Imaging, Biodistribution, and Dosimetry of Radionuclide-Labeled PD-L1 Antibody in an Immunocompetent Mouse Model of Breast Cancer

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Abstract

The programmed cell death ligand 1 (PD-L1) participates in an immune checkpoint system involved in preventing autoimmunity. PD-L1 is expressed on tumor cells, tumor-associated macrophages, and other cells in the tumor microenvironment. Anti–PD-L1 antibodies are active against a variety of cancers, and combined anti–PD-L1 therapy with external beam radiotherapy has been shown to increase therapeutic efficacy. PD-L1 expression status is an important indicator of prognosis and therapy responsiveness, but methods to precisely capture the dynamics of PD-L1 expression in the tumor microenvironment are still limited. In this study, we developed a murine anti–PD-L1 antibody conjugated to the radionuclide Indium-111 (111In) for imaging and biodistribution studies in an immune-intact mouse model of breast cancer. The distribution of 111In-DTPA-anti-PD-L1 in tumors as well as the spleen, liver, thymus, heart, and lungs peaked 72 hours after injection. Coinjection of labeled and 100-fold unlabeled antibody significantly reduced spleen uptake at 24 hours, indicating that an excess of unlabeled antibody effectively blocked PD-L1 sites in the spleen, thus shifting the concentration of 111In-DTPA-anti-PD-L1 into the blood stream and potentially increasing tumor uptake. Clearance of 111In-DTPA-anti-PD-L1 from all organs occurred at 144 hours. Moreover, dosimetry calculations revealed that radionuclide-labeled anti–PD-L1 antibody yielded tolerable projected marrow doses, further supporting its use for radiopharmaceutical therapy. Taken together, these studies demonstrate the feasibility of using anti–PD-L1 antibody for radionuclide imaging and radioimmuno-therapy and highlight a new opportunity to optimize and monitor the efficacy of immune checkpoint inhibition therapy.

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Introduction

The programmed cell death ligand 1 (PD-L1), also referred to as B7-H1 (1) and designated as CD274 (2), is part of an immune checkpoint system that is essential for preventing autoimmunity (3). Recent work has shown that this system is co-opted by tumor cells to suppress antitumor immunity. PD-L1 is expressed on tumor cells, tumor-associated macrophages (TAM), and other cells in the tumor microenvironment that can inhibit CD8+ T-cell effector function by its interaction with programmed cell death 1 (PD-1; ref. 4). Anti–PD-L1 antibodies (Ab) have been developed and are currently in clinical trial against a variety of cancers, including breast cancer (5–7).

PD-L1 overexpression is associated with a poorer prognosis in a variety of cancers, but patients with PD-L1 overexpression typically have a stronger response to anti–PD-L1 therapy. For example, patients identified to overexpress PD-L1 in melanoma have a 39% response rate to anti–PD-L1 therapy, compared with a 13% response rate in patients with PD-L1–negative melanoma (8).

Currently, immunohistochemistry (IHC) is utilized to evaluate PD-L1 expression in patients. PD-L1 is a dynamic biomarker, and its expression as determined by IHC is limited to a snapshot of the tumor environment (9). Accurately determining PD-L1 expression has the potential to identify patients who will best respond to anti–PD-L1 therapy, and monitoring changes in expression could provide information regarding treatment efficacy or potential toxicity. For example, high initial PD-L1 expression in the colon could warrant increased patient monitoring to avert irreversible colitis, one of the toxicities that has been observed in clinical trials.

The aims of this study were to develop and investigate a targeted anti–PD-L1 radiopharmaceutical for use as a molecular imaging agent. The resulting compound 111In-DTPA-anti-PD-L1 was evaluated in an immune-competent transgenic mouse model of breast carcinoma (10) to reflect the role of PD-L1 in the immune system. Furthermore, through dosimetry, we evaluated the potential of this agent to serve as a targeted radiopharmaceutical for PD-L1–targeted radionuclide therapy.

