Programmed Cell Death-1/ Ligand-1 PET Imaging A Novel Tool to Optimize Immunotherapy?

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KEYWORDS

• PD-1 • PD-L1 • Cancer • Molecular imaging • immunoPET • Immunotherapy
• Checkpoint inhibitors

KEY POINTS

- Immune checkpoint inhibitors (ICIs) targeting programmed cell death-1/programmed cell death ligand-1 (PD-1/PD-L1) have shown promising results in patients with various advanced stage solid tumors; however, response is limited to a subset of patients.
- Although the number of clinical studies with ICIs is rapidly increasing, there is still an unmet need for a robust biomarker to optimize response.
- PD-1/PD-L1 imaging using radiolabeled antibodies allows in vivo quantification of whole-body PD-1/PD-L1.
- Clinical PD-1/PD-L1 imaging can contribute to a better understanding of the dynamic complexity of PD-1/PD-L1 in the tumor microenvironment.

INTRODUCTION

Programmed Cell Death-1/Programmed Cell Death Ligand-1 Immune Checkpoint Inhibitors

Immune checkpoint inhibitors (ICIs) targeting the programmed cell death (ligand)-1 (PD-[L]1) axis have emerged at the forefront of cancer treatment.¹ Numerous ICIs have received Food and Drug Administration (FDA) and European Medicines Agency (EMA) approval as first-line or second-line treatment in patients with relapsed or metastatic malignancies, including non-small-cell lung cancer (NSCLC),^{2–4} melanoma,^{5,6} head and neck cancer,^{4,7} Merkel cell carcinoma,⁸ renal cell carcinoma,⁹ and urothelial cell cancer.^{10–12}

Currently, indications are rapidly expanding, including in the neo-adjuvant setting.¹³

Two key factors that determine success of ICI are the presence of PD-L1 and infiltration of distinct immune cell populations in the tumor microenvironment.^{14–18} Their dynamic and mobile nature highlights the complexity of ICI response. So far, favorable responses have been associated with high tumor PD-L1 expression and high T-cell infiltration. Despite promising clinical results, still only a subset of patients show durable responses to ICI targeting PD-1/PD-L1 and objective response rates are limited to 20% to 40% of patients.¹

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The immune histochemical analysis (IHC) of tumor PD-L1 expression currently is the only registered biomarker for treatment selection.^{13–15} It must be noted that patients with tumors stained negative for PD-L1 on a tumor biopsy can benefit from ICI.^{2,3} Thus, there is a need for a robust marker for ICI response.¹⁹ In this review, we focus on the prospect of clinical PD-1/PD-L1 imaging studies using radiolabeled antibodies as a tool to predict ICI response and improve our understanding of the tumor microenvironment to optimize the use of ICI.²⁰

Programmed Cell Death Ligand-1 Expression as a Marker for Immune Checkpoint Inhibitor Response

The association between high PD-L1 expression in the tumor and ICI response has resulted in the recommendation of the FDA/EMA to restrict the use of pembrolizumab (eg, as first-line treatment for advanced or metastasized NSCLC) to patients with a PD-L1 expressing tumors as assessed by IHC.² However, the IHC assessment of PD-L1 on tumor biopsies has several limitations that comprise its clinical use.

First, PD-L1 assessment using IHC on biopsies or single tissue specimen is limited by the heterogeneous tissue expression of PD-L1.²¹ This has been illustrated by heterogenic PD-L1 expression between primary tumors and metastases within one patient.^{22,23} Intratumoral heterogeneity, the discordant PD-L1 expression between tumor biopsy and surgical specimen from one tumor, adds the risk of sampling error and could lead to an overestimation or underestimation of the PD-L1 status of all tumor tissue in a patient.²⁴ Furthermore, PD-L1 expression varies over time, in response to a constantly evolving immune response¹ and to anticancer treatment as demonstrated by the upregulation of PD-L1 after radiotherapy treatment.²⁵

