

Targeting Tumor-Associated Glycans Lewis^{a/c/x} and sialyl-di-Lewis^a for Near-infrared Fluorescent (NIRF) Intraoperative Imaging of Pancreatic Ductal Carcinoma: a Preclinical Evaluation

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INTRODUCTION

Tumor-targeted molecular imaging may overcome current limitations in preoperative and intraoperative diagnosis and delineation of tumor tissue. Aberrant glycosylation is a hallmark of cancer, potentially providing a new set of tumor-specific targets for molecular imaging (Fuster et al. 2005, Houvast et al. 2020). Targeting of aberrant glycans on cancer-associated proteins present on the cell membrane may offer significant advantages for molecular imaging over traditional protein targeting, considering their strongly amplified, tumor-specific presence on the outermost layer of the cell and low abundance on healthy tissues (Houvast et al. 2020).

Tumor-associated glycans Lewis^{a/c/x} (Le^{a/c/x}) and sialyl-di-Lewis^a (sdi-Le^a), as described by Chua et al. and Tivadar et al., respectively, are highly expressed on gastrointestinal tumors including pancreatic, gastric, colon and rectal cancer, along with a limited expression on healthy surrounding tissues (Chua et al. 2015, Tivadar et al. 2020).

Previous research by our group showed the high potential of Le^{a/c/x} and sdi-Le^a as biomarkers for pancreatic ductal adenocarcinoma (PDAC) imaging (Houvast et al. 2021). Subsequently, chimeric (mouse/human) monoclonal antibodies (mAbs) CH88.2 and CH129 were developed to target Le^{a/c/x} and sdi-Le^a, respectively. In a proof-of-concept study, we showed that the Le^{a/c/x}-targeting tracer CH88.2-800CW allowed clear visualization of human colon carcinoma and pancreatic ductal adenocarcinoma (PDAC) xenografts in immune-compromised mice using near-infrared fluorescence (NIRF) imaging (Houvas et al. 2020). However, the potential of CH129 as a targeting vehicle for NIRF imaging is unknown.

The current study provides a comprehensive preclinical evaluation and comparison of CH88.2-800CW and CH129-800CW for NIRF imaging of PDAC, facilitating the clinical translation of Le^{a/c/x} and sdi-Le^a targeted NIRF imaging agents.

METHODS

- CH88.2 and CH129 mAbs were conjugated to the NIRF dye IRDye 800CW.
- Binding of CH88.2-800CW and CH129-800CW was evaluated on multiple human pancreatic cancer cell lines.
- Mice with subcutaneous or orthotopic BxPC-3_luc2 tumors were intravenously injected with 1 nmol (150 µg) of CH88.2-800CW, CH129-800CW or negative control tracer CD20-800CW (chimeric mAb rituximab).
- Mice were imaged daily until 1 week post injection (subcutaneous models) or at 4 days post injection (orthotopic models) using the preclinical PEARL and clinical Artemis NIRF camera systems.
- After sacrificing the mice, tumors and organs were resected after which tracer uptake was evaluated *ex vivo* using the PEARL NIRF imager.

