Production Of ^{18}F-AlF-RESCA-IL2

First, aqueous ^{18}F-fluoride was produced by irradiation of ^{18}O-water with a cyclotron (Cyclon 18 Twin, IBA) via the $^{18}O(p,n)^{18}F$ nuclear reaction. The ^{18}F-fluoride solution was passed through a QMA Sep-Pak Light anion exchange cartridge (Waters Chromatography Division, Millipore Corp, preconditioned with 3 mL metal free water) to recover the ^{18}O-water. The QMA was then washed with 10 mL of metal free water and 10 mL of air. The ^{18}F-fluoride was then eluted from the cartridge with 400 µL 0.9% sodium chloride (NaCl) (B. Braun). An ^{18}F-AlF solution was freshly prepared by adding ~10 GBq ^{18}F-fluoride in 400 µL 0.9% NaCl to 25 µL aluminum chloride (2 mM, 50 nmol) in 100 µL sodium acetate buffer 0.1 M, pH 4.5 (NaOAc, Sigma-Aldrich), and allowed to react at room temperature (RT) for 5 min. To the ^{18}F-AlF solution, 40 µL of the restrained complexing agent-tetrafluorophenol ester ((±)-H_3RESCA-TFP (50 nmol; NaOAc buffer, 0.1 M, pH 4.5; Leuven University, Belgium) was added. After 15 min of reaction at RT, the reaction mixture was diluted with 10 mL of water and transferred to an HLB cartridge (Waters Chromatography Division, Millipore Corp). ^{18}F-AlF-RESCA-TFP was eluted from the cartridge with 0.6 mL ethanol and 0.7 mL sodium acetate (pH 8.5) into a vial containing 100 µL IL2 (17 nmol) in dimethyl sulfoxide (DMSO, Sigma-Aldrich). The conjugation occurred at 50°C for 15 min, after which the reaction was quenched with 25 µL 25% phosphoric acid (H_3PO_4) and 48 µL 10% sodium dodecyl sulfate (SDS, Sigma-Aldrich). The product was diluted in 10 mL of water for injections (WFI) and passed through a tC2 Sep-Pak cartridge (Waters Chromatography Division, Millipore Corp), which was preconditioned with 5 mL ethanol (EtOH, Merck KGaA) followed by 5 mL of a solution of 5% EtOH containing 12 µL of 2.5% H_3PO_4 and 10 mL of air). The cartridge was washed three times with 2 mL 50% aqueous EtOH containing 23 µL 0.25% H_3PO_4. ^{18}F-AlF-RESCA-IL2 was eluted from the cartridge with 0.8 mL 100% EtOH containing 5 µL 0.25% H_3PO_4 and 3.5 mL 5% glucose (Baxter) and

collected in a vial containing 1.5 mL 5% glucose, 0.1% SDS and 0.5% human serum albumin (HSA, Sanquin) solution.

Radiochemical identity and purity were assessed by instant thin-layer chromatography (iTLC), eluted in a solution of 75% aqueous acetonitrile (R_f ^{18}F-AlF-RESCA-TFP=1 and R_f ^{18}F-AlF-RESCA-IL2=0) and by UPLC-ESI-HRMS using a Dionex Ultimate 3000 UPLC System (Thermo Fisher Scientific, Sunnyvale, USA) coupled in series to a UV detector and a radio-activity detector (Berthold FlowStar LB513, Mx50–6 flow cell. The identity of the product was confirmed using native IL2 as reference material.

