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PD-L1 PET/CT imaging with radiolabeled durvalumab in patients with advanced stage non-small cell lung cancer.

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Abstract

Background

Better biomarkers are needed to predict treatment outcome in NSCLC patients treated with anti PD-(L)1 checkpoint inhibitors. PD-L1 immunohistochemistry has limited predictive value, possibly due to tumor heterogeneity of PD-L1 expression. Noninvasive PD-L1 imaging using ^{89}Zr-durvalumab might provide a better reflection of tumor PD-L1 expression

Patients and methods

NSCLC patients eligible for second line immunotherapy treatment were enrolled. Patients received two injections of ^{89}Zr-durvalumab; one without a preceding dose of unlabeled durvalumab ('tracer dose only') and one with a preceding dose of 750 mg durvalumab, directly prior to tracer injection. Up to four PET/CT scans were obtained after tracer injection. Post-imaging acquisition, patients were treated with 750mg durvalumab every two weeks. Tracer biodistribution and tumor uptake were visually assessed and quantified as standardized uptake value (SUV) and both imaging acquisitions were compared. Tumor tracer uptake was correlated with PD-L1 expression and clinical outcome, defined as treatment response to durvalumab treatment.

Results

Thirteen patients were included and ten completed all scheduled PET scans. No tracer related adverse events were observed and all patients started durvalumab treatment. Biodistribution analysis showed ^{89}Zr-durvalumab accumulation in the blood pool, liver and spleen. Serial imaging showed that image acquisition 120 hours post injection delivered the best tumor to blood pool ratio. Most tumor lesions were visualized with the tracer-dose only versus the co-injection imaging acquisition (25% vs 13.5% of all lesions). Uptake heterogeneity was observed within (range SUVpeak 0.2 to 15.1) and between patients. Tumor uptake was higher in patients with treatment response or stable disease, compared to patients with disease progression according to RECIST 1.1. However, this difference was not statistically

significant (median SUVpeak 4.9 vs 2.4, p=0.06). SUVpeak correlated better with the combined tumor and immune cell PD-L1 score than with PD-L1 expression on tumor cells, although both were not statistically significant (p = 0.06 and p = 0.93, respectively).

Conclusions

^{89}Zr-durvalumab was safe without any tracer related adverse events and more tumor lesions were visualized using the tracer dose only imaging acquisition. ^{89}Zr-durvalumab tumor uptake was higher in patients with response to durvalumab treatment, but did not correlate with tumor PD-L1 IHC**.**

Word count: 350 words

Keywords: PET-imaging, PD-L1 inhibitor, non-small-cell lung cancer, immunotherapy

Introduction

With the introduction of immunotherapy, the treatment of non-small-cell lung cancer (NSCLC) changed dramatically. Multiple trials with PD-(L)1 checkpoint inhibitors in patients with (locally) advanced NSCLC have shown improved survival outcomes as compared to standard of care cytotoxic chemotherapy [1-5]. Unfortunately, not all patients with NSCLC are equally benefitting and the search for biomarkers that can predict treatment outcome is ongoing. Although PD-L1 immunohistochemistry (IHC) and tumor mutational burden (TMB) are associated with clinical benefit to checkpoint inhibitor therapy, they are far from perfect [6-9].

PD-L1 expression is a biopsy-based biomarker, with the disadvantage that a small biopsy specimen does not capture the full extent of tumor heterogeneity of PD-L1 expression and is associated with a higher chance of a false negative test result [6-8 10]. In addition, substantial heterogeneity of PD-L1 expression can be observed within and between tumor lesions of the same patient [11]. As a consequence of this lack of a good predictive biomarker, the majority of patients with advanced stage NSCLC patients are treated with a PD-(L)1 checkpoint inhibitor, with or without chemotherapy [2 5]. Inherently, a large patient group is treated with a potentially toxic treatment without clinical benefit.

Noninvasive biomarkers that are able to overcome the problem of intra-and intertumor heterogeneity are needed. Visualization and quantification of PD-L1 expression on all tumor cells could potentially be such a biomarker and recent clinical studies have shown that with the use of PD-(L)1 directed tracers like zirconium-89-labeled atezolizumab and nivolumab, ^{18}F-BMS-986192 and ^{99m}Tc-NM-01, tumor lesions could be visualized and that tracer uptake correlated with PD-L1 expression on tumor cells [12-14].

Following the results of the PACIFIC trial, adjuvant durvalumab was registered for stage III NSCLC patients treated with concurrent platinum-based chemotherapy and radiation therapy [15]. Adjuvant durvalumab prolonged progression free survival (PFS) significantly, and this also resulted in an overall survival (OS) benefit [16]. However, there is still a large group of patients with disease relapse despite

adjuvant durvalumab treatment. In the advanced disease setting, the phase III MYSTIC trial evaluated durvalumab with or without tremelimumab (anti-CTLA-4) and compared these treatments to standard chemotherapy as the first line treatment for patients with stage IV NSCLC [17]. Unfortunately, the primary endpoint of an improved OS was not met. This supports the need for a better biomarker that can select patients who can benefit from durvalumab or durvalumab tremelimumab combination treatment.

In this paper, we report the results of the first clinical PET imaging study conducted with ^{89}Zirconium labeled durvalumab, an anti-PD-L1 monoclonal antibody, in patients with advanced stage NSCLC. Imaging series were obtained after a single tracer dose injection and after a combined injection with a full dose of unlabeled durvalumab and the tracer dose. The aim of this study was to investigate the safety and feasibility of ^{89}Zr-durvalumab PET-CT and to explore the relation of the imaging results with PD-L1 IHC and treatment response. Due to the heterogeneity of PD-L1 expression in primary and metastatic lesions of individual patients, we hypothesize that ^{89}Zr-durvalumab PET-CT will show substantial differences in tracer uptake between lesions and to explore the relation of the imaging results with clinical parameters such as PD-L1 IHC and treatment response. This study was not powered to evaluate the predictive value of ^{89}Zr-durvalumab PET-CT for PD-L1 IHC or treatment outcome. To study the safety and feasibility, ten patients were required. The protocol allowed to enroll additional patients in case a patient did not complete the PET scan acquisition.

Materials and methods

Patients

Patients with stage IV NSCLC who had progressed after at least one line of platinum based doublet chemotherapy were asked to participate in this study. Earlier treatment with PD-(L)1 checkpoint inhibitors was not allowed. The study was conducted in accordance with the Declaration of Helsinki and

was approved by the Institutional Review Board of the Amsterdam UMC, location VU University Medical Centre, Amsterdam. Prior to inclusion, each patient signed a written informed consent, after receiving verbal and written explanation. The trial was registered at www.clinicaltrialsregister.eu (Clinical Trials Identifier: 2019-000670-37).

Key eligibility criteria were pathologically proven EGFR wild type and ALK fusion negative NSCLC, measurable disease according to RECIST 1.1 [18], ECOG performance status of 0–1, and the willingness to undergo a histological biopsy immediately prior to start of the study. Main exclusion criteria were symptomatic central nervous system (CNS) metastases, use of corticosteroids with an equivalent of >10 mg prednisone/day or active autoimmune disease.

Tumor biopsies

Histological tumor biopsies were obtained before the first ^{89}Zr-durvalumab injection and after the last line of systemic therapy. Biopsies were obtained from one lesion (metastasis or primary tumor, depending on the size and location of the individual lesions) per patient. An experienced thoracic pathologist (E.T.), blinded for clinical information, evaluated the histology slides. Tumor PD-L1 expression was scored for tumor cells, the tumor proportion score (TPS) and for both tumor and immune cells, the combined positive score (CPS) [19 20]. Details on histochemical stains are found in Table 1 of the supplementary data.

