European Journal of Nuclear Medicine and Molecular Imaging (2023) 50:1560–1563
https://doi.org/10.1007/s00259-023-06186-4

EDITORIAL

Optical image-guided therapy of pancreatic cancer with an ultra-small bispecific protein

Wenyu Song[1,2,3] · Jessica C. Hsu[3] · Xiaoli Lan[1,2] · Weibo Cai[3]

Published online: 8 March 2023

Bispecific proteins, such as bispecific antibodies (BsAbs), represent a promising class of biologics for the treatment of various diseases [1]. They are designed to target two different proteins/antigens simultaneously, allowing for more specific and effective targeting of the cells or molecules of interest [2]. Since tumor cells often express multiple antigens, BsAbs are more useful than monospecific antibodies particularly in cancer theranostic applications [3]. BsAbs can also redirect immune cells against tumor cells. For example, blinatumomab, a bispecific T-cell engager (BiTE), promotes T-cell-mediated cytotoxicity by directing $CD3^+$ T cells toward $CD19^+$ cancer cells [4–6]. In addition, BsAbs can overcome the limitations of monoclonal antibodies (mAbs), such as poor solubility, possible off-target effects, and rapid clearance from the body [7, 8]. Since the first development of BsAbs in 1961 [9], a burst of research activity surrounding this agent type has been seen over the years [10–12]. With two such therapies already approved by the FDA (emicizumab and blinatumomab) [13, 14], and many more undergoing clinical trials, they will soon become widespread in the fight against many diseases [15].

BsAbs can be classified into two main categories, namely, those with an Fc region (similar to IgG antibodies) and those without an Fc region (such as diabodies and nanobodies) [16]. The structure of IgG-like BsAbs is similar to that of traditional mAbs. They typically contain a complete Fc fragment and weigh over 100 kDa [17]. The Fc fragment prolongs the half-life of BsAbs, is responsible for antigen binding, and is involved in several processes, including cell-mediated cytotoxicity, complement-dependent cytotoxicity, and antibody-dependent cell-mediated phagocytosis [18]. Moreover, IgG-like BsAbs can be easily purified and are stable in nature. However, they have low permeability in tumor tissues, and their complex structures may result in chain mispairing during assembly.

Non-IgG-like BsAbs, on the other hand, lack an Fc region and possess only antigen-binding domains, thus avoiding chain-association problems [19]. They have a relatively simple structure and small molecular weight and can be highly expressed in cells. Owing to their small size, they exhibit strong tissue-penetrating ability and better delivery efficiency, making them highly effective in cancer therapy. Diabodies consist of two antigen-binding domains and have a molecular weight of approximately 50 kDa [20]. In addition, nanobodies [21], which are derived from camelid antibodies, are roughly one-tenth of the size of traditional antibodies and have good tissue-penetrating ability. Overall, non-IgG-like BsAbs offer several advantages over traditional IgG-like BsAbs and have demonstrated great promise particularly in the field of cancer therapy [16, 22–25]. Continued research in these areas may ultimately lead to improved treatment for widespread diseases [26].

Pancreatic ductal adenocarcinoma (PDAC) has long been a challenging disease to treat successfully. Highly specific mAbs have been evaluated as potential mono-targeted drugs for the treatment of PDAC, but they have failed to improve patient survival due to low intrinsic efficacy [27, 28]. The reasons for the treatment failures have been widely attributed to the high degree of molecular heterogeneity of PDAC cells, as well as the dense desmoplastic tumor stroma that is nearly impenetrable by traditional antibodies due to their large size [29]. Therefore, the development of highly multi-specific, small-sized therapeutic agents is urgently needed to provide an effective targeted therapy for PDAC.

This article is part of the Topical Collection on Preclinical Imaging.

✉ Xiaoli Lan
xiaoli_lan@hust.edu.cn; LXL730724@hotmail.com

✉ Weibo Cai
wcai@uwhealth.org

[1] Department of Nuclear Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

[2] Hubei Key Laboratory of Molecular Imaging, Wuhan, China

[3] Departments of Radiology and Medical Physics, University of Wisconsin – Madison, Madison, WI, USA

In this issue of the *European Journal of Nuclear Medicine and Molecular Imaging*, Wang et al. reported a superb treatment method for PDAC using an ultra-small bispecific fusion protein, Bi-fp50, labeled with fluorescent dyes [30]. By targeting both vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR), Bi-fp50 represents a promising new strategy for more effective targeted pathway therapy for PDAC. Bi-fp50 has demonstrated increased binding affinity for Bxpc3 PDAC cells compared to anti-VEGF scFv and anti-EGFR scFv alone. It has also significantly inhibited the proliferation and growth of Bxpc3 and Aspc1 PDAC cells, even at a relatively low concentration (0.3 μM).

