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INVITED PERSPECTIVE

Towards a better understanding of immune checkpoint inhibitor radiolabeled PET imaging studies

Running title:

Understanding ICI radiolabeled PET scans

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Treatment with immune checkpoint inhibitors (ICIs), such as anti-programmed death-1 (PD-1), anti-programmed-death ligand-1 (PD-L1), and anti-cytotoxic T-lymphocyte antigen-4 (CTLA-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, it is key to further improve its clinical efficacy.

Biomarkers play a central role to better understand the underlying mechanisms of (non)-response and acquired resistance(*4*), and thus tackle the next challenge in immuno-oncology. Biomarkers can be subdivided in 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 ICI, this requires a predictive biomarker that is preferably non-invasive, cheap, and standardizable. If the focus is on a deeper understanding of the mechanisms of action of ICIs to base the design of appropriate (combination) therapy on, biomarkers are required that answer questions in a cross-validated method. Here, expenses and the global applicability are less important but this type of research can accelerate future precision medicine advances, and most importantly, may improve upon 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*). The 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 single metastasis can be biopsied in each patient and there is a reasonable risk of sampling errors and misinterpretation. This is where ICI radiolabeled positron emission tomography (PET) imaging may play an important role. Data on whole-body PD-(L)1 expression obtained from PET radiolabeled antibodies may facilitate a more dynamic assessment of immuno-oncology treatment. Since several studies have now demonstrated the feasibility of [^{89}Zr]Zr-labeled ICI PET imaging in clinical setting(*9-11*), we urge to reflect 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 zirconium-89([^{89}Zr]Zr)-pembrolizumab, a radiolabeled anti-PD-1 monoclonal antibody(*9*). They showed that [^{89}Zr]Zr-pembrolizumab PET imaging in patients with advanced non-small-cell lung cancer (NSCLC) was safe, apart from one grade 3 myalgia after tracer injection, and feasible. Radiotracer uptake was correlated with efficacy of pembrolizumab, however, not statistically significant, which may be 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]Zr-labeled ICI PET imaging can make an important contribution to the field. It enables the in-vivo visualization of the biodistribution of ICI which allows 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 to study changes in intra-tumoral accumulation in combination treatments with other therapeutic antibodies or local/systemic treatments that may influence accumulation in the tumor, such as radiotherapy or anti-angiogenesis treatment(*13*).

Comparison of data at immune cell subset and lesion level remains difficult and is hard to interpret. As discussed by Niemeijer and colleagues(*9*), PD-1 is expressed by several immune cells, including exhausted effector cells, and antigen-presenting cells, such as dendritic cell subsets. Non-malignant lymph nodes also showed [^{89}Zr]Zr-pembrolizumab uptake, which was demonstrated in one patient with the impression of a non-malignant axillary lymph node on [^{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 PET radiolabeled PD-1 imaging is also that lesions are more difficult to delineate in patients treated with a pre-dose of the ICI therapy. This can be explained by low numbers of PD-1 positive cells in the tumor, migration of PD-1 positive T cells, and by PD-1 receptor occupation and saturation upon treatment, causing a loss of signal in [^{89}Zr]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 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. Due to 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]Zr-atezolizumab uptake to PD-L1 expression is difficult, since the presence of the tracer may be the result of favorable vascularization and/or permeability rather than target expression. This 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 three [^{89}Zr]Zr-labeled ICI PET imaging studies(*9-11*). Niemeijer et al.(*9*) reported that not even half of the lesions with a diameter ≥2 cm showed uptake on [^{89}Zr]Zr-pembrolizumab PET scan. In the [^{89}Zr]Zr-atezolizumab imaging trial uptake of [^{89}Zr]Zr-atezolizumab strongly correlated with clinical response, while [^{89}Zr]Zr -nivolumab uptake only correlated with lesional response(*10,11*). These above-mentioned phenomena may have significant implications for the interpretation of data from radiolabeled ICI PET imaging studies. This 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). [^{89}Zr]Zr-pembrolizumab and [^{89}Zr]Zr-nivolumab PET imaging were only performed in NSCLC patients, while [^{89}Zr]Zr-atezolizumab PET imaging was performed on a heterogenous patient population (e.g. bladder cancer, NSCLC 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 of [^{89}Zr]Zr-labeled ICI to clinical response. Also, PET analyses differed. Lesion tracer-uptake was quantified using peak standard uptake values (SUVpeak) in the NSCLC patients, while in the study of Bensch et al.(*10*) only SUVmax values are reported. The cut-off 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 unsure, but should be noted if we want to translate per lesion data to 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 to compare across different antibody-based PET imaging studies(*16*). In future [^{89}Zr]Zr-labeled ICI trials, it should be evaluated whether the differences in [^{89}Zr]Zr-labeled ICI uptake is correlated with overall survival, progression-free survival, and objective responses according to RECIST v1.1 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 will add biological relevance to the found heterogeneity on radiolabeled ICI

imaging. More importantly, this may also shed light on the question 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-(L)1 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]Zr-labeled ICI imaging can add value in better understanding clinical ICI responses, revealing ways of therapy resistance, and in future immuno-oncology drug selection and development. To achieve this, essential steps forward are: 1) obtaining histology for validation and in depth molecular- and immune cell profiling; 2) evaluation by whole-body PET radiolabeled antibodies to dynamically approach immunological responses. Here, different uptake parameters may correlate with clinical response; 3) knowledge on antibody distribution, binding characteristics, and metabolic pathways should be gathered in a data warehouse to increase understanding of molecular imaging and support holistic multi-dimensional research(*16*); and 4) ensuring 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.

DISCLOSURES

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Table. Main results of the published immune checkpoint inhibitor radiolabeled PET imaging studies.

Clinical trial	Imaging technique	Tumor type	Patients	Results
Niemeijer et al. [9] *NCT03065764*	**[^{89}Zr]Zr-pembrolizumab PET**	NSCLC	n=12	- [^{89}Zr]Zr-pembrolizumab PET is feasible and safe - [^{89}Zr]Zr-pembrolizumab uptake is higher in patients with treatment response - [^{89}Zr]Zr-pembrolizumab uptake was not correlated to PD-1/PD-L1 expression
Bensch et al. [10] *NCT02453984*	**[^{89}Zr]Zr-atezolizumab PET**	Locally advanced or metastatic solid tumors	n=22	- [^{89}Zr]Zr-atezolizumab PET is feasible and safe - [^{89}Zr]Zr-atezolizumab uptake correlates to PD-L1 expression - [^{89}Zr]Zr-atezolizumab SUVmax predicts response to atezolizumab including OS and PFS better than PD-L1 expression assessed by immunohistochemistry
Niemeijer et al. [11] 2015-004760-11	**[^{18}F]F-PD-L1 PET** **[^{89}Zr]Zr-nivolumab PET**	NSCLC	n=13	- [^{18}F]F-PD-L1 and [^{89}Zr]Zr-nivolumab PET is feasible and safe - [^{18}F]F-PD-L1 uptake correlates to tumor PD-L1 expression - [^{89}Zr]Zr-nivolumab uptake correlates to PD-L1 expression on lymphocytic aggregates - [^{89}Zr]Zr-atezolizumab SUVpeak correlates to treatment response (responders vs non-responders based on RECIST v1.1)