SUPPLEMENTARY INFORMATION FOR

TITLE: NON-INVASIVE PD-L1 QUANTIFICATION USING [^{18}F]DK222-PET IMAGING IN CANCER IMMUNOTHERAPY.

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Data availability statement

All data relevant to the study are included in the article, uploaded as online supplementary information. All data are available on reasonable request.

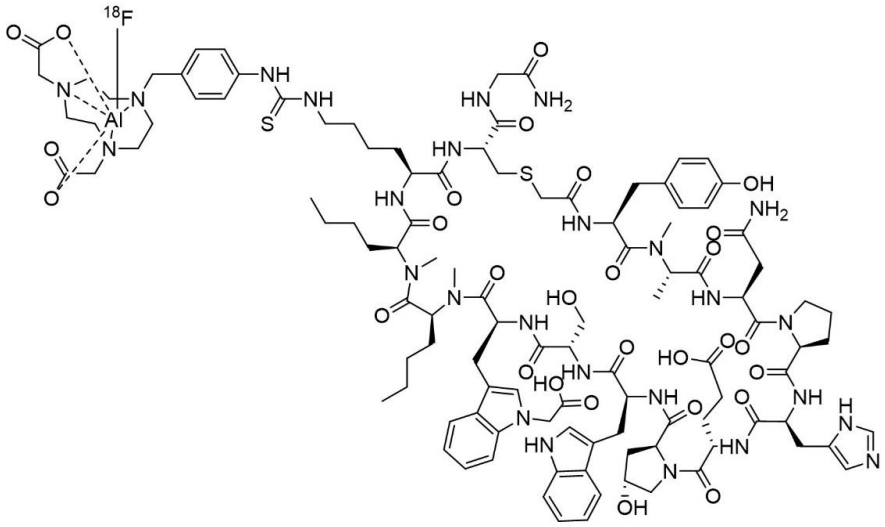


Figure S1. Structure of [^{18}F]DK222

Cell Line	Cancer type	RNASeq Score log2(TPM+1)
H2444	Lung Cancer	5.349
H226	Lung Cancer	3.875
A549	Lung Cancer	1.791
BFTC909	Urothelial Cancer	7.775
T24	Urothelial Cancer	4.259
SCABER	Urothelial Cancer	3.175

Table S1. $\log_2$(TPM+1) gene expression data extracted from Cancer Cell Line Encyclopedia for cell lines used in this study.

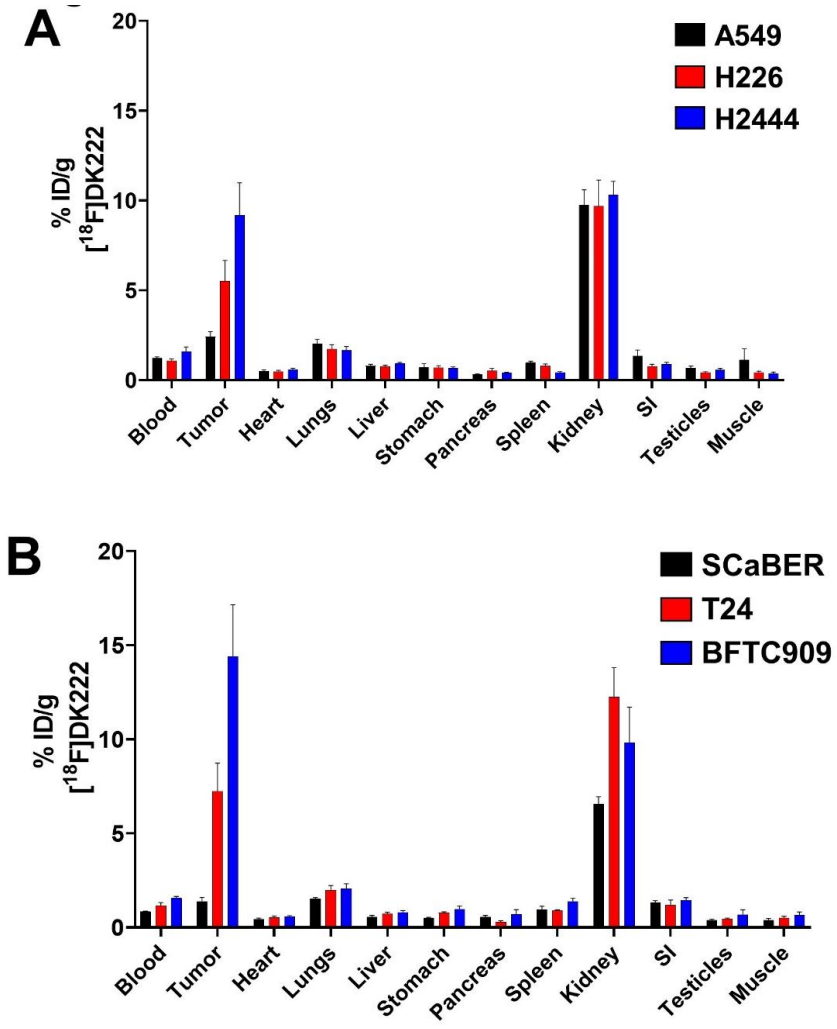


Figure S2. Ex vivo biodistribution in NSG mice bearing **(A)** NSCLC or **(B)** UC xenografts. Mice were injected with ~ 740 kBq (~20 µCi) [^{18}F]DK222 in 200 µL of 5% ethanol in saline and sacrificed after 60 minutes (n=5).

