

# **TE1PA as innovating chelator for $^{64}\text{Cu}$ immuno-TEP imaging: a comparative *in vivo* study with DOTA/NOTA by conjugation on 9E7.4 mAb in a syngeneic multiple myeloma model**

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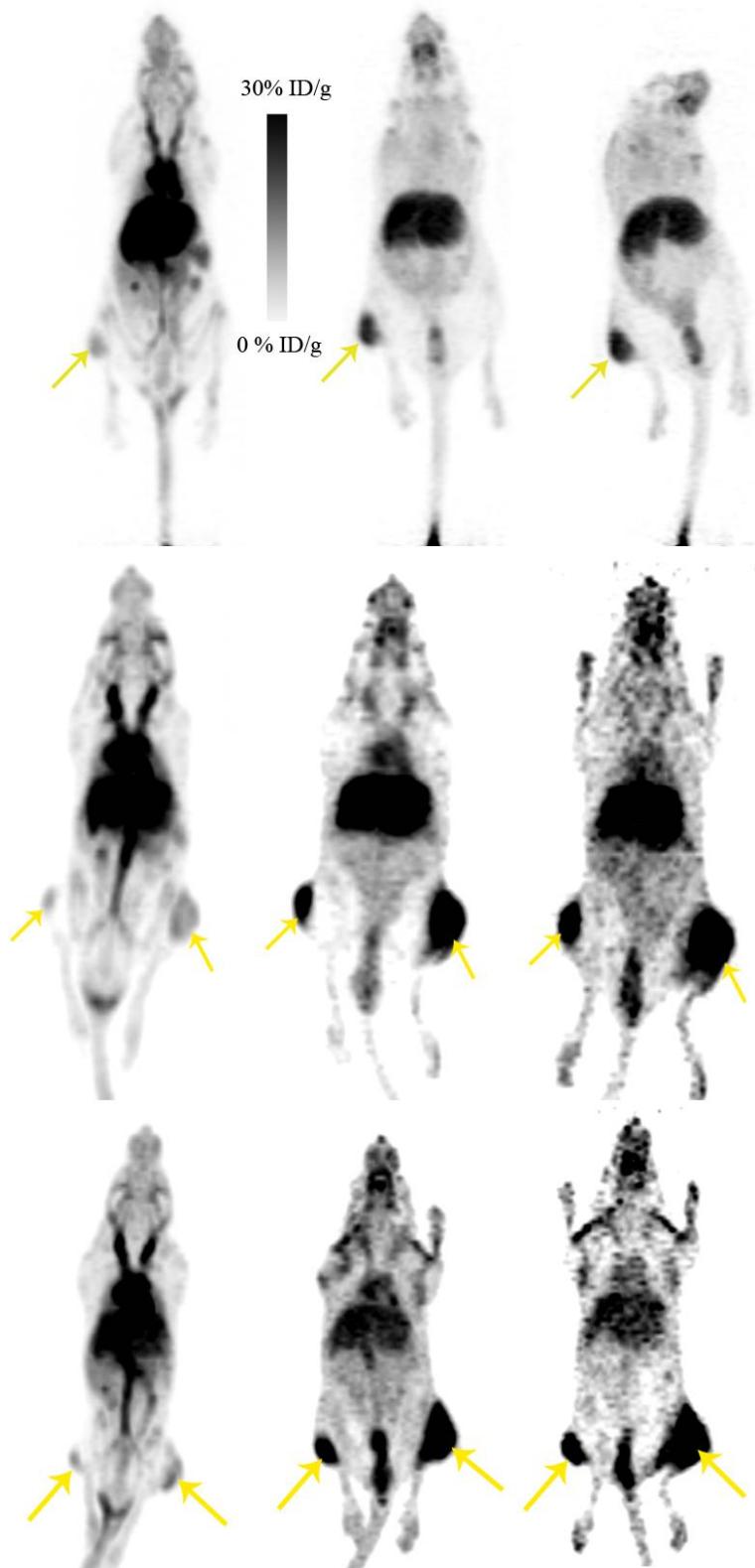
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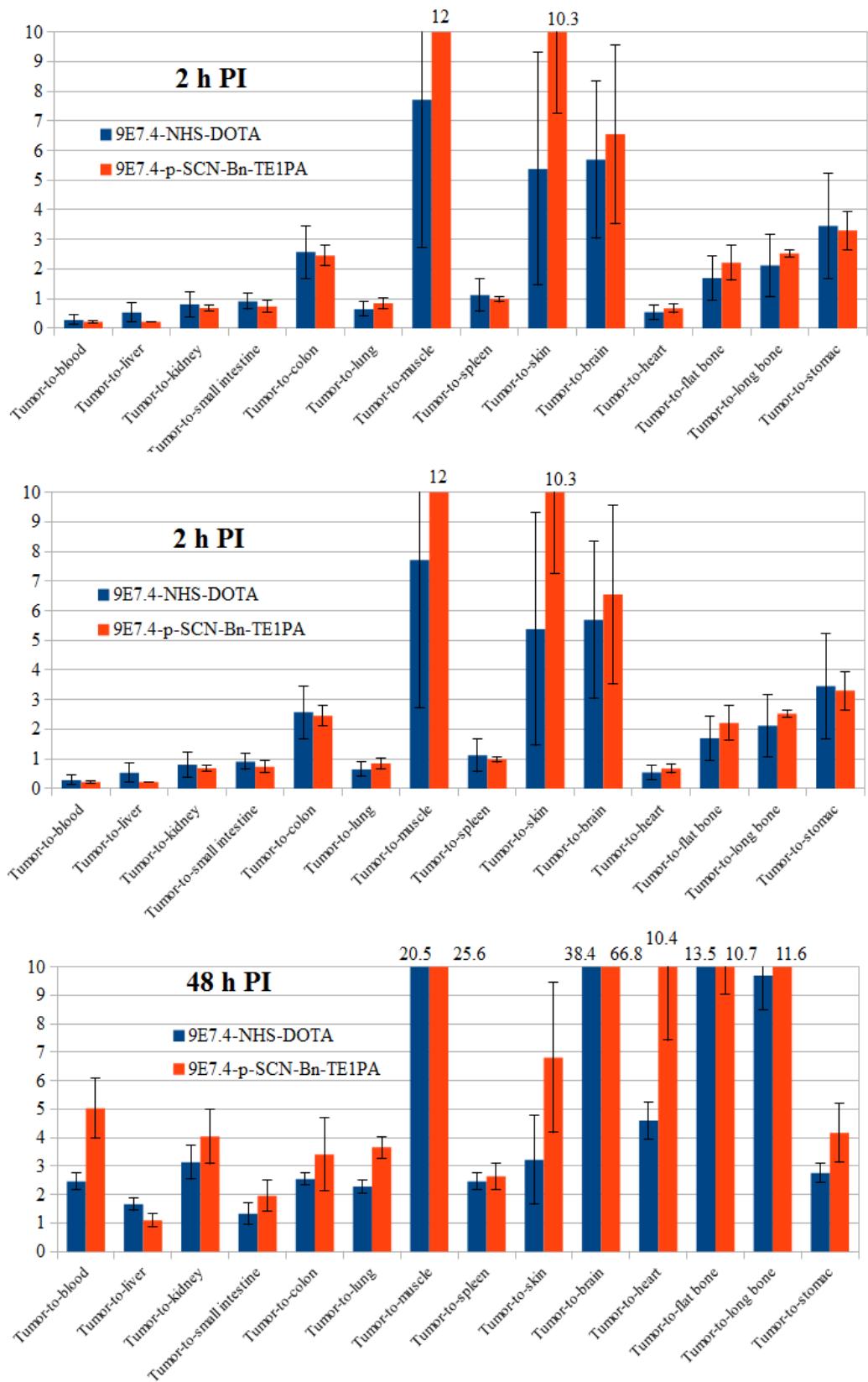
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| <sup>64</sup> Cu-9E7.4- <i>p</i> -SCN-Bn-NOTA<br>48 h PI |              |
|--|--------------|
| Tumor  | 14.33 ± 4.85 |
| Blood  | 8.06 ± 0.67  |
| Liver  | 6.94 ± 2.34  |
| Kidney   | 3.31 ± 0.09  |
| S. Intestine   | 6.07 ± 0.37  |
| Colon  | 3.23 ± 0.38  |
| Lung   | 5.91 ± 0.49  |
| Muscle   | 0.80 ± 0.15  |
| Spleen   | 3.42 ± 0.48  |
| Skin   | 2.60 ± 0.36  |
| Brain  | 0.29 ± 0.05  |
| Heart  | 2.63 ± 0.09  |
| Flat Bone  | 1.78 ± 0.5   |
| Stomach  | 3.89 ± 0.16  |

