized and tissues and organs were dissected for gamma counting and calculated as percent of injected dose/gram (ID/g%).

## Radiochemistry

	<sup>68</sup> Ga-	<sup>203</sup> Pb-	<sup>90</sup> Y-	<sup>177</sup> Lu-	<sup>212</sup> Pb-
Pentixafor	√☺	√☺	√☺	Not Tested	√☺
Pentixather			√☺	√☺	
Antagonist 1	√☺	xx	√☺	Not Tested	√☺
Antagonist 2	√☺	xx	Not Tested	Not Tested	√ but low efficiency





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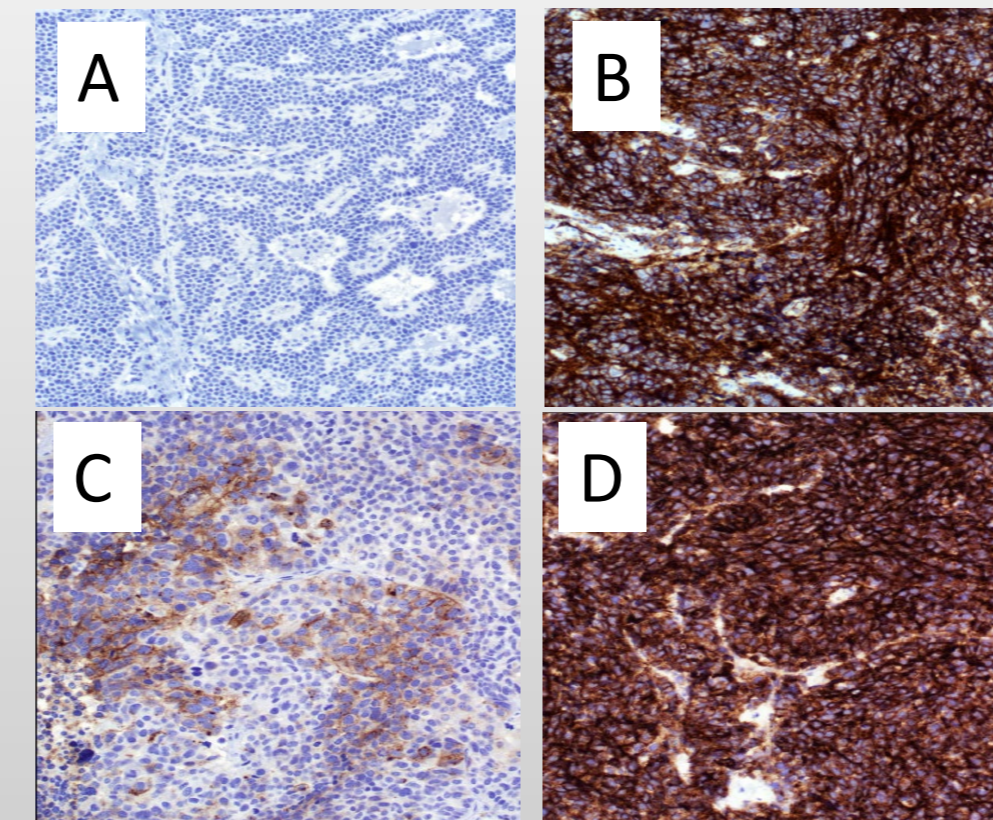
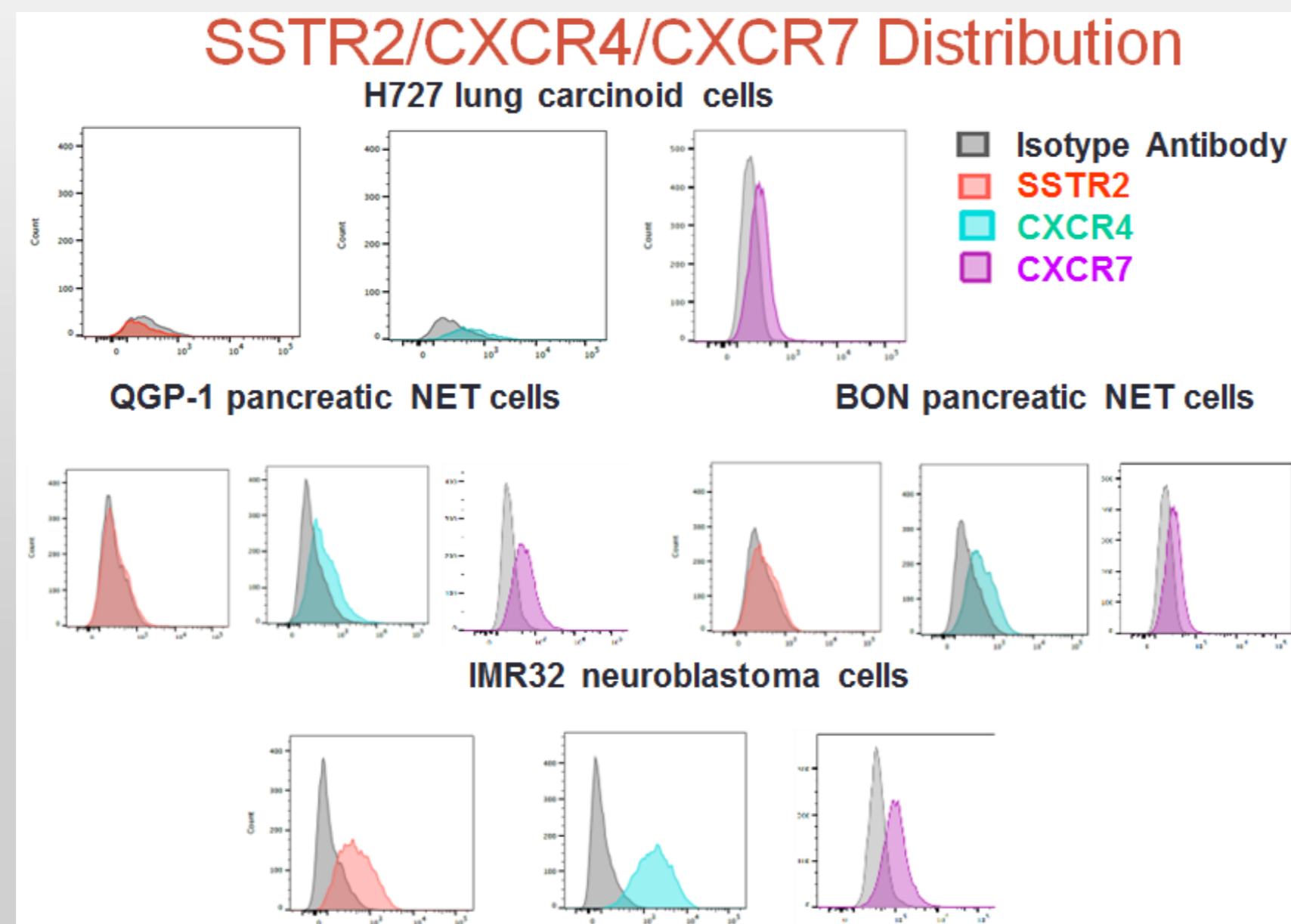
Departments of <sup>1</sup>Pediatrics and <sup>2</sup>Radiation Oncology, <sup>4</sup>Radiology, <sup>5</sup>Pathology, <sup>3</sup>State Hygienic Lab, University of Iowa, Iowa City, IA, USA