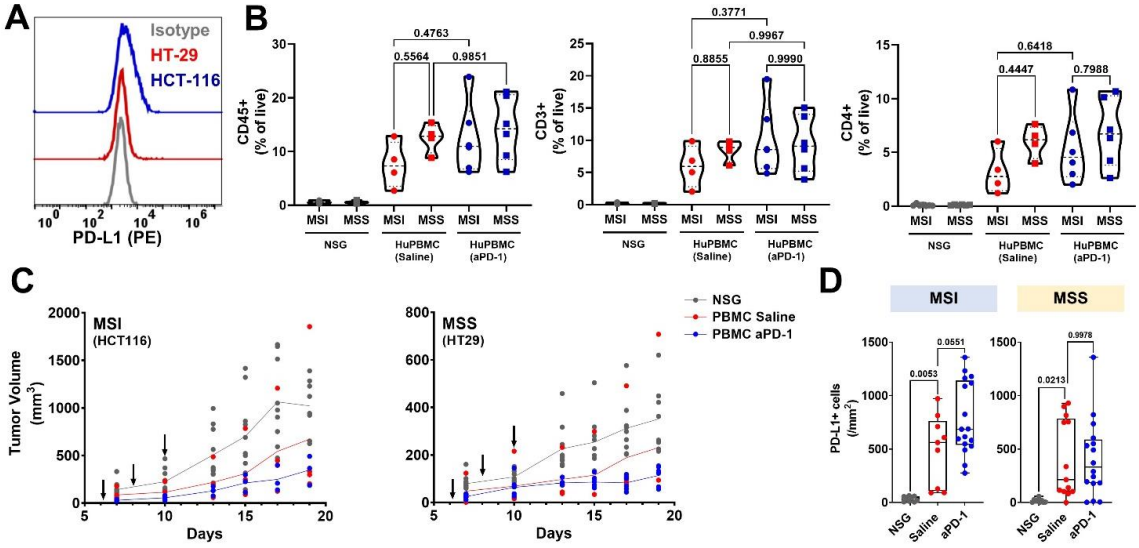


Figure S3. Flow cytometry analysis of MSI (HCT-116) and MSS (HT-29) tumors by flow cytometry. **A**, Baseline PD-L1 expression in MSI (HCT-116) and MSS (HT-29) cell lines. **B**, CD45+ immune cells, CD3+T cells and CD4+ T cells in tumors engrafted in huPBMC or NSG mice with and without aPD-1 treatment (4 mg/kg pembrolizumab *i.v.* on day 4, 6 and 8 as indicated by arrows). **B**, Tumor growth curves for MSI (HCT-116) and MSS (HT-29) tumors treated with either 4 mg/kg pembrolizumab *i.v.* on day 4, 6 and 8 or saline. P values by two-way ANOVA

