

Whole-body CD8 PET imaging captures changes in systemic lymphocyte distribution associated with cancer immunotherapy-related toxicity

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Abstract

CD8 lymphocytes are considered key effectors cells of immunotherapy-induced anti-tumour response and immune-related adverse events (irAEs). Local CD8 lymphocyte activity in tissues results from a multi-step process requiring trafficking of CD8 lymphocytes across several body compartments. Hence, *in vivo* distribution of CD8 lymphocytes is a critical asset of ensuing immune responses. Anti-CD8 α PET imaging enables non-invasive, quantitative evaluation of systemic CD8 lymphocyte distribution *in vivo*. This study investigated CD8 lymphocyte distribution in relation to occurrence of immune checkpoint inhibitor (ICI) therapy related irAEs. A cohort of 97 patients with metastatic cancer underwent PET/CT imaging 24 hours post injection of [^{89}Zr]Zr-crefmirlimab berdoxam, a CD8 α PET tracer, at baseline and after two courses of ICI therapy. Baseline CD8 lymphocyte distributions showed that lower lymph node uptake of uptake [^{89}Zr]Zr-crefmirlimab berdoxam correlated with increasing age. Upon treatment, different uptake patterns were observed. An increase in liver uptake was associated with occurrence of irAEs, independent of the affected organ. Prediction models trained on anti-CD8 α PET-derived features predicted irAEs with high accuracy. In this largest anti-CD8 α imaging cohort to date, we demonstrate that anti-CD8 α PET can capture shifts in whole body CD8 lymphocyte distribution in immune relevant organs and in organs affected by irAEs, providing new insights in CD8 lymphocyte biology during anti-cancer immunotherapy.

INTRODUCTION

Current anti-cancer immunotherapies, including immune checkpoint inhibitors (ICIs), adoptive T cell transfer and cancer vaccines mediate their cytotoxic effects in the tumour immune microenvironment (TIME) via CD8 lymphocytes as their critical effector cell population [1–4]. Local immune activity is determined by lymphocyte trafficking and successive immune-interactions in lymphoid organs and the TIME [5–8]. Compelling evidence in preclinical models demonstrated the crucial role in immune induction at immune relevant tissues at sites distant from the tumour, including tumour-draining lymph nodes [9–14] and spleen [15–17]. The pivotal role of efficient CD8 lymphocyte trafficking is mainly investigated in multicompartamental mathematical models describing the effect of immune cell trafficking on the development of immune responses [18, 19], or in multiplex immunohistochemistry studies small tissue samples obtained via invasive biopsies [20]. Large scale real-life *in vivo* data on whole-body CD8 cell distributions in cancer patients has not been published yet.

The systemic distribution of lymphocytes is dynamic over a life-time. Transcriptomic analyses of post-mortem tissue samples demonstrated the impact of host factors including age and disease status on residency and circulation of lymphocyte subsets [21–24]. The lymphocyte biodistributions described in these studies are in line with the concepts of immune aging [25–27] and exhaustion [28, 29]. These concepts are increasingly recognized to affect immune therapy induced anti-tumour responses [16, 30–32]. Furthermore, immune related adverse events (irAEs) resulting from immune therapies are associated with infiltration of CD8 lymphocytes in affected tissues [33–36] and might be related to aberrant peripheral CD8 lymphocyte trafficking.

Notwithstanding the invaluable contributions of preclinical models and the molecular level characterization of CD8 lymphocytes in tissue biopsies or blood samples, these technologies have limited potential to study CD8 localization and its dynamics in the context of immunotherapy induced effects in clinical studies. CD8 targeted PET/CT imaging with [⁸⁹Zr]Zr-crefmirlimab berdoxam provides whole body quantification of CD8 lymphocyte presence [37–39]. CD8 PET imaging has been applied for investigating tumour infiltrating lymphocytes in different tumour types [40–43]. Its non-invasive nature allows detection and quantification of longitudinal changes of CD8 lymphocyte presence at organ-levels. Here, we investigated which temporal changes on anti-CD8α PET-derived CD8 lymphocyte localization may associate with the development of irAEs in a cohort of patients with metastatic cancer, combined from two multi-centre studies (NCT03802123 and NCT05013099). In addition, we investigate how CD8 lymphocyte distributions differ between ICI therapy regimens and which host factors associate with *in vivo* CD8 cell distributions at baseline.

RESULTS

Study population

Table 1
Baseline characteristics of the study population

Age, years	Sample (total n = 97)
<i>Mean (range)</i>	64.9 (34–95)
Sex	65 (67.0%)
<i>n (%)</i>	32 (33.0%)
M	
F	
Tumour types	45
<i>n</i>	31
RCC	14
Melanoma	2
NSCLC	2
Merkel cell	1
Esophagogastric	1
Bladder	1
HNSCC	
Sarcoma	
ICI regimen	40
<i>n</i>	26
Anti-PD-1 & anti-CTLA-4	18
Anti-PD-1	4
+ TKI	1
+ chemo	6
+ anti-LAG-3	1
Anti-PD-L1	1
+ anti-VEGF	
+ chemo	
Interval between CD8 PET scans, days	40.3 (27–63)
<i>mean (range)</i>	

Age, years	Sample (total n = 97)
Mean (range)	64.9 (34–95)
BMI, kg/m ² <i>mean (range)</i>	27.5 (16.3–45.8)
RCC, renal cell carcinoma. NSCLC, non-small-cell lung cancer. HNSCC, head and neck squamous cell carcinoma. BMI, body mass index.	

The multi-centre phase II iCorrelate trial (NCT03802123) and phase IIb iPREDICT trial (NCT05013099) included a total of 122 patients. Of these enrolled patients, 97 underwent two anti-CD8α PET scans and were included in this analysis (Figure S1). The majority of patients had renal cell carcinoma (RCC, n = 45), melanoma (n = 31) or non-small cell lung cancer (NSCLC, n = 14) as primary tumour (Table 1). Inclusion was limited to patients scheduled for initial (combination) ICI therapy, with previous other treatments allowed. The most prevalent ICI regimens were anti-PD-1 in combination with anti-CTLA-4 and anti-PD-1 monotherapy. The interval between the baseline and on-treatment scan ranged from 27 to 63 days, which was influenced by timing of baseline imaging and therapy interval, however all on treatment scans were performed before initiation of the third course of ICI treatment.

Baseline uptake in normal tissues

We quantified uptake of [⁸⁹Zr]Zr-crefmirlimab berdoxam in 10 normal organs on 194 scans of 97 patients using both automated and manual segmentations, to correct for respiratory motion artifacts and exclude primary and metastatic tumour lesions. The pituitary was not in field of view in 4 patients. Inguinal and axillary lymph nodes that were not radiologically suspect or histologically confirmed metastatic sites were considered normal lymph nodes. On all PET images, uptake in bone marrow, healthy lymph nodes (LN), spleen and liver was pronounced (Fig. 1A-B). Spleen displayed the highest quantified uptake values (SUV_{mean} range 21.2–99.3) in all patients. The lowest uptake values were found in muscles (SUV_{mean} range 0.13–0.68). Baseline [⁸⁹Zr]Zr-crefmirlimab berdoxam uptake displayed heterogeneity among patients and specifically varied among different age groups. With increasing age, lymph node uptake decreased (Fig. 2A), the correlation between uptake in LN and age was stronger in females compared to males (Figure S3A). Increase in lymph node uptake upon ICI therapy was not correlated with age (figure S3B). The same pattern was seen for bone marrow uptake and patient age (Fig. 2C), but to a lesser extent. Baseline lymph node uptake was higher in patients with melanoma and renal cell carcinoma (in total n = 76) compared to other tumour types (in total n = 21). Despite that the melanoma and RCC groups contained the youngest patients, the observed age-related effect persisted across all tumour types (figure S3C). Blood pool uptake was positively and significantly correlated to uptake in well-perfused organs such as kidney, muscle and lungs, but not with total lymphocyte counts in peripheral blood (Fig. 2E-F). No trend related to patient sex or time of day of the scan acquisition was observed on SUV_{mean} of immune relevant organs (Figure S3, S4).

