ImmunoPET of CD38 with a radiolabeled nanobody: promising for clinical translation

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Immunotherapy, a therapeutic approach that activates and utilizes a patient's own immune system, has gathered tremendous attention from both patients and researchers, especially after the 2018 Nobel Prize in Physiology or Medicine was awarded jointly to James P. Allison and Tasuku Honjo “for their discovery of cancer therapy by inhibition of negative immune regulation.” More than 10,000 studies have been published within the past 2 years for understanding, improving, and clinically translating immunotherapy, based on keyword search in PubMed. For instance, immune checkpoint inhibitors hijacking programmed death-ligand 1 (PD-L1) and its receptor, programmed cell death protein 1 (PD-1), have emerged as the most promising candidates, and several monoclonal antibodies were approved by the Food and Drug Administration (FDA) for treating multiple types of cancer including melanoma, squamous cell lung cancer, renal cell carcinoma, etc. [1]. CD38, a member of the ribosyl cyclase family that is widely expressed on the surface of non-hematopoietic cells and diverse immune cells especially in treatment-resistant tumor microenvironment, has been recently recognized as a novel immune checkpoint protein [2, 3]. Anti-CD38 immunotherapy can be another promising

treatment for patients who cannot be cured by traditional methods. However, only a small subset of patients really benefits from immunotherapies, while nonresponders suffer from significant side effects [4]. Therefore, effectively distinguishing responders from nonresponders is a critical challenge confronting the clinicians and scientists, and hence, an area of significant research and innovation.

The conventional approaches to distinguishing responders from nonresponders rely heavily on either flow cytometry examination of blood samples or immunohistochemistry of biopsied tumors [5, 6], both of which are invasive, insensitive, and sometimes inaccurate due to the heterogeneous nature of cancer. Whole-body imaging techniques, especially immuno-positron emission tomography (immunoPET), can be a critical alternative for guiding patient selection. By administering small doses of radiolabeled antibody-based tracers that bind to the same receptors as that of the therapeutic agents, it is possible to noninvasively, quantitatively, and rapidly evaluate target expression level and hence predict therapeutic efficacy [7]. In addition to patient selection, PET can also be employed to longitudinally monitor the efficacy of immunotherapies, by imaging the activation and/or infiltration of different types of immune cells including T cells, B cells, and macrophages [8]. Thus, PET with suitable tracers can revolutionize treatment planning and monitoring, since repeated biopsy assays on multiple metastatic nodules or deep lesions are neither practical nor accurate.

Among the vast library of radiotracers for immunoPET, nanobody (also named as single-domain antibody, sdAb, or variable domain of a camelid heavy-chain only antibody, VHH), the smallest class of Fc-free antibody fragments, has shown distinct promise [9]. The most important merit of nanobodies for immunoPET is the short blood circulation half-life (usually <1 h), in comparison to intact antibodies (up to several days or weeks) [10], thereby allowing same-day imaging. Zirconium-89 (⁸⁹Zr, $t_{1/2}$ = 3.3 days) is typically required to label antibodies to match their long blood circulation [11, 12], which could be prone to unwanted bone

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accumulation due to the bone-seeking nature of ^{89}Zr . On the other hand, nanobodies allow the use of short-lived isotopes, such as ^{18}F ($t_{1/2} = 110$ min) and ^{68}Ga ($t_{1/2} = 68$ min), overcoming the concerns for nonspecific bone accumulation. Importantly, the use of ^{18}F and ^{68}Ga significantly expands the availability and feasibility of immunoPET, particularly in resource-strapped countries that do not have the capability to produce high-quality ^{89}Zr or ^{64}Cu . In addition, the short circulation of nanobodies significantly reduces the background signal in the blood pool and therefore enhances the signal-to-background contrast [4, 13], especially when imaging hematologic cancers, circulating immune cells, or immune organs such as the spleen that are immensely infiltrated with blood. The second merit of nanobody-based radiotracer is its potential renal clearance (due to its low molecular weight ~ 15 kDa), unlike whole antibodies (~ 150 kDa) which are cleared by the hepatic and splenic pathways. Because of renal clearance, nanobodies are especially suitable for imaging abdominal cancers and immune activation that involves spleen and lymph nodes in the abdomen. Thirdly, nanobodies may demonstrate enhanced tissue penetration owing to their smaller size, making them desirable for imaging poorly vascularized or dense tissues/tumors [14].