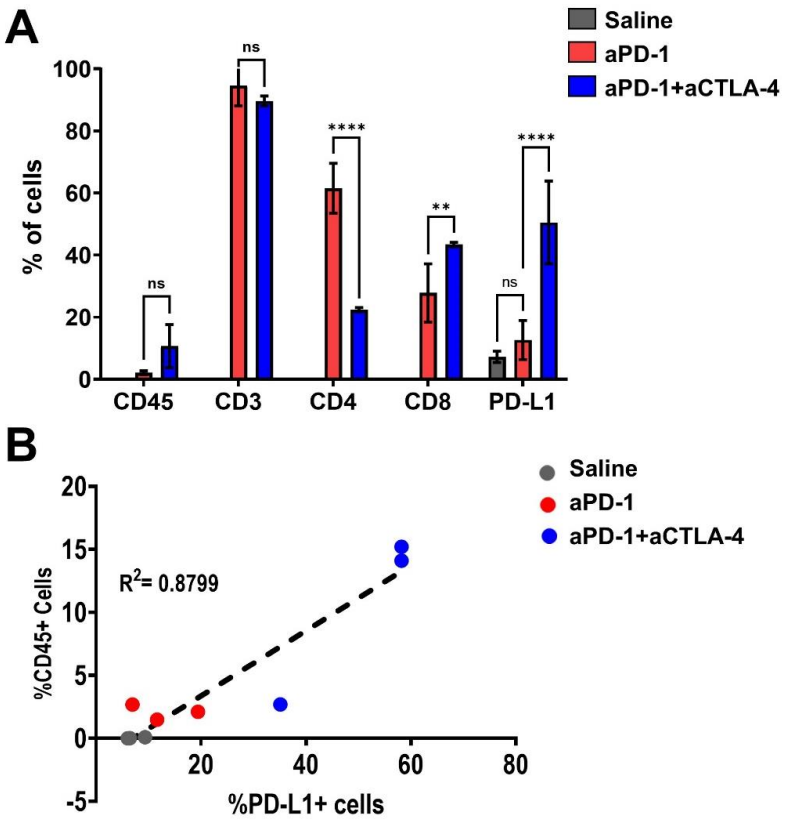


Figure S4. Flow cytometry data for A375 tumors engrafted in humanized mice (huCD34). **A,** Flow cytometry histograms showing increased immune cell infiltration and upregulated PD-L1 expression tumors of mice treated with different immunotherapy or saline. **B,** Immune cell analysis shows strong correlation between CD45+ immune cell infiltration and PD-L1 expression on tumor cells. **p<0.01, ****p<0.0001 by two-way ANOVA

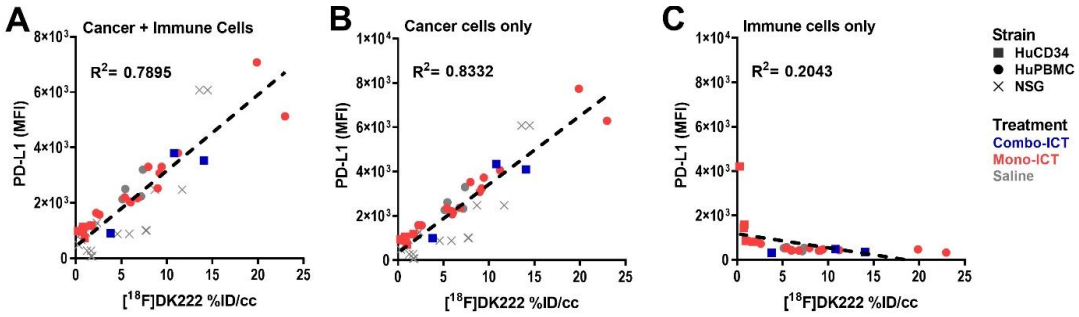
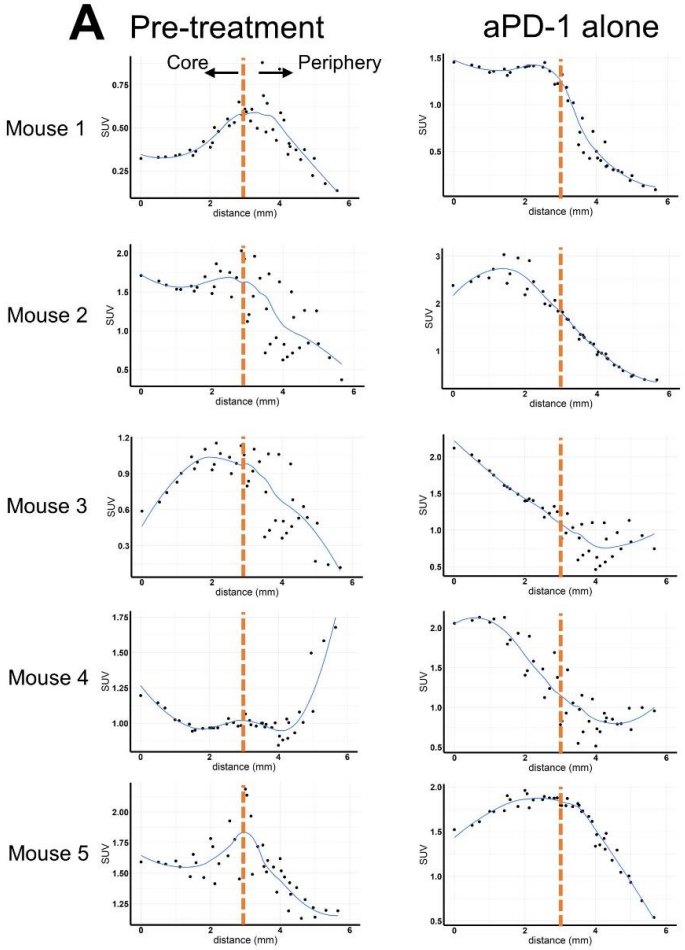


Figure S5. Combined correlation of all data points in different studies used in **Figure 5.** Flow cytometry derived PD-L1 MFI on **(A)** all cells, **(B)** cancer cells alone or **(C)** immune cells alone was used to correlate with [18F]DK222 %ID/cc on corresponding PET datasets. Simple linear regression and Pearson coefficient were used.