Second, each available validated IHC-assay uses different antibody clones, platforms, thresholds, and scoring algorithms.^{26–28} The feasibility of harmonizing the clinical use of 4 commercial PD-L1 IHC assays has been studied in NSCLC, concluding that 3 of 4 assays are able to similarly detect tumor PD-L1 expression.^{29,30} Nevertheless, interchanging assays and cutoffs leads to “misclassification” of PD-L1 status for 37% of patients, and this has important clinical consequences, as patients are potentially withheld from ICI treatment.^{29,30}

The pitfalls in the IHC analysis of PD-L1 have boosted the development of new tumor tissue, blood-based, and imaging biomarker research

strategies (Table 1). Examples are multiplex IHC that allows for simultaneous quantification of multiple immune checkpoint molecules differentially expressed on immune and tumor cells.^{31,32} Furthermore, the assessment of PD-L1 using next-generation sequencing (NGS) has shown comparable results to PD-L1 IHC analyses³³ and blood-based biomarkers such as PD-1+ CD8+ T cells have been associated with response to PD-1 targeting ICI.^{34,35} Also, PD-L1 expressing circulating tumor cells showed a good correlation with tumor PD-L1 and might serve as a predictor of early response to ICI targeting PD-1.^{36,37}

Molecular imaging modalities can function as a complementary tool to guide the development of effective ICI treatment strategies.³⁸ Using radiolabeled tracers, molecular imaging allows noninvasive visualization of all accessible PD-1/PD-L1,^{39–43} including its heterogeneous expression across metastases on a whole-body level. This approach also suits the dynamic nature of PD-L1/PD-1, as it reflects the actual PD-L1/PD-1 status instead of a historical PD-L1/PD-1 status that may have been subject to change. Repetitive imaging enables on-treatment assessment of PD-L1 expression and provides a unique insight into the therapy-induced changes⁴⁴ and might support alternative treatment planning (eg, radiotherapy before ICI in low PD-L1 expression tumor

Table 1 Summary of the characteristics of the currently explored biomarkers to visualize PD-1/PD-L1				
Ideal PD-1/ PD-L1 Biomarker Features	IHC	Flow cytometry ^a	NGS ^b	PD-1/ PD-L1 PET
Noninvasive	-	√√	-	√√
Cheap	√√	√	-	-
Fast	√	√	√	-
Longitudinal/ dynamic	-	√√	-	√√
Whole-body	-	-	-	√√
Applicable in clinic/as standard	√√	√√	√	√
Quantification	√	√	√	√
Sensitivity	√	√	√	√√

Abbreviations: IHC, immunohistochemistry; NGS, next-generation sequencing; PD-1/PD-L1, programmed cell death-1/programmed cell death ligand-1; √√, yes; √, moderate; -, no.
^a Blood based.
^b Tumor tissue based.

lesions). Finally, it allows for a better understanding of the in vivo distribution and behavior of targeted drugs; for example, drug delivery over time, drug dynamics related to dosing^{39,45}

In Vivo Molecular Imaging

In vivo molecular imaging requires radiolabeling of a compound of interest using a radionuclide that matches the compounds size and half-life, followed by visualization with Single Photon Emission computed tomography (SPECT) or PET imaging.⁴⁵ We describe the current status of pre-clinical and clinical PD-1/PD-L1 imaging studies, and discuss major challenges in analyzing and interpreting their results.

Preclinical programmed cell death-1/programmed cell death ligand-1 imaging studies

Several preclinical studies have demonstrated the feasibility of radionuclide imaging to noninvasively assess PD-1/PD-L1 expression. In preclinical studies, tracers can be directed against human PD-1/PD-L1 to validate tracers for subsequent clinical use. These tracers are evaluated in immune deficient mice bearing human xenografts or in humanized mice models; mice transplanted with human immune cells. Other tracers are directed against murine PD-1/PD-L1 to investigate the distribution of these agents in animals with a fully functioning immune system. Both strategies have provided valuable information about the potential role of PD-1/PD-L1 imaging to better understand and predict the efficacy of ICI.

