Supplemental Materials

Optimizing the Profile of $[^{99m}\text{Tc}]\text{Tc–NT(7–13)}$ Tracers in Pancreatic Cancer Models by Means of Protease Inhibitors

Panagiotis Kanellopoulos $^{1,2,*}$, Berthold A. Nock $^1$, Eric P. Krenning $^3$ and Theodosia Maina $^{1,*}$

$^1$ Molecular Radiopharmacy, INRATES, NCSR “Demokritos”, 15341 Athens, Greece; nock_berthold.a@hotmail.com
$^2$ Molecular Pharmacology, School of Medicine, University of Crete, Heraklion, 70013 Crete, Greece
$^3$ Cyclotron Rotterdam BV, Erasmus MC, 3015 CE Rotterdam, The Netherlands; erickrenning@gmail.com
* Correspondence: kanelospan@gmail.com (P.K.); maina_thea@hotmail.com (T.M.); Tel.: +30-210-650-3891 (P.K.); +30-210-650-3908 (T.M.)

Figure S1. Structure of (a) the angiotensin converting enzyme (ACE)-inhibitor lisinopril (Lis) and (b) the structure of sacubitril (AHU377) found in the drug Entresto, releasing the active substance sacubitrilat (LBQ657) in vivo upon ester-hydrolysis by esterases; sacubitrilat is a potent and specific inhibitor of neprilysin (NEP).
Table S1. Cellular uptake (membrane bound and internalized of total-added activity) of [\(^{99m}\text{Tc}\)]Tc-DT1 at 1 h incubation across cell lines, expressed as mean±SD, including specific and non-specific portions.

| Cell line | MB\(^1\) | I\(^2\) |
|-----------|----------|--------|
| AsPC-1    |          |        |
| specific  | 0.98±0.42| 14.11±2.28 |
| non-specific\(^3\) | 0.67±0.23 | 0.88±0.59 |
| PANC-1    |          |        |
| specific  | 0.76±0.39| 7.30±2.41  |
| non-specific | 0.44±0.20 | 0.29±0.12 |
| MiaPaca-2 |          |        |
| specific  | 0.37±0.23| 2.46±0.23  |
| non-specific | 0.87±0.49 | 0.50±0.36 |
| Capan-1   |          |        |
| specific  | 0.10±0.01| 0.28±0.13  |
| non-specific | 0.20±0.02 | 0.11±0.02 |

\(^1\) MB, membrane bound fraction; \(^2\) I, internalized fraction; non-specific values determined by incubation in the presence of 1 µM NT; these values were subtracted from the respective measured MB/I fractions to calculate the respective specific values.

Table S2. Biodistribution data for [\(^{99m}\text{Tc}\)]Tc-DT1, expressed as %IA/g mean±SD, in MiaPaca-2 xenograft-bearing SCID mice treated with the Entresto+Lis combination at 4 h pi.

| Tissue        | [\(^{99m}\text{Tc}\)]Tc-DT1 – 4 h pi | Entresto+Lis\(^2\) |
|---------------|----------------------------------|-------------------|
| Blood         | 0.06±0.01                        |                   |
| Liver         | 0.57±0.04                        |                   |
| Heart         | 0.08±0.02                        |                   |
| Kidneys       | 5.86±0.14                        |                   |
| Stomach       | 0.37±0.05                        |                   |
| Intestines    | 2.43±0.6                         |                   |
| Spleen        | 0.49±0.03                        |                   |
| Muscle        | 0.03±0.01                        |                   |
| Lungs         | 0.43±0.04                        |                   |
| Pancreas      | 0.1±0.02                         |                   |
| Tumor         | 1.97±0.25                        |                   |

All animals were injected with 185 kBq/10 pmol peptide; Entresto+Lis mice group (n = 4) with animals receiving 12 mg Entresto per os 30 min prior to radiotracer co-injection together with 200 µg Lis to in situ inhibit NEP and ACE, respectively.