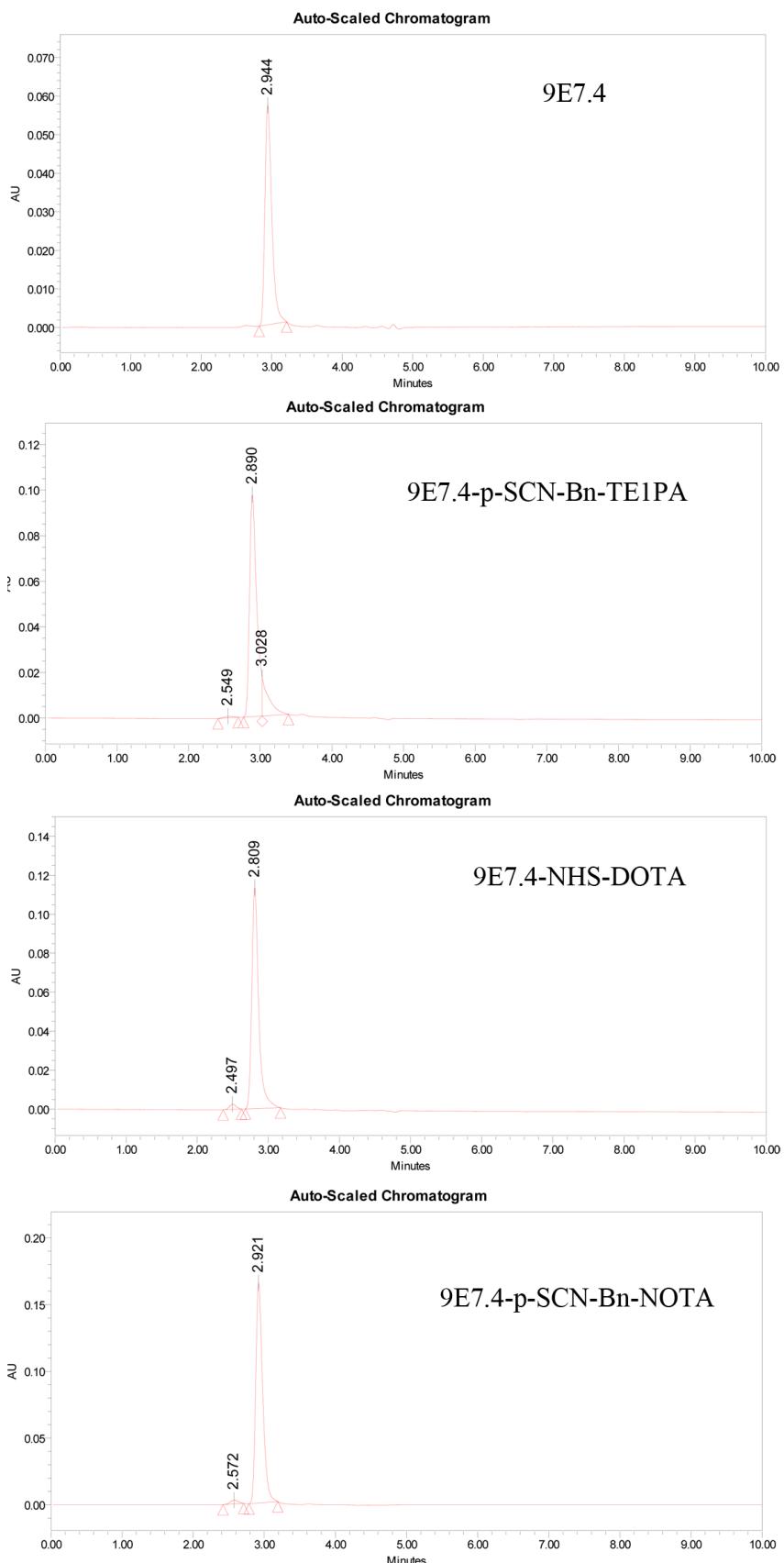
**Table S1:** % ID per gram of organ at 48 h post-injection for <sup>64</sup>Cu-9E7.4-*p*-SCN-Bn-NOTA (n = 3).