Durvalumab radiolabeling

^{89}Zr is purchased from Perkin-Elmer, Boston, USA and coupled to durvalumab (human immunoglobulin G1 kappa monoclonal antibody, primary route of elimination: protein catabolism. Half-life: 18 days) [21] via the bifunctional chelator N-succinyl-desferal-TFP ester (DFO) [22]. ^{89}Zr durvalumab is produced in compliance with current Good Manufacturing Practice at Amsterdam UMC, location Vrije Universiteit.

The procedures for radiolabeling of durvalumab with ^{89}Zr have been validated with respect to the final quality of the prepared conjugate and the production process. Details can be found in supplementary data.

Study design

Two imaging series were scheduled for all included patients (Figure 1). Whole body (vertex to mid-thigh) PET-CT with ^{89}Zr-durvalumab as radiotracer was performed after a single tracer dose injection (37 MBq, 2mg ^{89}Zr-durvalumab) on day one. Twelve days later, a therapeutic non-radiolabeled dose of 750 mg durvalumab was administered and followed within two hours by a tracer dose injection (37 MBq, 2mg ^{89}Zr-durvalumab). This time interval of two hours between the injection of the unlabeled durvalumab and the tracer dose resulted in a situation comparable to a co-administration at the same time, due to the slow tissue uptake from blood pool of large mAb's [23]. The second imaging series intended to overcome a possible sink effect; a small amount of radiotracer might be rapidly cleared from the circulation and accumulate in the liver, spleen or other organs/compartments. This might be overcome by pre-dosing with non-radiolabeled durvalumab that results in sufficient amounts of radiotracer in the circulation to be available for binding to PD-L1 receptors in tumor tissue. The first three enrolled patients were scanned 1, 72, 120 and 168 hours post-injection (for biodistribution purposes), both after the tracer only and after the combined radiolabeled and non-radiolabeled durvalumab injection. The subsequent patients underwent two PET scans after each tracer injection (T = 72 and T = 120 hours). The time interval between the first ^{89}Zr-durvalumab injection and the second combined tracer and non-radiolabeled durvalumab injection was 12 days, allowing for decay of radioactivity.

An ^{18}F-FDG PET scan, a diagnostic CT scan of thorax and upper abdomen and a brain MRI were obtained prior to the initiation of treatment. Following imaging acquisition, durvalumab (750 mg flat dose) was administered every 2 weeks until disease progression, unacceptable toxicity or withdrawal of consent.

Response assessment was performed with diagnostic contrast-enhanced CT scan of thorax and upper abdomen every 6 weeks during treatment and interpreted according to RECIST 1.1 [18].

PET-CT scan analysis

Tumor lesions were identified and segmented on the zirconium-PET images using in-house developed software [24], while also using the low dose CT scan. The baseline ^{18}F-FDG-PET-CT and diagnostic CT scans were used to differentiate between benign and malignant lesions. Volumes of interest (VOI) were manually delineated over the entire tumor lesions, when they could be distinguished from background on the attenuation corrected images of the PET scan. In the case of tumor lesions without evident visual zirconium uptake, a spherical VOI of 1 cc was drawn at the anatomical location of the tumor lesion, based on the low dose CT, ^{18}F-FDG-PET and diagnostic CT data. To quantify radiotracer uptake in normal tissue, a fixed VOI with a diameter of 2 cm (4.2 cc) was used. Tracer uptake in all delineated VOIs was semi-quantitatively assessed as standardized uptake value (SUV). From each VOI, the mean and peak activity concentrations (Bq/ml) were derived, normalized for body weight. SUVmean was reported for normal tissue tracer uptake and SUVpeak for tumor lesions. SUVpeak was used to minimize the noise effect of ^{89}Zr, as SUVmax is based on only one voxel [25]. To avoid partial volume effects, only tumor lesions exceeding 20 mm long axis diameter were included in the analysis.

Blood samples

Venous blood samples (7 mL each) were collected for determination of ^{89}Zr-durvalumab activity at 5, 30, 60 and 120 minutes post-injection (p.i.), and at day 3, 5 and 7 p.i. for the first 3 patients and at 5 and 30 minutes p.i. and at day 3 and 5 p.i. for the other patients.

Adverse events

Tracer related adverse events were recorded from the time of injection of the first tracer dose to the second full dose of durvalumab, which was two weeks after the second imaging series. Before the first and second dose of durvalumab, patients visited the outpatient clinic for a review of adverse events. This included a full physical examination and a laboratory assessment including complete blood count, comprehensive serum chemistry and TSH level. The National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 were used to score adverse events [26].

Statistical analysis

A Mann-Whitney U test was used to compare the SUVpeak of all lesions (long axis diameter ≥20 mm) in the different groups with and without progressive disease. Progressive disease was defined according to RECIST 1.1. The Kruskal-Wallis test was used to compare the SUVpeak in all response categories according to RECIST 1.1 (progressive disease (PD), stable disease (SD), partial response (PR) and complete response (CR)). Further, the relation between the lesion-based ^{89}Zr-durvalumab accumulation and PD-L1 expression as assessed with immunohistochemistry (PD-L1 expression 0%, 1-49%, ≥50%) was also explored with the Kruskal-Wallis test.

Median SUVpeak of all delineated lesions (long axis diameter ≥20 mm) in the entire cohort was calculated and used to divide the patients in groups with high and low uptake. PFS and OS were summarized using Kaplan-Meier plots.

P-values less than 0.05 were considered to be statistically significant. All statistical analysis were performed using SPSS statistics for Windows, version 25.0.

Results

Patients

Thirteen patients were enrolled between April 2018 and June 2019 (**Table 1**). All patients had pathologically confirmed NSCLC and confirmed progressive disease upon prior chemotherapy. All patients received their first tracer dose injection. Eleven out of 13 patients also received the second tracer injection according to study protocol. One patient died as a result of rapid progressive disease in-between scans, and two patients withdrew consent before second imaging series. For the first three patients the more extensive imaging protocol was followed (Table 2 of the supplementary data). Patients started durvalumab treatment on the day of the second tracer administration and received an average of 7 cycles of durvalumab (range 1 – 21, median 3). Response evaluation after 6 weeks was performed in 7 out of 13 patients, the other patients progressed earlier or died. Best observed response was a partial response in three patients, stable disease in two patients and progressive disease in one patient. One patient was not evaluable according to RECIST 1.1.

The reasons for treatment discontinuation were death or progressive disease in ten patients, durvalumab related pneumonitis in one patient, corona pandemic in one patient and at one patient's own request due to sicca symptoms grade II, probably related to durvalumab treatment. Median PFS was 1.3 months (95% CI 0.0 – 3.8) and median OS 4.8 months (95% CI 0.2– 9.4).

Biodistribution of ^{89}Zr-durvalumab

PET imaging 1 hour post injection (without pre-dose of unlabeled durvalumab) showed that ^{89}Zr-durvalumab uptake was mainly present in the blood pool (average SUVmean 7.2), liver (average SUVmean 6.7) and spleen (SUVmean 15.1). The ^{89}Zr-durvalumab activity in the blood pool decreased over time (average SUVmean 1.6 at 120 hours) and was stable in the liver and bone marrow. The spleen

showed the highest uptake with a peak at 72 hours p.i. (average SUVmean 20.0). Low uptake was seen in kidneys, non-tumor bearing lung tissue and brain (**Figures 2a and 2c and supplementary figure 1).**

When ^{89}Zr-durvalumab was administered after a non-radiolabeled therapeutic dose of durvalumab, a different pattern was observed. The presence of ^{89}Zr-durvalumab in the blood pool 1 hour post injection was comparable with the first imaging series, but remained higher during the following scans. This was a 2-fold higher amount compared to the first scan series. This large difference was confirmed by the venous plasma samples. **(Figure 3)**

Further, ^{89}Zr-durvalumab uptake in the second scan series was less pronounced in the organs such as spleen, bone marrow and liver. (**Figures 2b and d**).