Different tracer molecules have been developed to image PD-L1 and PD-1. Radiolabeled PD-L1 antibodies can distinguish tumors with different PD-L1 expression levels and allow for monitoring of therapy-induced changes in PD-L1 expression in preclinical tumor models.^{42–44,46} For example, Hettich and colleagues⁴³ developed a copper-64 (⁶⁴Cu) labeled anti-mouse PD-1 antibody to image PD-1 positive cells in murine tumor models, demonstrating specific tracer uptake in lymphoid organs (lymph nodes and spleen). In the same model, ⁶⁴Cu-labeled anti-PD-1 PET was able to measure the infiltration of PD-1 positive cells in melanoma lesions in mice that were treated with a combination of radiotherapy and CTLA-4/PD-L1 inhibitors, suggesting that PD-1 PET might be used for ICI treatment monitoring.

In another study, the clinically approved PD-1 inhibitor pembrolizumab labeled with zirconium-89 (⁸⁹Zr) was evaluated, demonstrating its potential to image tumor infiltration of adoptively transferred human peripheral blood mononuclear cells in humanized mice engrafted with melanoma

tumors.⁴⁷ Next to these studies, several other radiolabeled antibodies, nanobodies, and affibody molecules have preclinically demonstrated to be excellent tracers for noninvasive imaging of PD-L1 and PD-1.^{41,48,49}

Clinical programmed cell death-1/programmed cell death ligand-1 imaging studies

Most clinical PD-1/PD-L1 imaging studies use ⁸⁹Zr-labeled monoclonal antibodies, which combine the sensitivity of PET with the specificity of the antibody, resulting in whole-body PET imaging in a sensitive and quantitative manner.^{40,45,50,51}

Recently, Bensch and colleagues⁵² reported the first clinical PD-L1 PET imaging study with ⁸⁹Zr-labeled anti-PD-L1 monoclonal antibody atezolizumab in 22 patients with either locally advanced or metastatic bladder cancer, NSCLC, or triple negative breast cancer. Patients received 10 mg unlabeled atezolizumab followed by ⁸⁹Zr-atezolizumab (37 MBq, ~1 mg antibody) intravenously. Imaging was performed at 4 and 7 days postinjection, and subsequent atezolizumab treatment was started until disease progression (**Fig. 1**). The investigators concluded that ⁸⁹Zr-atezolizumab injection was safe, apart from one grade 3 infusion-related reaction. In normal tissue, increased ⁸⁹Zr-atezolizumab uptake over time was observed in intestines, kidney, liver, and bone marrow. Furthermore, ⁸⁹Zr-atezolizumab demonstrated high but variable uptake in nonmalignant lymph nodes and spleen. The standardized uptake value (SUV)_{max} ⁸⁹Zr-atezolizumab tumor uptake was 10.4 (95% confidence interval [CI] 8.5–12.7; range 1.6–46.1), with major intratumoral and intertumoral heterogeneity (**Fig. 2**). ⁸⁹Zr-atezolizumab tumor uptake was related to clinical response; patients with complete response had a 2.35-fold higher SUV_{max} than patients with immediate progression (95% CI 98%–476%; $P < .001$).

Niemeijer and colleagues⁵³ published a study using the anti-PD-1 monoclonal antibody nivolumab radiolabeled with ⁸⁹Zr. A total of 13 patients with advanced NSCLC received ⁸⁹Zr-nivolumab (37 MBq ± 10%, 2 mg antibody) followed by PET/computed tomography at 162 hours (~7 days) postinjection. Subsequently, nivolumab treatment was initiated. The tracer injection was considered safe in the absence of grade ≥3 tracer-related adverse events. Quantitative PET-analyses revealed high tracer accumulation in spleen and liver. ⁸⁹Zr-nivolumab tumor uptake was higher in patients with immunohistochemically proven PD-1 positive tumor-infiltrating immune cells as compared with PD-1 negative tumors (median SUV_{peak} 7.0 vs 2.7, $P = .03$).

Fig. 1. Examples of PET/CT images of 4 patients illustrating ^{89}Zr -atezolizumab tumor uptake in 5 different locations on day 7 postinjection (white arrows indicate tumor lesions; PET scans were performed once per patient and time point). Images (A) and (B) are from the same patient, whereas images (C), (D), and (E) are from a separate patient each. (From Bensch F, van der Veen EL, Lub-de Hooge MN, et al. (89)Zr-atezolizumab imaging as a non-invasive approach to assess clinical response to PD-L1 blockade in cancer. *Nature medicine*. 2018;24(12):1852-1858⁵³, with permission.)

