Toward a Better Understanding of Immune Checkpoint Inhibitor Radiolabeled PET Imaging Studies

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Treatment with immune checkpoint inhibitors (ICIs), such as antiprogrammed death-1 (PD-1), antiprogrammed-death ligand-1 (PD-L1), and anticytotoxic T-lymphocyte antigen-4 monoclonal antibodies, have dramatically changed the treatment landscape for a wide range of malignancies (1,2). ICIs aim to restore antitumor immunity by blocking immunosuppressive checkpoints, which are hijacked by cancer cells to avoid destruction by the immune system (3). Although ICIs are the breakthrough in cancer therapy of the last decade, a key effort is to further improve their clinical efficacy.

Biomarkers play a central role in allowing us to better understand the underlying mechanisms of response, nonresponse, and acquired resistance (4) and thus tackle the next challenge in immunooncology. Biomarkers can be subdivided into blood-based, tissue-based (immunohistochemistry or sequencing), exhaled breath analysis (5), and imaging-based biomarkers. As each biomarker has its strengths and limitations, these profiles define their respective roles in the optimization of overall ICI efficacy. For example, if the strategy is to preselect patients who may respond to ICIs, a predictive biomarker is required that is preferably noninvasive, inexpensive, and standardizable. If the focus is a deeper understanding of the mechanisms of action of ICIs, on which to base the design of appropriate (combination) therapy, biomarkers are required that answer questions in a cross-validated method. Here, expenses and global applicability are less important, but this type of research can accelerate future precision medicine advances and, most importantly, may improve on current drug development pipelines.

One of the challenges we encounter in ICI optimization is to define the optimal assessment of the dynamics of tumor PD-L1 expression and PD-1 expression on immune cell subsets. Our knowledge of the regulatory mechanisms controlling PD-L1 expression, and its interplay with other checkpoint molecules (6), is incomplete and complicates the interpretation of static ex vivo assessments (7). Assessment of the expression of targeted immune checkpoint molecules on protein level on tumor tissue, such as PD-L1 expression, has become clinical practice even though its predictive value is moderate at best. Methods to classify and quantify tumor PD-L1 expression vary greatly (8). Histopathology and immunohistochemistry seemingly fail in providing a complete picture, since not every metastasis can be biopsied in each patient and there is a reasonable risk of sampling errors and misinterpretation. This is where ICI radiolabeled PET imaging may play an important role. Data on whole-body PD-1 or PD-L1 expression obtained from PET radiolabeled antibodies may facilitate a more dynamic assessment of immunooncology treatment. Since several studies have now demonstrated the feasibility of ^{89}Zr-labeled ICI PET imaging in the clinical setting (9–11), we urge reflection on the questions that can be addressed by imaging of the PD-1/PD-L1 axis with radiolabeled full antibodies. In other words, should clinical PD-1/PD-L1 imaging be used as a predictive biomarker for preselection of patients, or might another role better match the biomarker profile?

In this issue of The Journal of Nuclear Medicine, Niemeijer et al. report on the safety and biodistribution of ^{89}Zr-pembrolizumab, a radiolabeled anti-PD-1 monoclonal antibody (9). They showed that ^{89}Zr-pembrolizumab PET imaging in patients with advanced non–small cell lung cancer was safe, apart from 1 grade 3 myalgia after tracer injection, and feasible. Radiotracer uptake correlated with the efficacy of pembrolizumab, but this correlation was not statistically significant, as might have been due to the small number of events ($n = 3$) and corresponding low power.

To increase our current understanding of response and (primary) resistance to ICI treatment, ^{89}Zr-labeled ICI PET imaging can make an important contribution to the field. It enables the in vivo visualization of the biodistribution of ICI, allowing us to address questions on the relation between antibody dose and tumor uptake, the relevance of heterogeneous antibody accumulation across different regions within the tumor, and the role of Fc-tail modification or antibody isotypes (12). Furthermore, antibody-based PET imaging may visualize different inhibitory and stimulatory immune checkpoints on tumors, immune cells, and healthy tissue. These tools allow us to study changes in intratumoral accumulation in combination treatments with other therapeutic antibodies or local or systemic treatments that may influence accumulation in the tumor, such as radiotherapy or antiangiogenesis treatment (13).

Comparison of data at the immune cell subset and lesion levels remains difficult and is hard to interpret. As discussed by

Niemeijer et al. (9), PD-1 is expressed by several immune cells, including exhausted effector cells, and by antigen-presenting cells such as dendritic cell subsets. Nonmalignant lymph nodes also showed ^{89}Zr-pembrolizumab uptake, which was demonstrated in 1 patient with the impression of a nonmalignant axillary lymph node on an ^{18}F-FDG PET scan and biopsy-proven PD-1–positive lymphocytes. The authors do not specify whether the PD-1 expression was seen on antigen-presenting cells or T-cell subsets. The difficulty in radiolabeled PD-1 PET imaging is also that lesions are more difficult to delineate in patients treated with a predose of the ICI therapy. This effect can be explained by low numbers of PD-1–positive cells in the tumor, migration of PD-1–positive T cells, and PD-1 receptor occupation and saturation on treatment, causing a loss of signal in ^{89}Zr-labeled anti–PD-1 imaging. PET with radiolabeled ICIs may therefore contribute to an improved understanding of the on-treatment antibody behavior in blood, tumor, and normal tissue such as secondary lymphoid tissues. If baseline or early identification of responding patients is conceivable, this contribution is of great importance to prevent unnecessary immune-related adverse events and costs (14).