RESULTS

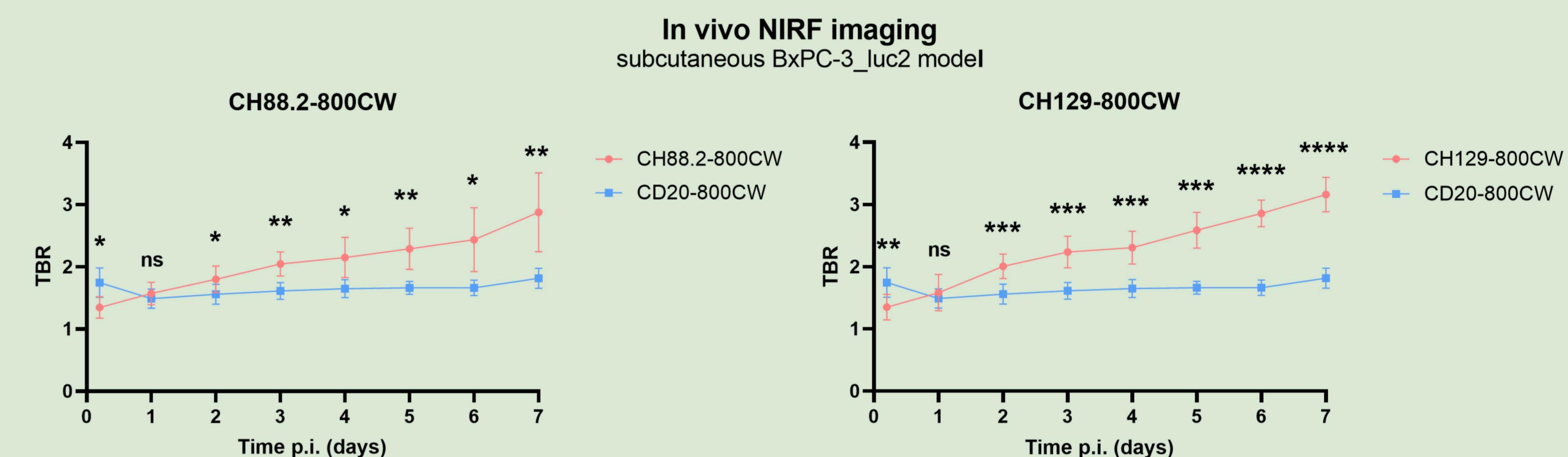


Figure 1 Mean TBRs in BxPC-3_luc2 tumor-bearing mice after injection of 1 nmol CH88.2-800CW and CH129-800CW, along with their standard deviations

CH88.2-800CW and CH129-800CW showed high binding on BxPC-3_luc2 cells (data not shown). Subsequently, administration of CH88.2-800CW and CH129-800CW to BxPC-3_luc2 tumor-bearing mice demonstrated clear tumor delineating fluorescence signal and high tumor-to-background ratios (TBRs) compared to CD20-800CW, suggesting specific CH88.2 and CH129 tumor binding. Next, the NIRF imaging potential of both tracers was evaluated in clinically more relevant orthotopic PDAC models.

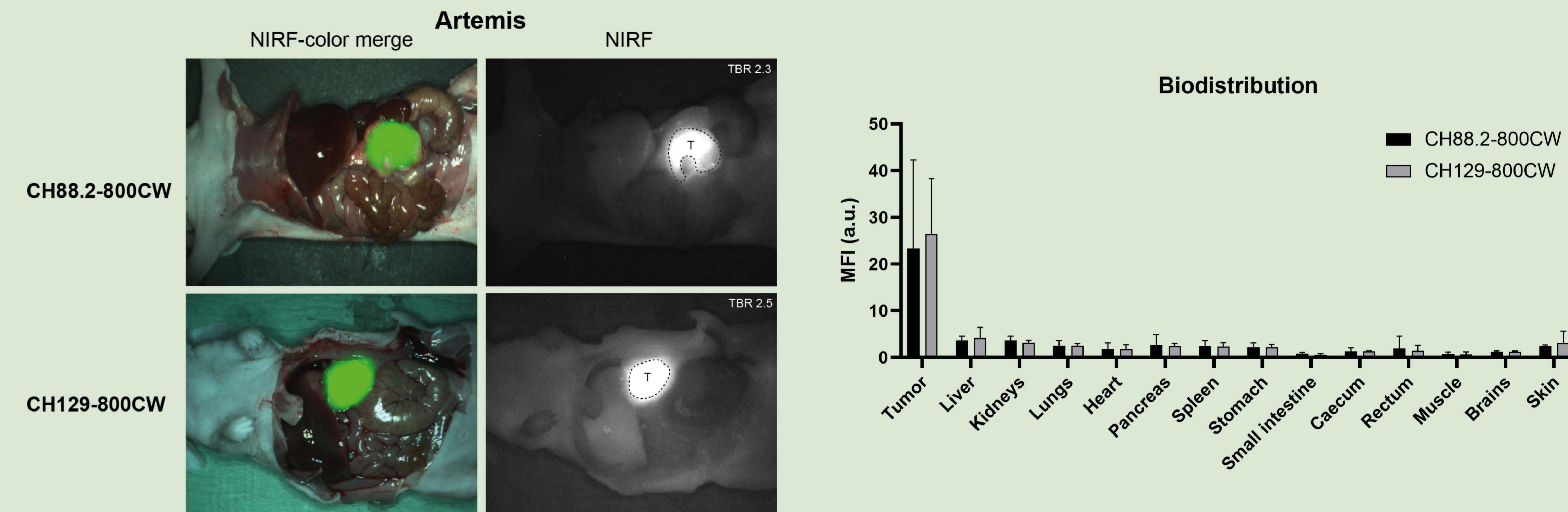


Figure 2 Representative NIRF-color and NIRF images of orthotopic BxPC-3_luc2 tumors 4 days after injection with 1 nmol of CH88.2-800CW or CH129-800CW. Images were generated with the clinical Artemis NIRF imager.

Using the clinical Artemis imager, orthotopic PDAC tumors could be clearly delineated with mean TBRs of 2.9 ± 0.4 and 2.5 ± 0.3 at 4 days post injection for CH88.2-800CW and CH129-800CW, respectively.