On-treatment changes in normal tissue uptake upon ICI treatment and corticosteroids

Upon ICI therapy, when patients underwent imaging after two courses of ICI, we observed differential changes in [^{89}Zr]Zr-crefmirlimab berdoxam uptake among ICI regimen groups. Patients who received anti-PD-1 monotherapy showed a general trend of a stable mean uptake in most organs of interest. Regarding spleen uptake, patients treated with anti-PD-(L)1 and TKI exhibited significantly higher change in SUV_{mean} compared to patients on anti-PD-1 in combination with anti-CTLA-4. In patients treated with PD-1 inhibitors combined with chemotherapy, a trend towards increased uptake was observed in lymph nodes, blood pool, bone marrow and spleen. Lymph node change in uptake was significantly higher in patients treated with anti-PD-1 and chemotherapy compared to those treated with anti-PD-(L)1 and TKI.

At baseline, four patients were on corticosteroids and continued this medication use throughout the study. These included three patients on dexamethasone for brain metastases or pain from metastases (2 or 4 mg bidaily) and one patient on 20 mg prednisone daily, indicated for adrenal insufficiency. Eleven patients started corticosteroid use between the two imaging timepoints. This included five patients starting dexamethasone at 4 mg for brain metastases ($n = 4$) or metastatic pain ($n = 1$). Six patients started prednisone at different dosages, which were all indicated for irAEs except one patient with biopsy complications. All patients who started use of dexamethasone after the first, but before the second scan, showed a reduction in mean bone marrow ($p < 0.05$) uptake values compared to those who did not use immunosuppressants ($n = 80$), or used immunosuppressants during both imaging timepoints ($n = 4$) (Fig. 3B). A non-statistically significant trend was observed for reduction in blood pool and spleen. This effect was not observed for patients using prednisone during the second imaging timepoint.

Correlations with immune-related adverse events

During the course of treatment, 78 irAEs of any grade were reported in 43 patients, of which 23 were grade 2 or higher and occurred within 33 days after the second scan. Of those, 14 were labelled as clinically relevant because they were either grade 3 or resulted in treatment discontinuation or initiation of immunosuppressive treatment. Most reported irAEs (any grade) were skin-related toxicity, hepatitis, gastrointestinal toxicity and/or endocrine toxicity. Visible increase in uptake in affected organ was observed in several cases of any grade irAEs, including a case of uveitis (Fig. 4A). In all cases except one patient with hyperthyroidism (CTCAE grade 1), increase in SUV_{mean} in the involved organ was detected. However, similar increases in uptake were also observed in some patients for whom the irAE was not reported within 33 days after last imaging timepoint (Fig. 4B).

The abovementioned analyses on group-level illustrate the multifactorial interplay between [^{89}Zr]Zr-crefmirlimab berdoxam PET features of CD8 cell distributions and host factors, therapy and type of irAE. Using [^{89}Zr]Zr-crefmirlimab berdoxam PET-derived metrics, we employed machine learning to predict the occurrence of irAE on an individual patient level. To this end, we developed an explainable machine learning model to investigate the associations between uptake in immune relevant organs and clinically

relevant irAEs within 30 days after the second imaging timepoint, to explore which features that contribute most to prediction accuracy.

Machine learning model development

Three increasingly complex models were trained with a selection of 32 imaging derived and 5 clinical features (sex, tumour type, age, therapy and BMI, Table S1). In addition to organ volumes, imaging features included both baseline SUV_{mean} values for organs of interest and the engineered feature ΔSUV_{mean} of the same organs. Intercorrelations between the features was tested by a multicollinearity test, but none were excluded for passing the Variance Inflation Factor (VIF) threshold of 10. As the distribution of most feature values was skewed, Youden-optimal thresholds were calculated per model, resulting in improved specificity measures (Fig. 5B). Model performance was evaluated by AUC, the best AUC retrieved was 0.793, by the RF classifier. Classifier XGB performed best in terms of F1-score, balancing precision and recall (Fig. 5B).

IrAEs were labelled clinically relevant when they were grade 3 or were grade 2 and resulted in medication changes. Evaluation of models that predict clinically relevant labelled irAEs compared to overall occurrence of any grade ≥ 2 irAE outcome, learned that the same features were relevant for both outcomes, but with lesser magnitudes (Figure S5). Furthermore, as the dataset was imbalanced in group size between the two clinically relevant irAE groups ($n = 14$ clinically relevant irAEs versus $n = 83$ no clinically relevant irAEs), we evaluated the effect of data augmentation, which showed only minor effects on AUCs (figure S4A).

Model outcome

To learn which anti-CD8 α PET/CT derived features and clinical features are most predictive in our model, Shapley Additive Explanations was applied, showing that ΔSUV_{mean} features – particularly for blood pool, lymph nodes, bone marrow, and spleen – ranked among the most important predictors (Fig. 5C). Baseline SUV_{mean} of lymph nodes, bone marrow and spleen contributed to the prediction of the occurrence, suggesting pre-treatment CD8 lymphocyte distribution patterns modulate clinically relevant irAE risk. Additionally, baseline spleen volume was also identified as a positive predictor of irAE risk, whereas younger age was related to lesser risk of developing clinically relevant irAE. Upon treatment initiation, increased uptake in liver and lungs contributed to the development of irAE. Interestingly, an increase in SUV_{mean} of the blood pool, and to much lesser extent an increase spleen uptake, were negative predictors for clinically relevant grade ≥ 2 irAEs. Unlike baseline SUV_{mean} of the lymph nodes, changes in tracer uptake in lymph node did not contribute to the prediction of irAE risk. Baseline uptake or changes upon treatment in endocrine organs showed ambiguous impact on prediction outcome, as observed from the cohort analyses; increased uptake values in thyroid gland or pancreas also occurred in patients who did not develop clinically relevant irAE. From the clinical factors, receiving combined PD-1 and CTLA-4 inhibition positively contributed to irAE risk, in line with clinical trials reporting increased irAE occurrence in combination treatments as compared to monotherapy anti-PD-1 [44].

DISCUSSION

CD8 lymphocytes are associated with ICI therapy related irAEs [34, 36, 45], and assessment of their *in vivo* distribution across body compartments can be highly informative for understanding aberrant immune responses. In the current study we show the potential of anti-CD8 α PET imaging to quantify inter- and intra-patient variation of CD8 lymphocyte systemic distribution patterns associated with irAEs related to ICI treatment. By employing explainable machine learning models, we show that both baseline and changes in SUV_{mean} features in normal, immune relevant, organs are predictive the likelihood of clinically relevant irAE development. Additionally, increase in tracer uptake in organs affected by irAEs compared to baseline was shown in all cases except one case of thyroiditis. However, this extent of increase in PET uptake in irAE cases was also observed in some patients who did not develop clinically apparent toxicity within the reporting period.

Our machine learning analysis revealed baseline healthy lymph node and bone marrow SUV_{mean} as potential predictive features for subsequent clinically relevant toxicity, which suggest that already before treatment initiation, CD8 lymphocyte signatures may be present that co-determine irAE risk. In addition, change in organs including liver and bone marrow SUV_{mean} were associated with grade ≥ 2 clinically relevant toxicity suggesting that dynamics of CD8 lymphocytes upon ICI treatment indeed play a role in irAE development. Interestingly, this association between increased liver uptake and occurrence of irAEs was irrespective of the organ involved, which could be explained by CD8 lymphocyte infiltration in the liver parenchyma or an increased tracer retention in the liver due to perfusion or increased clearance rate of the radiopharmaceutical. Despite possible clinical variables that were unaccounted for in the current prediction modelling analysis – which include previous treatment lines, on-study radiotherapy or co-medication – our models perform reasonably well with anti-CD8 α PET and CT derived features alone, especially considering the relatively small study size for machine learning models.