One of the most notable features of Bi-fp50 is its ultra-small size, which measures approximately 50 kDa in molecular weight or 5–6 nm in hydrodynamic diameter. This uniquely designed characteristic enables effective penetration through the dense tumor barrier and allows for accumulation deep within the tumor tissue. In fact, after intravenous injection, Bi-fp50 was found to be widely distributed throughout the entire tissue and primarily enriched in the tumor, with nearly twice the accumulation compared to scFv2 in an orthotopic PDAC tumor model. The broad distribution and deep tissue penetration of Bi-fp50 led to apoptosis in the whole tumor. These exciting results provide guidance for further development of novel therapeutic approaches for PDAC.

Bi-fp50's high bispecificity and ultra-small size also make it an ideal candidate for combination therapy with other treatment modalities. For example, it could be combined with immunotherapy or radiotherapy to further enhance its therapeutic effects. While safety and off-target toxicity are potential concerns with immunotherapy, previous studies have shown that bispecific antibodies and their derivatives can significantly reduce the risk of off-target side effects because they can selectively bind to T cells and cancer cells at two terminals [31]. Without posing a safety risk, Bi-fp50 provides great prospect for dual-targeted immunotherapy of PDAC. In addition, the design of Bi-fp50 allows for the attachment of other molecular agents, such as drugs used in radiotherapy and chemotherapy, to achieve synergistic therapeutic effects. Thus, the emergence of ultra-small bispecific proteins, such as Bi-fp50, has opened up new opportunities for combination therapies and created a new frontier for improved cancer treatment strategies.

The applicability of bispecific proteins in nuclear medicine has been increasingly investigated in recent years [32, 33]. For instance, Stergiou et al. evaluated ^{89}Zr-labeled mAbAdu-scFab8D3 (Adu-8D3) for amyloid-β imaging and targeting in a preclinical Alzheimer's disease (AD) mouse model [34]. Adu-8D3 is a BsAb consisting of aducanumab with bivalent binding to human Aβ plaques and with a single-chain Fab (scFab) of the 8D3 mAb targeting murine TfR1 attached to the heavy c-terminal. Their study demonstrated the highly specific uptake of Adu-8D3 in the brain, which was seven times higher than that of Adu with single-target specificity. This indicates that Adu-8D3 utilizes the brain shuttle mechanism through TfR1 to enhance brain uptake. Adu-8D3 could serve as a powerful tool in reducing the risk of constructing novel brain shuttle antibodies and developing biopharmaceuticals for neurological diseases. Another study reported the use of ^{89}Zr-IBI322 (anti-CD47 and PD-L1) to evaluate the safe and effective therapeutic dose of a BsAb [35]. Preliminary pharmacodynamics studies suggested that dose-escalation PET imaging using ^{89}Zr-BsAbs is a suitable strategy for PK/PD modeling as well as safety prediction. This approach allows for the determination of rational dosing of BsAbs in preclinical and clinical trials. Furthermore, the effectiveness of using an anti-carcinoembryonic antigen (CEA) recombinant bispecific monoclonal antibody (TF2) and ^{68} Ga-HSG for pre-targeted immunological PET (or immuno-PET) was assessed in patients with metastatic colorectal carcinoma [36]. In this pilot study, the use of pre-targeted immuno-PET with anti-CEA/anti-IMP288 BsAbs and ^{68} Ga-haptens demonstrated positive results in 9 out of 11 patients with good diagnostic performance. After analyzing lesions on a per-lesion basis, immuno-PET demonstrated higher sensitivity, specificity, positive predictive value, and negative predictive value compared to the combined diagnosis of EUS/CT/MRI and FDG-PET. It further demonstrated its excellent diagnostic performance.