Materials and Methods

Reagents

All chemicals were purchased from Sigma-Aldrich Chemical Co. or Thermo Fisher Scientific, unless otherwise specified. Aqueous solutions were prepared using ultrapure water (resistivity, 18 MΩ cm) treated with Chelex resin purchased from Bio-Rad Laboratories, Inc. p-SCN-Bn-DTPA was purchased from Macro cyclics, Inc. 111InCl3 was purchased from MDS Nordion. The antimurine PD-L1–reactive antibody used is described in ref. 11.
Radiolabeling of DTPA-anti-PD-L1 antibodies with $^{111}$In

The anti–PD-L1 Ab was conjugated to $N$-[2-amino-3-[(p-isothiocyanatophenyl)propyl]-trans-cyclohexane-1,2-diamine-N,N',N"-pentaacetic acid (SCN-CHX-A$^5$-DTPA) using previously described standard methods yielding DTPA-anti-PD-L1 (12, 13). Indium-111 ($^{111}$InCl$_3$) (37–74 MBq) was added to an acid washed 1.5 mL eppendorf tube containing 0.25 mL of 0.2 M HCl and 0.03 mL of 3 mol/L NH$_4$OAc, pH = 7. After a minute, 0.2 mg of DTPA-anti-PD-L1 Ab was added to the mixture. The mixture was allowed to set at room temperature for 45 to 60 minutes and then transferred to an Amicon Ultra 10 K centrifugal filter device. PBS was added and the device was centrifuged for 15 minutes at 3,000 rpm to remove free $^{111}$InCl$_3$ (1×). Radiochemical purity of >98% was determined by radio thin-layer chromatography (TLC), and the protein concentration was determined by Nanodrop.

In vitro studies

Cell lines. The NT2.5 cell line was established from spontaneous mammary tumors in female neu-N mice (14, 15). A frozen stock of these cells was obtained from Dr. Elisabeth Jaffe’s lab at Johns Hopkins University. The PD-L1 expression of NT2.5 cells was compared with 4T1, a murine mammary carcinoma cell line, and HBL100, a human breast cancer cell line, both provided by Dr. Saraswati Sukumar at Johns Hopkins University. The EL4 murine B7H1 (EL4), a murine lymphoma cell line, transfected to express murine PD-L1, served as a positive control (16). Frozen EL4 cells were obtained from Ambion, Inc. ATCC and HBL100 cell lines obtained from Sukumar Lab in 2014 were purchased from the ATCC (authenticated using STR profile analysis). NT2.5 and EL4 cell lines obtained from the Jaffe and Ambion in 2014 were not authenticated by the Sgouros lab. Cells were cultured for a maximum of 4 weeks before thawing fresh, early passage cells. HER2 status in the NT2.5 cell line was confirmed by Western blot analysis and real-time RT-qPCR. All cells were confirmed to be mycoplasma negative (Hoechst stain and PCR; tested in 2014). NT2.5 cells were grown in RPMI1640 media with 20% FBS + 1.2% HEPES + 1% t-glutamine + 1% non-essential amino acids + 1% sodium pyruvate + 0.2% insulin + 0.02% gentamicin. 4T1 cells were grown in RPMI 1640 media supplemented with 10% FBS. EL4 cells were grown in DMEM media + 10% horse serum + 1% penicillin/streptomycin (PS). HBL100 cells were grown in DMEM media + 10% FBS + 1% PS. All cell lines were incubated at 37°C in 5% CO$_2$. Cell incubation with IFN$\gamma$ was performed by removing the growth medium and incubating the cells overnight in media with 1% serum and 200 ng/mL recombinant mouse IFN$\gamma$ (EMD Millipore).