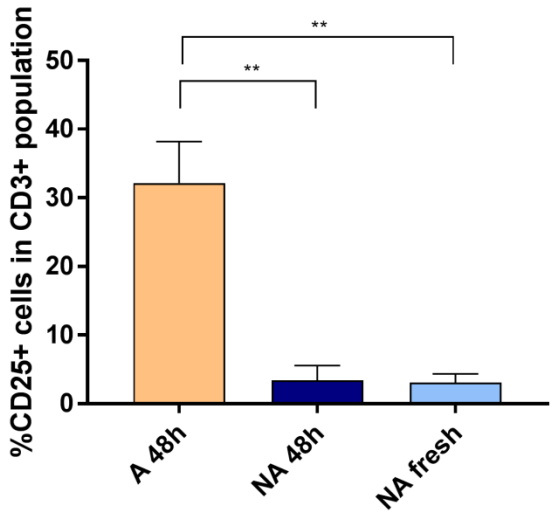
Production Of ^{68}Ga-Ga-NODAGA-IL2

Synthesis of NODAGA-IL2. To a solution of IL2 in DMSO (100 µL, 14 nmol), a 4-fold molar excess of a solution of NODAGA-NHS ester in DMSO (40 µL-55 nmol, Chematech) was added, followed by 5 µL N,N-diisopropylethylamine (pH 8.5). This mixture was incubated for 2 h at RT with slow stirring. The reaction was quenched with 25 µL 25% H_3PO_4 and 48 µL 10% SDS. The product was diluted in 10 mL of WFI and purified with a tC2 cartridge, as described above. The final product was eluted with 0.5 mL 100% EtOH, containing 5 µL 0.25% H_3PO_4, and 0.5 mL 100% EtOH. The conjugate was kept at -80^{0}C until the day of radiolabeling.

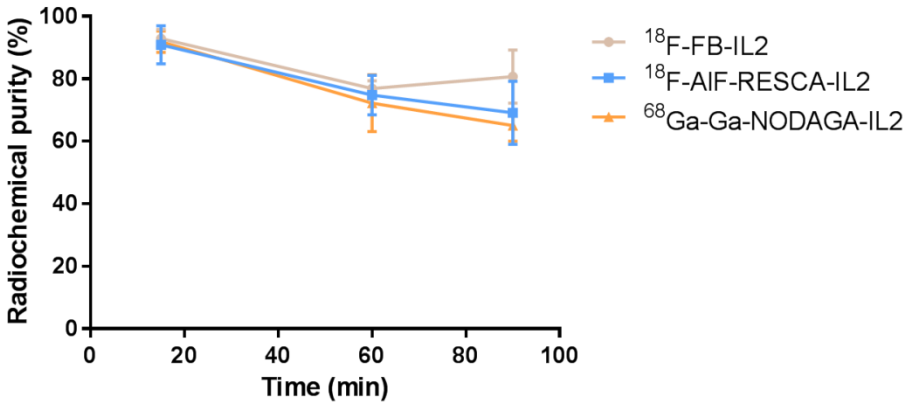
Radiolabeling of ^{68}Ga-Ga-NODAGA-IL2 (Method 1). ^{68}Ga-Cl_3 was eluted from a > 9 month old GMP 1110 MBq grade ^{68}Ge/^{68}Ga-generator was used (Eckert & Ziegler). $^{68}Ga^{3+}$ was trapped in a PS-H^+ cartridge (ABX) and subsequently eluted from this cartridge with 1.5 mL 5M sodium chloride (NaCl). To the defrosted solution of NODAGA-IL2, 100 µL ^{68}Ga-Cl_3 (20-100 MBq) and 300 µL 1.5M HEPES buffer (ABX) were added (pH between 3-5). After 15 min of conjugation at 50^{o}C, the mixture was quenched with 25 µL 25% H_3PO_4 and 48 µL 10% SDS. The

product was purified with a tC2 cartridge as described above. ^{68}Ga-Ga-NODAGA-IL2 was eluted from the cartridge with 0.8 mL 100% EtOH, containing 5 µL 0.25% H_3PO_4, and 3.5 mL of 5% glucose and collected in a vial containing 1.5 mL of 5% glucose, 0.1% SDS and 0.5% HSA solution. The final product was analyzed by iTLC eluted with a 0.1M citric acid solution (R_f ^{68}Ga-Cl_3=1 and R_f ^{68}Ga-NODAGA-IL2=0) and by ultra-performance liquid tomography (UPLC).

Radiolabeling of ^{68}Ga-Ga-NODAGA-IL2 (Method 2). ^{68}Ga-Cl_3 was eluted from a ^{68}Ge/^{68}Ga-generator with 6 mL 0.1M hydrochloric acid (HCl, Rotem Industries) into a vial containing 4 mL 37% HCl to form a final HCl concentration of 4 M. The eluate was then passed through a PS-HCO_3^- cartridge (Synthra®), preconditioned with successively 5 M HCl, 1 M HCl, WFI and again 5 M HCl). The cartridge was washed with 2 mL 4 M HCl and dried under a strong flow of nitrogen to eliminate the excess 4 M HCl. [^{68}Ga]Cl_3 was subsequently eluted with 300 µL water (100-360 MBq) into an Eppendorf (metal free) containing 75 mg HEPES and 10 µL of 25% ammonia (Merck KGaA). This solution (pH 3-4) was added to the solution of NODAGA-IL2 and the pH was adjusted to 4-5 with 25% ammonia. After 15 min of conjugation at 50^{o}C, the mixture was quenched with 25 µL H_3PO_4 25% and 48 µL 10% SDS. The product was purified, formulated and analyzed as mentioned above.