In addition to the imaging functions, the therapeutic potential of bispecific proteins in combination therapies have been shown in a number of studies. For example, ^{90}Y-CHX-A″-C6.5 diabody (with specificity toward HER2/neu) can be used for targeted cancer therapy by inhibiting the growth of established MDA-361/DYT2 tumor xenografts in immunodeficient mice [37]. This suggests that diabody molecules having excellent antitumor properties can serve as effective therapeutic agents for radioimmunotherapy. Cheal et al. recently reported that ^{177}Lu-DOTA-BsAb (anti-HER2-C825) is beneficial for treating mice with human solid tumor xenografts (GPA33 and GD2) [38]. The agent demonstrated great treatment outcome, with 62.5% histological cure (5/8) and 37.5% microscopic residual disease (3/8) at 85 days, while avoiding severe radiotoxicity in critical organs. From these studies, BsAbs are both active for immuno-PET imaging and also provide targeted therapy, indicating their suitability for cancer theranostic applications. Moreover, a novel three-step pretargeted radioimmunotherapy strategy utilizing a glycoprotein A33 (GPA33)-targeting BsAb and a small-molecule radioactive hapten (^{177}Lu-DOTA-Bn) demonstrated excellent tumor-to-background ratios [39]. In the GPA33-positive human colorectal cancer xenograft mouse model, this approach led to a 100% histological cure without any discernible radiation damage to crucial organs. These

findings highlight the potential of utilizing bispecific antibodies in pretargeted radioimmunotherapy.

Most BsAbs used in current diagnostic and therapeutic studies are generally large in size with very limited tumor-penetrating capability. The lack of intratumoral enrichment and deep penetration may affect their clinical relevance [40]. To improve the outlook of BsAbs, the recent work done by Wang et al., which is highlighted herein, is of great importance. The development of ultra-small-sized multispecific proteins presents a new strategy for more effective targeted pathway therapy and represents a paradigm-shifting treatment regimen for a broad spectrum of hard-to-treat solid tumors. Nonetheless, future research should focus on identifying more specific molecular targets in tumor cells and other relevant constituents, and designing rational conjugation or combination with other drugs. These improvements will further increase the significance of ultra-small BsAbs in clinical applications.

In conclusion, the recent advances in BsAbs design and production hold immense promise for the future of cancer theranostics. While there are still challenges to overcome, the potential of these multispecific therapeutic proteins to revolutionize cancer treatment and nuclear medicine is clear, and continued research and development will pave the path for newly improved diagnostic and therapeutic methods for a wide range of diseases.

Funding The authors are grateful for financial support from the National Natural Science Foundation of China (No. 82030052), the University of Wisconsin–Madison, and the National Institutes of Health (P30CA014520 and T32CA009206).

Declarations

Studies with human participants or animals This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest WC declares conflict of interest with the following corporations: Actithera, Inc.; Rad Source Technologies, Inc.; Portrai, Inc.; rTR Technovation Corporation; and Four Health Global Pharmaceuticals, Inc. All other authors declare that they have no conflict of interest.