Building on these advantages, in this issue of *European Journal of Nuclear Medicine and Molecular Imaging*, Wei, Zhao, Liu, and colleagues reported a ^{68}Ga -labeled CD38-targeted nanobody, [^{68}Ga]Ga-NOTA-Nb1053, to predict the therapeutic outcomes of anti-CD38 immunotherapy in multiple myeloma, a hematological neoplasm of B cell lineage [15]. Good radiochemical yield ($> 50\%$), excellent radiochemical purity ($> 99\%$), and immunoreactivity ($> 95\%$) were achieved, and sharp images of CD38-overexpressing tumors were acquired, outperforming the traditional [^{18}F]FDG PET. [^{68}Ga]Ga-NOTA-Nb1053 exhibited excellent specificity, confirmed with the blocking study by pre-injection of daratumumab (therapeutic anti-CD38 antibody). The imaging results correlated well with CD38 expression levels assessed by cellular and histological analyses, manifesting its potential for early diagnosis and effective patient selection.

Not limited to multiple myeloma, anti-CD38 therapy can be potentially applied to other solid and liquid tumors. CD38 is highly expressed on cancer cells and tumor-associated immune cells, and inhibits CD8 $^{+}$ T cell proliferation, antitumor cytokine secretion, and tumor cell killing capability [2, 3]. Thus, anti-CD38 therapies may potentially benefit patients who are resistant to currently approved immune checkpoint inhibitors (such as PD-1/PD-L1). Towards this goal, [^{68}Ga]Ga-NOTA-Nb1053-based immunoPET could play an important role in selecting patients who are suitable for anti-CD38 therapy, and predicting and evaluating consequent therapeutic efficacy by longitudinal monitoring of CD38 expression. Therefore, [^{68}Ga]Ga-NOTA-Nb1053 immunoPET promises wide applicability and significant potential for clinical translation.

Radiolabeled nanobodies have also been employed in various other studies to noninvasively evaluate the status of the immune system. Ploegh and colleagues from Boston Children's Hospital published a series of articles reporting the applications of radiolabeled anti-CD8 and anti-CD11b nanobodies to accurately track the in vivo biodistribution of CD8 $^{+}$ T cells and CD11b $^{+}$ myeloid cells [5, 16–18]. The results revealed that the infiltration of CD8 $^{+}$ T cells in colorectal tumors correlated with effective immunotherapy, and homogeneous distribution of CD11b $^{+}$ myeloid cells in tumors may indicate positive immune responses. The responders exhibited a dominant population of CD11b $^{+}$ cells with an M1-like signature, while the nonresponders displayed an M2-like transcriptional signature [17]. Imaging of tumor-associated macrophages was also investigated in several other studies, with radiolabeled nanobodies targeting the macrophage mannose receptor (MMR, CD206) [19, 20]. Since recruitment of MMR $^{+}$ tumor-associated macrophages is generally related to poor responsiveness to immunotherapy [21], MMR-targeted imaging may be beneficial in predicting and longitudinally monitoring the response of immunotherapy.

