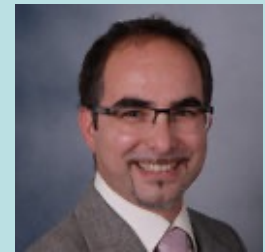# Development of imaging approaches to monitor tumor-reactive T cells in vivo

Angela Krackhardt and Calogero D'Alessandria

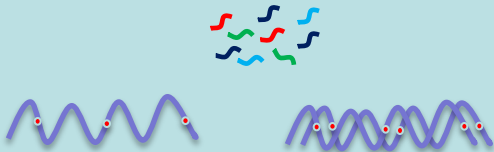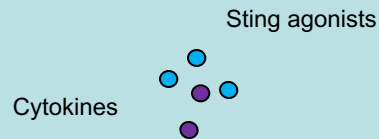Dr. Sabine Mall    Dr. Nahid Yusufi    Kristine Mayer    Dario Gosmann    Lisa Russelli    Theresa Käsbauer    Sandro Bissenberger
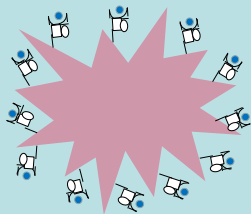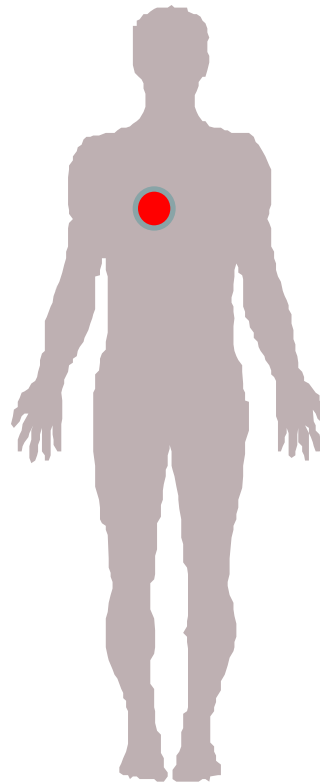
# Cancer Immunotherapy

### Therapeutic vaccination

### Immune checkpoint modulation

PD-1
TIM3
CD4
CD8
LAG-3
CTLA-4

## Patient with cancer

### Enhancing innate immune signaling

Sting agonists

Cytokines

### CAR/TCR-transgenic T cells

T cell

TCR

T cell

CAR

### Dendritic cell vaccination
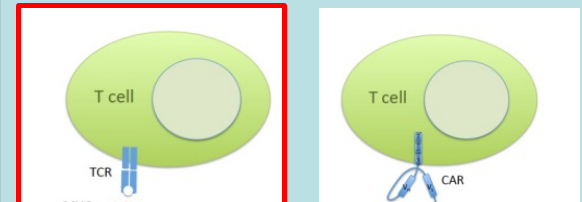
### Adoptive transfer of non-modifiedT/NK cells
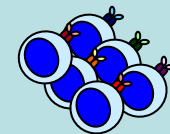
# Unique characteristics of Immunotherapy
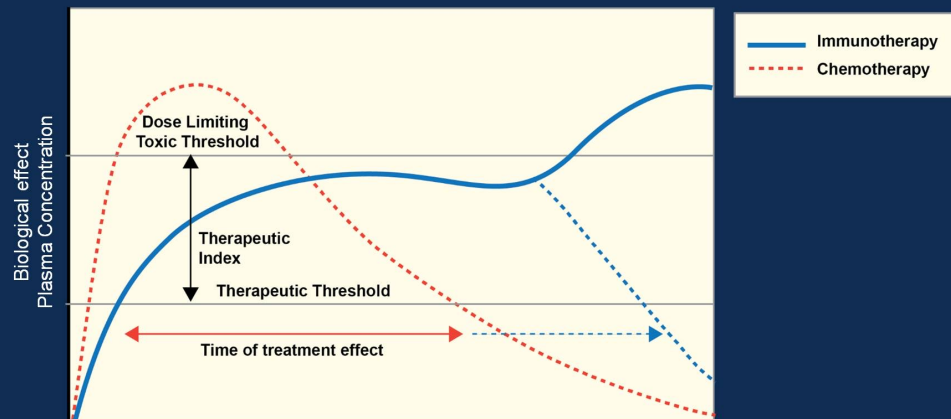