Real-time RT-qPCR

Total RNA was extracted with Trizol reagent (Invitrogen), and cDNA was synthesized from total RNA (2 μg) using an M-MLV Reverse Transcriptase (Promega). Aliquots of cDNA were used as templates for real-time RT-qPCR procedure using a PD-L1-specific primer (forward: 5’ CGCTTCTACATGGACACCA3’; reverse: 5’ GAGGGAGCCGCTTGACACTA 3’). Relative quantities of mRNA expression were analyzed using real-time PCR (Applied Biosystem 7500 Real-Time PCR system; Applied Biosystems). The Maxima SYBR Green/ROX Master Mix (Fermentas) was used according to the manufacturer’s instruction.

Flow cytometry

Cell lines were cultured for 24 hours in the presence or absence of IFN$\gamma$. Cells were trypsinized, washed with PBS solution, and blocked with PBS containing 10% FBS. Cells (2 × 10$^5$) were incubated with anti-mouse B7-H1 (CD274)-PE clone M1H5 (ebioscience) for 30 minutes at 4°C. After repeated washing, cells were resuspended in 500 μL of PBS and analyzed on a FACs flow cytometer (BD Biosciences).

IHC

IHC staining was performed on 8-μm-thick cryo-sectioned tissue samples of the NT2.5 tumor, spleen, thymus, liver, and kidneys. The tissue samples were fixed by acetone (4°C) for 10 minutes. Endogenous peroxide activity was quenched by 10-minute incubation in 3% H$_2$O$_2$, and nonspecific binding was blocked with serum. The dilution of 1:50 of primary Ab (anti–PD-L1; Abcam) was incubated at room temperature (16 hours). Diluted biotinylated anti-rabbit IgG (Vectastain kit; Vector Laboratories) was added to the tissue samples and incubated for 30 minutes. Vercatine ABC reagent and DAB were used for staining color development, and the counterstaining was performed with hematoxylin solution.

Binding assay

Cell experiments were performed to determine the binding affinity of $^{111}$In-DTPA-anti-PD-L1 in NT2.5 cells. Cells were seeded in 24-well plates (50,000–100,000 cells) 36 hours prior to the experiment. IFN$\gamma$ (400 ng) was added to each well 24 hours prior to the experiment, to induce PD-L1 expression. Before the experiment, cells were washed (1×) with PBS (1 mL), and 0.5 mL growth media (RPMI 1640 with 0.1% PS and 10% FBS) were added to each well. To determine nonspecific binding, 5 μg of anti–PD-L1 was added to half of the wells as a cold block 30 minutes prior to $^{111}$In-DTPA-anti-PD-L1. $^{111}$In-DTPA-anti-PD-L1 was added to all the wells in increasing concentrations (1.0–25 nmol/L). The samples were incubated for 2.5 hours on ice. After incubation, the radioactive media were removed. Cell pellets were rinsed twice with PBS (1 mL) and dissolved in 0.5% SDS solution. The radioactivity in each fraction was measured in a gamma well counter (Perkin-Elmer 2470 WIZARD2 Automatic Gamma Counter). The protein content of each cell lysate sample was determined (BCA Protein Assay Kit; Pierce). The measured radioactivity associated with the cells was normalized to the amount of cell protein present (cpm/mg protein). The $K_d$ and $B_{max}$ were calculated using PRISM 5 (Graphpad).

In vivo studies

Animals. Mouse imaging and biodistribution studies were performed using the rat HER-2/neu expressing mouse mammary cell line, NT2.5, in neu-N transgenic (17) healthy female mice. 8 to 12 weeks old, both obtained courtesy of Dr. Elizabeth Jaffee at Johns Hopkins University. All animal studies were approved by the Animal Care and Use Committee of the Johns Hopkins University, School of Medicine. All mice were s.c. injected in the left flank with $10^5$ cells in 50 μL Matrigel. In addition, mice used for SPECT imaging were also injected s.c. with $10^6$ cells/50 μL Matrigel in the right flank. All injections were done 3 weeks prior to experiments. SPECT imaging of $^{111}$In-labeled anti–PD-L1 antibodies in tumor-bearing neu-N mice. Tumor-bearing healthy female neu-N mice were injected with a solution of $^{111}$In-DTPA-anti-PD-L1 in PBS. Animals were imaged on a small-animal SPECT camera (eBioscience) for 30 minutes at 4°C.
transgenic mice were each injected i.v. with 7.4 MBq \(^{111}\)In-DTPA-anti-PD-L1. The mice (\(n = 3\)) were imaged at 1, 24, and 72 hours post-injection (p.i.) with the VECTor\(^4\) SPECT imaging system (MiLabs) using a general purpose mouse collimator, with 0.6-mm pinholes and imaging resolution of 0.4 mm. Images were acquired for 60 minutes at the 1- and 24-hour time points, and for 90 minutes at the 72-hour time point. Images were reconstructed with voxel side length 0.2 mm using pixel-based ordered subsets expectation maximization (POSEM) (18), a vendor-supplied iterative algorithm. After reconstruction, a 3D-Gaussian filter with a 0.6-mm full width at half maximum (FWHM) was applied to each image.