SUPPLEMENTAL FIGURE 1. FACS analysis for *in vitro* experiments. Expression of CD25+ was determined in lymphocyte, CD3 positive, population of activated (A) and non-activated (NA) hPBMCs. Data expressed as mean±SD; *P≤0.05, **P≤0.01.



SUPPLEMENTAL FIGURE 2. Stability *in vivo* determined by TCA precipitation assay in plasma samples of immunocompetent BALB/c mice collected 15, 60 and 90 min after tracer injection (n=6 per time point for each tracer). Data expressed as mean±SD.

Supplemental TABLE 1 *Ex vivo* biodistribution of three radiolabeled IL2 tracers. 15, 60 and 90 min post-injection in BALB/c mice.

Organs	^{18}F-FB-IL2 (%ID/g)			^{18}F-AlF-RESCA-IL2 (%ID/g)			^{68}Ga-Ga-NODAGA-IL2 (%ID/g)		
	15 min	60 min	90 min	15 min	60 min	90 min	15 min	60 min	90 min
Whole blood	7.7 ± 2.1	3.1 ± 2.0	2.3 ± 1.4	7.9 ± 3.7	1.8 ± 0.4	1.2 ± 0.3	3.9 ± 1.2	0.9 ± 0.3	0.8 ± 0.4
Plasma	10.0 ± 1.8	4.0 ± 2.6	2.9 ± 1.5	6.0 ± 2.2	2.1 ± 0.7	1.4 ± 0.3	6.7 ± 2.2	1.2 ± 0.5	1.3 ± 0.9
Heart	6.5 ± 2.5	3.1 ± 2.1	2.8 ± 2.2	6.8 ± 5.3	2.9 ± 1.9	3.1 ± 2.8	2.1 ± 0.7	0.8 ± 0.1	0.8 ± 0.3
Lung	13.7 ± 5.2	6.9 ± 4.8	8.8 ± 6.7	11.8 ± 9.5	8.0 ± 8.7	5.3 ± 5.8	5.6 ± 3.4	2.9 ± 1.9	2.7 ± 1.1
Salivary gland	1.5 ± 0.5	1.1 ± 0.7	0.9 ± 0.3	1.7 ± 0.8	0.9 ± 0.1	0.7 ± 0.2	1.8 ± 0.4	1.1 ± 0.3	1.1 ± 0.4
Thymus	2.1 ± 0.6	1.8 ± 1.6	1.4 ± 1.0	2.1 ± 0.7	1.6 ± 0.5	1.8 ± 1.6	2.0 ± 0.9	1.1 ± 0.4	1.3 ± 0.5
Liver	25.9 ± 8.2	9.6 ± 7.8	4.2 ± 2.9	34.8 ± 10.6	31.3 ± 6.3	25.9 ± 8.7	22.0 ± 3.8	22.9 ± 4.3	26.6 ± 4.7
Kidney	18.4 ± 5.9	18.9 ± 9.4	14.4 ± 4.3	41.0 ± 11.7	49.6 ± 22.2	39.1 ± 14.1	114.9 ± 21.0	162.8 ± 18.9	197.2 ± 25.6
Urine	33.7 ± 13.3	111.6 ± 62.3	432.0 ± 325.6	4.8 ± 3.1	38.7 ± 12.0	71.0 ± 20.9	16.7 ± 22.7	15.8 ± 9.4	20.1 ± 8.5
Bladder	5.9 ± 3.0	27.2 ± 23.9	126.9 ± 102.5	4.0 ± 3.6	10.3 ± 5.3	14.4 ± 6.4	7.8 ± 6.3	2.7 ± 1.2	2.2 ± 1.4
Stomach	1.2 ± 0.3	1.1 ± 0.7	1.2 ± 1.0	1.6± 0.3	1.1 ± 0.4	1.8 ± 1.5	1.5 ± 0.3	0.8 ± 0.2	0.8 ± 0.3
Pancreas	1.4 ± 0.5	1.4 ± 0.9	1.4 ± 1.5	1.3 ± 0.5	1.0 ± 0.9	0.8 ± 0.6	0.9 ± 0.2	0.5 ± 0.1	0.8 ± 0.5
Spleen	22.4 ± 11.8	13.9 ± 10.0	9.2 ± 6.3	41.0 ± 30.9	43.6 ± 26.7	33.6 ± 31.7	19.8 ± 3.2	19.8 ± 3.5	22.8 ± 4.4
Small intestine	1.8 ± 0.6	3.2 ± 2.4	1.7 ± 0.8	4.5 ± 1.5	4.3 ± 2.9	4.7 ± 4.3	1.6 ± 0.4	0.8 ± 0.1	0.9 ± 0.3
Colon	1.3 ± 0.4	1.1 ± 0.7	1.8 ± 2.9	1.4 ± 0.4	1.0 ± 0.5	0.9 ± 0.5	2.0 ± 0.5	1.1 ± 0.3	1.4 ± 0.7
Lymph node (axillary)	1.9 ± 1.6	3.0 ± 2.5	0.5 ± 0.4	4.6 ± 2.9	3.0 ± 2.3	6.6 ± 6.3	3.7 ± 4.0	2.4 ± 2.6	3.5 ± 3.1
Lymph node (mesenteric)	1.6 ± 0.4	1.8 ± 0.9	3.5 ± 5.0	2.2 ± 0.6	2.0 ± 1.1	1.9 ± 1.1	3.5 ± 1.2	3.3 ± 1.6	7.0 ± 7.7
Muscle	0.6 ± 0.1	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.8 ± 1.3	0.3 ± 0.1	0.5 ± 0.1	0.2 ± 0.1	0.3 ± 0.3
Skin	0.7 ± 0.1	1.0 ± 0.7	2.6 ± 2.3	1.0 ± 0.5	0.7 ± 0.4	1.5 ± 1.9	1.0 ± 0.3	0.9 ± 0.2	0.8 ± 0.3
Brain	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.2
Bone	2.1 ± 1.0	0.7 ± 0.4	0.7 ± 0.2	3.3 ± 1.1	3.9 ± 1.2	4.9 ± 1.4	1.1 ± 0.3	0.7 ± 0.3	0.9 ± 0.5
Bone marrow	38.7 ± 19.7	8.8 ± 8.6	14.3 ± 24.8	26.8 ± 16.5	26.9± 12.4	19.2 ± 7.3	11.7 ± 1.3	9.3 ± 2.7	12.3 ± 3.7