Safety

The most frequently reported adverse events from the time of injection of the first tracer dose to the second full dose of durvalumab were anemia and pain (**Table 2**), which are most likely related to previously administered chemotherapy and/or disease progression. No tracer related adverse events were recorded.

Tumor uptake

Visual analyses

In total, 102 lesions from 13 patients were detected on the baseline ^{18}F-FDG PET-CT scans of which 33 lesions had a long axis diameter of ≥20 mm.

From the 102 lesions, 26 (25%) lesions were visualized on the ^{89}Zr-durvalumab PET-CT scans using the tracer only imaging acquisition. Of the lesions with a long axis diameter of ≥20 mm, 10 out of 33 (30%) were visible.

In the imaging series of the first three patients, the tumor to background ratio was highest in the PET-CT scan obtained 120 hours post injection and tumor uptake was heterogeneous within and between patients, as shown in **Figure 4**.

The ^{89}Zr-durvalumab PET-CT scans that were obtained after the unlabeled therapeutic dose of durvalumab revealed a total of 14 (14%) lesions. Only three lesions appeared on the second imaging series that were not visible on the first imaging series. Two of these were small (<20mm) lesions while one was a large lung tumor (62mm).

In total, 50 lesions with ^{89}Zr-durvalumab uptake (malignant and non-malignant) were seen at 120 hours p.i of the 'tracer dose only' scan series, while 15 ^{89}Zr-durvalumab positive lesions (malignant and non-malignant) were seen at 120 hours p.i. in the second scan series (tracer dose after unlabeled therapeutic dose of durvalumab). Of the 50 ^{89}Zr-durvalumab positive lesions, 52% were also ^{18}F-FDG positive and thus regarded as malignant. The ^{89}Zr-durvalumab positive and ^{18}F-FDG negative (non-malignant) lesions were mostly mediastinal lymph nodes, but also axillary, abdominal and supraclavicular lymph nodes were seen. Interestingly, most of these did not show stable uptake. At 72 hours p.i. 23 ^{89}Zr-durvalumab positive, ^{18}F-FDG negative (non-tumor) lesions were seen. At 120 hours this was 24. Only 12 of these lesions were seen both on the 72 hour and the 120 hour scans.

Quantitative analyses

Average SUVpeak tumor for all delineated tumor lesions / SUVpeak aorta at 72-hours and at 120 hours was 4.1/2.2= 1.8 and 3.9/1.9= 2.1 respectively.

For subsequent quantitative analyses of tumor uptake, only ^{18}F-FDG positive lesions ≥20 mm in size of the 'tracer dose only' acquisition were included and delineated on the PET scan at 120 hours after first tracer injection. The range of tracer uptake within patients with >1 lesion varied from SUVpeak 0.2 (patient 3 with 2 lesions) to 15.2 (patient 9 with 6 lesions). This large range is caused by one lesion with

high uptake that was close to the spleen, with spill in of splenic tracer activity in the tumor VOI. Without this outlier, the range varied between 0.2 and 4.1, with an average range of 2.4. In large tumors heterogeneous uptake was observed, most often with uptake in the periphery of the tumor. This might be due to impaired vascularization in the core of the tumor (due to necrosis) as this was also observed on the ^{18}F-FDG-PET. However the periphery of the tumor showed a different uptake pattern on the ^{89}Zr-durvalumab-PET as compared to the ^{18}F-FDG-PET (**Figure 5**).

Response

There were three patients who had a PR and two with SD lasting 3 and 5 months, respectively. Median SUVpeak of tumor lesions in patients without progressive disease at 6 weeks was 4.9 compared to 2.4 in patients with progressive disease at 6 weeks. The difference was not statistically significant (p=0.06). Median SUVpeak in patients with PD, SD and PR was 2.4, 4.6 and 5.9, respectively. These differences however, were not statistically significant (p=0.12) either (**Figure 6**). Patients with an average SUVpeak higher than the median (SUVpeak 3.0) had a PFS of 7.3 months. Those with a SUVpeak lower than the median had a PFS of 5.5 months (P=0.46). For OS, patients with a SUVpeak higher than the median, the mean OS was 18.4 months. In patients with a SUVpeak lower than the median, mean OS was 5.9 months. This difference was not statistically significant (p = 0.13). The outlier near the spleen in patient 9 as mentioned above was excluded from all response calculations.

Immunohistochemistry

Eleven out of 13 patients were evaluable for PD-1 and PD-L1 expression on immune and tumor cells. Our cohort only contained biopsies with a PD-L1 TPS of 0, 1 and 100% (example **supplementary figure 2**). There was no correlation between PD-L1 TPS and the median ^{89}Zr-durvalumab uptake of all tumor lesions (≥20 mm) per patient (p = 0.93). Although not statistically significant, median ^{89}Zr-durvalumab

uptake increased with higher PD-L1 CPS (p=0.06). Again in this calculation the outlier near the spleen was excluded (**Figure 7**). No significant difference was observed in average ^{89}Zr-durvalumab uptake and PD-1 IHC (p = 0.10).

Discussion

In this study we show that ^{89}Zr-durvalumab is safe and well tolerated, without any reported tracer related adverse events. Biodistribution of ^{89}Zr-durvalumab was comparable to results observed in previous studies using ^{89}Zr labeled immune checkpoint inhibitors [12 13]. High uptake was seen in the liver (likely due to tracer catabolism) and spleen where ^{89}Zr-durvalumab binds to PD-L1 receptors on lymphocytes and dendritic cells. As there are PD-L1 positive lymphocytes in bone marrow, uptake here was slightly higher than blood pool. Low uptake was observed in the kidneys, lungs and brain.

We showed the difference between two imaging acquisitions, one without a pre-dose and one with a therapeutic pre-dose of unlabeled durvalumab. The imaging series after the co-injection with unlabeled durvalumab showed a much lower uptake in target tissues (tumor, spleen and bone marrow) compared to the imaging series without a pre-dose; likely due to saturation of the available PD-L1 receptors by the much higher therapeutic dose compared to the tracer dose (750mg vs 2mg). Further, likely due to saturation of the catabolic capacity of the monoclonal antibody durvalumab, the liver also showed lower uptake in the second imaging series. Consequently, also less tumor lesions were delineable on the PET scans that were made after the co-injection with unlabeled durvalumab.

The use of this unlabeled durvalumab intended to overcome the so called sink effect, where a substantial amount of the ^{89}Zr labeled tracer accumulates in non-tumor tissues with high specific (e.g. spleen) or non-specific (e.g. liver) uptake. As a result, insufficient amounts of radiotracer are left in the circulation, available to bind to PD-L1 receptors on tumor cells. We demonstrated however that the imaging series without the unlabeled pre-dose identified more tumor lesions than the imaging series

with the pre-dose. As a result of the co-injection of the tracer with a full dose of unlabeled durvalumab, the latter occupies the majority of the PD-L1 receptors in normal tissue and tumor lesions and a larger fraction of the tracer remains present in the blood pool (**Figure 5b**). This might also explain why a large tumor in the lung was not visualized on the first imaging series, but showed uptake compared to background in the second series. Based on contrast enhanced CT evaluation this was a well-vascularized tumor and the tumor PD-L1 IHC in this specific patient (patient 6) was 1%. The tumor was visualized in the second series due to higher amount of tracer in the blood pool instead of binding to the tumor cells. Selection of the optimal tracer strategy for imaging of tumor lesions remains challenging. In this study we show adequate uptake in tumor lesions and target tissues using the tracer only strategy. However, we only studied either a tracer only dose or co-injection with a full dose of unlabeled durvalumab and the optimal imaging strategy might be co-injection of the tracer with a lower unlabeled dose. Further research is needed to explore if such a strategy is better or not.