*G. Pennock, et.al, American Journal of Clinical Oncology. 2012*
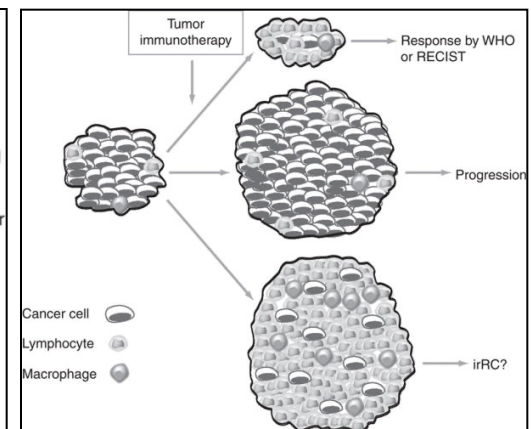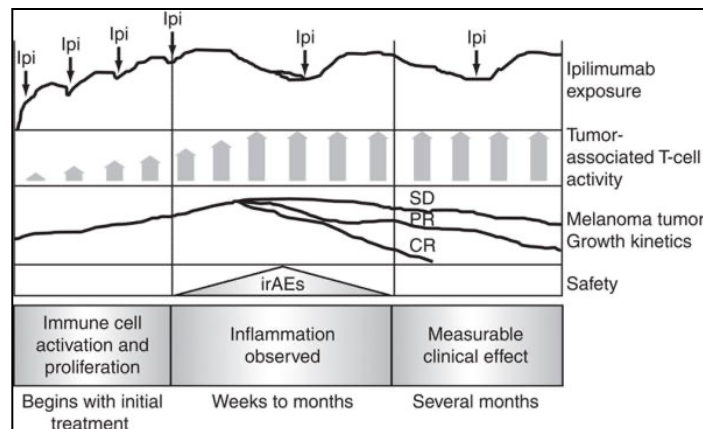
# Research project C10