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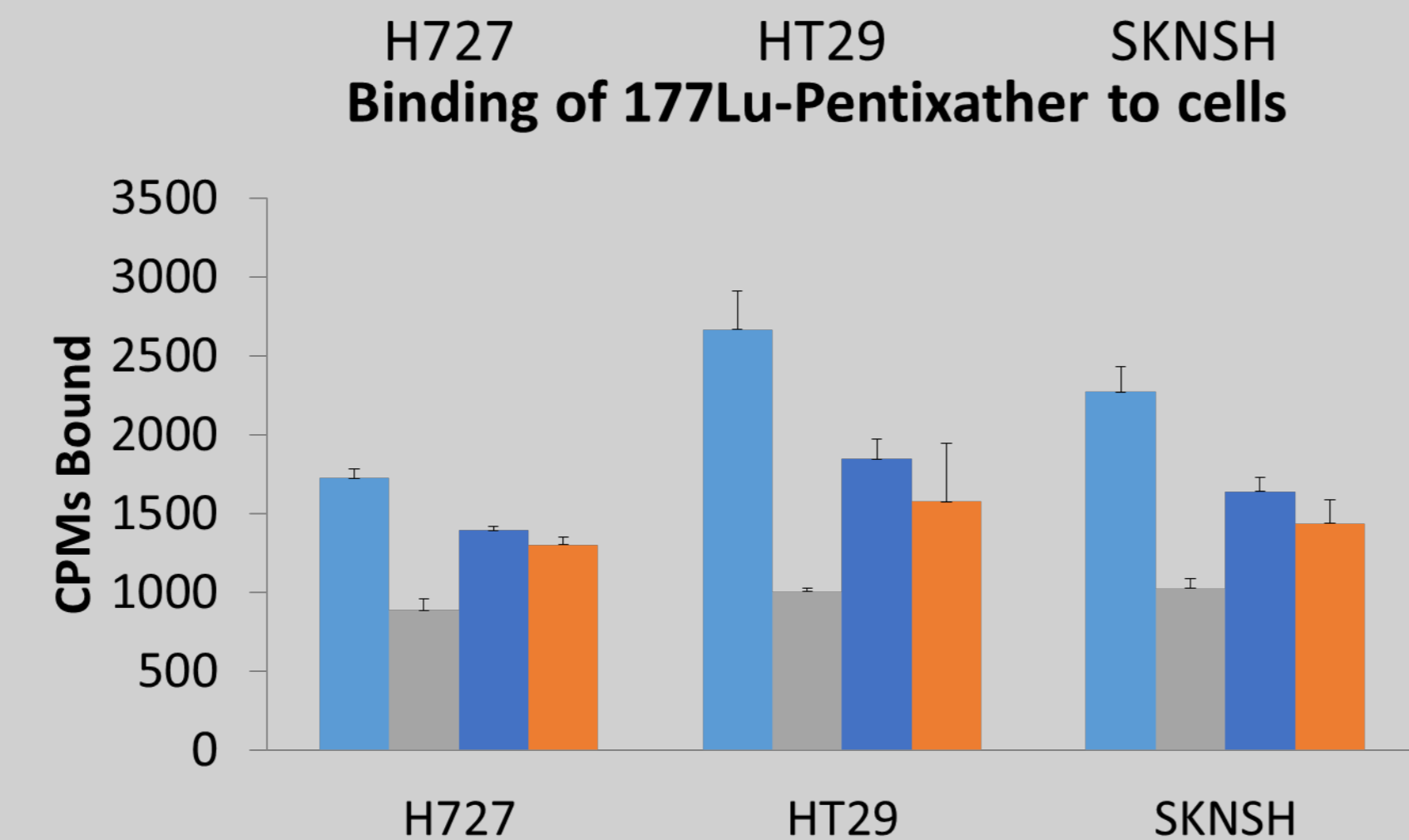