Figure S6


A
Pre-treatment
aPD-1 alone
Core
Periphery
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
SUV
distance (mm)

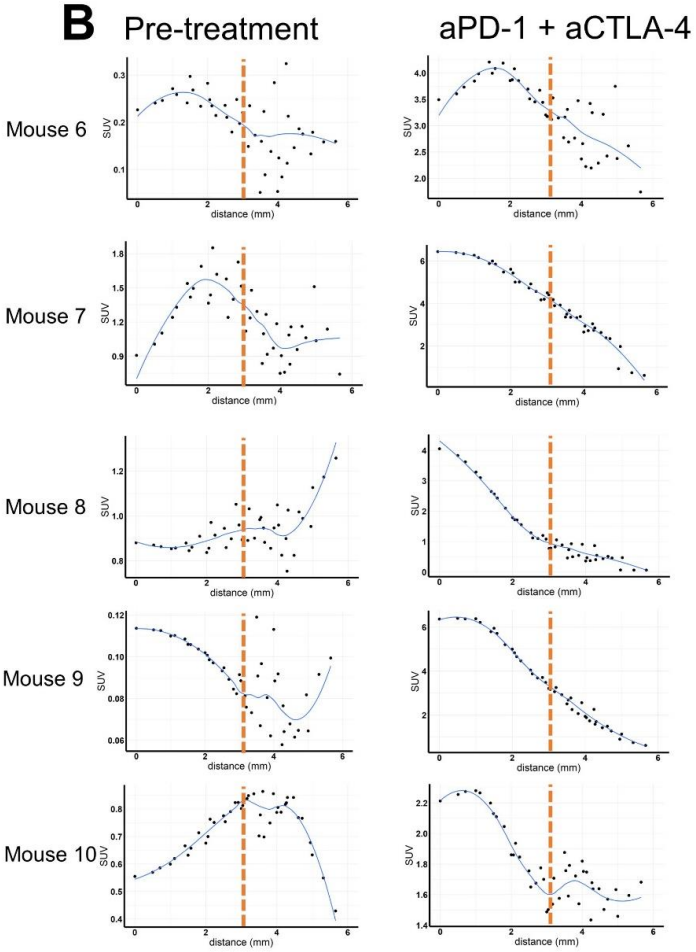


Figure S6 Individual SUV [^{18}F]DK222 vs distance of each mice before and after mono- **(A)** or combination-treatment **(B)**.

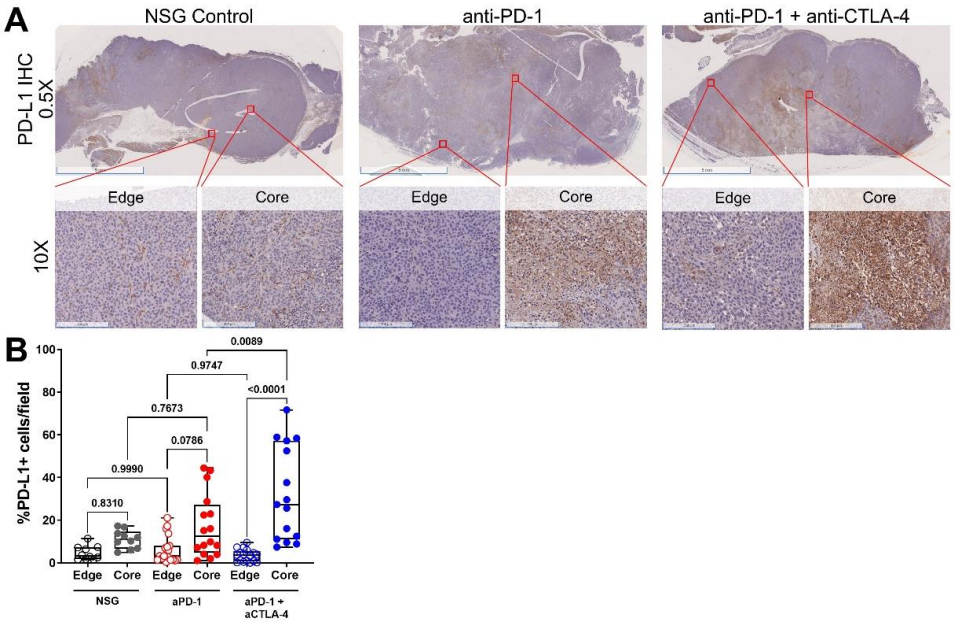


Figure S7. A, Representative PD-L1 immunohistochemistry of A375 tumors treated with aPD-1 monotherapy or aPD-1+aCTLA-4 combination therapy. **B:** Quantification of immunoreactivity for PD-L1 at edges and cores of tumors treated with mono- or combination therapy.