References

1. Fan G, Wang Z, Hao M, Li J. Bispecific antibodies and their applications. J Hematol Oncol. 2015;8:130. https://doi.org/10.1186/s13045-015-0227-0.
2. Segués A, Huang S, Sijts A, Berraondo P, Zaiss DM. Opportunities and challenges of bi-specific antibodies. Int Rev Cell Mol Biol. 2022;369:45–70. https://doi.org/10.1016/bs.ircmb.2022.05.001.
3. Li H, Er Saw P, Song E. Challenges and strategies for next-generation bispecific antibody-based antitumor therapeutics. Cell Mol Immunol. 2020;17(5):451–61. https://doi.org/10.1038/s41423-020-0417-8.
4. Sedykh SE, Prinz VV, Buneva VN, Nevinsky GA. Bispecific antibodies: design, therapy, perspectives. Drug Des Devel Ther. 2018;12:195–208. https://doi.org/10.2147/dddt.S151282.
5. Goebeler ME, Knop S, Viardot A, Kufer P, Topp MS, Einsele H, et al. Bispecific T-cell engager (BiTE) antibody construct blinatumomab for the treatment of patients with relapsed/refractory non-Hodgkin lymphoma: final results from a phase I study. J Clin Oncol. 2016;34(10):1104–11. https://doi.org/10.1200/jco.2014.59.1586.
6. Zhao L, Li S, Wei X, Qi X, Liu D, Liu L, et al. A novel CD19/CD22/CD3 trispecific antibody enhances therapeutic efficacy and overcomes immune escape against B-ALL. Blood. 2022;140(16):1790–802. https://doi.org/10.1182/blood.2022016243.
7. Suurs FV, Lub-de Hooge MN, de Vries EGE, de Groot DJA. A review of bispecific antibodies and antibody constructs in oncology and clinical challenges. Pharmacol Ther. 2019;201:103–19. https://doi.org/10.1016/j.pharmthera.2019.04.006.
8. Arndt MA, Krauss J, Kipriyanov SM, Pfreundschuh M, Little M. A bispecific diabody that mediates natural killer cell cytotoxicity against xenotransplantated human Hodgkin's tumors. Blood. 1999;94(8):2562–8.
9. Nisonoff A, Rivers MM. Recombination of a mixture of univalent antibody fragments of different specificity. Arch Biochem Biophys. 1961;93(2):460–2. https://doi.org/10.1016/0003-9861(61)90296-X.
10. Velasquez MP, Bonifant CL, Gottschalk S. Redirecting T cells to hematological malignancies with bispecific antibodies. Blood. 2018;131(1):30–8. https://doi.org/10.1182/blood-2017-06-741058.
11. Kauer J, Vogt F, Hagelstein I, Hörner S, Märklin M, Maurer S et al. CD18 antibody application blocks unwanted off-target T cell activation caused by bispecific antibodies. Cancers (Basel). 2021;13(18). https://doi.org/10.3390/cancers13184596.
12. Krishnamurthy A, Jimeno A. Bispecific antibodies for cancer therapy: a review. Pharmacol Ther. 2018;185:122–34. https://doi.org/10.1016/j.pharmthera.2017.12.002.
13. Husain B, Ellerman D. Expanding the boundaries of biotherapeutics with bispecific antibodies. BioDrugs. 2018;32(5):441–64. https://doi.org/10.1007/s40259-018-0299-9.
14. Blair HA. Emicizumab: a review in haemophilia A. Drugs. 2019;79(15):1697–707. https://doi.org/10.1007/s40265-019-01200-2.
15. Szijj P, Chudasama V. The renaissance of chemically generated bispecific antibodies. Nat Rev Chem. 2021;5(2):78–92. https://doi.org/10.1038/s41570-020-00241-6.
16. Du Y, Xu J. Engineered bifunctional proteins for targeted cancer therapy: prospects and challenges. Adv Mater. 2021;33(48):2103114. https://doi.org/10.1002/adma.202103114.
17. Zhang X, Yang Y, Fan D, Xiong D. The development of bispecific antibodies and their applications in tumor immune escape. Exp Hematol Oncol. 2017;6:12. https://doi.org/10.1186/s40164-017-0072-7.
18. Chames P, Baty D. Bispecific antibodies for cancer therapy: the light at the end of the tunnel? MAbs. 2009;1(6):539–47. https://doi.org/10.4161/mabs.1.6.10015.
19. Labrijn AF, Janmaat ML, Reichert JM, Parren P. Bispecific antibodies: a mechanistic review of the pipeline. Nat Rev Drug Discov. 2019;18(8):585–608. https://doi.org/10.1038/s41573-019-0028-1.
20. Kontermann RE, Brinkmann U. Bispecific antibodies. Drug Discov Today. 2015;20(7):838–47. https://doi.org/10.1016/j.drudis.2015.02.008.
21. Bannas P, Hambach J, Koch-Nolte F. Nanobodies and nanobody-based human heavy chain antibodies as antitumor therapeutics. Front Immunol. 2017;8:1603. https://doi.org/10.3389/fimmu.2017.01603.
22. Chang C-H, Wang Y, Li R, Rossi DL, Liu D, Rossi EA, et al. Combination therapy with bispecific antibodies and PD-1 blockade enhances