Development of imaging techniques to monitor tumor reactive

T cells in vivo based on antibody-derived constructs

Tracer development

Metabolic imaging of T cell activity in tumors

BLI imaging of T cells

# Adoptive T-cell transfer of TCR-transgenic T cells



TCR Transfer

Isolation of T cells

PET-CT/MRT monitoring of
T-cell therapy efficiency

Expansion

Transfusion

# Principle of Immuno-Imaging of TCR-transgenic T cells
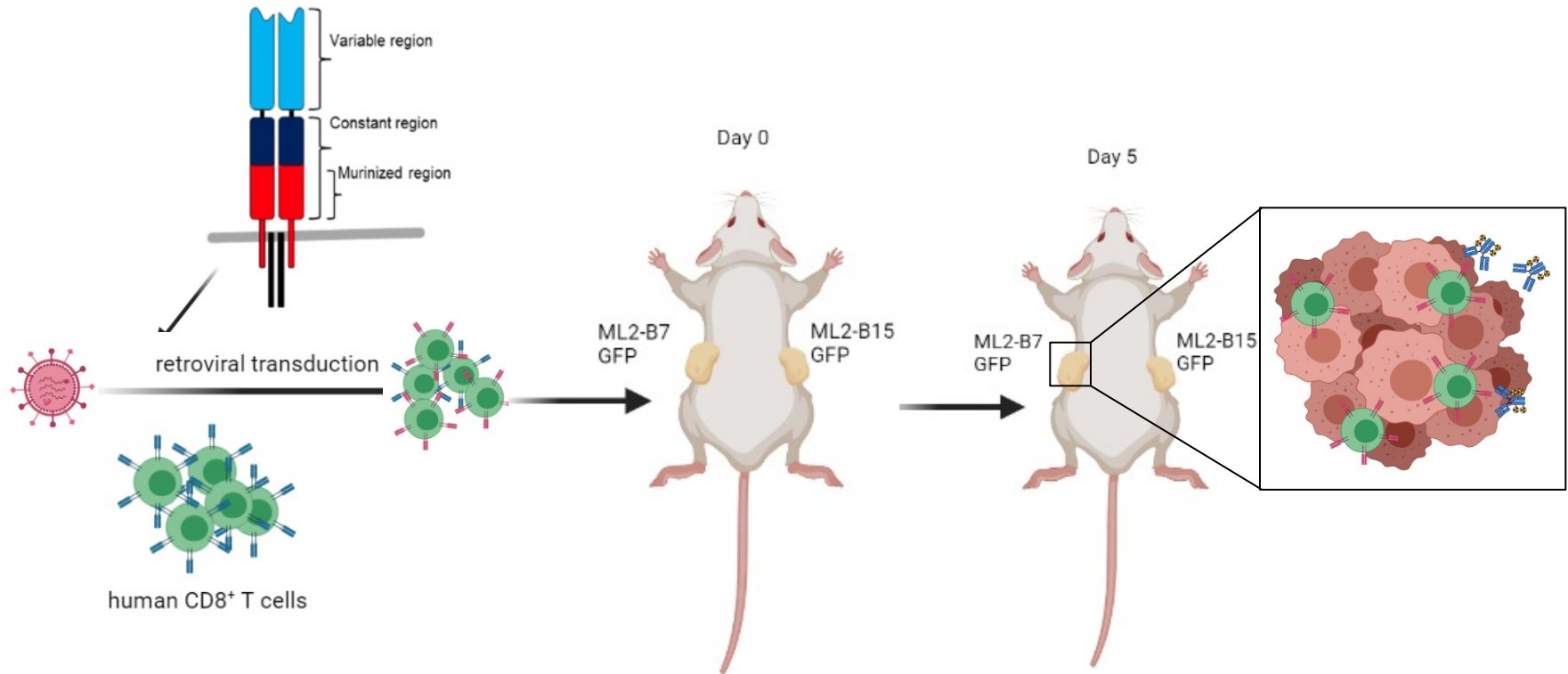
# In vivo imaging of T cells by $^{89}$Zr-aTCRmu-F(ab`)$_2$ by Immuno-PET