Current research on radiolabeling and immunoPET of nanobodies is almost exclusively preclinical. Many challenges remain and more studies are needed to further improve the nanobodies for clinical translation. Firstly, random-site radiolabeling is the most commonly used labeling method in which the chelators randomly react with amine or other functional groups. This method is suitable for antibodies, because of their large molecular weight and abundant functional groups. However, it could be problematic when labeling nanobodies that have limited number of functional groups. Chelator conjugation with the functional groups within or near the complementarity determining region may interfere with antigen binding. Although one study reported that random-site radiolabeling did not significantly hamper the binding affinity of nanobodies [6], it is necessary to confirm the biological function of each radiolabeled nanobody before clinical translation, since every type of nanobody has a unique structure. Site-specific radiolabeling may serve as an alternative and superior radiolabeling approach for nanobodies. However, the increased cost of site-specific radiolabeling should also be considered during good manufacturing practices (GMP) production for potential clinical applications.

Secondly, although renal clearance of nanobodies remarkably reduces the radiation burden on the liver and spleen, high kidney uptake should be noted which may lead to potential renal toxicity. However, one study pointed out that the highest dose to the kidneys (up to 90 mGy) after radiolabeled nanobody administration stayed well below the threshold of 7–8 Gy for potential deterministic effects [20]. Several approaches have been reported to further reduce the renal uptake. In this highlighted study, Wang et al. reported that pretreatment of mice with sodium maleate or fructose

significantly decreased kidney retention of the PET tracers [15]. Potential health concerns from such strategies that interfere with tubular reabsorption should be considered when applying to patients in the clinical settings. Another study reported the use of PEGylated nanobodies for reduced renal retention without inducing additional side effects [5, 17]. However, such a strategy may decrease the antigen-binding affinity of the nanobodies.

Thirdly, many reported studies on immunoPET imaging were conducted in immunocompromised nude mice bearing xenograft tumors. Higher signal-to-background ratio was achieved in these proof-of-concept studies by avoiding the significant influence from native immune cells. However, the pharmacokinetics of radiolabeled nanobodies in immunodeficient mice may not represent the real in vivo scenario, since the abundant T cells, B cells, and myeloid cells in blood circulation and peripheral tissues may massively interact with nanobodies and lead to significantly different pharmacokinetics and biodistribution. Therefore, immunocompetent mouse models and non-human primate models are recommended for robust testing of nanobody-based immunoPET imaging before clinical investigation.

Immune response is a complex network of interactions involving multiple types of immune cells and cytokines. Even the same lineage of immune cells may behave heterogeneously in different biological processes and pathologies [22]. Therefore, imaging a single biomarker may not be sufficient for robust prediction and evaluation of immunotherapy outcomes. For instance, Rashidian et al. reported that while the intratumoral infiltration of CD11b⁺ cells in responders positively correlated with the efficacy of anti-PD-1 immunotherapy, CD11b⁺ cells in nonresponders were believed to stop the entry of CD8⁺ T cells into the tumor and negatively impacted the therapeutic outcomes [17]. Multi-biomarker imaging may hold the key to precision immunotherapy. Multi-isotope single-photon emission computed tomography (SPECT), which can distinguish signals from different isotopes, can be used to target multiple biomarkers for a better understanding of the complex immune system. In addition, multi-color optical imaging and multispectral optoacoustic tomography can serve as complementary imaging modalities for simultaneously imaging different biomarkers, although they are not as sensitive and quantitative as radionuclide-based imaging. By integrating the concerted efforts from radiologists, oncologists, radiochemists, immunologists, medical physicists, biologists, etc., multimodal molecular imaging approaches may lead the way to potentiate more effective precision immunotherapy at both the preclinical and clinical settings.

As a relatively new cancer marker and immune checkpoint protein, there are still many unknowns about CD38-related biology and potential clinical applications. In this work, radiolabeling of anti-CD38 nanobodies for immunoPET was achieved with excellent radiolabeling quality, imaging

contrast, and targeting specificity [15]. Because of the unique advantages of nanobodies and critical role of CD38 in cancer therapy, anti-CD38 nanobody-based immunoPET holds great potential for clinical translation to improve clinical outcomes for cancer patients.

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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.

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