Our data also shows that different ICI regimens impact distribution of CD8 lymphocytes in lymph nodes and spleen. It is known that chemo-immunotherapy combinations associate with more toxicity likely due to the combination of tissue damage resulting in increased antigen presentation. This might result in increased CD8 trafficking, which is consequently reflected in increased SUV_{mean} in healthy lymph nodes in chemo-immunotherapy treated patients compared to anti-PD-1 and TKI treated patients in our analysis. However, as chemo-immunotherapy is indicated only for NSCLC in the current cohort, it is unknown to what extent the tumour type plays a role in this effect. Additionally, our data suggest impact of dexamethasone use on change in bone marrow uptake. We found that concomitant dexamethasone use (4 mg/day) during the second scan was associated with a decrease in uptake in bone marrow, which is in line with the known effects of dexamethasone on lymphocytes [46, 47]. In patients who used prednisone during the second, but not first scan, this effect was not observed, suggesting differential effects between dexamethasone and prednisone. Others have previously shown dexamethasone and prednisone modulate T cells differently [47, 48]. However, clinical confounders cannot be ruled out; the dexamethasone-treated group possibly represents a more advanced disease state as dexamethasone was indicated for brain metastases and pain relief. Beyond these available clinical parameters, our

immune system is shaped over time by many other factors, such as infections and vaccinations [49], which could not be accounted for. In addition, host factors including the composition of the gut microbiome are known correlates of irAEs [34, 50–52], which were not taken into account in this analysis. We did not observe any apparent sex differences, apart from a stronger decline in baseline lymph node uptake with increasing age in females, which aligns with known age-related sex differences [53].

The discrepancy between anti-CD8 α PET uptake and reported irAEs in some cases can partly be explained by the fact that presence of CD8 lymphocyte in organs and tissues does not per se reflect their effector function. CD8 lymphocytes traverse through tissues, but to unleash their cytotoxic capacity requires sufficient stimulation of the T-cell receptor, both in magnitude and duration [54, 55]. In this respect, the development of tracers targeting the activation [13] or release of effector molecules such as granzyme B [56, 57] or interferon [58] can be complementary to localizing and quantifying CD8 lymphocytes. Moreover, CD8 lymphocytes are considered critical actors, but not the sole contributors of irAEs as other cell types are reported to be involved [59–61] opening doors for radiotracers targeting other immune cells [62–64].

At baseline, we demonstrate higher peripheral lymph node uptake in patients of younger age. It is generally acknowledged that immune function declines with age [65, 66], which is reflected in lower CD8 lymphocyte numbers in peripheral lymph nodes [22, 23]. We found that baseline blood uptake correlates with uptake in kidney and muscle; as these organs in general normally contain little to no lymphocytes, this most likely reflects the blood pool in these organs. Additionally, due to hepatic excretion of [^{89}Zr]Zr-crefmirlimab berdoxam, uptake in liver cannot be exclusively attributed to CD8 α target presence but includes a component of tracer excretion and metabolism as well. To avoid misinterpretation due to spill-over from excreted activity in faecal content, the intestinal wall was not included in our analyses although it represents a large mucosal surface harbouring a significant proportion of the total lymphocyte pool [67]. Likewise, quantification of PET uptake in skin was not included in our analysis while skin events were the most often reported irAE in our dataset.

Another limitation of this study is the predefined imaging timepoints after two courses of treatment, which may not capture transient ICI-induced inflammatory events between scan timepoints (i.e. 4–6 weeks) or cover the temporal heterogeneity in immune induction between and within patients in general. Although there is a peak in irAE occurrences during the first 6 to 12 weeks of treatment [68, 69], corresponding with the time frame of this study, events occurring longer after treatment initiation are also reported [70–72].

Quantification of ^{89}Zr -immunoPET images requires consideration of the properties of the radioisotope ^{89}Zr which includes image noise due to the combination of low positron emission yield and gamma scatter, limited spatial resolution, as well as consequences of uneven distribution across organs, in addition to respiratory artifacts [73–77]. We choose SUV_{mean} as it is least sensitive to reconstruction settings [78]. Additionally, for smaller organs, such as lymph nodes and the pituitary gland, partial

volume effects can impact uptake values [79]. Alternative measures may be considered, for example relative distributions or total tracer uptake per organ to quantify the total pool of CD8 lymphocytes in the body [67], and deserve exploration in future studies.

[⁸⁹Zr]Zr-crefmirlimab berdoxam is currently under investigation in phase I and II clinical trials such as NCT07030712, NCT06534190, NCT06650163 and NCT06863233 and has proven CD8 specificity and safety [37, 41, 80]. Other tracers targeting CD8 lymphocytes have different kinetics and perhaps whole-body distribution, but this has not yet been systematically investigated [40, 81, 82]. The long half-life of ⁸⁹Zr would also allow acquiring images at repeated timepoints after a single injection, providing insights in the kinetics of *in vivo* labelled CD8 lymphocytes. Longitudinal quantitative evaluation of CD8 lymphocyte distribution in this setting relates closest to studying CD8 lymphocyte trafficking, which is inherent to lymphocyte function. Smaller radiopharmaceuticals, such as nanobodies [81], are favourable in terms of effective dose and rapid background clearance. This allows repeated injections and longitudinal evaluation of CD8 lymphocyte distributions by providing serial snapshots over time. IrAEs are driven by aberrant trafficking of lymphocytes to normal organs, and both these imaging protocols can contribute to understanding the dynamics of immune trafficking during immunotherapy treatment.

This study evaluated whole-body CD8 lymphocyte distributions at baseline and early on-treatment with [⁸⁹Zr]Zr-crefmirlimab berdoxam PET/CT imaging in the largest oncological cohort to date. We demonstrate associations of uptake in peripheral lymph nodes with age at baseline and increased liver uptake with the occurrence of irAEs. Our explainable machine-learning approach to use the multidimensional data derived from anti-CD8α PET scans for predicting irAEs indicates that anti-CD8α PET imaging has potential as a biomarker technology to understand host CD8 immunotypes.

METHODS

Study design

Data from patients from two multi-centre clinical trials (NCT03802123, phase 2a and NCT05013099, Phase 2b) were included. The study protocols were approved by the review boards from all institutions involved and patients provided written informed consent. Eligible patients had histopathological confirmed advanced or metastatic solid tumour (including melanoma, non-small cell lung cancer, renal cell carcinoma, Merkel cell carcinoma) and were scheduled to start immune checkpoint inhibitor (ICI) therapy. This includes EMA- and FDA-approved standard-of-care regimens with anti-CTLA-4 or anti-PD-(L)1 agents, either as a monotherapy or combined with another ICI, chemotherapy or a tyrosine kinase inhibitor (Table 1). All adverse events were prospectively reported for at least 33 days after the last ⁸⁹Zr]Zr-crefmirlimab berdoxam PET imaging (Figure S1B). Adverse events were identified and reported as immune-related adverse events by experienced oncologists using Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0).