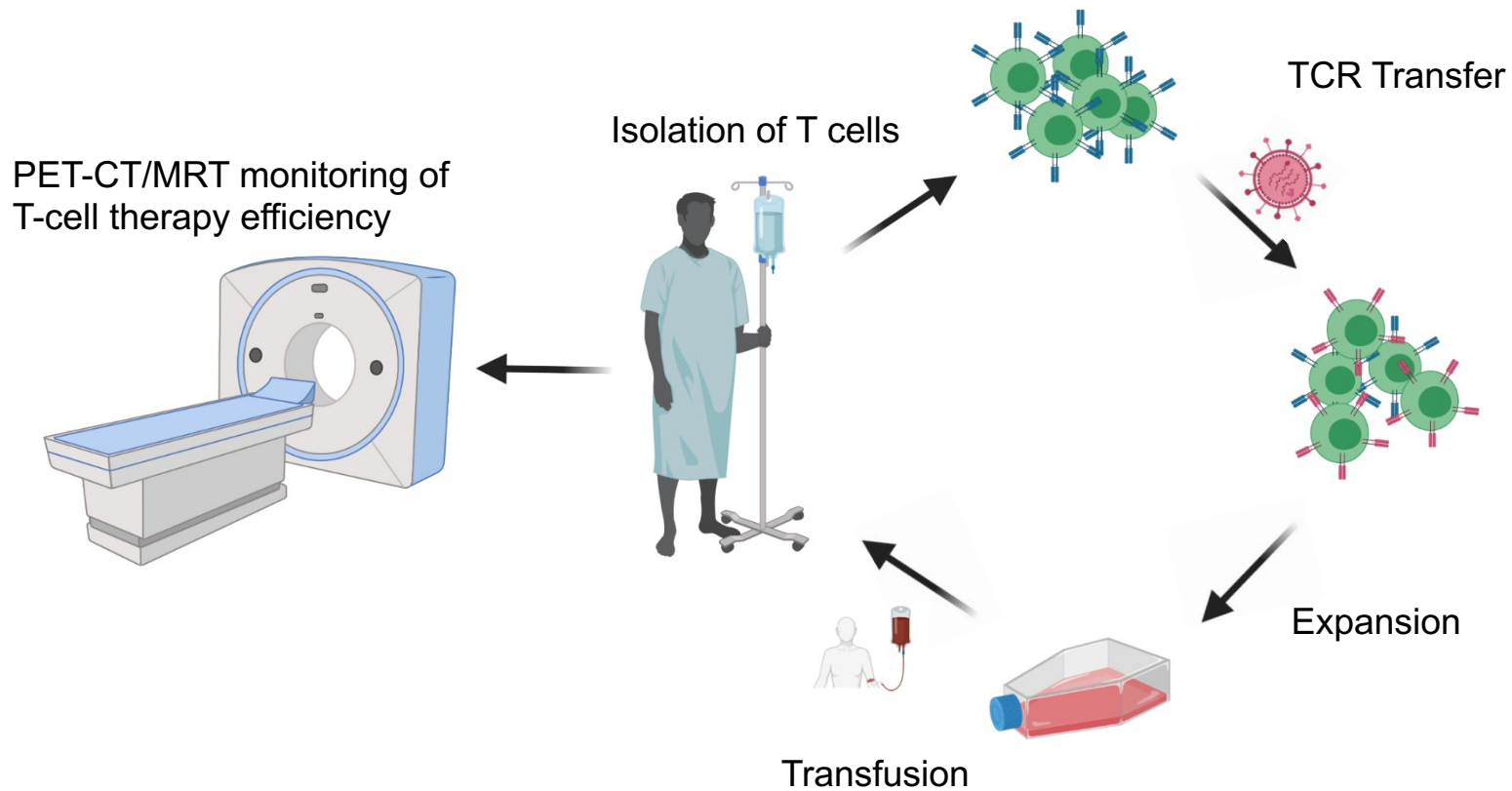Tumor cells s.c. 1x10$^7$ — Total body irradiation — TCR2.5D6iRFP Tcm i.v. 2x107 — $^{98}$Zr-F(ab´)$_2$ i.v. injection — PET/CT Imaging

Day -8 — Day -1 — Day 0 — Day 3 — Day 5

$^{89}$Zr-aTCRmu-F(ab`)$_2$ – 48h p.i.