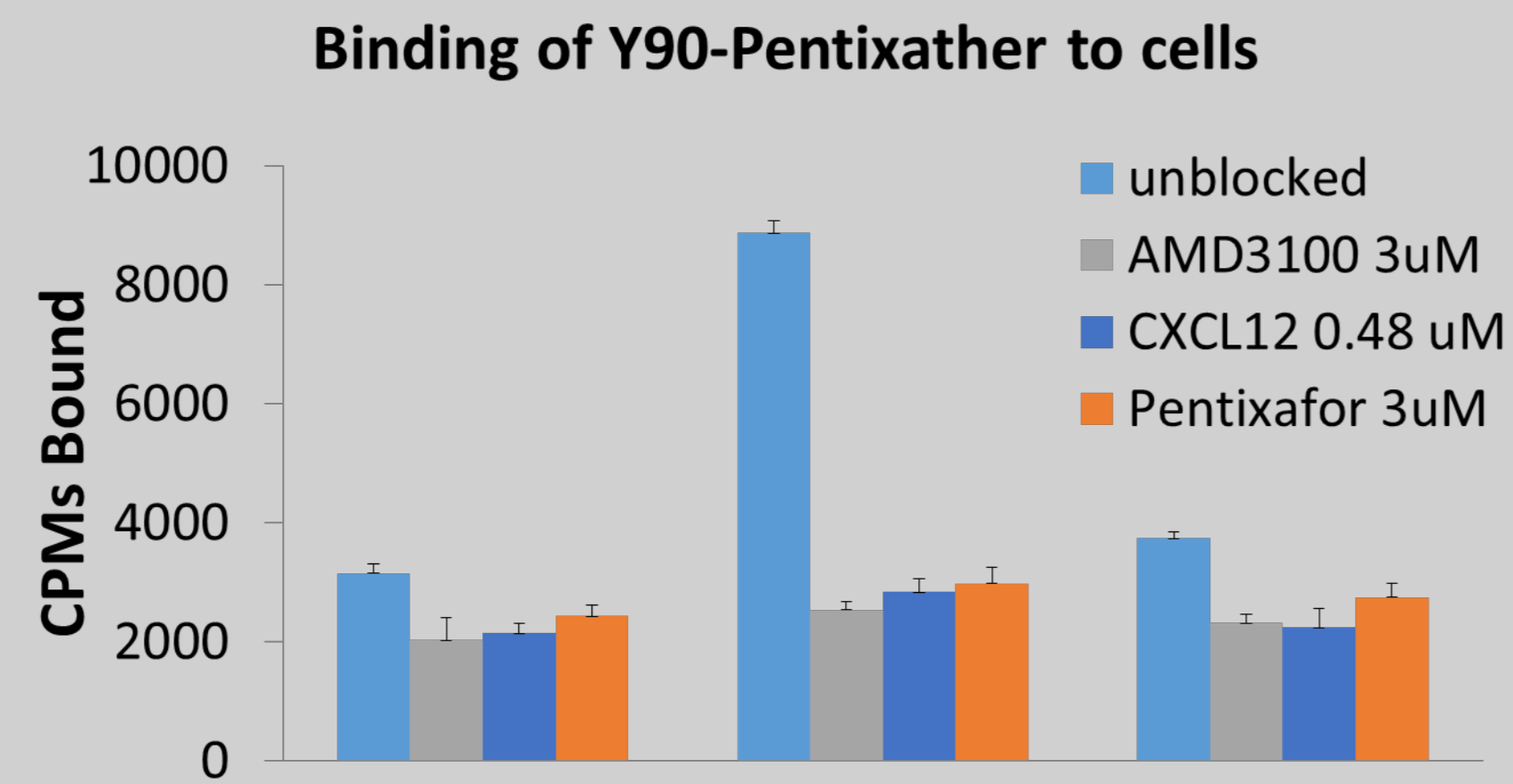
## CXCR4 Expression