^{89}Zr -nivolumab uptake did not correlate with high tumor PD-L1 expression. Visual ^{89}Zr -nivolumab tumor uptake revealed significant heterogeneity, both between and within patients. ^{89}Zr -nivolumab tumor uptake was related to clinical response; of lesions with a diameter of ≥ 20 mm, the SUV_{peak} was numerically higher in responding lesions compared with nonresponding lesions (median SUV_{peak} 6.4 vs 3.0, $P = .019$).

In addition to the first clinical studies with radio-labeled PD-1/PD-L1 monoclonal antibodies, Xing

and colleagues⁵⁴ were the first to demonstrate clinical single-domain antibodies (sdAbs) or nanobody SPECT-imaging. They reported results from 16 patients with advanced NSCLC using NM-01 (anti-PD-L1), which was radiolabeled site-specifically with $^{99\text{m}}\text{Tc}$. The administered dose of 3.8 to 10.4 MBq/kg, corresponding to 100 or 400 μg of NM-01, was considered to be safe. SPECT scans were acquired at 1 and 2 hours postinjection, resulting in visible tumor uptake at 2 hours postinjection. Average tumor to blood

Fig. 2. PET/CT images of lesions of 3 patients with heterogeneous ^{89}Zr -atezolizumab uptake between lesions on day 7 postinjection (PET scans were performed once per patient and time point). Mediastinal lesion of a patient with NSCLC (SUV_{max} 19.9) (left), an abdominal wall metastases of a patient with bladder cancer (SUV_{max} 36.4) (middle), and a bone metastasis of a patient with triple negative breast cancer (SUV_{max} 7.1) (right). (From: Bensch F, van der Veen EL, Lub-de Hooge MN, et al. (89)Zr-atezolizumab imaging as a non-invasive approach to assess clinical response to PD-L1 blockade in cancer. *Nature medicine*. 2018;24(12):1852-1858⁵³, with permission.)

pool ratios (T:BP) at 2 hours varied from 1.24 to 3.53 and correlated well with PD-L1 expression measured immunohistochemically. Visual intratumoral and intertumoral heterogeneity of tracer uptake was noted in some primary tumors and among several patients. Patients with a PD-L1 expression $\leq 1\%$ tend to show a lower T:BP ratio (mean 1.89 vs 2.49, $P = .048$).

Finally, Niemeijer and colleagues⁵³ reported clinical PET imaging data with ^{18}F -anti-PD-L1 adnectin (BMS-986192) in patients with advanced NSCLC. Patients received 3 MBq/kg ^{18}F -BMS-986192 (specific activity ≥ 6.1 GBq/ μmol) and a PET scan was acquired at 1 hour postinjection. SUV_{peak} for lesions with $\geq 50\%$ tumor PD-L1 expression was significantly higher compared with lesions with less than 50% tumor PD-L1 expression, according to IHC (8.2 vs 2.9, $P = .018$). Visual ^{18}F -BMS-986192 uptake heterogeneity was observed both between and within patients. Again, SUV_{peak} for responding lesions was higher than nonresponding lesions (median 6.5 vs 3.2, $P = .03$).

Limitations and Challenges of Programmed Cell Death-1/Programmed Cell Death Ligand-1 Imaging

Currently, multiple clinical PD-1/L1 imaging studies are awaited or ongoing (Table 2). Their main hypothesis is that PD-1/PD-L1 imaging will enable us to (1) study the tumor and normal tissue targeting of PD-1/L1 ICI, (2) determine the correlation between PD-1/PD-L1 targeting of various metastases and their response to ICI, (3) understand the mechanism of action of ICI, and (4) assess the dynamics of PD-1/PD-1 on anticancer treatment. First clinical studies have demonstrated that PD-1/PD-L1 imaging using radiolabeled antibodies, small peptides or sdAbs is feasible and safe. Their results suggest a relation between tracer uptake and IHC PD-L1 expression, as well as a relation between tracer uptake and ICI response. However, they were conducted in a limited number of patients and besides technical limitations, the correct interpretation of tracer uptake is challenging and various contributing factors should be taken into account.