**Biodistribution of \(^{111}\)In-labeled anti–PD-L1 antibodies in tumor-bearing neu-N mice.** Biodistribution experiments were conducted as previously described with minor modifications (19). Briefly, healthy NT2.5 tumor-bearing female neu-N transgenic mice (\(n = 4\) (time point) were injected i.v. with \(^{111}\)In-DTPA-anti-PD-L1 (\(\sim 0.93\) MBq, 8.4 \(\mu\)g). At 1, 24, 72, and 144 hours p.i., the mice were sacrificed. The blood, heart, lungs, liver, kidneys, spleen, stomach (with content), intestine (with content), femur, thymus, muscle, and tumors were harvested, weighed, and measured in a gamma well counter. In addition, competitive blocking studies were performed at 24 hours p.i. The mice were coinjected with \(^{111}\)In-DTPA-anti-PD-L1 and unlabeled (cold) anti–PD-L1 Ab (30× and 100×). The percentage of injected dose per gram (\%ID/g) was calculated by comparison with a weighed, diluted standard.

**Normal tissue and tumor dosimetry.** Normal tissue and tumor absorbed dose (AD) calculations were performed for \(^{177}\)Lu and \(^{90}\)Y (candidates radionuclides for therapy), and \(^{111}\)In (used in diagnostic imaging). The organ activity concentrations obtained from the murine biodistribution studies using \(^{111}\)In-labeled Ab (\(\%ID/g_{(\text{M})}\)) were translated to human whole-organ percentage of injected dose (\(\%ID/\text{organ}\left[H\right]\)) based on the principle that the organ to whole-body activity concentration ratio of a radiopharmaceuticals in mice would equal that in humans. This principle is implemented in the following expression:

\[
\%ID/\text{organ}_{(H)} = \left(\frac{\%ID}{g}\right)_{(\text{M})} \cdot \frac{\text{TBW}_{(H)}}{\text{TBW}_{(\text{M})}}
\]

where \(\text{OW}_{(\text{H})}\) is the human organ weight, \(\text{TBW}_{(\text{M})}\) is the average total body weight of the mice (\(\text{TBW}_{(\text{M})} = 25\) g), and \(\text{TBW}_{(\text{H})}\) is the average total body weight for an adult male (\(\text{TBW}_{(\text{H})} = 73.7\) kg; refs. 20, 21). The activity concentration in human red marrow was estimated using a previously described blood-based method (22) wherein the activity concentration obtained at each time point in the murine biodistribution studies (\(A_{(\text{M})}\)) was related to the red marrow activity concentration in a human (\(A_{(\text{H})}\)) using the following expression:

\[
A_{(\text{H})} = A_{(\text{M})} \cdot \text{RMECFF} \cdot \frac{1}{(1 - \text{HCT})}.
\]

\(\text{RMECFF}\) is the red marrow extracellular fluid fraction (\(\text{RMECFF} = 0.19\)), and \(\text{HCT}\) is the volume fraction of red blood cells in blood (\(\text{HCT} = 0.47\)) in humans.