[⁸⁹Zr]Zr-crefmirlimab berdoxam administration and PET/CT image acquisition

PET images were acquired at 21 centres. A third-generation Cancer Therapy (CTN3) Oncology phantom was scanned in all centres to allow for calibration between centres. Patients underwent PET imaging twice; maximum 14 days before initiation of ICI therapy and after two courses of ICI therapy. PET images were acquired 24 ± 3 hours after intravenous administration of [^{89}Zr]Zr-crefmirlimab berdoxam at 1.5 milligram protein dose. Planned radioactivity dose was 37 MBq, a cohort of $n = 16$ patients received 111 MBq, as per specific study protocol. Low-dose CT images were acquired with the PET images for anatomic localization and attenuation correction. PET and CT scans were acquired from skull to mid-thigh, with 6–8 minutes per bed position for the 37 MBq injected patients, and 4 minutes for the 111 MBq administered cohort. PET reconstruction settings were compatible with EANM guidelines and considered differences in acquisition times, attenuation corrected PET images were used for analysis. On the day of baseline [^{89}Zr]Zr-crefmirlimab berdoxam administration or PET image acquisition, a venous blood sample was acquired and processed for analysis of total lymphocyte counts, according to local laboratory protocols.

[^{89}Zr]Zr-crefmirlimab berdoxam PET/CT image analysis

Organ (system)	Segmentation method		
	Automatic	Manual	Methods
Blood pool	1		Combined segmentation of aorta, vena cava, atria, ventricles and iliac veins and arteries
Spleen	1		
Muscle	1		Combined segmentation of gluteus, iliopsoas and autochthonous back musculature
Lungs	1		Automated correction: All lung lobes 10 voxels medial direction, lower lung lobes 10 voxels from the inferior direction, middle lung lobe 5 voxels inferior direction. Combined segmentation of the 5 lung lobes.
Kidneys	1		Automated correction: erosion of 3 voxels from superior direction
Lymph nodes		1	3 lymph nodes selected per region (axillary and inguinal left and right), individual nodes linked baseline and on treatment
Bone marrow	1		Combined segmentation of all vertebrae, sternum, hips and 1st through 6th ribs
Liver	1		
Pituitary gland		1	10mm sphere
Thyroid	1		

Segmentations of regions of interest were generated using both automatic and manual segmentation methods (Table 1). Multiple-organ objective segmentation (MOOSE) software version 3.1 [83] was applied to generate automated segmentations. To prevent overestimation of activity in lung and kidney segments, corrections for motion artefacts and spillover from mediastinal lymph nodes, spleen and liver were made by adjusting the automated segmentations (Figure S2, Table 1). Researchers were blinded for irAEs during analyses of the images. All segmentations were visualized in 3D Slicer software version 5.8.1 superimposed on the PET and CT images and checked visually case by case. Reported tumour lesions were manually excluded from the segmentations. Bone marrow, muscle and blood pool segmentations were combined from multiple segmentations (Table 2).

Table 2. Methods of segmentation of the organ groups of interest

In addition to automated segmentations, the pituitary gland and a selection of lymph nodes were manually segmented using Segment Editor tool in 3D Slicer in the following way. In order to quantify the uptake in peripheral lymph nodes, in the left and right inguinal and axillary regions, 3 lymph nodes per

region were segmented. This was done while assuring the same lymph nodes on baseline and follow up scan were selected. Of patients for whom the pituitary gland was in field of view, a sphere of 10 mm was drawn that contained the gland. Of all resulting segments, PET uptake was assessed and standardized uptake values (SUV) were calculated from mean absolute uptake values using a custom R script, corrected for body weight.

Machine learning model development

Toxicity outcome was defined as reported grade 2 or higher irAE with clinical consequences, and with start date between baseline scan and 33 days after follow-up scan. Univariate logistic regression was performed to assess associations between individual PET derived features and toxicity. Patient level PET parameters and clinical parameters were modelled using three classification algorithms with the following parameters. (1) Logistic Regression (LR) (L1/L2, $C \in \{0.0001-10\}$); (2) Random Forest (RF) ($n_estimators \in \{100,300,500\}$, $max_depth \in \{3,5,7,10,None\}$); (3) XGBoost (RandomizedSearchCV, 150 iterations, continuous distributions for learning rate, subsample, colsample_bytree, reg_alpha, reg_lambda; discrete for n_estimators, max_depth, min_child_weight). All models: inner 5-fold stratified cross-validation (CV) for hyperparameter selection (AUC-ROC criterion); outer 5-fold stratified CV for unbiased performance estimation. SMOTE variants used 75 XGBoost random iterations and full grids for LR/RF.

Model performance was assessed using area under the receiver operating characteristic curve (AUC-ROC), accuracy, precision, recall, and F1-score. Decision thresholds were optimised using Youden's J criterion (maximising sensitivity + specificity - 1) derived from out-of-fold predictions. Feature importance was evaluated using Gini importance (Random Forest with 200 trees, max depth 5 and balanced class weights) and SHAP values (XGBoost, 100 estimators, max depth 4 and learning rate 0.1). All analyses were performed in Python 3 using packages scikit-learn, XGBoost, imbalanced-learn, and SHAP.

Statistical analyses

For all continuous variables, means and ranges were reported. Categorical variables were reported in percentages of population total. All statistical analyses were performed using R version 4.4.1 or GraphPad Prism software version 10 (GraphPad Software Inc., San Diego, CA), employing a P value threshold of 0.05. Inter-group analyses and correlations were performed without assuming Gaussian distributions. Kruskal-Wallis tests were run when comparing multiple groups, employing correction for multiple testing with Dunn's correction method. Mann-Whitney U test was performed when comparing two group means.

Declarations

Ethics approval declaration

The study was approved by the Medical Ethical Committee METC Oost-Nederland.

Data and code availability

Clinical data cannot be made public in accordance with Dutch legislation. Processed imaging data may be made available to researchers upon reasonable request.

Author contributions

Data collection: EP, EvG

Data analysis: EP, EvG, GS, EA

Drafting paper: EP, EA, HW

Revising paper: SH, CvH, KS, KM, MAP, EA, HW

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Competing interests

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References

1. Ribas, A. and J.D. Wolchok, *Cancer immunotherapy using checkpoint blockade*. Science, 2018. 359(6382): p. 1350–1355.
2. Cáceres-Morgado, P. and A. Lladser, *Tumor-Specific CD8(+) T-Cell Responses Induced by DNA Vaccination*. Methods Mol Biol, 2021. 2197: p. 225–239.

3. Dudley, M.E. and S.A. Rosenberg, *Adoptive-cell-transfer therapy for the treatment of patients with cancer*. *Nature Reviews Cancer*, 2003. 3(9): p. 666–675.
4. Raskov, H., et al., *Cytotoxic CD8 + T cells in cancer and cancer immunotherapy*. *British journal of cancer*, 2021. 124(2): p. 359–367.
5. Spitzer, M.H., et al., *Systemic immunity is required for effective cancer immunotherapy*. *Cell*, 2017. 168(3): p. 487–502. e15.
6. Schenkel, J.M. and K.E. Pauken, *Localization, tissue biology and T cell state—implications for cancer immunotherapy*. *Nature Reviews Immunology*, 2023. 23(12): p. 807–823.
7. Chen, D.S. and I. Mellman, *Oncology meets immunology: the cancer-immunity cycle*. *immunity*, 2013. 39(1): p. 1–10.
8. Mazo, I.B., et al., *Bone marrow is a major reservoir and site of recruitment for central memory CD8 + T cells*. *Immunity*, 2005. 22(2): p. 259–270.
9. Prokhnevskaya, N., et al., *CD8(+) T cell activation in cancer comprises an initial activation phase in lymph nodes followed by effector differentiation within the tumor*. *Immunity*, 2023. 56(1): p. 107–124.e5.
10. Fransen, M.F., et al., *Tumor-draining lymph nodes are pivotal in PD-1/PD-L1 checkpoint therapy*. *JCI Insight*, 2018. 3(23).
11. Tsui, C., et al., *Lymph nodes fuel KLF2-dependent effector CD8 T cell differentiation during chronic infection and checkpoint blockade*. *Nature Immunology*, 2025. 26: p. 1752–1765.
12. Rahim, M.K., et al., *Dynamic CD8 + T cell responses to cancer immunotherapy in human regional lymph nodes are disrupted in metastatic lymph nodes*. *Cell*, 2023. 186(6): p. 1127–1143. e18.
13. Levi, J., et al., *Imaging of Activated T Cells as an Early Predictor of Immune Response to Anti-PD-1 Therapy*. *Cancer Research*, 2019. 79(13): p. 3455–3465.
14. Van Pul, K.M., et al., *Immunotherapy goes local: the central role of lymph nodes in driving tumor infiltration and efficacy*. *Frontiers in Immunology*, 2021. 12: p. 643291.
15. Morgan, D.M., et al., *Expansion of tumor-reactive CD8 + T cell clonotypes occurs in the spleen in response to immune checkpoint blockade*. *Science immunology*, 2024. 9(99): p. eadi3487.
16. Hiam-Galvez, K.J., B.M. Allen, and M.H. Spitzer, *Systemic immunity in cancer*. *Nature reviews cancer*, 2021. 21(6): p. 345–359.
17. Schwenck, J., et al., *Cancer immunotherapy is accompanied by distinct metabolic patterns in primary and secondary lymphoid organs observed by non-invasive in vivo (18)F-FDG-PET*. *Theranostics*, 2020. 10(2): p. 925–937.
18. Palsson, S., et al., *The development of a fully-integrated immune response model (FIRM) simulator of the immune response through integration of multiple subset models*. *BMC Syst Biol*, 2013. 7: p. 95.
19. Smith, A.M., *Decoding immune kinetics: unveiling secrets using custom-built mathematical models*. *Nature methods*, 2024. 21(5): p. 744–747.
20. Gupta, T., et al., *Tracking in situ checkpoint inhibitor-bound target T cells in patients with checkpoint-induced colitis*. *Cancer Cell*, 2024. 42(5): p. 797–814. e15.