Table 2
Ongoing and awaited clinical PD-1/PD-L1 imaging studies

Clinical Trial	Phase	Imaging Technique	Tumor Type	Patients, n
2015-004760-11	Completed	^{18}F -PD-L1 PET and ^{89}Zr -nivolumab PET	NSCLC	10
NCT02453984	Completed	^{89}Zr -atezolizumab PET	Locally advanced or metastatic solid tumors	22
NCT03514719 (PINNACLE)	Recruiting	^{89}Zr -avelumab PET	NSCLC	37
NCT03829007 (PINCH)	Recruiting	^{89}Zr -durvalumab PET and ^{18}F -FDG PET	HNSCC	58
2015-005765-23	Recruiting	^{89}Zr -durvalumab PET	NSCLC	10
NCT03520634	unknown	^{18}F -PD-L1 PET	Melanoma	15
NCT02760225	Active, not recruiting	^{89}Zr -pembrolizumab PET	Melanoma/NSCLC	18
NCT03065764	Active, not recruiting	^{89}Zr -pembrolizumab PET	NSCLC	10
NCT03843515 (NeoNivo)	Recruiting	^{18}F -FDG PET and ^{18}F -PD-L1 PET	Oral cavity squamous cell carcinoma	15
NCT02978196	Recruiting	$^{99\text{m}}\text{Tc}$ -anti-PD-L1 (sdAb) SPECT	NSCLC	50
NCT03850028	Not yet recruiting	^{89}Zr -atezolizumab PET	DLBCL	20
NCT03853187 (DONAN)	Recruiting	^{89}Zr -durvalumab PET and ^{111}In -CD8 T-cell SPECT	NSCLC	20

Abbreviations: DLBCL, diffuse large B-cell lymphoma; ^{18}F -FDG PET, PET with fludeoxyglucose F 18; NSCLC, non-small-cell lung cancer; PD-1/PD-L1, programmed cell death-1/programmed cell death ligand-1; sdAb, single-domain antibody; ^{89}Zr , zirconium 89.

For example, the image quality of PET imaging is limited by the low spatial resolution that results in a partial volume effect mainly affecting small and low-contrast tumor lesions. Also, measurement errors can occur due to a low signal-to-noise ratio, caused by low injected activity doses and the low positron abundance of ^{89}Zr .⁵⁵ Therefore, heterogeneous tracer uptake should be interpreted with caution in the absence of histologic confirmation of target expression, as visual variable uptake may not reflect true heterogeneous PD-L1 expression but noise-induced variability of the quantitative target-uptake measurements as previously described by Jauw and colleagues.⁵⁵ Variable tracer uptake may also result from differences in perfusion and accessibility in tumor and normal tissues.⁵⁶ Thus, it could be that PD-1/PD-L1 expression is present, but due to limited perfusion, the radiolabeled antibody is not able to reach its target.

Third, knowledge on the target expression levels is not only essential to accurately interpret the acquired scan, but also to find optimal dosing levels for imaging. Immune cells in the spleen often express PD-L1 because of their physiologic role in regulating the immune response. Therefore, the spleen acts as a sink organ for PD-L1 radiotracers. At low antibody doses, most of the injected tracer will accumulate in the spleen, resulting in rapid blood clearance and minimal targeting to other PD-L1-positive tissues like the tumor.^{44,57} By increasing the antibody dose, spleen uptake saturates, resulting in restored circulation time and increased targeting to tumor and other PD-L1-positive tissue.⁵² Contrary to PD-L1, expression levels of PD-1 on immune cells are much lower and there is no sink organ affecting the biodistribution of PD-1 targeting tracers. Therefore, lower doses should be used to prevent saturation of all PD-1 and to obtain high-contrast images.^{43,53}

The physiologic presence of PD-1/PD-L1 in various types of cells (eg, tumor, immune, or other cell types), challenges the interpretation of visual tracer uptake.^{43,44,57} Histologic confirmation is therefore advisable and, for example, multiplex IHC could be used for the identification of each cell type, taken all previously mentioned limitations of IHC on tumor biopsies into account. The addition of another imaging technique (eg, PET with fludeoxyglucose F 18) is also worth considering to distinguish between physiologic targeting from receptor-mediated targeting, or identify different cell types. However, this remains extremely challenging.

