**A Novel Engineered Small Protein for Positron Emission Tomography Imaging of Human Programmed Death Ligand-1: Validation in Mouse Models and Human Cancer Tissues**

Arutselvan Natarajan1, Chirag B. Patel1,2,&, Sindhuja Ramakrishnan1,&, Paramjyot S Panesar1,

Steven R. Long3, Sanjiv S. Gambhir1,4,5,\*

**Supplementary information**

**Supplementary Table S1**

Summarizes the demographic and pathology characteristics of patients from who the human tissue specimens were obtained.

**Supplementary Figure S2**

Measurement of DOTA chelates per FN3hPD-L1 molecule by mass spectrometry. Representative MALDI-TOF MS spectra of FN3hPD-L1 (unmodified), and DOTA-FN3hPD-L1 (modified). The conjugation reaction was performed with FN3hPD-L1 and DOTA-NHS in a 1:5 ratio. The intensity peaks in the 12 kDa region occurred at 11824 Da and 13071 Da for FN3hPD-L1 and DOTA-FN3hPD-L1, respectively.

**Supplementary Video S3**

Three-dimensional microPET-CT visualization of hPD-L1 expressing CT26 syngeneic tumor-bearing mice [CT26/hPD-L1-nblk and CT26-hPD-L1-blk] at 1 h post-injection of 64Cu-FN3hPD-L1. **Nblk** = No pre-blocking with non-radioactive FN3hPD-L1 prior to radiotracer injection. **Blk** = Pre-blocking with non-radioactive FN3hPD-L1, 2 h prior to the tracer administration. White arrow indicates the location of the tumor implanted into the left shoulder (please see video online).

**Supplementary Figure S4**

Human cancer tissue hPD-L1 expression at the time of biopsy based on results from an FDA-approved send-out lab, and at the time of surgical resection based on validated antibodies of two hPD-L1 clones (SP263 and E1L3N) and the FN3hPD-L1 binder.

**Supplementary Figure S5**

**(A)** 64Cu-FN3hPD-L1 PET of a NSG mouse bearing a subcutaneously-implanted MDA-MB-231 human triple negative breast cancer xenograft in the left shoulder. **(B)** 4 and 24 hours p.i. 64Cu-FN3hPD-L1 tumor uptake (%ID/g mean±SD) under blocking (blk) and non-blocking (nblk) conditions. **(C)** Xenografts were harvested 4 days after 64Cu-FN3hPD-L1 injection and processed for histology. From left to right: hematoxylin and eosin (H&E) and immunohistochemistry (IHC) staining (validated hPD-L1 antibody clones E1L3N and SP263, respectively) shown at 40× magnification (scale bars represent 50 µm). The hPD-L1 IHC in the xenograft was scored by a pathologist (SRL) by focusing on areas of tumor and excluding areas of necrosis or tumor-infiltrating inflammatory cells such as lymphocytes. In the MDA-MB-231 xenograft previously exposed to 64Cu-FN3hPD-L1, hPD-L1 staining was scored as 55% using clone E1L3N and 85% using clone SP263.

**Supplementary Figure S6**

Differential hPD-L1 expression in MDA-MB-231 hPD-L1-expressing triple negative breast cancer xenograft and Raji hPD-L1-non-expressing Burkitt’s lymphoma xenograft. These xenografts were not exposed to the 64Cu-FN3hPD-L1 radiotracer. From left to right: hematoxylin and eosin (H&E) and immunohistochemistry (IHC) staining (validated hPD-L1 antibody clones E1L3N and SP263, respectively) shown at 40× magnification (scale bars represent 50 µm). The hPD-L1 IHC in the xenografts was scored by a pathologist (SRL) by focusing on areas of tumor and excluding areas of necrosis or tumor-infiltrating inflammatory cells such as lymphocytes. In the MDA-MB-231 xenograft, hPD-L1 staining was 60% using clone E1L3N and 90% using clone SP263. In the Raji xenograft, hPD-L1 staining was 0% using clone E1L3N and 5% using clone SP263.

**Table S1**

|  |  |  |  |
| --- | --- | --- | --- |
| **Case** | **Age at Diagnosis (y)** | **Sex** | **Pathology Diagnosis** |
|
| 1 | 68 | Female | Poorly differentiated lung adenocarcinoma |
| 2 | 79 | Male | Lung adenocarcinoma |
| 3 | 79 | Male | Metastatic lung adenocarcinoma to brain |
| 4 | 73 | Female | Metastatic lung adenocarcinoma to brain |
| 5 | 44 | Male | Metastatic lung adenocarcinoma to brain |
| 6 | 43 | Male | Oncocytic thyroid carcinoma |