the antitumor potency of T cells. Cancer Res. 2017;77(19):5384–94. https://doi.org/10.1158/0008-5472.Can-16-3431.
23. Huang S, van Duijnhoven SMJ, Sijts AJAM, van Elsas A. Bispecific antibodies targeting dual tumor-associated antigens in cancer therapy. J Cancer Res Clin Oncol. 2020;146(12):3111–22. https://doi.org/10.1007/s00432-020-03404-6.
24. Watts RJ, Dennis MS. Bispecific antibodies for delivery into the brain. Curr Opin Chem Biol. 2013;17(3):393–9. https://doi.org/10.1016/j.cbpa.2013.03.023.
25. Chen Z, Kankala RK, Yang Z, Li W, Xie S, Li H, et al. Antibody-based drug delivery systems for cancer therapy: mechanisms, challenges, and prospects. Theranostics. 2022;12(8):3719–46. https://doi.org/10.7150/thno.72594.
26. Deshaies RJ. Multispecific drugs herald a new era of biopharmaceutical innovation. Nature. 2020;580(7803):329–38. https://doi.org/10.1038/s41586-020-2168-1.
27. Adiseshaiah PP, Crist RM, Hook SS, McNeil SE. Nanomedicine strategies to overcome the pathophysiological barriers of pancreatic cancer. Nat Rev Clin Oncol. 2016;13(12):750–65. https://doi.org/10.1038/nrclinonc.2016.119.
28. Ho WJ, Jaffee EM, Zheng L. The tumour microenvironment in pancreatic cancer – clinical challenges and opportunities. Nat Rev Clin Oncol. 2020;17(9):527–40. https://doi.org/10.1038/s41571-020-0363-5.
29. Grünwald BT, Devisme A, Andrieux G, Vyas F, Aliar K, McCloskey CW, et al. Spatially confined sub-tumor microenvironments in pancreatic cancer. Cell. 2021;184(22):5577-92.e18. https://doi.org/10.1016/j.cell.2021.09.022.
30. Wang Q, Wang J, Yan H, Li Z, Wang K, Kang F, et al. An ultrasmall bispecific protein augments tumor penetration and treatment for pancreatic cancer. Eur J Nucl Med Mol Imaging. 2023. https://doi.org/10.1007/s00259-023-06115-5.
31. Oberg HH, Janitschke L, Sulaj V, Weimer J, Gonnermann D, Hedemann N, et al. Bispecific antibodies enhance tumor-infiltrating T cell cytotoxicity against autologous HER-2-expressing high-grade ovarian tumors. J Leukoc Biol. 2020;107(6):1081–95. https://doi.org/10.1002/jlb.5ma1119-265r.
32. Kaur S, Venktaraman G, Jain M, Senapati S, Garg PK, Batra SK. Recent trends in antibody-based oncologic imaging. Cancer Lett. 2012;315(2):97–111. https://doi.org/10.1016/j.canlet.2011.10.017.
33. Tavaré R, Escuin-Ordinas H, Mok S, McCracken MN, Zettlitz KA, Salazar FB, et al. An effective immuno-PET imaging method to monitor CD8-dependent responses to immunotherapy. Cancer Res. 2016;76(1):73–82. https://doi.org/10.1158/0008-5472.Can-15-1707.
34. Stergiou N, Wuensche TE, Schreurs M, Mes I, Verlaan M, Kooijman EJM et al. Application of 89Zr-DFO*-immuno-PET to assess improved target engagement of a bispecific anti-amyloid-ß monoclonal antibody. Eur J Nucl Med Mol Imaging. 2023. https://doi.org/10.1007/s00259-023-06109-3.
35. Wang Y, Pan D, Huang C, Chen B, Li M, Zhou S, et al. Dose escalation PET imaging for safety and effective therapy dose optimization of a bispecific antibody. MAbs. 2020;12(1):1748322. https://doi.org/10.1080/19420862.2020.1748322.
36. Touchefeu Y, Bailly C, Frampas E, Eugène T, Rousseau C, Bourgeois M, et al. Promising clinical performance of pretargeted immuno-PET with anti-CEA bispecific antibody and gallium-68-labelled IMP-288 peptide for imaging colorectal cancer metastases: a pilot study. Eur J Nucl Med Mol Imaging. 2021;48(3):874–82. https://doi.org/10.1007/s00259-020-04989-3.
37. Adams GP, Shaller CC, Dadachova E, Simmons HH, Horak EM, Tesfaye A, et al. A single treatment of yttrium-90-labeled CHX-A″-C6.5 diabody inhibits the growth of established human tumor xenografts in immunodeficient mice. Cancer Res. 2004;64(17):6200–6. https://doi.org/10.1158/0008-5472.Can-03-2382.
38. Cheal SM, Xu H, Guo HF, Patel M, Punzalan B, Fung EK, et al. Theranostic pretargeted radioimmunotherapy of internalizing solid tumor antigens in human tumor xenografts in mice: curative treatment of HER2-positive breast carcinoma. Theranostics. 2018;8(18):5106–25. https://doi.org/10.7150/thno.26585.
39. Cheal SM, Fung EK, Patel M, Xu H, Guo H-f, Zanzonico PB et al. Curative multicycle radioimmunotherapy monitored by quantitative SPECT/CT-based theranostics, using bispecific antibody pretargeting strategy in colorectal cancer. J Nucl Med. 2017;58(11):1735–1742. https://doi.org/10.2967/jnumed.117.193250.
40. Thakur A, Huang M, Lum LG. Bispecific antibody based therapeutics: strengths and challenges. Blood Rev. 2018;32(4):339–47. https://doi.org/10.1016/j.blre.2018.02.004.