Finally, knowledge of the (differences in) makeup of currently available antibodies targeting PD-1/PD-L1 is essential for correct interpretation

of (variable) tracer uptake.⁵⁸ Especially the interaction between the Fc-region of the antibody with the Fc-gamma receptors (FcγRs) presenting immunoglobulin (Ig)G subtypes. Vivier and colleagues⁵⁹ suggested that the binding of humanized IgG1 by FcγRI contributes to the uptake of radiolabeled immuno-conjugates in nontarget tissues during antibody-based PET imaging. Based on their findings, the use of deglycosylated radiolabeled immuno-conjugates is proposed to decrease unwanted uptake in healthy organs, thereby optimizing the imaging quality and interpretation.

FUTURE PERSPECTIVES

The prospect of ICI treatment strategies lies in our ability to translate our increasing understanding of the human immune responses in the tumor micro-environment to more effective treatments. PD-L1 assessment on tumor biopsies using IHC is unlikely to play a role in this respect.^{2,3} PD-1/PD-L1 imaging has shown to visualize whole-body PD-1/PD-L1 expression and accessibility. However, the presence of single immune checkpoints is likely an oversimplification of factors determining ICI therapy response. Therefore, PD-1/PD-L1 imaging may give a more accurate representation of whole-body PD-1/PD-L1 expression compared with IHC PD-L1, but its role as predictor of response remains to be determined. Next to pre-treatment imaging, it would be very relevant to explore the feasibility of (repetitive) on-treatment imaging to assess the dynamics of PD-L1/PD-1 on anticancer treatment.

Different types of radiotracers have already demonstrated their potential in the (pre)clinical setting to image PD-1/PD-L1, including intact monoclonal antibodies, nanobodies, and adnectins. Which one is most suitable depends on the research question. For example, smaller proteins, such as nanobodies and peptides, can be of growing interest because they are rapidly eliminated from the bloodstream and can reach the core of tumors, whereas antibodies may be more limited in their tissue-penetrating capacity because of their size.⁴⁸ Therefore, these molecules may be more suitable to answer fundamental research questions about the expression levels of immune checkpoints in tumors. On the other hand, intact therapeutic antibodies provide us with important information of the whole-body distribution of ICI and the accessibility of tumors for monoclonal antibodies in the clinical setting.

In addition to PD-1/PD-L1 imaging, further insights into the fundamental mechanisms that regulate early aspects of T-cell activation will provide key information to better understand the

variable ICI response rates. In this respect, some promising molecular imaging approaches have been reported recently.^{16,60–62} Imaging CD8+ T cells using antibody fragments allows for direct assessment of the location, density, and proximity of CD8+ T cells, which is relevant because ICI invigorates CD8+ T-cell responses to specific tumor antigens.^{63,64} Several clinical studies in lymphoma and solid malignancies (NCT03802123, NCT03610061) are ongoing; however, a tolerogenic tumor microenvironment can inhibit the activity of cytotoxic T cells. Granzyme B is a serine-protease released from CD8+ T cells and natural killer cells, and is one of the key markers for immune cell activation. Granzyme B PET imaging using a targeted peptide, GZP, can visualize and quantify granzyme B; therefore, it allows for the identification of active tumor infiltrating lymphocytes to monitor the therapeutic efficacy of ICI treatment.⁶⁵ Currently, the in vivo visualization of active TILs is being evaluated using [¹⁸F]F-AraG PET/MR in patients with urothelial carcinoma receiving neo-adjuvant anti-PD-1/PD-L1 ICI treatment (NCT03007719).

SUMMARY

The rapidly evolving landscape of immunoncology is promising, but also leaves many yet unanswered questions. Recent clinical studies have demonstrated feasibility of PD-1/PD-L1 imaging to visualize PD-L/PD-L1 expression in clinical studies. These emerging noninvasive, whole-body and quantitative tools serve to improve our understanding of the dynamic tumor microenvironment and enable optimization of ICI-based strategies.

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