

Supplemental Table 1. PET ROI %ID/g values (\pm S.D.) for ^{89}Zr -Df-Pembrolizumab in ICR mice (n = 4).

| Organ | ^{89}Zr -Df-Pembro accumulation (%ID/g) at time post-injection (h) | | | | | | | |
|--------|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | 0.5 h | 6 h | 12 h | 24 h | 48 h | 72 h | 120 h | 168 h |
| Blood | 16.4 \pm 0.8 | 10.2 \pm 0.8 | 8.90 \pm 0.60 | 8.48 \pm 0.22 | 7.20 \pm 0.55 | 6.95 \pm 0.15 | 6.24 \pm 0.41 | 5.48 \pm 0.51 |
| Liver | 8.48 \pm 0.79 | 6.90 \pm 0.22 | 5.43 \pm 0.90 | 5.05 \pm 0.23 | 4.50 \pm 0.35 | 4.25 \pm 0.30 | 4.32 \pm 0.33 | 3.40 \pm 0.68 |
| Spleen | 6.20 \pm 0.31 | 5.10 \pm 0.24 | 4.53 \pm 0.11 | 4.75 \pm 0.23 | 4.55 \pm 0.30 | 4.30 \pm 0.37 | 4.39 \pm 0.57 | 4.10 \pm 0.41 |
| Kidney | 8.03 \pm 1.28 | 5.95 \pm 0.49 | 4.30 \pm 1.21 | 3.70 \pm 1.08 | 3.80 \pm 0.30 | 3.15 \pm 1.11 | 3.45 \pm 0.84 | 3.65 \pm 0.05 |
| Bone | 3.85 \pm 0.73 | 3.23 \pm 0.31 | 3.45 \pm 0.21 | 3.43 \pm 0.18 | 3.90 \pm 0.35 | 4.53 \pm 0.31 | 4.76 \pm 0.62 | 5.15 \pm 0.48 |
| Muscle | 0.65 \pm 0.14 | 1.07 \pm 0.09 | 1.05 \pm 0.11 | 1.28 \pm 0.18 | 1.06 \pm 0.20 | 0.99 \pm 0.25 | 0.94 \pm 0.17 | 0.97 \pm 0.04 |

Supplemental Table 2. PET ROI %ID/g values (\pm S.D.) for ^{89}Zr -Df-Pembrolizumab in Sprague Dawley rats (n = 4).

| Organ | ^{89}Zr -Df-Pembro accumulation (%ID/g) at time post-injection (h) | | | | | | | |
|-----------|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | 0.5 h | 6 h | 12 h | 24 h | 48 h | 72 h | 120 h | 168 h |
| Blood | 4.09 \pm 0.31 | 3.60 \pm 0.44 | 2.92 \pm 0.11 | 2.19 \pm 0.14 | 1.74 \pm 0.06 | 1.51 \pm 0.07 | 1.27 \pm 0.06 | 1.16 \pm 0.10 |
| Liver | 2.14 \pm 0.15 | 1.79 \pm 0.20 | 1.39 \pm 0.08 | 1.13 \pm 0.14 | 0.97 \pm 0.04 | 0.90 \pm 0.04 | 0.97 \pm 0.04 | 0.87 \pm 0.06 |
| Spleen | 1.89 \pm 0.07 | 1.84 \pm 0.30 | 1.03 \pm 0.21 | 0.95 \pm 0.09 | 0.87 \pm 0.08 | 0.85 \pm 0.11 | 0.72 \pm 0.13 | 0.71 \pm 0.05 |
| Kidney | 1.02 \pm 0.23 | 1.02 \pm 0.14 | 1.02 \pm 0.19 | 0.80 \pm 0.09 | 0.76 \pm 0.07 | 0.70 \pm 0.08 | 0.69 \pm 0.01 | 0.64 \pm 0.08 |
| Lungs | 0.36 \pm 0.03 | 0.51 \pm 0.11 | 0.45 \pm 0.05 | 0.45 \pm 0.05 | 0.45 \pm 0.02 | 0.47 \pm 0.03 | 0.52 \pm 0.05 | 0.42 \pm 0.02 |
| Intestine | 0.74 \pm 0.06 | 0.66 \pm 0.03 | 0.63 \pm 0.03 | 0.47 \pm 0.05 | 0.45 \pm 0.03 | 0.44 \pm 0.04 | 0.42 \pm 0.04 | 0.42 \pm 0.04 |
| Muscle | 0.20 \pm 0.03 | 0.20 \pm 0.03 | 0.20 \pm 0.01 | 0.22 \pm 0.03 | 0.23 \pm 0.02 | 0.20 \pm 0.02 | 0.19 \pm 0.01 | 0.19 \pm 0.02 |
| Ovaries | 1.13 \pm 0.21 | 1.06 \pm 0.31 | 0.88 \pm 0.07 | 0.83 \pm 0.18 | 0.92 \pm 0.16 | 0.94 \pm 0.23 | 0.86 \pm 0.19 | 0.86 \pm 0.19 |
| Brain | 0.33 \pm 0.04 | 0.25 \pm 0.07 | 0.24 \pm 0.04 | 0.25 \pm 0.05 | 0.26 \pm 0.06 | 0.19 \pm 0.02 | 0.23 \pm 0.03 | 0.23 \pm 0.05 |