Although tumor lesions could be visualized and quantified, not every patient showed tracer uptake in tumor lesions. Absence of tracer uptake however did not rule out a treatment response. For example, the only tumor lesion of patient 12 did not show higher uptake then background, while the availability of tracer was sufficient (**Figure 3**) and a partial response was achieved. Tumor uptake within and between patients was heterogeneous. There are numerous causes for this, such as heterogeneous presence of PD-L1 positive malignant cells or density of these cells in the tumor stroma. Also immune cells can be more prevalent in one part of the tumor, while the other part can be an immune desert. Also PD-(L)1 expression on these immune cells can be heterogeneous [6-8]. In our data, especially in larger tumor lesions, uptake was more pronounced at the periphery of the tumor. This can be caused by the binding site barrier effect where less penetration into the tumor mass occurs as a result of binding of the relatively large size monoclonal antibody to receptors in the periphery of tumor lesions. Further, a higher

perfusion rate at the edge of the tumor compared to the center, or the higher prevalence of immune cells at the periphery of immune-infiltrate excluded tumor lesions could explain this observation [27]. Using the ^{18}F-FDG PET-CT obtained at baseline as reference, we were able to differentiate between malignant and benign lesions that were visualized on the ^{89}Zr-durvalumab PET. To interpret ^{89}Zr-durvalumab PET results in future studies we would advise to make an ^{18}F-FDG PET scan as reference. An interesting difference was observed in ^{89}Zr-durvalumab uptake of non-malignant lesions, mostly lymph nodes, between the scans obtained at 72 and 120 hours post-injection. Some of these lesions showed higher uptake at the scan 72 hours post injection, others at 120 hours post-injection. Since these lesions are lymphoid tissue, the change in uptake over time might be related to the immune cells assembling in lymph nodes and leaving them at the next point in time. Dendritic cells are known to travel from tissue to lymph nodes and T-cells the other way around [28].

Three patients developed an adverse event attributed to durvalumab treatment, predictive signs were not visible on ^{89}Zr-durvalumab PET-CT. Two patients were diagnosed with pneumonitis and one patient experienced sicca symptoms. A higher ^{89}Zr- durvalumab uptake was not observed in lung tissue of patients who developed pneumonitis during durvalumab treatment compared to patients that did not develop pneumonitis. Further, the patient with sicca symptoms did not show uptake on the ^{89}Zr-durvalumab PET-CT in the parotid glands.

Previous studies showed a correlation between progression free survival and a high tumor uptake on immuno-PET. In a clinical study with anti-PD-L1 ^{18}F-BMS-986192, uptake expressed as SUVpeak in tumors was significantly correlated to PD-L1 expression [12]. In the same study, patients were scanned with ^{89}Zr-nivolumab and tumor uptake was significantly higher in patients whose tumor biopsies showed aggregates of PD-1 positive tumor-infiltrating immune cells. Further, the uptake of ^{89}Zr-nivolumab and ^{18}F-BMS-986192 was higher in responding lesions than in lesions that were stable in size or showed an increase. Another ^{89}Zirconium labeled drug study was conducted with the PD-L1 checkpoint inhibitor

atezolizumab [13]. Comparable results as with the ^{89}Zr-nivolumab tracer were found: no correlation with PD-L1 expression on tumor cells, but significantly higher ^{89}Zr-atezolizumab uptake in responding patients. In our study we found that there was not a difference of median SUVpeak between the PD-L1 TPS groups (0%, 1-49% and ≥50%). However, for PD-L1 CPS (both tumor and immune cell PD-L1 expression), a trend between the PD-L1 CPS groups and SUVpeak was found. Due to the spatial resolution of PET, the SUVpeak value is composed of tracer binding to PD-L1 positive tumor and immune cells. Therefore, PD-L1 CPS might be a better tissue correlative for PET than PD-L1 TPS.

In our results there was no significant correlation between response and SUVpeak; although a trend was seen. Since this study was not powered for treatment outcome, as the sample size was too small, future studies need to evaluate the predictive value of ^{89}Zr-durvalumab for durvalumab treatment outcome. Also, a relatively large number of patients deteriorated quickly. As known, the clinical situation in patients with progressive NSCLC after first line of chemotherapy often declines rapidly. Also the study design could have possibly affected the clinical outcome. Due to the extensive imaging protocol, there was a study related delay in the start of treatment.

Immuno-PET is a promising step forward in prediction of response to checkpoint inhibitors. Identifying the best treatment strategy is of great importance to prevent unnecessary toxicity and costs [29-31]. The group of patients who receive PD-(L)1 checkpoint inhibitors is growing. Recently, adjuvant durvalumab in stage III has been approved [15 16] Neo-adjuvant immunotherapy for early stage NSCLC might follow in the near future [32-34]. However, in a substantial number of these patients, the disease will relapse. A one size fits all strategy feels like a step backwards in time. Immuno-PET tracers such as the ^{89}Zr-durvalumab tracer could potentially guide patient selection in the clinical setting and assist in the development of new treatment strategies.

Conclusion

This study shows that ^{89}Zr-durvalumab PET-CT imaging is safe and feasible. Tumor lesions could be visualized and quantified, and more tumor lesions could be delineated with the use of only the tracer dose of durvalumab compared to the use of an unlabeled therapeutic pre-dose of durvalumab. ^{89}Zr-durvalumab uptake did not correlate with PD-L1 TPS. Non-significant correlations were found between clinical outcome during durvalumab treatment and tracer uptake and between PD-L1 CPS and tracer uptake. Further research is needed to investigate the potential role and the optimal dose of ^{89}Zr-durvalumab as a biomarker in cancer patients treated with durvalumab.

KEY POINTS

QUESTION: Is ^{89}Zr-durvalumab PET-CT a safe and feasible tool to visualize and quantify PD-L1 positive malignant lesions in NSCLC.

PERTINENT FINDINGS: In this single arm open label exploratory pilot 13 patients underwent one or more ^{89}Zr-durvalumab PET-CT scans. There were no serious adverse events and uptake of the ^{89}Zr-durvalumab was visualized and quantified in malignant lesions. Uptake showed heterogeneity within and between lesions. ^{89}Zr-durvalumab uptake showed a better correlation to PD-L1 CPS then PD-L1 TPS IHC although both not statically significant.

IMPLICATIONS FOR PATIENT CARE: Further research is needed to investigate the potential role of ^{89}Zr-durvalumab as a biomarker in cancer patients treated with durvalumab.

