

## Altered splenic [<sup>89</sup>Zr]Zr-rituximab uptake in nonresponding ILD patients

**Table S1.** Description of all patients prior to treatment with rituximab

Patient number	Age	M/F	Diagnosis	Year of diagnosis	HRCT diagnosis	medication Pre-rituximab
1	69	M	RA	2013	UIP	azathioprine, prednisone
2	67	M	RA	2010	UIP	prednisone, cyclophosphamide, azathioprine
3	65	M	RA	2013	NSIP	cyclophosphamide, methotrexate
4	44	M	ASS	2012	UIP	prednisone, azathioprine
5	57	F	ASS	2015	fNSIP	prednisone, azathioprine
6	69	F	ASS	2009	fNSIP	prednisone, azathioprine
7	59	F	cEAA	2015	EAA	azathioprine cyclophosphamide
8	63	F	cEAA	2015	EAA	cyclophosphamide, prednisone
9	57	M	Scleroderma	2014	fNSIP	cyclophosphamide, azathioprine
10	69	F	Connective tissue disease	2010	fNSIP	cyclophosphamide, prednisone
11	67	F	RA	2008	UIP	prednisone, azathioprine
12	63	F	cEAA	2007	EAA	prednisone, azathioprine, mycophenolic acid
13	71	M	ASS	2013	fNSIP	prednisone, azathioprine, methotrexate
14	30	V	ASS	2015	fNSIP	prednisone, azathioprine, cyclophosphamide
15	61	V	ASS	2012	fNSIP with OP	prednisone, azathioprine
16	70	M	cEAA	2014	EAA	prednisone, azathioprine
17	63	V	cEAA	2014	EAA	prednisone, azathioprine
18	68	M	cEAA	1991	EAA	prednisone, cyclophosphamide, azathioprine
19	55	V	cEAA	2006	EAA	prednisone, azathioprine
20	49	V	Dermatomyositis	2016	fNSIP with OP	prednisone, cyclophosphamide
21	51	M	Scleroderma	2014	fNSIP	prednisone, cyclophosphamide, azathioprine

Rheumatic arthritis (RA), antisynthetase syndrome (ASS), chronic extrinsic allergic alveolitis (cEAA), usual interstitial pneumonia (UIP), fibrotic (f) non-specific interstitial pneumonia (NSIP) organizing pneumonia (OP).

**Table S2.** Detailed production and QC procedures of [<sup>89</sup>Zr]Zr-rituximab

Rituximab (0.5 mL of 10 mg/mL (33 nmol) was diluted to 0.5 mg/mL with 0.9% NaCl (470 µL), after which the pH was adjusted to 9.5-9.7. Two equivalents of Fe-TFP-*N*-suc-desferal in acetonitrile (20 µL of 3.3 nmol/µL, 66 nmol) were added, mixed carefully and reacted for 30 minutes at room temperature. Next, 50 µL of 100 mg/mL gentisic acid pH 4.0-4.2 were added, followed by adjustment of the pH to 4.20-4.50 with 0.25 M sulfuric acid. Hereafter, EDTA (50 µL, 25 mg/mL) was added and reacted for 30 minutes at 35 °C to remove Fe, after which the conjugated *N*-suc-DFO-rituximab was purified by size exclusion chromatography (PD10, GE Healthcare) and the product collected in 5 mg/mL gentisic acid in 0.9% NaCl pH 4.9-5.3. Hereafter, *N*-suc-DFO-rituximab was radiolabeled. To this end, 600 µL 1 M oxalic acid containing the required amount of <sup>89</sup>Zr were mixed with 1800 µL 2M Na<sub>2</sub>CO<sub>3</sub> and reacted for 3 minutes. Next 3 mL 0.5 M Hepes and 2 mL *N*-suc-DFO-rituximab were added and reacted for 60 minutes at room temperature while slowly shaken. After the incubation period [<sup>89</sup>Zr]Zr-rituximab was purified by size exclusion chromatography using a PD10 column. The product was eluted in 5 mg/mL gentisic acid in 0.9% NaCl pH 4.9-5.3. The product was formulated to arrive at an injection dose of 18 MBq-5 mg-10 mL [<sup>89</sup>Zr]Zr-Rituximab. The mean of the product pH was 6.02±0.26. The mean radiochemical purity as assessed by iTLC was 99.1±0.3%. To this end 2 µL of product was applied on a TLC strip (Biodex, cat nr. 150-771) and developed in 10% acetonitrile in 20 mM citric acid+ 50 mM EDTA pH 4.8-5.0 as described by the supplier. [<sup>89</sup>Zr]Zr-rituximab remains on the baseline, while impurities such as free Zr-89 and <sup>89</sup>Zr-DFO run with the solvent front. The mean protein integrity was 99.6±1.2% as determined by size exclusion HPLC using a superdex 200 10/300 GL column (GE) and a mixture of 0.1 M phosphate, 0.15 M NaCl and 0.01 M NaN<sub>3</sub> pH 6.2-7.0 in water as the eluent at a flow rate of 0.5 mL/min. The mean immune reactive fraction as assessed by a binding assay was 90.5±2.3% using SU-DHL-4 cells fixed in 2% paraformaldehyde. Sterility of each [<sup>89</sup>Zr]Zr-rituximab batch was assured by performing a media fill immediately after final filter sterilisation of each batch. These procedures resulted in a sterile final product with endotoxin levels <0.5 EU/mL. The radiopharmaceutical consists of 10 mg rituximab labeled with 18 MBq Zr-89 in a total injection volume of 10 mL. Zr-89 was obtained at >740 MBq/mL in 1 M oxalic acid from Perkin Elmer (Boston, MA, USA). Reference: Adams H, van de Garde EMW, van Moorsel CHM, Vugts DJ, van Dongen GAMS, Grutters JC, Keijsers RG. [<sup>89</sup>Zr]Zr-rituximab PET/CT activity in patients with IMID-IP: a feasibility study. *Am J Nucl Med Mol Imaging* 2019; 9: 296-308.