Whole-organ time-integrated activity coefficients (TIAC) were calculated for \(^{177}\)Lu and \(^{90}\)Y by applying the half-life of these two radionuclides to the decay-corrected pharmacokinetics obtained using \(^{111}\)In. The resulting radioactivity concentrations versus time curves for each organ were integrated using a hybrid numerical integration/analytical integration method. If the data could be fit to a one or two exponential expressions, then the curves were integrated analytically from zero to infinity. Alternatively, numerical integration was performed over the measured data, and the last two time points were used to derive a single exponential function that was analytically integrated beyond the last measurement. The Medical Internal Radiation Dose (MIRD) Committee methodology (23, 24) as implemented in OLINDA/EXM (25) was used to calculate organ AD. Tumor AD was estimated using the sphere module in OLINDA/EXM to calculate the AD to a sphere from photon and electron emissions originating within the sphere.

**Statistical analysis**

Statistical analysis was performed using the software, GraphPad. All data are presented as the mean value ± SD. Groups were compared using two tailed Student \(t\) test, and \(P\) values were considered significant if \(P \leq 0.05\).

**Results**

**Radiochemistry**

The \(^{111}\)In-DTPA-anti–PD-L1 conjugate was radiolabeled in 45 to 60 minutes at room temperature in ammonium acetate buffer at a specific activity of 110 to 122 MBq/\(\mu\)g with >98% radiochemical purity following purification.

**In vitro studies**

**Real-time RT-qPCR.** To examine the level of PD-L1 mRNA in the breast cancer cell lines treated with IFN\(\gamma\), we performed real-time RT-qPCR for the cell lines 4T1, NT2.5, and HBL100 using designed human or mouse PD-L1–specific primers. In addition, EL4 was evaluated to serve as a positive control. The results showed that PD-L1 mRNA levels were significantly increased in the IFN\(\gamma\)-treated murine cell lines, 4T1 and NT2.5, but not HBL100, a human breast cancer cell line (Fig. 1). Interestingly, PD-L1 mRNA
in HER2-positive NT2.5 cells was 4.5 times higher than in the triple-negative 4T1 cells.

**Flow cytometry.** Cell-surface expression of PD-L1 with and without IFNγ treatment was evaluated by flow cytometry (Fig. 2A–D). PD-L1 expression on the positive control line, EL4, was not significantly increased with IFNγ. PD-L1 expression on 4T1 cells increased 2.7-fold with IFNγ incubation. The highest, 7.5-fold, increase in PD-L1 expression was obtained with NT2.5 cells.

**IHC.** IHC was performed on tissue samples from NT2.5 tumor-bearing neu-N mice (Fig. 3). The NT2.5 tumors showed high expression of PD-L1 in the cytoplasm and on the membranes. The spleen showed high expression of PD-L1 on the membranes, the kidneys showed low expression, the thymus showed low expression, and the liver had very low to no expression of PD-L1.

**Binding assay.** Saturation binding assay shows that 111In-DTPA-anti-PD-L1 binds with high affinity to PD-L1, with a Kd of 8.3 ± 3.2 nmol/L and a Bmax of 65.4 ± 10.1 fmol/mg (approximately 1.4 × 10^12 sites/cell). It should be noted that without IFNγ in the binding assay, NT2.5 cells do not show binding of 111In-DTPA-anti-PD-L1.

**In vivo studies**

**SPECT imaging of 111In-labeled anti-PD-L1 antibodies in tumor-bearing neu-N mice.** Whole-body coronal SPECT image slices show the distribution of 111In-DTPA-anti-PD-L1 at 1, 24, and 72 hours p.i. (Fig. 4). At 1 hour p.i., signal intensity was highest in the liver, spleen, and thymus, but was also observed in the region of the spinal column. The signal intensity within the spinal column likely reflects the high concentration of 111In-DTPA-anti-PD-L1 in the circulation. At 24 and 72 hours p.i., clearance of 111In-DTPA-anti-PD-L1 from nontarget tissues, including the blood and accumulation in PD-L1-rich sites, allowed visualization of the isografts. Relative to 1 hour, liver intensity was reduced, whereas signal intensity in the spleen and thymus persisted.