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Figures

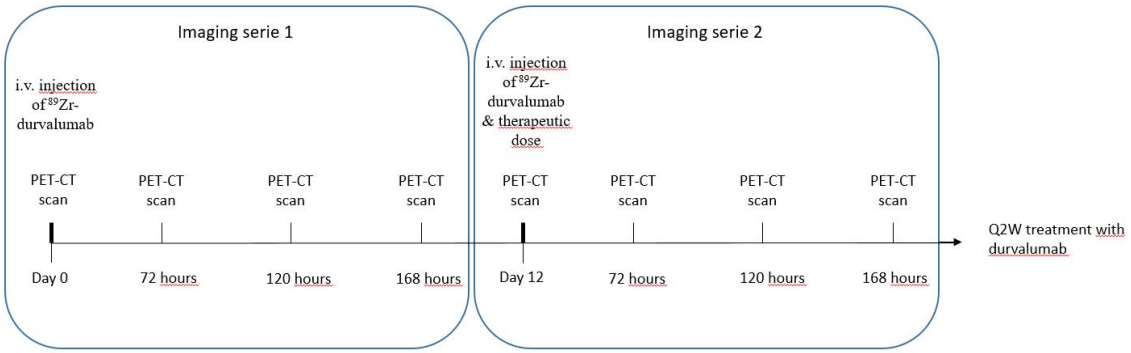


Figure 1

The first 3 included patients received 4 PET-CT scans after each tracer injection (1, 72, 120 and 168 hours post injection). Subsequent patients were scanned at 72 and 120-hours post injection.

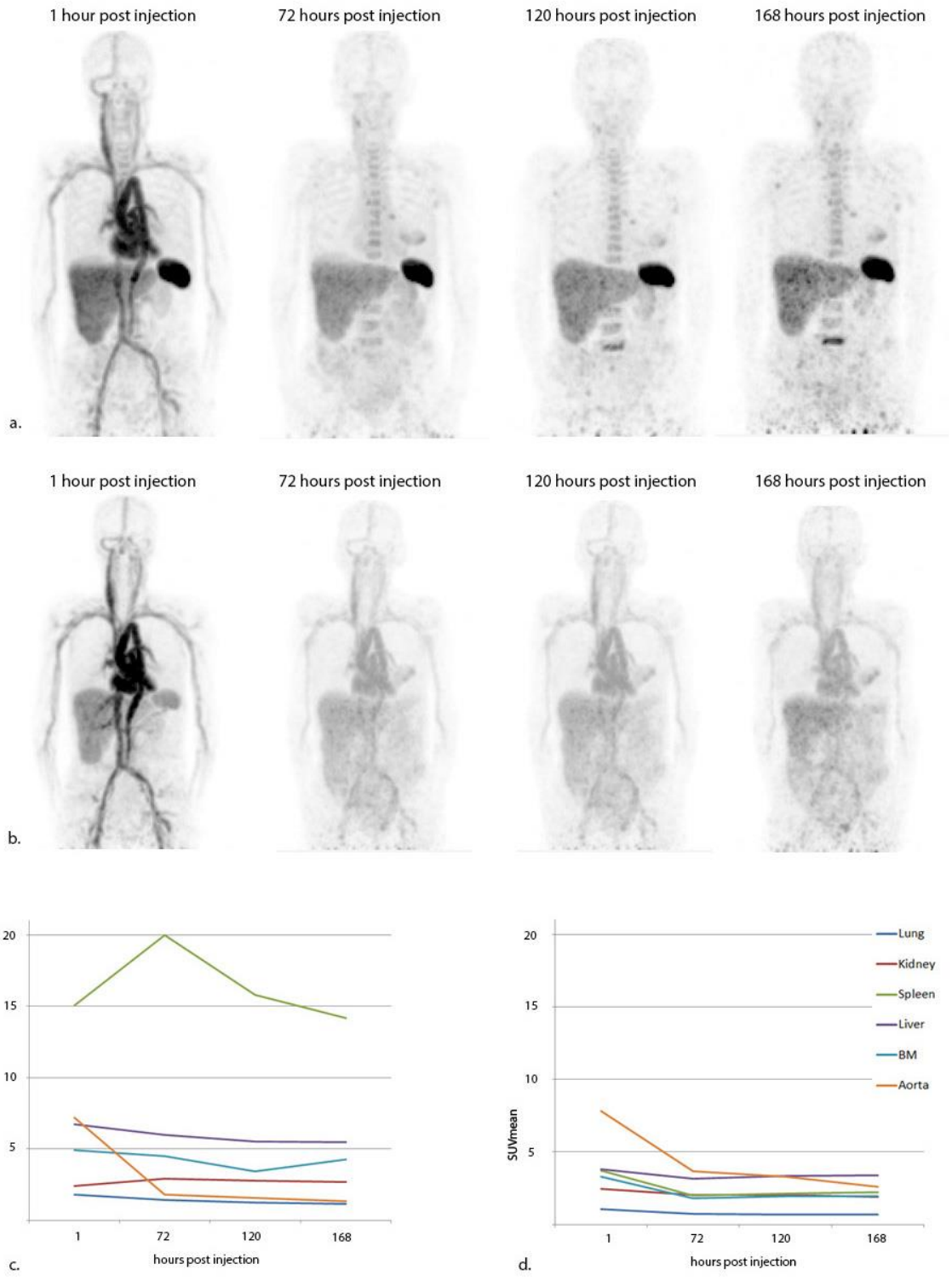


Figure 2

a. *Biodistribution at 1, 72, 120 and 168 hours post injection of tracer dose (2mg) only; b. Biodistribution at 1, 72, 120 and 168 hours post injection of tracer dose (2mg) with unlabeled predose (750mg) of durvalumab; c. Average SUVmean of first 3 patients per organ without unlabeled predose of durvalumab; d. Average SUVmean of patient 2 and 3 per organ with unlabeled predose of durvalumab.*

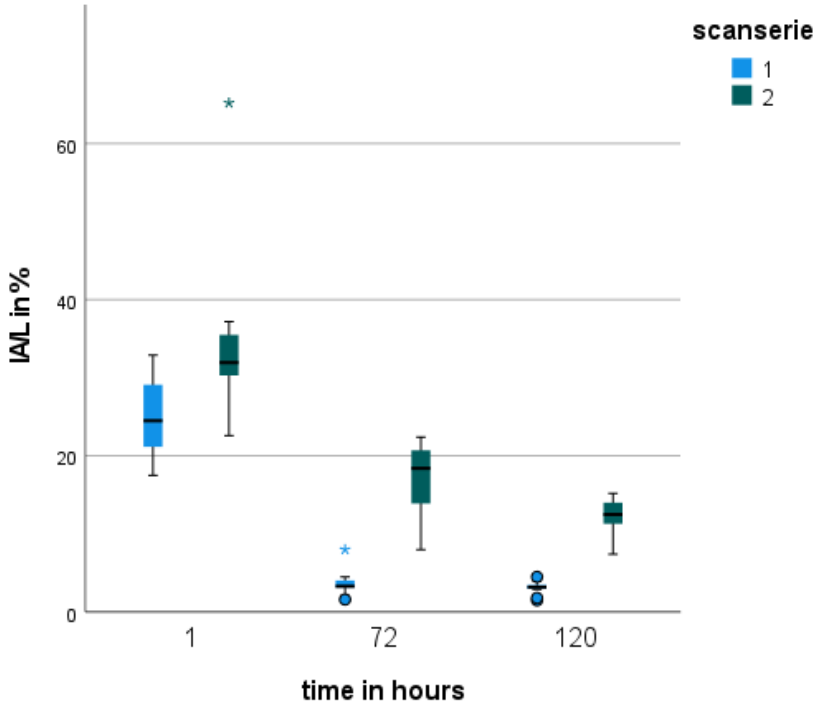


Figure 3

Venous plasma samples at 1, 72 and 120 hours after injection of ^{89}Zr-durvalumab in average *radioactivity in %IA/L. Scanserie 1: without pre-dose of unlabeled durvalumab. Scanserie, 2: with 750mg pre-dose of unlabeled durvalumab.*