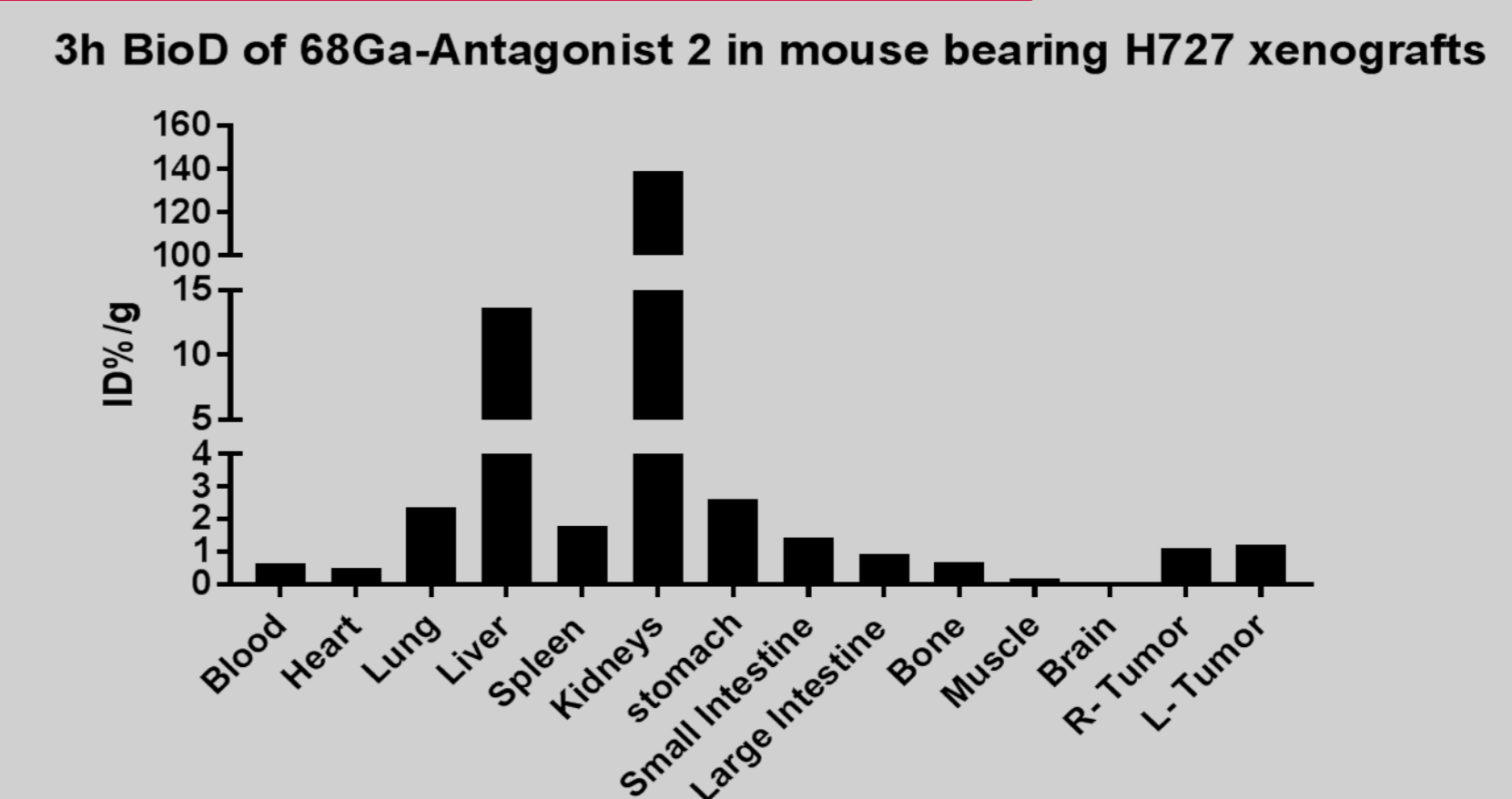
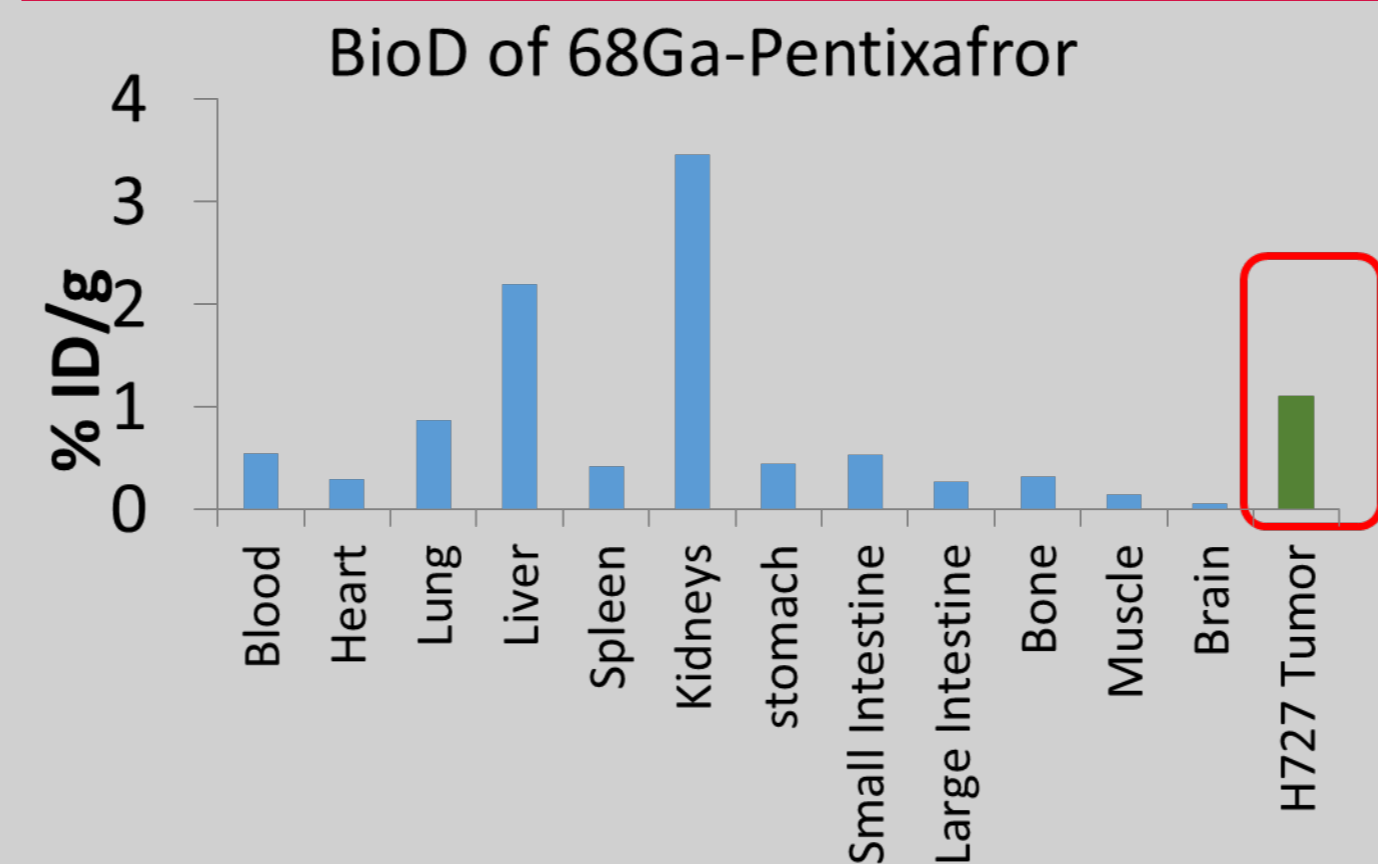
Iowa Clinical Validation	Positive	Mean H-score (if positive)
Well Differentiated NET	4.5% (3/66)	3
Poorly Differentiated NEC	<b>85% (82/97)</b>	<b>112</b>