21. Domínguez Conde, C., et al., *Cross-tissue immune cell analysis reveals tissue-specific features in humans*. Science, 2022. 376(6594): p. eabl5197.
22. Thome, J.J., et al., *Spatial map of human T cell compartmentalization and maintenance over decades of life*. Cell, 2014. 159(4): p. 814–828.
23. Carpenter, D., et al., *Human immunology studies using organ donors: Impact of clinical variations on immune parameters in tissues and circulation*. American Journal of Transplantation, 2018. 18(1): p. 74–88.
24. Wells, S.B., et al., *Multimodal profiling reveals tissue-directed signatures of human immune cells altered with age*. Nature Immunology, 2025: p. 1–14.
25. Kaiser, M., et al., *Immune Aging and Immunotherapy in Cancer*. Int J Mol Sci, 2021. 22(13).
26. Han, S., et al., *Age-associated remodeling of T cell immunity and metabolism*. Cell Metab, 2023. 35(1): p. 36–55.
27. Childs, B.G., et al., *Cellular senescence in aging and age-related disease: from mechanisms to therapy*. Nat Med, 2015. 21(12): p. 1424–35.
28. McLane, L.M., M.S. Abdel-Hakeem, and E.J. Wherry, *CD8 T Cell Exhaustion During Chronic Viral Infection and Cancer*. Annu Rev Immunol, 2019. 37: p. 457–495.
29. Philip, M. and A. Schietinger, *Heterogeneity and fate choice: T cell exhaustion in cancer and chronic infections*. Current opinion in immunology, 2019. 58: p. 98–103.
30. Elias, R., et al., *Aging, immune senescence, and immunotherapy: A comprehensive review*. Semin Oncol, 2018. 45(4): p. 187–200.
31. Miller, B.C., et al., *Subsets of exhausted CD8(+) T cells differentially mediate tumor control and respond to checkpoint blockade*. Nat Immunol, 2019. 20(3): p. 326–336.
32. Bruni, D., H.K. Angell, and J. Galon, *The immune contexture and Immunoscore in cancer prognosis and therapeutic efficacy*. Nature Reviews Cancer, 2020. 20(11): p. 662–680.
33. Hone Lopez, S., et al., *Immune checkpoint inhibitor treatment induces colitis with heavy infiltration of CD8 + T cells and an infiltration pattern that resembles ulcerative colitis*. Virchows Archiv, 2021. 479(6): p. 1119–1129.
34. Lechner, M.G., et al., *Clonally expanded, thyrotoxic effector CD8(+) T cells driven by IL-21 contribute to checkpoint inhibitor thyroiditis*. Sci Transl Med, 2023. 15(696): p. eadg0675.
35. Grabie, N., et al., *Endothelial Programmed Death-1 Ligand 1 (PD-L1) Regulates CD8 + T-Cell Mediated Injury in the Heart*. Circulation, 2007. 116(18): p. 2062–2071.
36. Matson, D.R., et al., *Fatal myocarditis following treatment with the PD-1 inhibitor nivolumab*. Journal of forensic sciences, 2018. 63(3): p. 954–957.
37. Farwell, M.D., et al., *CD8-Targeted PET Imaging of Tumor-Infiltrating T Cells in Patients with Cancer: A Phase I First-in-Humans Study of 89Zr-Df-IAB22M2C, a Radiolabeled Anti-CD8 Minibody*. Journal of Nuclear Medicine, 2022. 63(5): p. 720–726.

38. Omidvari, N., et al., *First-in-human immunoPET imaging of COVID-19 convalescent patients using dynamic total-body PET and a CD8-targeted minibody*. Science Advances, 2023. 9(41): p. eadh7968.
39. Koenen, H.J., et al., *Preliminary evidence of localizing CD8 + T-cell responses in COVID-19 patients with PET imaging*. Frontiers in Medicine, 2024. 11: p. 1414415.
40. Kist de Ruijter, L., et al., *Whole-body CD8 + T cell visualization before and during cancer immunotherapy: a phase 1/2 trial*. Nature Medicine, 2022.
41. Postow, M.A., et al., *CD8 PET imaging with [(89)Zr]Zr-berdoxam-crefmirlimab in patients with solid tumors: the Phase II iCorrelate study*. J Immunother Cancer, 2026. 14(3).
42. de Boer, J.W., et al., *Whole-body CD8(+) T-cell PET imaging in patients with large B-cell lymphoma before and during CD19-directed CAR T-cell therapy: a phase 2 study*. Nat Commun, 2025. 16(1): p. 11629.
43. Wang, Y., et al., *Pilot study of a novel nanobody (68) Ga-NODAGA-SNA006 for instant PET imaging of CD8(+) T cells*. Eur J Nucl Med Mol Imaging, 2022. 49(13): p. 4394–4405.
44. Arnaud-Coffin, P., et al., *A systematic review of adverse events in randomized trials assessing immune checkpoint inhibitors*. International journal of cancer, 2019. 145(3): p. 639–648.
45. Sasson, S.C., et al., *Interferon-Gamma–Producing CD8 + Tissue Resident Memory T Cells Are a Targetable Hallmark of Immune Checkpoint Inhibitor–Colitis*. Gastroenterology, 2021. 161(4): p. 1229–1244.e9.
46. Tyan, K., et al., *Cytokine changes during immune-related adverse events and corticosteroid treatment in melanoma patients receiving immune checkpoint inhibitors*. Cancer Immunology, Immunotherapy, 2021. 70: p. 2209–2221.
47. Fauci, A., *Mechanisms of corticosteroid action on lymphocyte subpopulations. II. Differential effects of in vivo hydrocortisone, prednisone and dexamethasone on in vitro expression of lymphocyte function*. Clinical and experimental immunology, 1976. 24(1): p. 54.
48. Okoye, I.S., et al., *The glucocorticoids prednisone and dexamethasone differentially modulate T cell function in response to anti-PD-1 and anti-CTLA-4 immune checkpoint blockade*. Cancer Immunol Immunother, 2020. 69(8): p. 1423–1436.
49. Halper-Stromberg, A. and B. Jabri, *Maladaptive consequences of inflammatory events shape individual immune identity*. Nat Immunol, 2022. 23(12): p. 1675–1686.
50. Andrews, M.C., et al., *Gut microbiota signatures are associated with toxicity to combined CTLA-4 and PD-1 blockade*. Nat Med, 2021. 27(8): p. 1432–1441.
51. Kang, J.H., J.A. Bluestone, and A. Young, *Predicting and Preventing Immune Checkpoint Inhibitor Toxicity: Targeting Cytokines*. Trends in Immunology, 2021. 42(4): p. 293–311.
52. Valpione, S., et al., *Sex and interleukin-6 are prognostic factors for autoimmune toxicity following treatment with anti-CTLA4 blockade*. Journal of Translational Medicine, 2018. 16(1): p. 94.
53. Klein, S.L. and K.L. Flanagan, *Sex differences in immune responses*. Nat Rev Immunol, 2016. 16(10): p. 626–38.

