Supplementary Data

Tracer synthesis

Commercially available deferoxamine (DFO) was pre-loaded using iron(III) chloride. In a single pot reaction, boc-L-glutamic acid 1 tert-butyl ester was activated using dipyrrolidino(N-succinimidyloxy)carbenium hexafluorophosphate (HSPyU) and DIEA to produce an NHS-ester. The pre-loaded DFO was then added to the reaction pot to produce Boc-Glu(OtBu)-DFO[Fe]. The use of iron prevents HSPyU from reacting with the three hydroxamate groups of DFO. Protecting groups on the amino acid were removed using a combination of TFA and DCM at room temperature. The target molecule was purified using solid phase extraction. A small molar equivalency of $FeCl_3$ was used during a wash step to ensure that the DFO is complexed to iron(III). The near-infrared contrast agent ZW800F was conjugated to the L-GLU-DFO[Fe] compound using a pre-activated NHS-ester. The target compound ZW800F-Glu(COOH)-DFO[Fe] was purified using solid phase extraction and concentrated under vacuum. The remaining carboxyl group on the glutamic acid was converted into a TFP-ester using EDC/TFP in a MES buffer. The TFP-ester product was purified using solid phase extraction and concentrated under vacuum. The TFP ester was used due to its increased stability to moisture during the drying process. The TFP-ester was reacted with commercially available cRGDyK to produce ZW800F-Glu(cRGDyK)-DFO[Fe]. The peptide bond is formed in DMSO under a basic environment. During purification using solid phase extraction, EDTA is used to remove the iron from the DFO. The synthesis of cRGD-ZW800-1 was previously described [1].

Preparation of ZW800F-cRGD-[^{89}Zr]Zr-DFO

100 µL 1 M oxalic acid containing 56.3 MBq of zirconium was mixed with 37 µL 0.9% NaCl and 45 µL 2 M Na_2CO_3 and reacted for 10 minutes. Hereafter 0.49 mL 0.5 M HEPES and 20 µL 2 mM cRGD-DFO-ZW800F were added bringing the total volume to 1 mL which was reacted for 60 minutes at room temperature. The reaction mixture was applied to a tC18 Sep-Pak, which was preconditioned with 10 mL ethanol and 10 mL water. Next the Sep-Pak was washed twice with 8 mL water, followed by elution of the Sep-Pak with 60/40 ethanol/50 mM NaOAc pH 5.0. The product was collected in 0.5 mL (fraction 3 and 4 from eluted cartridge) in a radiochemical yield of 88%. Next the product was formulated to achieve a dose of per mouse of 10 nmol (dose chosen based on previous experience [2])/3 MBq ZW800F-cRGD-[^{89}Zr]Zr-DFO in 150 µL injection volume and was immediately injected. The radiochemical purity was 99.5% base on iTLC using 50 mM EDTA as eluent.

Preparation of ZW800F-cRGD-Zr-DFO

100 µL 1 M oxalic acid containing 400 nmol (2 equivalents) of zirconium was mixed with 345 µL 0.9% NaCl and 45 µL 2 M Na_2CO_3 and reacted for 10 minutes. Hereafter, 0.5 mL 0.5 M HEPES and 100 µL 2 mM cRGD-DFO-ZW800F (200 nmol) were added bringing the total volume to 1090 µL which was reacted for 60 minutes at room temperature. The reaction mixture was applied to a tC18 Sep-Pak, which was preconditioned with 10 mL ethanol and 10 mL water. Next the Sep-Pak was washed twice with 8 mL water, followed by elution of the Sep-Pak with 60/40 ethanol/50 mM NaOAc pH 5.0. The product was collected in 0.5 mL (fraction 3 and 4 from eluted cartridge) in a yield of 78% (determined by HPLC using an Alltima C-18 column). Next the product was formulated to achieve a dose of per mouse of 10 nmol ZW800F-cRGD-Zr-DFO in 120 µL injection volume. The stability of the product was assessed for 7 days by high-performance liquid chromatography (HPLC) (**Figure 2**).

References

[1] Choi HS, Gibbs SL, Lee JH, Kim SH, Ashitate Y, Liu F, Hyun H, Park G, Xie Y, Bae S, Henary M, Frangioni JV. Targeted zwitterionic near-infrared fluorophores for improved optical imaging. Nat Biotechnol 2013; 31: 148-53.

[2] Handgraaf HJM, Boonstra MC, Prevoo HAJM, Kuil J, Bordo MW, Boogerd LSF, Sibinga Mulder BG, Sier CFM, Vinkenburg-van Slooten ML, Valentijn ARPM, Burggraaf J, van de Velde CJH, Frangioni JV, Vahrmeijer AL. Real-time near-infrared fluorescence imaging using cRGD-ZW800-1 for intraoperative visualization of multiple cancer types. Oncotarget 2017; 8: 21054-66.