Supplemental Table 3. PET ROI %ID/g values (\pm S.D.) for ^{89}Zr -Df-Pembrolizumab in NSG mice (NSG mice reconstituted with h-PBMCs; n = 4).

| Organ | ^{89}Zr -Df-Pembro accumulation (%ID/g) at time post-injection (h) | | | | | | | |
|----------------|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | 0.5 h | 6 h | 12 h | 24 h | 48 h | 72 h | 120 h | 168 h |
| Blood | 21.8 \pm 2.23 | 16.0 \pm 1.56 | 13.2 \pm 0.66 | 11.8 \pm 0.81 | 9.78 \pm 0.78 | 9.73 \pm 0.54 | 8.01 \pm 0.45 | 6.83 \pm 0.80 |
| Liver | 12.9 \pm 1.96 | 11.2 \pm 0.71 | 9.73 \pm 0.58 | 8.45 \pm 0.96 | 7.18 \pm 0.19 | 6.48 \pm 0.20 | 6.25 \pm 0.62 | 6.33 \pm 0.68 |
| Spleen | 5.48 \pm 0.71 | 6.26 \pm 1.50 | 6.08 \pm 1.49 | 5.05 \pm 1.73 | 6.75 \pm 0.65 | 5.33 \pm 0.53 | 4.88 \pm 0.72 | 4.33 \pm 0.18 |
| Kidney | 8.81 \pm 1.91 | 8.53 \pm 0.75 | 7.73 \pm 0.62 | 6.75 \pm 0.50 | 6.15 \pm 0.53 | 5.15 \pm 0.11 | 5.9 \pm 0.70 | 5.08 \pm 0.43 |
| Salivary Gland | 4.13 \pm 0.13 | 4.10 \pm 0.46 | 4.55 \pm 0.50 | 5.25 \pm 0.48 | 4.65 \pm 0.49 | 5.23 \pm 0.36 | 4.93 \pm 0.46 | 5.03 \pm 0.59 |
| Muscle | 0.74 \pm 0.22 | 0.88 \pm 0.10 | 0.24 \pm 0.04 | 1.05 \pm 0.18 | 1.22 \pm 0.17 | 1.10 \pm 0.20 | 1.04 \pm 0.22 | 0.94 \pm 0.09 |

Supplemental Table 4. PET ROI %ID/g values (\pm S.D.) for ^{89}Zr -Df-Pembrolizumab in PBL mice (NSG mice reconstituted with h-PBMCs; n = 4).