54. Viola, A. and A. Lanzavecchia, *T cell activation determined by T cell receptor number and tunable thresholds*. Science, 1996. 273(5271): p. 104–106.
55. Conley, J.M., M.P. Gallagher, and L.J. Berg, *T cells and gene regulation: the switching on and turning up of genes after T cell receptor stimulation in CD8 T cells*. Frontiers in immunology, 2016. 7: p. 76.
56. Larimer, B.M., et al., *The Effectiveness of Checkpoint Inhibitor Combinations and Administration Timing Can Be Measured by Granzyme B PET Imaging* Granzyme B PET Immunotherapy Response Quantification. Clinical Cancer Research, 2019. 25(4): p. 1196–1205.
57. Shen, X., et al., *(68)Ga-grazytracer PET for noninvasive assessment of response to immunotherapy in solid tumors and lymphomas: a phase 1/2 clinical trial*. Nat Commun, 2024. 15(1): p. 8791.
58. Gibson, H.M., et al., *IFN γ PET Imaging as a Predictive Tool for Monitoring Response to Tumor Immunotherapy*. Cancer Res, 2018. 78(19): p. 5706–5717.
59. Esfahani, K., et al., *Moving towards personalized treatments of immune-related adverse events*. Nat Rev Clin Oncol, 2020. 17(8): p. 504–515.
60. Ramos-Casals, M., et al., *Immune-related adverse events of checkpoint inhibitors*. Nature reviews Disease primers, 2020. 6(1): p. 1–21.
61. Sullivan, R.J. and J.S. Weber, *Immune-related toxicities of checkpoint inhibitors: mechanisms and mitigation strategies*. Nature Reviews Drug Discovery, 2022. 21(7): p. 495–508.
62. Salehi Farid, A., et al., *CD45-PET is a robust, non-invasive tool for imaging inflammation*. Nature, 2025. 639(8053): p. 214–224.
63. Traenkle, B., et al., *Single-Domain Antibodies for Targeting, Detection, and In Vivo Imaging of Human CD4(+) Cells*. Front Immunol, 2021. 12: p. 799910.
64. Boughdad, S., et al., *(68)Ga-DOTATOC PET/CT to detect immune checkpoint inhibitor-related myocarditis*. J Immunother Cancer, 2021. 9(10).
65. Jang, I.H., et al., *The ageing immune system as a driver of systemic ageing*. Nature Reviews Immunology, 2026: p. 1–18.
66. Mogilenko, D.A., I. Shchukina, and M.N. Artyomov, *Immune ageing at single-cell resolution*. Nature Reviews Immunology, 2022. 22(8): p. 484–498.
67. Sender, R., et al., *The total mass, number, and distribution of immune cells in the human body*. Proceedings of the National Academy of Sciences, 2023. 120(44): p. e2308511120.
68. Postow, M.A., R. Sidlow, and M.D. Hellmann, *Immune-Related Adverse Events Associated with Immune Checkpoint Blockade*. N Engl J Med, 2018. 378(2): p. 158–168.
69. Martins, F., et al., *Adverse effects of immune-checkpoint inhibitors: epidemiology, management and surveillance*. Nature Reviews Clinical Oncology, 2019. 16(9): p. 563–580.
70. Călugăreanu, A., et al., *Late onset of nivolumab-induced severe gastroduodenitis and cholangitis in a patient with stage IV melanoma*. Immunotherapy, 2019. 11(12): p. 1005–1013.
71. Ghisoni, E., et al., *Late-onset and long-lasting immune-related adverse events from immune checkpoint-inhibitors: an overlooked aspect in immunotherapy*. European Journal of Cancer, 2021.

- 149: p. 153–164.
72. Durbin, S.M., et al., *Late-Onset Immune-Related Adverse Events After Immune Checkpoint Inhibitor Therapy*. JAMA Network Open, 2025. 8(3): p. e252668-e252668.
73. Keyes, J.W., *SUV: Standard Uptake or Silly Useless Value?* Journal of Nuclear Medicine, 1995. 36(10): p. 1836–1839.
74. Mohr, P., et al., *Quantification of ⁸⁹Zr-ImmunoPET: Methodological considerations*. Nuclear Medicine and Biology, 2025: p. 109050.
75. Mohr, P., et al., *Advances and challenges in immunoPET methodology*. Frontiers in Nuclear Medicine, 2024. 4: p. 1360710.
76. Miedema, I.H.C., et al., *⁸⁹Zr-Immuno-PET with Immune Checkpoint Inhibitors: Measuring Target Engagement in Healthy Organs*. Cancers, 2023. 15(23): p. 5546.
77. Boellaard, R., et al., *Effects of noise, image resolution, and ROI definition on the accuracy of standard uptake values: a simulation study*. Journal of Nuclear Medicine, 2004. 45(9): p. 1519–1527.
78. Rogasch, J.M., et al., *Influences on PET quantification and interpretation*. Diagnostics, 2022. 12(2): p. 451.
79. Chomet, M., et al., *Performance of nanoScan PET/CT and PET/MR for quantitative imaging of (¹⁸F and (⁸⁹Zr as compared with ex vivo biodistribution in tumor-bearing mice*. EJNMMI Res, 2021. 11(1): p. 57.
80. Pandit-Taskar, N., et al., *First-in-humans imaging with ⁸⁹Zr-Df-IAB22M2C anti-CD8 minibody in patients with solid malignancies: preliminary pharmacokinetics, biodistribution, and lesion targeting*. Journal of Nuclear Medicine, 2020. 61(4): p. 512–519.
81. De Groof, T.W., et al., *Specific imaging of CD8 + T-Cell dynamics with a nanobody radiotracer against human CD8 β* . European Journal of Nuclear Medicine and Molecular Imaging, 2024. 52(1): p. 193–207.
82. Wang, Y., et al., *Pilot study of a novel nanobody ⁶⁸Ga-NODAGA-SNA006 for instant PET imaging of CD8 + T cells*. European Journal of Nuclear Medicine and Molecular Imaging, 2022. 49(13): p. 4394–4405.
83. Sundar, L.K.S., et al., *Fully automated, semantic segmentation of whole-body ¹⁸F-FDG PET/CT images based on data-centric artificial intelligence*. Journal of Nuclear Medicine, 2022. 63(12): p. 1941–1948.