Supplemental TABLE 2 *Ex vivo* biodistribution of three radiolabeled IL2 tracers in immunodeficient SCID mice, inoculated with human activated PBMCs, 15 min before tracer injection. After tracer injection a 60 min dynamic PET scan was made, followed by *ex vivo* biodistribution studies. Activity in each organ was measured and the percentage of the injected dose (%ID/g) was calculated.

Organs	^{18}F-FB-IL2 (%ID/g)			^{18}F-AlF-RESCA-IL2 (%ID/g)			^{68}Ga-Ga-NODAGA-IL2 (%ID/g)		
	PBMC	PBMC block	Matrigel	PBMC	PBMC block	Matrigel	PBMC	PBMC block	Matrigel
Whole blood	6.4 ± 2.9	3.8 ± 2,6	3.6 ± 1.4	1.2 ± 0.6	1.1 ± 0.8	1.5 ± 0.9	2.3 ± 0.6	2.0 ± 0.7	2.6 ± 1.8
Plasma	7.8 ± 2.8	3.2 ± 2,0	3.7 ± 0.7	1.7 ± 1.1	0.6 ± 0.9	1.4 ± 0.5	3.2 ± 0.9	2.9 ± 1.4	4.5 ± 1.9
Heart	8.9 ± 4.7	8.2 ± 6,4	6.6 ± 6.4	0.9 ± 0.3	8.7 ± 18.7	0.9 ± 0.3	1.2 ± 0.3	1.3 ± 0.6	1.3 ± 0.4
Lung	13.6 ± 5.5	23.0 ± 19.9	8.3 ± 6.8	2.8 ± 1.0	5.7 ± 3.2	3.2 ± 1.9	2.8 ± 1.3	4.9 ± 4.8	2.8 ± 1.4
Salivary gland	2.1 ± 0.7	1.5 ± 1.0	1.4 ± 0.6	0.6 ± 0.3	0.6 ± 0.2	0.6 ± 0.3	1.1 ± 0.2	1.2 ± 0.6	1.1 ± 0.2
Thymus	5.9 ± 3.6	4.2 ± 1.6	2.9 ± 1.1	1.9 ± 1.3	2.4 ± 1.7	2.0 ± 1.7	1.7 ± 0.8	1.7 ± 0.6	1.2 ± 0.9
Liver	23.1 ± 14.2	16.4 ± 12.5	14.3 ± 7.3	32.9 ± 11.7	39.0 ± 16.9	37.7 ± 17.3	30.5 ± 16.2	29.0 ± 13.5	36.5 ± 13.1
Kidney	29.9 ± 9.2	15.9 ± 14.1	17.7 ± 4.6	42.7± 42.9	32.2 ± 26.6	42.6 ± 40.4	40.2 ± 44.9	38.9 ± 25.0	52.2 ± 13.7
Urine	499.3 ± 383.7	253.7 ± 122.7	231.0 ± 160.7	16.8 ± 18.0	17.4 ± 9.3	24.1 ± 26.4	7.5 ± 10.1	17.1 ± 17.4	11.6 ± 4.4