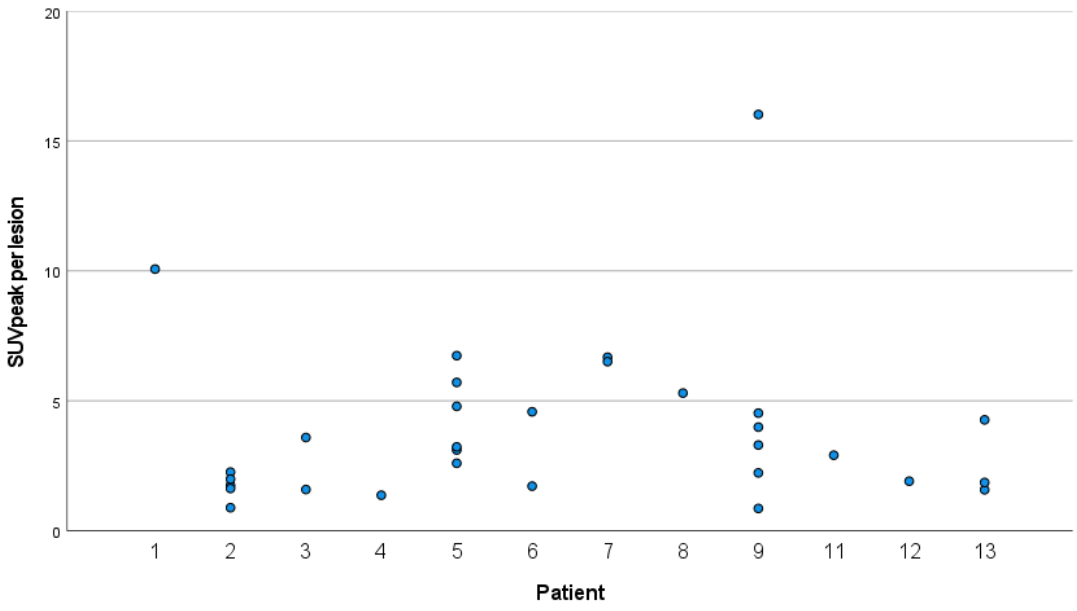


Figure 4

Tracer uptake for all patients per delineated tumor ≥20mm. Without pre-dose of durvalumab at 120 hours post tracer injection. (patient 12 scan at 72-hours as the 120 hours scan was not available).

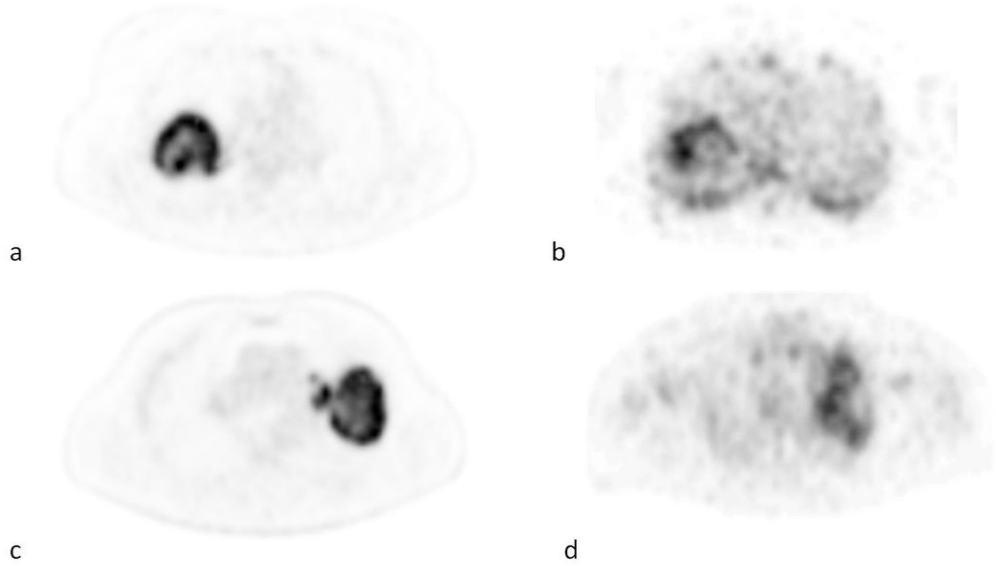


Figure 5

a. *^{18}F-FDG-PET of a large, malignant lesion in the right lung;* b. *Same patient as a. with heterogeneous uptake of ^{89}Zr-durvalumab in a large malignant lesion in the right lung;* c. *^{18}F-FDG-PET uptake in a large malignant lesion in the left upper lobe;* d. *Same patient as c. with heterogeneous uptake of ^{89}Zr-durvalumab in a large malignant lesion in the left upper lobe.*

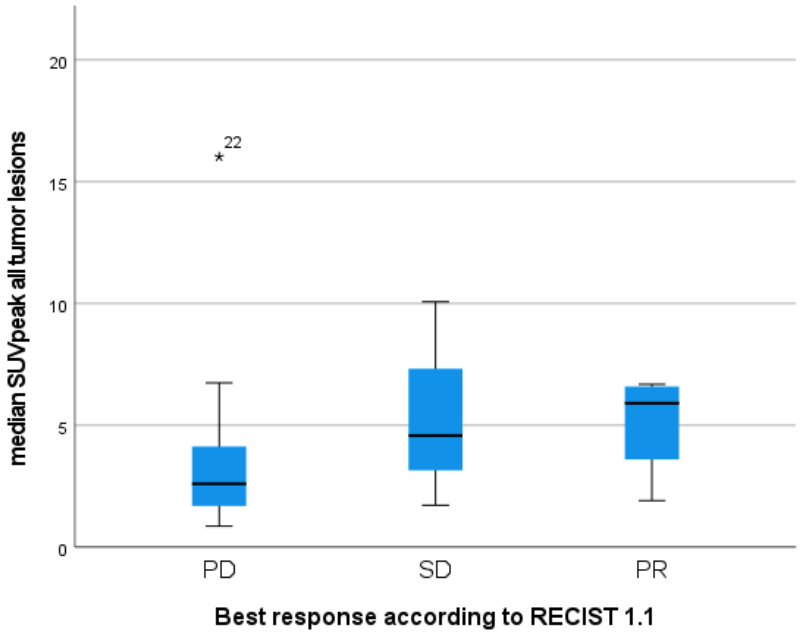


Figure 6

Median ^{89}Zr-durvalumab uptake 120 hours p.i for all tumor lesions (≥20 mm) per best RECIST response category.

**22 represents a tumor lesion close to the spleen, with spill in of splenic tracer activity in the tumor VOI*

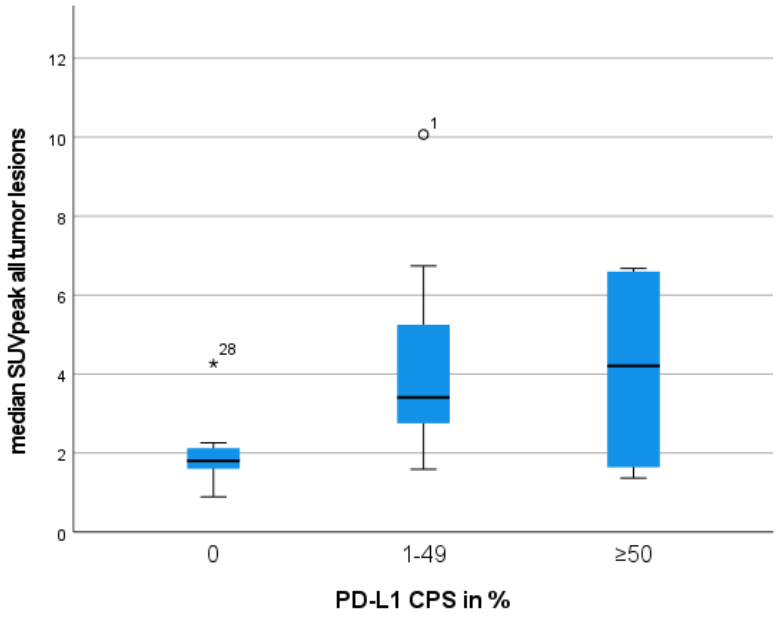


Figure 7

Correlation between median ^{89}Zr-durvalumab uptake 120 hours post injection for all tumor lesions (≥20 mm) and PD-L1 CPS in %.