Figures

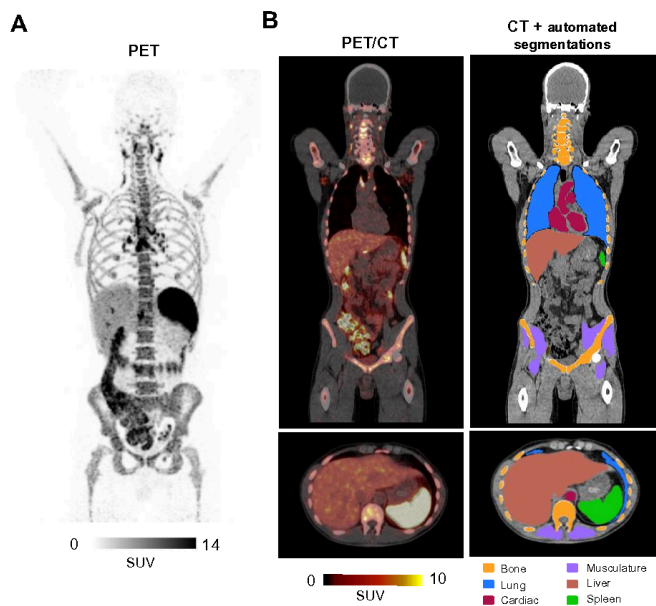


Figure 1 | [^{89}Zr]Zr-crefmirlimab berdoxam PET imaging allows quantification of whole-body CD8 lymphocyte distribution including spleen, bone marrow and healthy lymph nodes.

A: Maximum intensity projection of a representative baseline [^{89}Zr]Zr-crefmirlimab berdoxam PET scan with visible uptake in bone marrow, spleen, liver and axillary and mediastinal lymph nodes. **B:** Of the same patient, coronal PET/CT fused image and CT image with projections of automated segmentations used to quantify per-organ uptake values.

Figure 1

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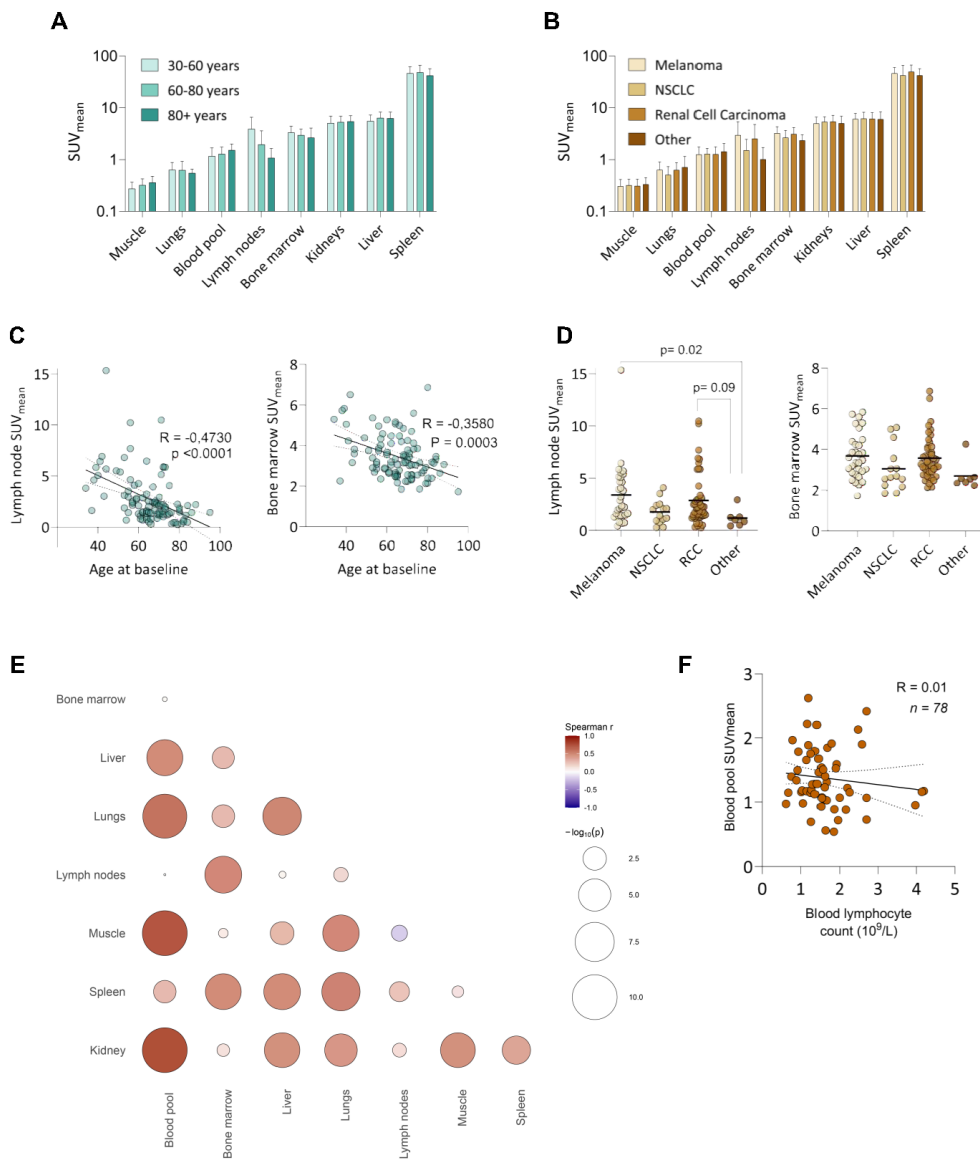


Figure 2 | Baseline uptake of $[^{89}\text{Zr}]Zr\text{-crefmirlimab berdoxam}$ in healthy lymph nodes and bone marrow is correlated with age and blood pool uptake correlates with uptake in muscle and kidney but not with peripheral blood lymphocytes.

A, B: SUV_{mean} in organs of interest for different age groups (**A**) and tumour types (**B**) of the baseline (pre-immunotherapy) PET images of 97 patients, bars indicate group means and error bars standard deviations. **C:** Spearman correlations of baseline lymph node and bone marrow SUV_{mean} against patient age, spearman R and P values of correlations. **D:** Lymph node and bone marrow uptake (SUV_{mean}) per tumour type, black bars indicate group means. **E:** Spearman correlations indicating intercorrelations between organ uptake (SUV_{mean}). Circle size represents $-\log_{10}(p)$ values, color indicating spearman R. **F:** Spearman correlation of blood pool SUV_{mean} and peripheral blood lymphocyte counts of $n = 78$ patients.

Figure 2

See image above for figure legend

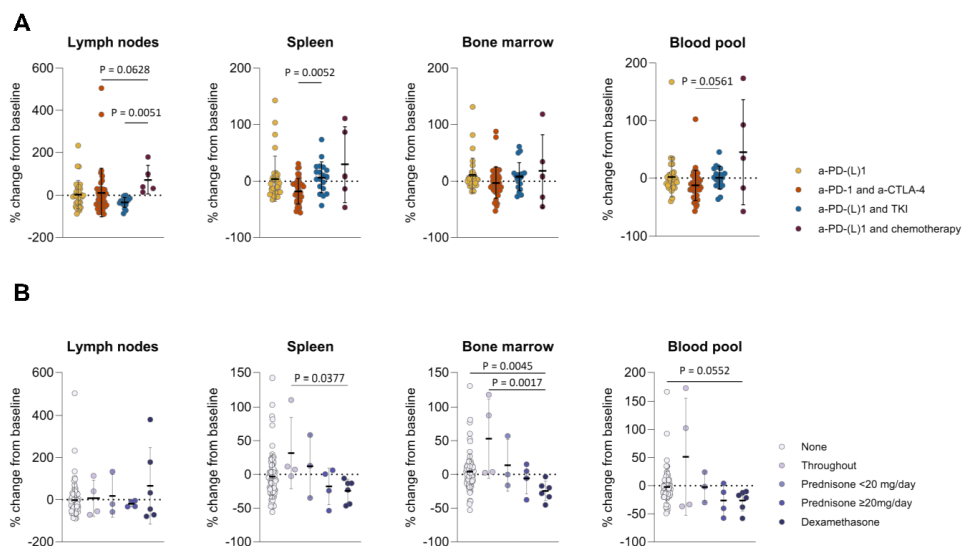


Figure 3 | Upon therapy, patients on combination with TKI and chemotherapy display differential uptake in healthy lymph nodes and dexamethasone use is associates with a decrease in uptake in bone marrow.