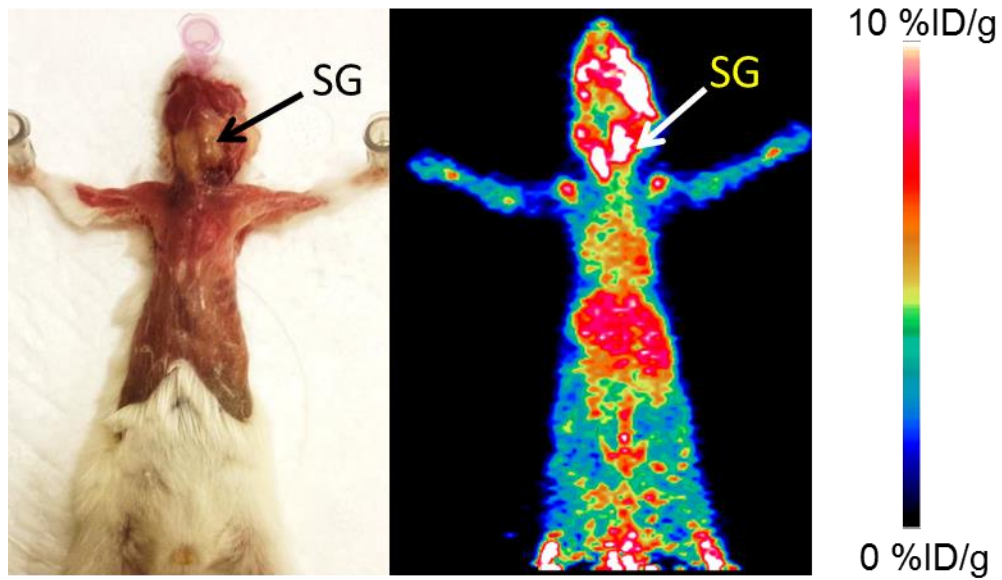
| Organ | ^{89}Zr -Df-Pembro accumulation (%ID/g) at time post-injection (h) | | | | | | | |
|----------------|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | 0.5 h | 6 h | 12 h | 24 h | 48 h | 72 h | 120 h | 168 h |
| Blood | 24.5 \pm 1.35 | 17.2 \pm 1.63 | 13.8 \pm 0.93 | 9.30 \pm 0.51 | 6.13 \pm 0.41 | 4.60 \pm 0.56 | 3.20 \pm 0.41 | 3.65 \pm 0.59 |
| Liver | 14.6 \pm 0.40 | 12.0 \pm 0.83 | 11.0 \pm 0.38 | 7.68 \pm 0.64 | 7.65 \pm 0.57 | 7.13 \pm 0.33 | 7.33 \pm 0.84 | 5.48 \pm 0.76 |
| Spleen | 10.3 \pm 1.31 | 8.05 \pm 0.69 | 7.65 \pm 0.30 | 5.10 \pm 0.68 | 4.85 \pm 0.40 | 4.50 \pm 0.53 | 4.15 \pm 0.80 | 3.85 \pm 0.47 |
| Kidney | 11.5 \pm 0.53 | 8.18 \pm 1.27 | 6.68 \pm 0.48 | 5.33 \pm 0.66 | 5.13 \pm 0.13 | 4.23 \pm 0.44 | 3.73 \pm 0.45 | 3.33 \pm 0.66 |
| Salivary Gland | 3.43 \pm 0.55 | 5.48 \pm 0.40 | 7.23 \pm 0.20 | 8.23 \pm 0.33 | 9.65 \pm 0.66 | 10.7 \pm 0.77 | 10.8 \pm 0.72 | 11.1 \pm 0.83 |
| Muscle | 0.85 \pm 0.15 | 0.95 \pm 0.11 | 0.88 \pm 0.18 | 0.90 \pm 0.31 | 0.95 \pm 0.23 | 0.75 \pm 0.15 | 0.88 \pm 0.26 | 0.95 \pm 0.21 |

Supplemental Table 5. Estimated radiation absorbed doses to an adult human after intravenous injection of ^{89}Zr -Df-Pembrolizumab, based on PET imaging data obtained in ICR mice (n = 4).