Data interpretation is different when a PD-L1 checkpoint inhibitor is used. PD-L1 is, for example, highly expressed by splenic cells. Because of the sink organ capacity of the spleen, there is a dose-dependent targeting of other PD-L1–positive cells, in particular cancer cells (15). Also, translating the quantification of ^{89}Zr-atezolizumab uptake to PD-L1 expression is difficult, since the presence of the tracer may be the result of favorable vascularization or permeability rather than target expression. This tracer presence can still be beneficial for the response to ICI treatment, but tracer uptake should be interpreted cautiously. The intra- and interlesional heterogeneity in tumor tracer uptake was described in all 3 ^{89}Zr-labeled ICI PET imaging studies (9–11). Niemeijer et al. (9) reported that not even half the lesions with a diameter of at least 2 cm showed uptake on ^{89}Zr-pembrolizumab PET scans. In the ^{89}Zr-atezolizumab imaging trial, uptake of ^{89}Zr-atezolizumab strongly correlated with clinical response, whereas ^{89}Zr-nivolumab uptake correlated only with lesional response (10,11). These phenomena may have significant implications in the interpretation of data from radiolabeled ICI PET imaging studies and should be kept in mind, depending on the respective research question.

It should further be noted that there are differences in the design of the studies (Table 1). ^{89}Zr-pembrolizumab and ^{89}Zr-nivolumab PET imaging were performed only on non–small cell lung cancer patients, whereas ^{89}Zr-atezolizumab PET imaging was performed on a heterogeneous patient population (e.g., bladder cancer, non–small cell lung cancer, and triple-negative breast cancer). The variable PD-L1 expression and ICI treatment response across patients with different tumor types may impact the potential correlation between ^{89}Zr-labeled ICIs and clinical response. Also, the PET analyses differed. Lesion tracer uptake was quantified using SUV_{peak} in non–small cell lung cancer patients, whereas in the study of Bensch et al. (10) only SUV_{max} was reported. The cutoff of 2 cm to correct for partial-volume effect was performed in all studies. The interpretation and relevance of tracer uptake in the smaller lesions is not certain but should be noted if we want to translate per-lesion data to the patient level. Ultimately, all collected PET data, preferably including metabolic features assessed by ^{18}F-FDG PET, should be combined in a data warehouse to identify the best approach for analyses and may help in comparisons across different antibody-based PET imaging studies (16).

Future ^{89}Zr-labeled ICI trials need to evaluate whether the differences in ^{89}Zr-labeled ICI uptake correlate with overall survival, progression-free survival, and objective responses according to RECIST, version 1.1, using a diagnostic CT scan. Furthermore, baseline and on-treatment biopsies should be included in a mechanism-based trial program. Here, multiplex immunohistochemistry can be used to quantify and localize corresponding immune cell subsets and their immune checkpoint expression in biopsy samples (17). This

TABLE 1
Main Results of Published ICI Radiolabeled PET Imaging Studies

Clinical trial	Imaging technique	Tumor type	Patients (n)	Results
Niemeijer et al. (9) (NCT03065764)	^{89}Zr-pembrolizumab PET	Non–small cell lung cancer	12	^{89}Zr-pembrolizumab PET is feasible and safe; ^{89}Zr-pembrolizumab uptake is higher in patients with treatment response; ^{89}Zr-pembrolizumab uptake did not correlate with PD-1/PD-L1 expression
Bensch et al. (10) (NCT02453984)	^{89}Zr-atezolizumab PET	Locally advanced or metastatic solid tumors	22	^{89}Zr-atezolizumab PET is feasible and safe; ^{89}Zr-atezolizumab uptake correlates with PD-L1 expression; ^{89}Zr-atezolizumab SUV_{max} predicts response to atezolizumab, including overall and progression-free survival, better than PD-L1 expression assessed by immunohistochemistry
Niemeijer et al. (11) (2015-004760-11)	^{18}F-PD-L1 PET; ^{89}Zr-nivolumab PET	Non–small cell lung cancer	13	^{18}F-PD-L1 and ^{89}Zr-nivolumab PET is feasible and safe; ^{18}F-PD-L1 uptake correlates with tumor PD-L1 expression; ^{89}Zr-nivolumab uptake correlates to PD-1 expression on lymphocytic aggregates; ^{89}Zr-atezolizumab SUV_{peak} correlates with treatment response (responders vs. nonresponders based on RECIST, version 1.1)

information will add biologic relevance to the found heterogeneity on radiolabeled ICI imaging and, more importantly, may shed light on the question of why patients with a biopsy-based low PD-L1 expression by immunohistochemistry are able to respond to ICI therapy.

We are back to our main question: should clinical PD-1 or PD-L1 axis imaging be used as a predictive biomarker for preselection of patients, or might another role better match the biomarker profile? We believe ^{89}Zr-labeled ICI imaging can add value through allowing us to better understand clinical ICI responses, through revealing ways of therapy resistance, and through promoting future immunooncology drug selection and development. The essential steps forward in achieving this goal are, first, to perform histologic evaluation for validation and in-depth molecular and immune cell profiling; second, to perform a whole-body PET evaluation of radiolabeled antibodies to dynamically approach immunologic responses (here, different uptake parameters may correlate with clinical response); third, to gather—into a data warehouse—knowledge of antibody distribution, binding characteristics, and metabolic pathways to increase our understanding of molecular imaging and support holistic multidimensional research (16); and fourth, to ensure prospective standardization based on international guidelines.

Taken together, we believe radiolabeled ICI imaging is valuable in current and future mechanism-driven ICI studies to improve ICI treatment.

DISCLOSURE

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