



Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

journal homepage: www.elsevier.com/locate/jpa

Commentary

Comment on: “Development of a CLDN18.2-targeting immuno-PET probe for non-invasive imaging in gastrointestinal tumors”

Cuicui Li ^{a, b, c}, Xiaoyuan Chen ^{a, b, d, e, f, *}, Jingjing Zhang ^{a, b, d, **}^a Department of Diagnostic Radiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, 119074, Singapore^b Clinical Imaging Research Centre, Centre for Translational Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, 117599, Singapore^c Department of Nuclear Medicine, Beijing Friendship Hospital Affiliated to Capital Medical University, Beijing, 100050, China^d Nanomedicine Translational Research Program, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, 117597, Singapore^e Departments of Surgery, Chemical and Biomolecular Engineering, and Biomedical Engineering, Yong Loo Lin School of Medicine and College of Design and Engineering, National University of Singapore, Singapore, 119074, Singapore^f Institute of Molecular and Cell Biology, Agency for Science, Technology, and Research (A*STAR), 61 Biopolis Drive, Proteos, Singapore, 138673, Singapore

ARTICLE INFO

Article history:

Received 3 July 2023

Accepted 13 July 2023

Available online 20 July 2023

In the ever-evolving landscape of cancer research and treatment, the quest for novel and non-invasive imaging techniques has become crucial for accurate diagnosis and effective therapy. This study [1] successfully developed a good manufacturing practices (GMP) grade ⁸⁹Zr-labeled anti-Claudin18.2 (CLDN18.2) recombinant humanized antibody TST001. ⁸⁹Zr-DFO-TST001 exhibited high radiochemical purity (>99%) and specific activity (24.15 ± 1.34 GBq/ μ mol). It demonstrated good specificity and rapid tumor accumulation in vivo and in vivo. Through immuno-PET imaging, it enables non-invasive visualization and quantification of CLDN18.2 expression level in CLDN18.2-positive gastrointestinal tumor models.

CLDN18.2 is a member of the tight junction protein family of CLDN18, which plays a critical role in regulating tissue permeability, paracellular transport, and signal transduction processes. Research has revealed that CLDN18.2 is predominantly expressed in the gastric mucosa and is retained during malignant transformation. CLDN18.2 demonstrates high expression levels in various cancers, particularly in gastrointestinal cancers, while its expression in normal healthy tissues is limited, making it a potential target for cancer theranostics [2]. With the development of precision

medicine, immunotherapy has achieved great success. Recently published Phase III clinical trial data demonstrated that in patients with CLDN18.2-positive, HER2-negative, locally advanced unresectable, or metastatic gastric or gastroesophageal junction adenocarcinoma, immunotherapy targeting CLDN18.2 using zolbetuximab combined with mFOLFOX6 treatment significantly prolonged median progression-free survival (10.61 months vs. 8.67 months) and median overall survival (18.23 months vs. 15.54 months) compared with placebo plus mFOLFOX6 treatment [3]. The TST001 antibody used in this study was less fucosylated and demonstrated more potent complement-dependent cytotoxicity (CDC) and antibody-dependent cellular phagocytosis (ADCP) in CLDN18.2-positive cells and patient-derived xenograft models than zolbetuximab [4]. This study is also the first published article on the use of radiolabeled TST001 for immuno-PET imaging in gastrointestinal tumor models.

As mentioned in the article [1], the current main detection methods of CLDN18.2 protein, such as immunohistochemistry (IHC), are invasive, and due to the heterogeneity of tumors, they cannot provide real-time comprehensive information about the distribution and expression levels of CLDN18.2 in patients. Immuno-PET imaging is a specific molecular imaging technique newly developed in recent years, which uses radiolabeled specific monoclonal antibodies or their fragments as molecular tracer probes. Immuno-PET combines the high specificity and affinity of antibody targeting with the high sensitivity and quantitative analysis of PET imaging, allowing non-invasive visualization and evaluation of target antigen expression and distribution in vivo, by

Peer review under responsibility of Xi'an Jiaotong University.

* Corresponding author. Departments of Diagnostic Radiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, 119074, Singapore.

** Corresponding author. Departments of Diagnostic Radiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, 119074, Singapore.

E-mail addresses: chen.shawn@nus.edu.sg (X. Chen), j.zhang@nus.edu.sg (J. Zhang).<https://doi.org/10.1016/j.jpha.2023.07.010>2095-1779/© 2023 The Authors. Published by Elsevier B.V. on behalf of Xi'an Jiaotong University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

facilitating precise diagnosis, staging, and monitoring of treatment response, immuno-PET emerges as a highly promising imaging modality with great application potential. In this study, ^{89}Zr -DFO-TST001 was used to detect the expression and distribution of CLDN18.2 in gastrointestinal tumors. This novel molecular probe holds promise as a tool for the screening of beneficiary groups and efficacy evaluation of CLDN18.2 targeted therapy.

Although ^{89}Zr -DFO-TST001 allows visualization and precise quantification of tumor CLDN18.2 expression, some limitations remain. Due to the large molecular weight of intact monoclonal antibodies (about 150 kDa), the circulation and retention time of ^{89}Zr -DFO-TST001 in vivo are prolonged, resulting in a peak tumor uptake occurring several days post injection (p.i.) (48 h p.i. in this study). Additionally, the imaging agent is primarily eliminated through the liver, leading to significant hepatic radioactivity accumulation, which significantly affects the diagnostic accuracy of liver metastases. In another study published by the same team, it was observed that the tumor-to-liver ratios of the ^{124}I -labeled humanized anti-CLDN18.2 antibody 18B10(10L) and ^{124}I /Cy5.5-labeled murine anti-CLDN18.2 antibody 5C9, were significantly higher than that reported here [5,6]. It is unclear whether such discrepancy comes from the differences in radiolabels and/or the antibodies.