CXCR4 is more preferentially expressed in NECs than NETs by IHC staining. Panels A to D are representative staining of CXCR4 in human NET ileal (A), human small cell lung origin NEC (B), mice xenograft bearing human lung carcinoid H727 NET cells (C) and mice xenograft bearing human neuroblastoma IMR32 cells (D). The tables summarize the CXCR4 level of TMA from the patients at the University of Iowa with low grade well differentiated NETs or high grade poorly differentiated NECs

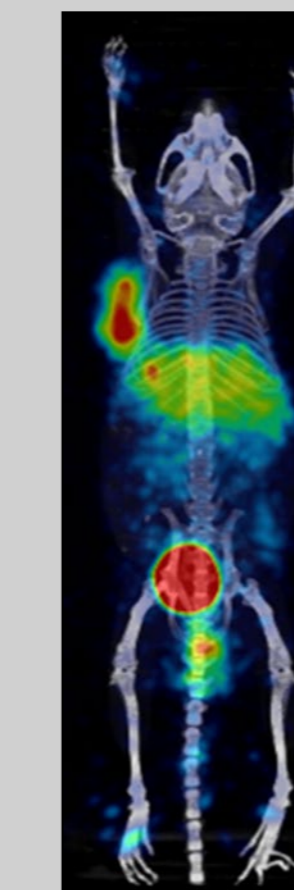
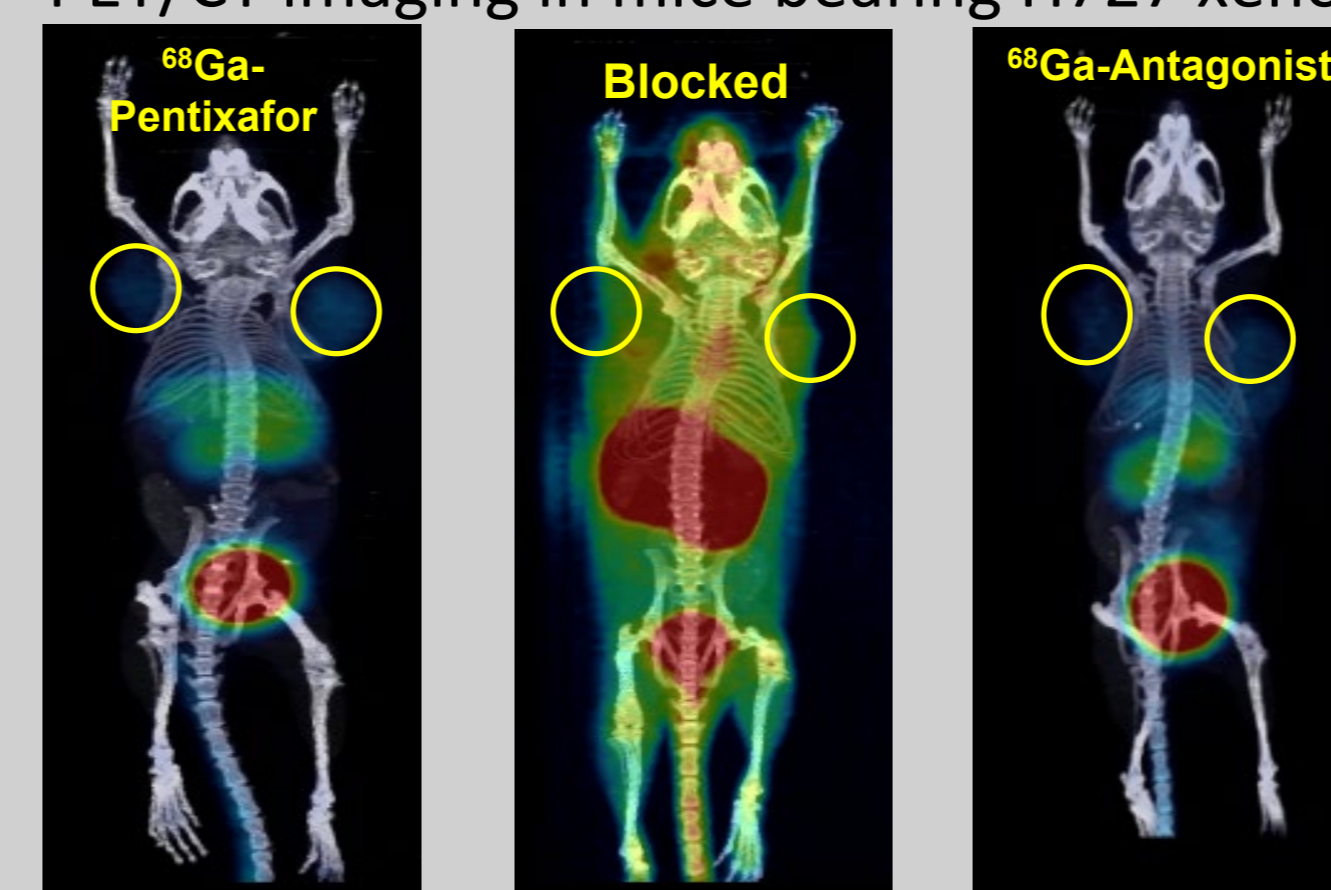
## CXCR4 Antagonists



## Biodistribution & Molecular Imaging



PET/CT imaging in mice bearing H727 xenograft



SPECT/CT imaging demonstrated strong tumor uptake of 177Lu-Pentixather in mouse bearing IMR32 xenograft (1 hr post-injection of 470  $\mu$ Ci). The medium to high kidney and liver uptake suggested the renal and hepatic excretion of the 177Lu-labeled radiopharmaceutical.



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## Conclusion

**<sup>68</sup>Ga-CXCR4 antagonists are specific PET/CT radiotracers for high-grade NETs and NECs diagnosis. Radio-therapeutic antagonists need further investigation of their safety and efficacy in G3 NETs and NECs, especially renal and hematotoxicity.**

## References

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