**Biodistribution of 111In-DTPA-anti-PD-L1 antibodies in tumor-bearing neu-N mice.** The distribution of 111In-DTPA-anti-PD-L1 in NT2.5 tumor-bearing neu-N mice was obtained by ex vivo counting of tissues collected from mice sacrificed at different time points p.i. (Fig. 5A). The results obtained from these studies were generally consistent with the imaging observations of Fig. 4. At 1 hour p.i., 111In-DTPA-anti-PD-L1 was mainly in the blood (28.7 ± 15.6%ID/g) and spleen (24.8 ± 6.3%ID/g). Tumor concentration at this time was 3.9 ± 2.3%ID/g. By 24 hours, blood concentration decreased to 12.6 ± 2.2%ID/g. Tumor concentration increased to 56.5 ± 16.7%ID/g at 72 hours. Accumulation of 111In-DTPA-anti-PD-L1 peaked in the tumor at 56.5 ± 16.7%ID/g with moderate tumor to muscle/blood ratios (23 ± 8, ± 1). The spleen concentration decreased to (102.4 ± 12.8%ID/g), liver (29.7 ± 5.8%ID/g), thymus (31.0 ± 17.6%ID/g), heart (8.7 ± 1.5%ID/g), and lung (12.1 ± 2.0%ID/g) also had the highest accumulation at 72 hours. Accumulation in other organs at this time was low, with uptake decreasing or remaining constant. Clearance of 111In-DTPA-anti-PD-L1 from all organs at this time was low, with uptake decreasing or remaining constant. Clearance of 111In-DTPA-anti-PD-L1 from all organs at this time was low, with uptake decreasing or remaining constant.
organs was seen at 144 hours. At this time, the concentration in tumor (21.1 ± 11.2%ID/g) was significantly greater than other organs, except the spleen (63.5 ± 25.4%ID/g), liver (14.9 ± 4.2%ID/g), and thymus (16.8 ± 16.2%ID/g).

In the presence of excess unlabeled anti-PD-L1 Ab, the 24-hour distribution of 111In-DTPA-anti-PD-L1 was significantly altered (Fig. 5B). Coinjection of the labeled Ab with 30- or 100-fold unlabeled Ab reduced the 24-hour spleen uptake to 16.2 ± 1.8%ID/g ($P \leq 0.0002$, relative to unblocked) and 10.7 ± 5.0%ID/g ($P \leq 0.0002$, relative to unblocked), respectively; the concentration in blood increased to 45.2 ± 5.5 ($P \leq 0.0001$) and 43.0 ± 13.0 ($P \leq 0.004$)%ID/g, respectively. 111In-DTPA-anti-PD-L1 concentrations in the tumor at 30 and 100 times the blocking dose were 21.2 ± 3.8 ($P \leq 0.09$, relative to unblocked) and 17.6 ± 5.4%ID/g ($P \leq 0.04$, relative to unblocked), respectively. The 24-hour uptake in the thymus was not blocked.

**Normal organ and tumor dosimetry.** AD calculations for selected tissues are listed in Table 1. As expected from the biodistribution data, the highest AD for each radionuclide is to the spleen. 90Y-labeled anti-PD-L1 Ab would deliver 8.2 mGy/MBq, while 177Lu labeled anti-PD-L1 Ab would deliver 2.5 mGy/MBq to the spleen corresponding tumor ADs are 3.0 and 1.1 mGy/MBq, respectively. The tumor to red marrow AD ratio is about 10 for both therapeutic radionuclides. The
blockade treatment strategy (3). Patients with PD-L1 overexpression have typically demonstrated a greater response to anti–PD-L1 Ab therapy compared with patients with low or negative PD-L1 expression. However, mixed responses to anti–PD-L1 therapy highlight the need to develop methods that better predict whether a patient will respond to anti–PD-L1 therapy (8). Furthermore, the response to therapy targeting the PD-L1/PD-1 axis has been shown to improve when combined with external-beam radiation therapy in preclinical studies (26, 27). In this work, we examined the feasibility of using anti–PD-L1 Ab for radionuclide imaging and radioimmunotherapy.