A & B: Changes in $[^{68}\text{Zr}]\text{-crefmirumab berdoxam}$ uptake upon two courses of treatment in a selection of immune relevant organs expressed as percent change in SUV_{mean} in lymph nodes, bone marrow, spleen and blood pool per ICI regimen group (**A**) and corticosteroid treatment group (**B**). Both with two-sided P values from Kruskal-Wallis tests with Dunn's multiple testing correction.

Figure 3

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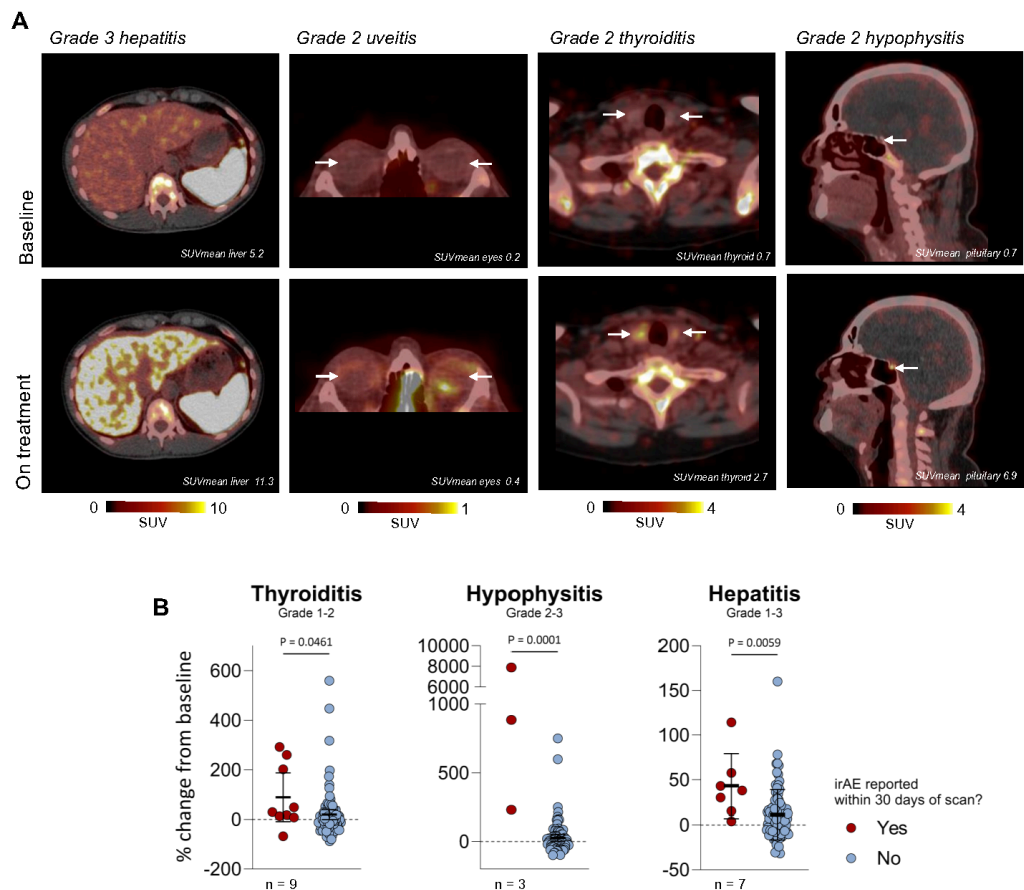


Figure 4 | Increased uptake of [^{89}Zr]Zr-crefmirlimab berdoxam in irAE affected organs was observed in most cases.

A: PET/CT fusion images of organs affected by irAEs with visible increase in uptake compared to baseline image. SUVmean was quantified using either automated (liver, thyroid) or manual segmentation methods (eyes, pituitary). **B:** Quantification of percent increase in SUV_{mean} in affected and non-affected thyroid, pituitary and liver, respectively. P values from Mann-Whitney U tests.

Figure 4

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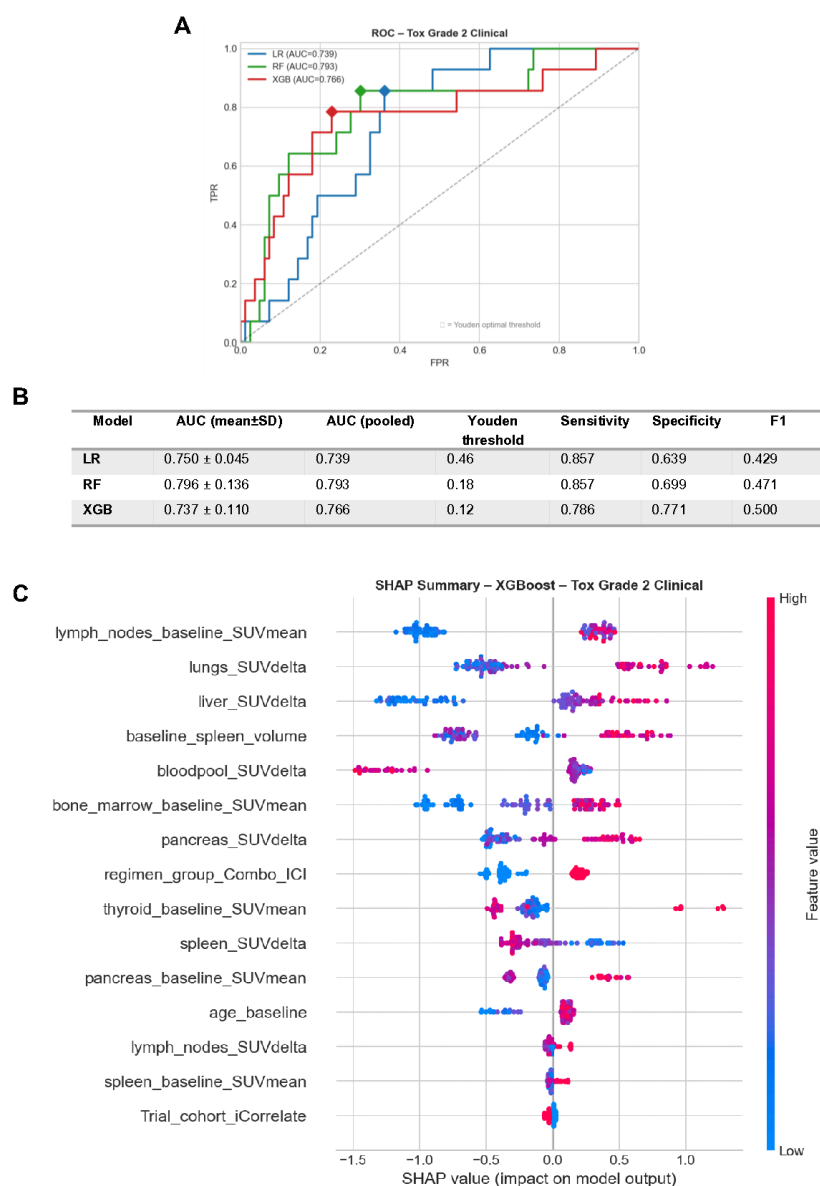


Figure 5 | Three machine learning prediction models predict grade ≥ 2 irAE clinically relevant with high accuracy, revealing baseline lymph node SUVmean and Δ SUVmean of lungs and liver as predictive features.

A: Area Under the Curve (AUC) of the Receiver Operating Characteristic (ROC) for the three models. **B:** performance of the three models employing the optimized Youden threshold. **C:** SHAP values for the top 15 predictive features indicating the impact of high and low feature values on model output. LR = Linear Regression, RF = Random Forest, XGB = XGBoost.

Figure 5

See image above for figure legend

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