Bladder	39.7 ± 22.9	52.1 ± 21.9	134.1 ± 136.5	7.0 ± 5.5	3.9 ± 3.9	8.9 ± 8.3	3.0 ± 3.3	3.2 ± 1.5	3.6 ± 2.6
Stomach	2.3 ± 1.1	1.4 ± 0.5	1.1 ± 0.4	0.6 ± 0.2	0.8 ± 0.3	0.6 ± 0.2	0.8 ± 0.2	0.7 ± 0.4	6.8 ± 10.2
Pancreas	2.2 ± 0.7	2.0 ± 0.5	1.4 ± 0.5	0.6 ± 0.2	0.5 ± 0.3	0.5 ± 0.2	0.8 ± 0.3	0.6 ± 0.2	1.0 ± 0.5
Spleen	18.6 ± 13.4	20.9 ± 15.5	10.5 ± 5.9	12.3 ± 7.0	14.3 ± 6.6	15.2 ± 7.5	17.0 ± 12.0	18.8 ± 8.1	11.4 ± 0.4
Small intestine	4.7 ± 0.9	2.5 ± 1.2	3.3 ± 1.6	2.1 ± 1.2	2.1 ± 1.1	2.8 ± 1.5	1.1 ± 0.2	0.7 ± 0.3	1.6 ± 0.7
Colon	2.2 ± 0.9	1.9 ± 1.0	1.3 ± 0.3	0.9 ± 0.4	0.7 ± 0.3	1.0 ± 0.6	1.1 ± 0.2	0.6 ± 0.1	1.2 ± 0.6
Lymph node (axillary)	4.4 ± 4.4	1.6 ± 0.9	1.9 ± 1.9	6.6 ± 11.3	1.0 ± 1.1	1.0 ± 0.9	1.9 ± 1.6	4.4 ± 5.7	1.2 ± 1.3
Lymph node (mesenteric)	2.6 ± 1.1	2.8 ± 2.0	2.9 ± 1.4	3.2 ± 5.9	0.7 ± 0.4	1.3 ± 0.7	1.1 ± 0.4	0.8 ± 0.6	0.8 ± 0.3
Muscle	0.9 ± 0.3	0.6 ± 0.2	0.5 ± 0.1	0.4 ± 0.2	0.3 ± 0.2	0.3 ± 0.1	0.5 ± 0.3	0.4 ± 0.1	0.4 ± 0.2
Skin	2.4 ± 1.6	1.0 ± 0.4	0.9 ± 0.7	0.6 ± 0.2	0.6 ± 0.3	0.6 ± 0.3	1.0 ± 0.4	0.7 ± 0.2	1.3 ± 0.4
Brain	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0
Brown adipose tissue	1.9 ± 0.8	1.1 ± 0.6	0.8 ± 0.3	0.5 ± 0.3	0.9 ± 0.7	0.3 ± 0.1	0.5 ± 0.2	0.6 ± 0.3	0.7 ± 0.3

Bone	1.7 ± 0.7	1.1 ± 0.5	0.7 ± 0.2	4.3 ± 2.6	3.2 ± 1.2	3.9 ± 2.6	1.7 ± 1.0	1.0 ± 0.6	1.9 ± 0.8
Bone marrow	15.7 ± 5.9	11.2 ± 6.4	10.3 ± 5.7	17.2 ± 11.3	17.6 ± 16.5	12.1 ± 9.8	7.7 ± 4.8	14.3 ± 11.9	9.8 ± 6.7
PBMCs	2.0 ± 0.5	1.2 ± 0.4	0.9 ± 0.3	2.0 ± 1.4	0.7 ± 0.3	0.5 ± 0.4	1.1 ± 0.5	0.8 ± 0.4	0.5 ± 0.2