| Organ | mGy/MBq (S.D.) | | rad/mCi (S.D.) | |
|-------------------------------------|----------------|---------|----------------|---------|
| Adrenals | 0.025 | (0.001) | 0.093 | (0.001) |
| Brain | 0.240 | (0.006) | 0.889 | (0.023) |
| Breasts | 0.159 | (0.002) | 0.588 | (0.006) |
| Small Intestine | 0.022 | (0.001) | 0.080 | (0.001) |
| Stomach Wall | 0.533 | (0.005) | 1.970 | (0.019) |
| Kidneys | 0.025 | (0.001) | 0.091 | (0.005) |
| Liver | 0.811 | (0.036) | 2.999 | (0.135) |
| Lungs | 0.494 | (0.004) | 1.829 | (0.016) |
| Muscle | 0.019 | (0.001) | 0.069 | (0.001) |
| Ovaries | 1.425 | (0.018) | 5.273 | (0.065) |
| Pancreas | 0.024 | (0.001) | 0.090 | (0.001) |
| Red Marrow | 0.574 | (0.012) | 2.122 | (0.046) |
| Skin | 0.344 | (0.005) | 1.271 | (0.018) |
| Spleen | 0.016 | (0.001) | 0.058 | (0.001) |
| Thymus | 0.021 | (0.001) | 0.078 | (0.001) |
| Thyroid | 0.565 | (0.009) | 2.091 | (0.033) |
| Urinary Bladder Wall | 0.512 | (0.007) | 1.894 | (0.027) |
| Uterus | 0.023 | (0.001) | 0.086 | (0.001) |
| Total Body Effective Dose (mSv/MBq) | 0.515 (0.005) | | | |

Supplemental Table 6. Estimated radiation absorbed doses to an adult human after intravenous injection of ⁸⁹Zr-Df-Pembrolizumab, based on PET imaging data obtained in Sprague Dawley rats (n = 4).

| Organ | mGy/MBq (S.D.) | | rad/mCi (S.D.) | |
|-------------------------------------|----------------|---------|----------------|---------|
| | Adrenals | 0.017 | (0.006) | 0.062 |
| Brain | 0.538 | (0.882) | 1.991 | (0.263) |
| Breasts | 0.179 | (0.001) | 0.662 | (0.005) |
| Small Intestine | 0.019 | (0.008) | 0.072 | (0.028) |
| Stomach Wall | 0.621 | (0.009) | 2.299 | (0.033) |
| Kidneys | 0.017 | (0.007) | 0.061 | (0.025) |
| Liver | 0.958 | (0.077) | 3.543 | (0.286) |
| Lungs | 0.523 | (0.010) | 1.934 | (0.038) |
| Muscle | 0.012 | (0.005) | 0.045 | (0.018) |
| Ovaries | 1.363 | (0.010) | 5.041 | (0.038) |
| Pancreas | 0.018 | (0.007) | 0.065 | (0.025) |
| Red Marrow | 0.524 | (0.009) | 1.938 | (0.033) |
| Skin | 0.365 | (0.006) | 1.350 | (0.022) |
| Spleen | 0.109 | (0.103) | 0.402 | (0.382) |
| Thymus | 0.015 | (0.006) | 0.054 | (0.021) |
| Thyroid | 0.593 | (0.011) | 2.192 | (0.042) |
| Urinary Bladder Wall | 0.578 | (0.006) | 2.137 | (0.021) |
| Uterus | 0.017 | (0.007) | 0.062 | (0.024) |
| | | | | |
| Total Body Effective Dose (mSv/MBq) | 0.540 (0.008) | | | |



SUPPLEMENTAL FIGURE 1. The salivary glands were visualized by extraction and removal of skin. PET imaging was performed on the euthanized PBL mouse. The salivary glands clearly showed high PET uptake. Additional signal was attributed to a combination of the lacrimal glands and saliva.