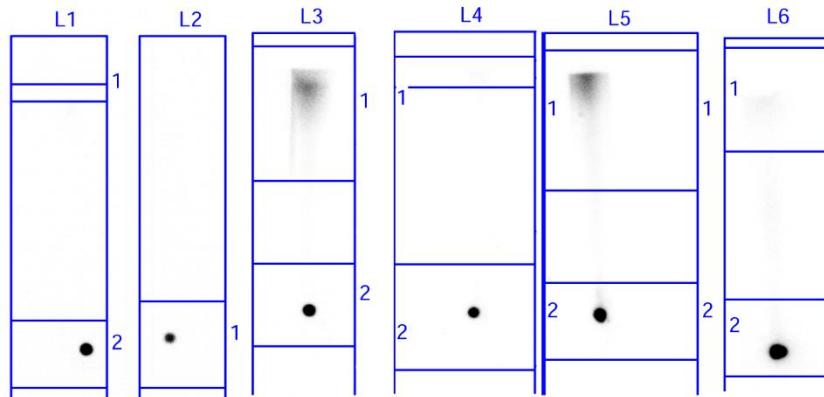
**Figure S2:** PET images of subcutaneous 5T33 grafted mouse at 2 h, 24 h and 48 h post-injection of  $^{64}\text{Cu}$ -9E7.4-*p*-NCS-Bn-TE1PA (top),  $^{64}\text{Cu}$ -9E7.4-NHS-DOTA (middle) and  $^{64}\text{Cu}$ -9E7.4-*p*-SCN-Bn-NOTA (bottom). Yellow arrows indicate tumors.



**Figure S3:** Tumor-to-organ ratios calculated for  $^{64}\text{Cu}$ -9E7.4-NHS-DOTA and  $^{64}\text{Cu}$ -9E7.4-p-SCN-Bn-TE1PA, presented at 2, 24 and 48 h post-injection.



**Figure S4:** Size exclusion-HPLC of non-modified 9E7.4 antibody, 9E7.4-*p*-SCN-Bn-TE1PA, 9E7.4-NHS-DOTA and 9E7.4-SCN-Bn-NOTA



| Lane #1<br>ID   | Gross DLU   | % Sum | Lane #4<br>ID   | Gross DLU   | % Sum |
|-----------------|-------------|-------|-----------------|-------------|-------|
| <b>1 - Prof</b> | 6,769.1     | 1.7   | <b>1 - Prof</b> | 18,304.1    | 5.1   |
| <b>2 - Prof</b> | 394,718.4   | 98.3  | <b>2 - Prof</b> | 342,057.5   | 94.9  |
| <b>Lane</b>     | 474,304.5   |       | <b>Lane</b>     | 434,032.9   |       |
| <b>UnRes</b>    | 72,817.0    |       | <b>UnRes</b>    | 73,671.3    |       |
| Lane #2<br>ID   | Gross DLU   | % Sum | Lane #5<br>ID   | Gross DLU   | % Sum |
| <b>1 - Prof</b> | 108,874.3   | 100.0 | <b>1 - Prof</b> | 1,310,573.0 | 55.4  |
| <b>Lane</b>     | 175,639.2   |       | <b>2 - Prof</b> | 1,054,598.2 | 44.6  |
| <b>UnRes</b>    | 66,764.9    |       | <b>Lane</b>     | 2,482,075.0 |       |
| Lane #3<br>ID   | Gross DLU   | % Sum | Lane #6<br>ID   | Gross DLU   | % Sum |
| <b>1 - Prof</b> | 893,575.1   | 58.9  | <b>1 - Prof</b> | 114,290.9   | 3.6   |
| <b>2 - Prof</b> | 622,800.9   | 41.1  | <b>2 - Prof</b> | 3,066,264.8 | 96.4  |
| <b>Lane</b>     | 1,573,807.7 |       | <b>Lane</b>     | 3,297,803.5 |       |
| <b>UnRes</b>    | 57,431.7    |       | <b>UnRes</b>    | 117,247.9   |       |

**Figure S5:** Radiochromatograms obtained for  $^{64}\text{Cu}$ -9E7.4-*p*-SCN-Bn-TE1PA (lane 1),  $^{64}\text{Cu}$ -9E7.4-NHS-DOTA (lane 2),  $^{64}\text{Cu}$ -9E7.4-NHS-NODA-GA (lane 3)  $^{64}\text{Cu}$ -9E7.4-*p*-SCN-Bn-NOTA (lane 4),  $^{64}\text{Cu}$ -9E7.4-SNHS-TE1PA (lane 5) and  $^{64}\text{Cu}$ -9E7.4-*p*-SCN-Bn-NOTA (lane 6). Radiolabeled antibodies stay at the bottom of the chromatography while free  $^{64}\text{Cu}$  migrates at the top.