**22 represents a tumor lesion close to the spleen, with spill in of splenic tracer activity in the tumor VOI.*

°1 represents a tumor lesion in the right middle lobe with very high tracer uptake.

Tables

Table 1 *Baseline characteristics of patients included in the study.*

Patient	Age (y)	M/F	Histology	PD-L1 TPS (%)	PD-L1 CPS (%)	Treatment cycles (n)	BOR	Reason treatment discontinuation	PFS (days)	OS (days)
1	59	F	Adenocarcinoma	0	12.5	7	SD	PD	86	823
2	53	M	Adenocarcinoma	0	0	1	PD	PD	19	19
3	75	F	Adenocarcinoma	0	7,5	1	PD	PD	<15	63
4	79	M	Adenocarcinoma	100	100	2	PD	PD	34	40
5	77	M	Adenocarcinoma	0	5	2	PD	PD	22	147
6	57	F	Squamous	1	5	10	SD	PD	154	182
7	54	M	Adenocarcinoma	100	90	14	PR	PD	183	NR
8	70	M	Squamous	NE	NE	9	PR	Toxicity	684	NR
9	70	M	Adenocarcinoma	0*	NE	1	PD	PD	9	15
10	64	M	Adenocarcinoma	0	0	1	PD	PD	2	2
11	72	M	Adenocarcinoma	1	25	22	NE	COVID-19 pandemic	NR	NR
12	72	F	NOS	100	90	12	PR	Toxicity	NR	NR
13	69	M	Squamous	0	0	3	PD	PD	41	78

**PD-L1 TPS derived from cytology, NR: not reached, NOS: not otherwise specified, SD: stable disease, PR: partial response, PD: progressive disease, NE; not evaluable, BOR: best observed response*

Table 2 *Adverse events.*

Adverse event	Any grade	Grade 3 or 4
Anemia	8 (62%)	1 (8%)
Thrombocytopenia	5 (38%)	
AF increased	6 (46%)	
GGT increased	3 (23%)	
ASAT increased	1 (8%)	
Hypercalcemia	1 (8%)	
Hypomagnesemia	2 (15%)	
Cough	3 (23%)	
Dyspnea	3 (23%)	1 (8%)
Pneumonia	1 (8%)	1 (8%)
Pain	7 (54%)	1 (8%)
Anorexia	4 (31%)	1 (8%)
Constipation	1 (8%)	
Epistaxis	1 (8%)	
Acute kidney injury	1 (8%)	
Vena cava superior syndrome	1 (8%)	1 (8%)

All adverse events recorded from the time of injection of the first tracer dose to the second full dose of durvalumab in 13 patients.

Supplementary material

Production of ^{89}Zr-durvalumab

^{89}Zr-Durvalumab has been produced in compliance with current Good Manufacturing Practice at the Amsterdam UMC, location Vrije Universiteit. ^{89}Zr-durvalumab has been produced according to the previously reported method of Verel et al.[22] In short, 5 mg durvalumab (0.1 mL 50 mg/mL) was diluted with 870 µL 0.9% NaCl. The pH was adjusted to 9.5-9.7 with ± 60 µL 0.1 M Na2CO3. Next, 2 equivalents of TFP-Fe-N-suc-DFO ester in MeCN (20 µL) were added and reacted for 30 minutes at room temperature followed by 50 uL 100 mg/mL gentisic acid pH 4.0-4.2. Subsequently the pH was adjusted to 4.2-4.5, 50 µL of 25 mg/mL EDTA added and the reaction mixture heated to 35C for 30 minutes to remove Fe from DFO. Next, the conjugated DFO-durvalumab was purified by size exclusion chromatography (PD10, GE Healthcare) and the product collected in 0.9% NaCl. Finally DFO-durvalumab was radiolabelled. To this end 150 µL 1M oxalic acid containing the required amount of ^{89}Zr was mixed with 67.5 µL 2M Na2CO3 and reacted for 3 minutes. Next 0.75 mL 0.5 M Hepes and 0.53 mL DFO-durvalumab (~1.7 mg) were added and reacted for 60 minutes at room temperature while slowly shaken. After the incubation period, ^{89}Zr-durvalumab was purified by size exclusion chromatography using a PD10 column and 0.9% NaCl as eluent. The product was formulated to arrive at an injection dose of 37 MBq – 2 mg – 20 mL ^{89}Zr-durvalumab with 5% coverage to compensate for losses during sterile filtration. The mean of the product pH was 6.4 ± 0.3. The mean radiochemical purity as assessed by iTLC was 99.4 ± 0.3%. To this end 2 µL of product was applied on a iTLC strip (Biodex, product nr 150-771) and developed with TLC eluent (450 µL 20 mM citric acid/50 mM EDTA pH 4.8-5.0 and 50 µL MeCN). After the solvent front has reached the top of the strip, the strip is cut at the indicated line. The top part of the strip will then contain free ^{89}Zr/^{89}Zr-DFO, while the bottom part contains ^{89}Zr-durvalumab. The mean radiochemical purity was 99.8 ± 0.4% and the mean protein integrity was 98.8 ± 0.8% as determined by size exclusion HPLC using a superdex 200 10/30 GL increase size exclusion column (GE healthcare Life sciences) including a guard column using

a mixture of 0.05 M sodium phosphate, 0.15 M sodium chloride (pH 6.8) and 0.01 M NaN3 as the eluent at a flow rate of 0.5 mL/min. The mean immune reactive fraction as assessed by a binding assay was 89.9 ± 3.8%. Sterility of each ^{89}Zr-durvalumab batch was assured by performing a media fill immediately after final filter sterilisation of each batch. These procedures resulted in a sterile final product with endotoxin levels <0.2 EU/mL.