Wei et al. [7] used positron-emitting radionuclide-labeled anti-CLDN18.2 nanobody (12–15 kDa; the variable region of the heavy chain of heavy chain-only antibody [VHH]) and found significant accumulation of radioactivity in tumors 1 h p.i. But due to the small molecular weight, it resulted in a heavy burden on the kidneys. The anti-CLDN18.2 VHH-Fc developed by Hu et al. [8] and the hu7v3-Fc developed by Zhong et al. [9] (recombinant VHH fused with human IgG1-Fc), were labeled with ^{89}Zr and imaged in CLDN18.2-positive tumor models, with persistent tumor radioactivity accumulation and favorable tumor-to-liver ratios. Antibody fragments such as F(ab')_2 and Fab labeled with radionuclides can be used instead of intact antibodies to prepare molecular probes, which can reduce the molecular weight to 110 kDa and 50 kDa while retaining the specific binding of antigen-antibody. This not only avoids the interference of the Fc region with the immune system but also allows faster pharmacokinetic properties [10]. It might be possible that in this case, the use of radiolabeled antibody fragments instead of intact antibodies will solve the issue of low tumor-to-liver ratio.

Overall, the development of a CLDN18.2-targeting immuno-PET probe represents a significant advancement in non-invasive imaging for gastrointestinal tumors and other CLDN18.2-positive tumors. Based on the favorable anti-tumor efficacy and relatively low toxicity of TST001 antibody observed in CLDN18.2-positive cells and patients [4], it is possible to identify patients with high expression of CLDN18.2 as potential candidates through immuno-PET for subsequent radioimmunotherapy, such as ^{177}Lu -labeled TST001. This approach may provide a novel treatment option for

cancer patients who are intolerant to chemotherapy or require repeated administration in the future.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported by the National University of Singapore (Grant Nos.: NUHSRO/2021/097/Startup/13, NUHSRO/2020/133/Startup/08, NUHSRO/2023/008/NUSMed/TCE/LOA, NUHSRO/2021/034/TRP/09/Nanomedicine, and NUHSRO/2021/044/Kickstart/09/LOA), National Medical Research Council (Grant Nos.: OFYIRC23jan-0025, OFYIRC23jan-0017, MOH-001254-01, and CG21APR1005), Singapore Ministry of Education Academic Research Fund (Grant Nos.: NUHSRO/2022/093/T1/Seed-Sep/06 and MOE-000387-01), National Research Foundation (Grant No.: CRP28-2022RS-0001), National Natural Science Foundation of China (Grant No.: 82202206), and Beijing Natural Science Foundation (Grant No.: 7224365).

References

- [1] Y. Chen, X. Hou, D. Li, et al., Development of a CLDN18.2-targeting immuno-PET probe for non-invasive imaging in gastrointestinal tumors, *J. Pharm. Anal.* 13 (2023) 367–375.
- [2] Y. Kubota, A. Kawazoe, S. Mishima, et al., Comprehensive clinical and molecular characterization of claudin 18.2 expression in advanced gastric or gastroesophageal junction cancer, *ESMO Open* 8 (2023), 100762.
- [3] K. Shitara, F. Lordick, Y.J. Bang, et al., Zolbetuximab plus mFOLFFOX6 in patients with CLDN18.2-positive, HER2-negative, untreated, locally advanced unresectable or metastatic gastric or gastro-oesophageal junction adenocarcinoma (SPOTLIGHT): A multicentre, randomised, double-blind, phase 3 trial, *Lancet* 401 (2023) 1655–1668.
- [4] W. Cao, H. Xing, Y. Li, et al., Claudin18.2 is a novel molecular biomarker for tumor-targeted immunotherapy, *Biomark. Res.* 10 (2022), 38.
- [5] S. Wang, C. Qi, J. Ding, et al., First-in-human CLDN18.2 functional diagnostic pet imaging of digestive system neoplasms enables whole-body target mapping and lesion detection, *Eur. J. Nucl. Med. Mol. Imag.* 50 (2023) 2802–2817.
- [6] C. Zhao, Z. Rong, J. Ding, et al., Targeting claudin 18.2 using a highly specific antibody enables cancer diagnosis and guided surgery, *Mol. Pharmaceutics* 19 (2022) 3530–3541.
- [7] W. Wei, D. Zhang, Y. Zhang, et al., Development and comparison of $^{68}\text{Ga}/^{18}\text{F}/^{64}\text{Cu}$ -labeled nanobody tracers probing Claudin18.2, *Mol. Ther. Oncolytics* 27 (2022) 305–314.
- [8] G. Hu, W. Zhu, Y. Liu, et al., Development and comparison of three ^{89}Zr -labeled anti-CLDN18.2 antibodies to noninvasively evaluate CLDN18.2 expression in gastric cancer: A preclinical study, *Eur. J. Nucl. Med. Mol. Imag.* 49 (2022) 2634–2644.
- [9] W. Zhong, Y. Lu, Z. Ma, et al., Development of a humanized VHH based recombinant antibody targeting claudin 18.2 positive cancers, *Front. Immunol.* 13 (2022), 885424.
- [10] L. Kang, C. Li, Z.T. Rosenkrans, et al., Noninvasive evaluation of CD20 expression using ^{64}Cu -labeled F(ab')_2 fragments of obinutuzumab in lymphoma, *J. Nucl. Med.* 62 (2021) 372–378.