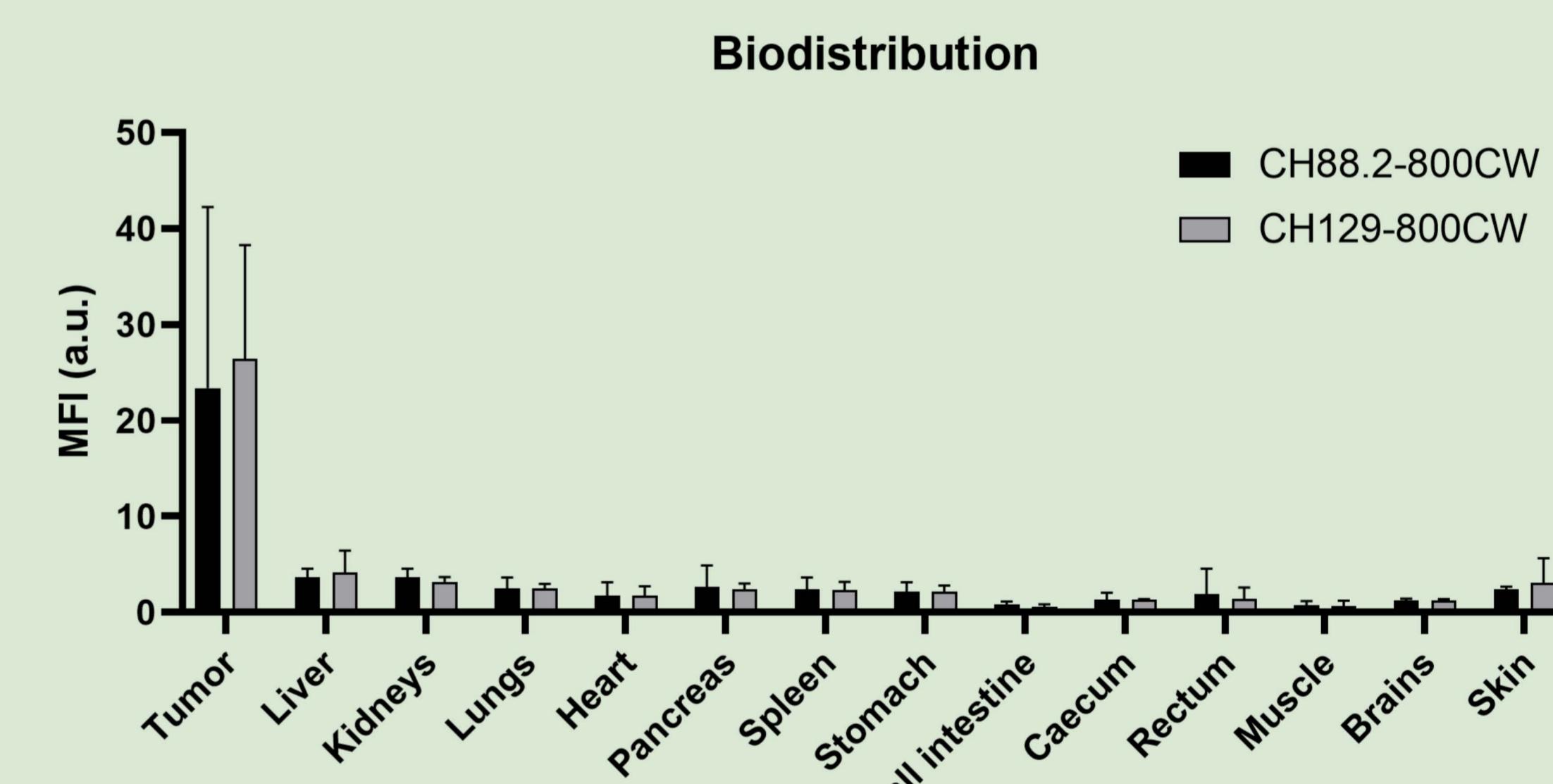


Figure 3 Mean fluorescence signals of CH88.2-800CW and CH129-800CW in orthotopic BxPC-3_luc2 tumors and healthy organs at 4 days after injection.

Ex vivo analysis revealed that fluorescent signals in all tumor lesions were higher compared to healthy organ signals, including the liver and kidneys.

CONCLUSION

Our study showed that Le^{a/c/x} and sdi-Le^a-targeting tracers CH88.2-800CW and CH129-800CW are highly promising agents for NIRF imaging of PDAC. Through this research, we pave the way for a clinical translation of CH88.2-800CW and CH129-800CW, which could improve real-time intraoperative delineation of PDAC and clinical outcomes.