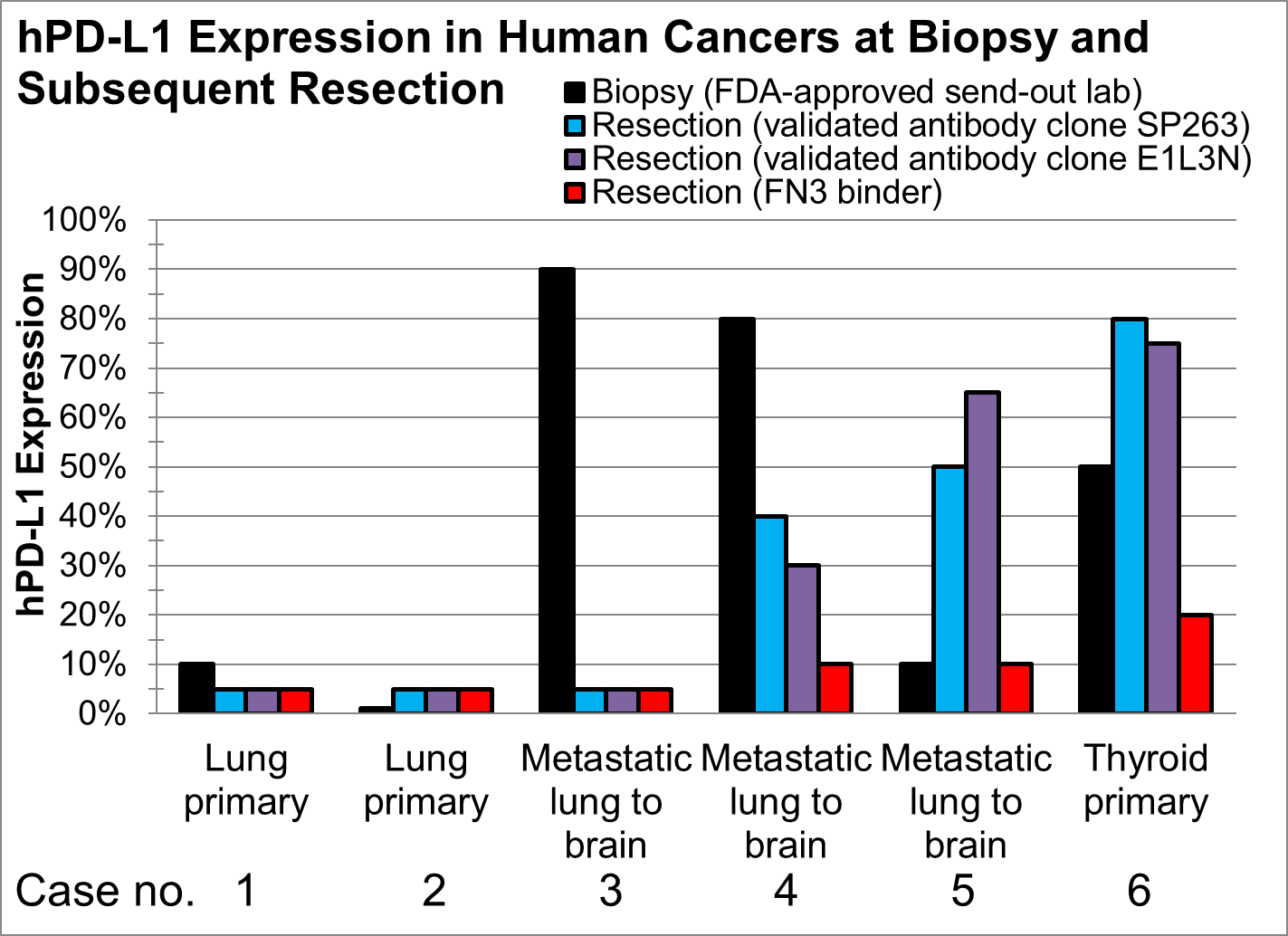
**Figure S2**



**Video S3**

Please see the video online.

**Figure S4**



**Figure S5**

****

**Figure S6**

|  |  |  |  |
| --- | --- | --- | --- |
| **Specimen** | **H&E (40×, scale bar = 50 µm)** | **hPD-L1 clone E1L3N** | **hPD-L1 clone SP263** |
| MDA-MB-231 xenograft (hPD-L1 expressing) | F:\00 FILES to XFER\20180823 MDAMB231 and Raji hPD-L1 IHC\1_breastcold_40x_h&e.bmp | **F:\00 FILES to XFER\20180823 MDAMB231 and Raji hPD-L1 IHC\1_breastcold_40x_ihc_pdl1_e1l3n.bmp** | **F:\00 FILES to XFER\20180823 MDAMB231 and Raji hPD-L1 IHC\1_breastcold_40x_ihc_pdl1_sp263.bmp** |
| Raji xenograft (hPD-L1 negative) | F:\00 FILES to XFER\20180823 MDAMB231 and Raji hPD-L1 IHC\4_rajicold_40x_ihc_h&e.bmp | F:\00 FILES to XFER\20180823 MDAMB231 and Raji hPD-L1 IHC\4_rajicold_40x_ihc_ihc_pdl1_e1l3n.bmp | F:\00 FILES to XFER\20180823 MDAMB231 and Raji hPD-L1 IHC\4_rajicold_40x_ihc_ihc_pdl1_e1l3n.bmp |