PD-L1 is a dynamic biomarker with its expression varying in response to the immune system. Our in vitro studies helped highlight the dynamic nature of PD-L1 expression and its response to immune signals, such as IFN-γ. IFN-γ has been reported to induce PD-L1 expression in a variety of cells (28, 29). The real-time RT-qPCR and flow cytometry results confirm that the PD-L1 expression in the murine-derived mammary carcinoma cells is highly dependent on inflammatory signaling. In the presence of IFN-γ, the highest PD-L1 upregulation at both the mRNA and cell surface levels was seen in the endogenously derived NT2.5 tumor cell line. IHC confirmed the high expression of PD-L1 in NT2.5 tumors. In addition, IHC demonstrated high PD-L1 expression in the spleen, whereas expression in liver, kidneys, and thymus was substantially lower in the neu-N model.

The in vivo imaging and biodistribution data provided in this work illustrate a number of principles that could be used to guide and potentially optimize immune checkpoint therapy. Whole-body imaging helps identify cross-reactive normal organs. Whole-body SPECT images of 111In-DTPA-anti-PD-L1 showed high signal intensity in the PD-L1–positive isografts and in potentially cross-reactive organs, such as the spleen and thymus. The biodistribution data supported the SPECT imaging and demonstrated that tumor, spleen, and thymus had the highest uptake at 72 hours p.i. The imaging and biodistribution results were generally consistent with IHC staining. However, the thymus demonstrated high uptake of 111In-DTPA-anti-PD-L1 Ab, but was identified by IHC to have medium to low expression of PD-L1. Although PD-L1 expression in the thymus and spleen has been previously noted (30), the impact of such cross-reactivity on overall kinetics could not be appreciated without the in vivo studies presented here.

Recently, Heskamp and colleagues used a human tumor (MB231) xenograft in an athymic mouse model to demonstrate that a human PD-L1 Ab can be imaged with minimal cross-reactivity to other normal organs (31), the animal model used lacked a thymus and, correspondingly, the ability to produce T cells, a key component in the PD-1/PD-L1 axis. In an immunologically intact model, using an Ab against murine PD-L1, we show substantial uptake in the spleen and thymus, which was not demonstrated in the immune-deficient mouse model. Furthermore, the mouse model used in our experiments demonstrated that the spleen is a sink for 111In-DTPA-PD-L1, confirmed by the coadministration of 111In-DTPA-PD-L1 and excess unlabeled Ab. The coadministration of excess unlabeled anti–PD-L1 Ab effectively and dramatically blocked PD-L1 sites in the spleen, shifting the concentration of 111In-DTPA-PD-L1 into the blood. In addition, reduced blocking was seen in the tumor but not to the extent seen in the spleen. Surprisingly, significant blocking was not observed in the thymus, suggesting a large pool of PD-L1–positive cells in this tissue. It is also possible that the Fc portion of the antibody is binding to sites on the thymus. These results suggest thymus had high ADs of 0.7 and 2.2 mGy/MBq, and the liver’s ADs were 0.8 and 2.7 mGy/MBq for 177Lu and 186Y-Ab, respectively.

### Discussion

Antibodies against PD-L1 have shown great promise in patients with a variety of cancers (5), as part of a novel immune checkpoint therapy. However, mixed responses to anti–PD-L1 therapy highlight the need to develop methods that better predict whether a patient will respond to anti–PD-L1 therapy (8). Furthermore, the response to therapy targeting the PD-L1/PD-1 axis has been shown to improve when combined with external-beam radiation therapy in preclinical studies (26, 27). In this work, we examined the feasibility of using anti–PD-L1 Ab for radionuclide imaging and radioimmunotherapy.

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