Supplementary tables

Supplemental Table 1. *Details immunohistochemistry*

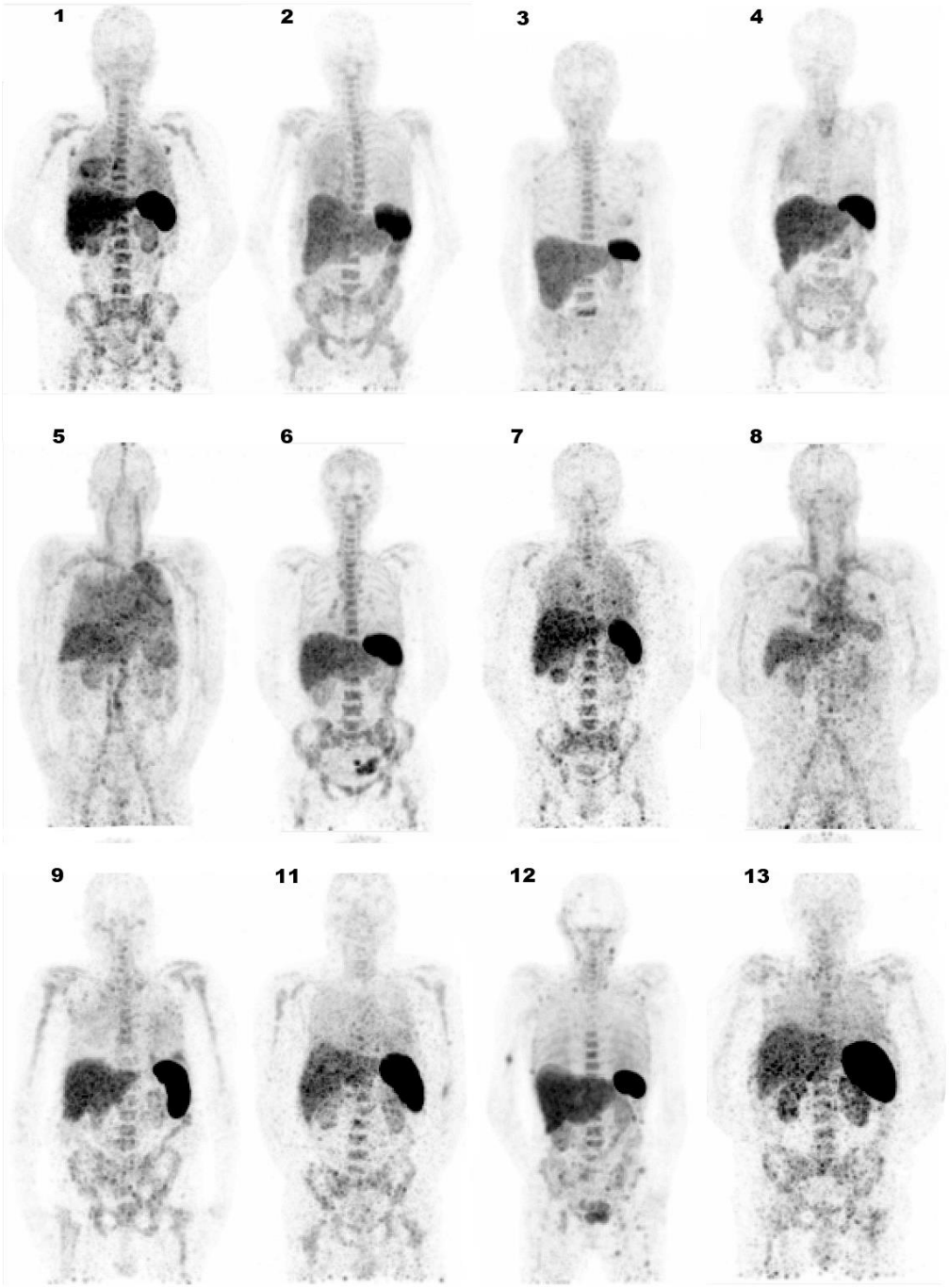
Antibody	**Company**	**Clone**	**Species**	**Epitope retrieval**	**Dilution**	**Incubation time**	**Detection method**
PD1	Cell Marque Corporation	NAT105	Mouse	24 min CC1	1/100 in Dako reduc ab dilution	32 min	Optiview
PD-L1 SP263	Ventana	SP263	Rabbit	68 min CC1	RTU	16 min	Optiview

Supplemental Table 2 *Acquired PET-CT scans.*

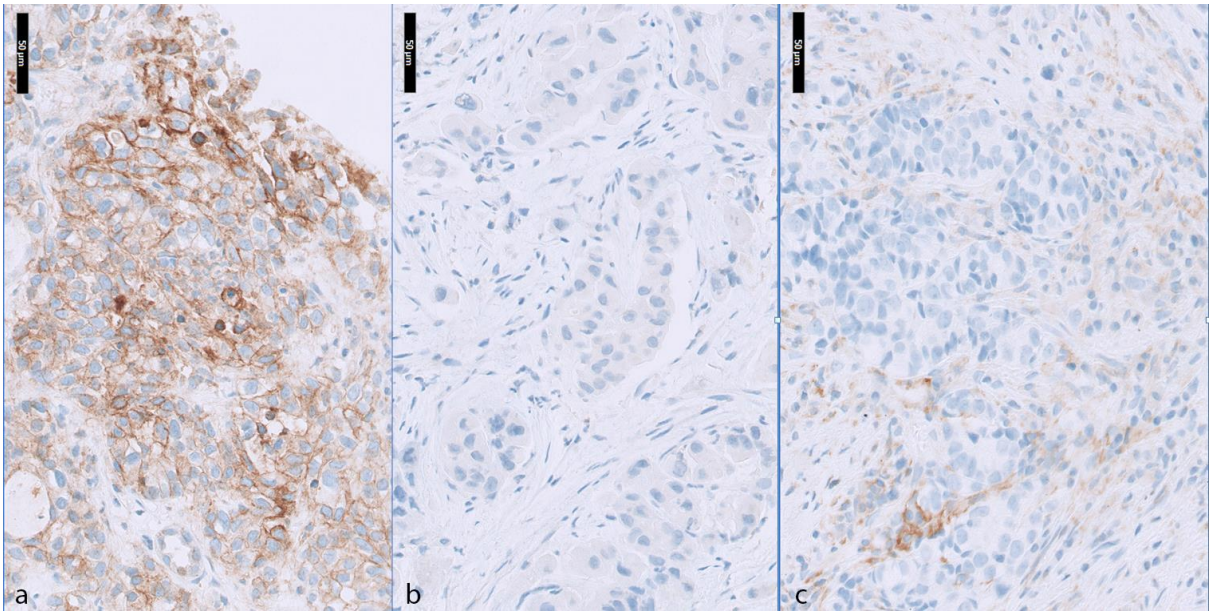
Patient	Tracer dose 1	1 hour	72 hours	120 hours	168 hours		Tracer dose 2	1 hour	72 hours	120 hours	168 hours
1	X	X	X	X	X		-	-	-	-	-
2	X	X	X	X	X		X	X	X	X	X
3	X	X	X	X	X		X	X	X	X	X
4	X	-	X	X	-		X	-	X	X	-
5	X	-	X	X	-		X	-	X	X	-
6	X	-	X	X	-		X	-	X	X	-
7	X	-	X	X	-		X	-	X	X	-
8	X	-	X	X	-		X	-	X	X	-
9	X	-	X	X	-		X	-	X	X	-
10	X	-	X	X	-		X	-	-	-	-
11	X	-	X	X	-		X	-	X	X	-
12	X	-	X	-	-		-	-	-	-	-
13	X	-	X	X	-		X	-	X	X	-

PET-CT scans were planned at 1, 72, 120 and 168 hours post-injection for the first 3 patients, and at 72 and 120 hours post injection for the other patients. Patient one withdrew consent after the 4th PET-CT was obtained and did not receive second tracer dose. Patient 10 died of progressive disease and patient 12 withdrew consent after the PET-CT 72 hours post-injection of the first tracer dose.

Supplementary figures



Supplementary figure 1
MIP (maximum intensity projection) per patient 120 hours post tracer injection, without pre-dose of durvalumab. (patient 12 scan at 72-hours as the 120 hours scan was not available).



Supplementary figure 2

Examples PD-L1 immunohistochemical staining is shown for 3 needle biopsies. a. 100% membranous expression of PD-L1 in all tumour cells. b. No membranous PD-L1 in tumor cells. c. PD-L1 positivity in some stromal lymphocytes and histiocytes with focal (1%) membranous expression in tumor cells (lower side).