TCR2.5D6 — Non-transduced — PBS

Mall et al. Cancer Research 2016
Yusufi et al. Theranostics 2017
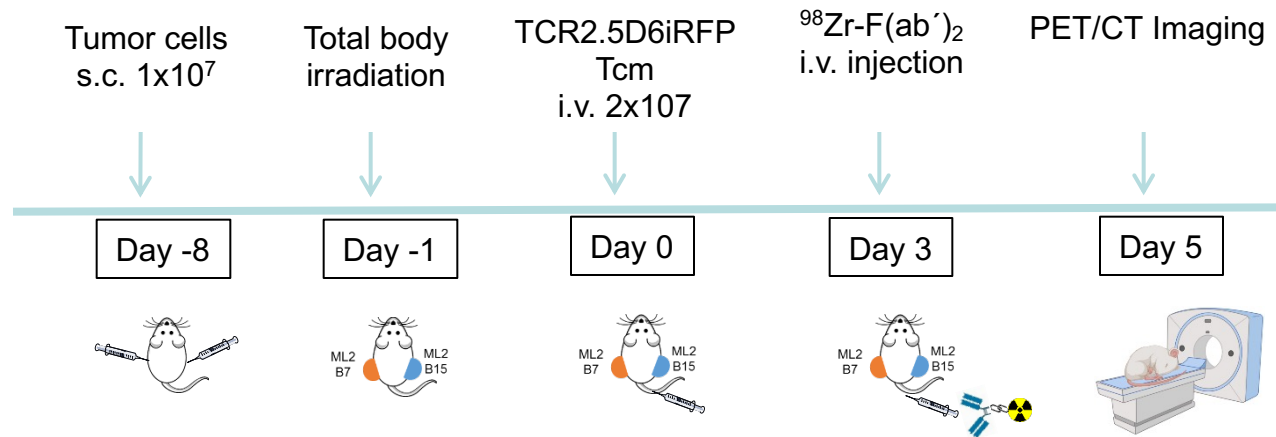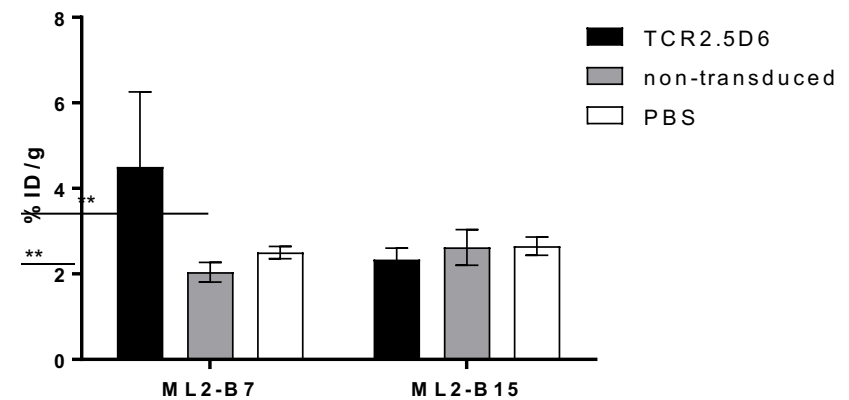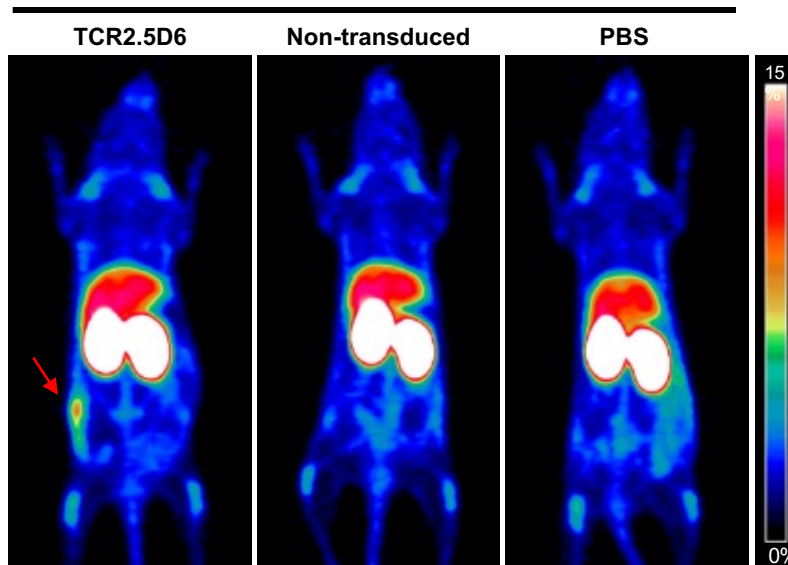
# Identification of heterogeneity of T-cell infiltration by Immuno-PET



Mall et al. Cancer Research 2016
Yusufi et al. Theranostics 2017

# Cancer Immunotherapy

# General T-Cell monitoring by Immuno-PET

In vitro

In vivo

**Target selection**

**Probe selection**

**Nuclide selection**

**Specific binding to target**

**High affinity binding to target**

**Impact on functionality after binding to T cells**

Apoptosis

Proliferation

Cytokine production

$Ca^{2+}$ influx

Cytotoxic activity against target cells

**Biodistribution**

**Secretion**

**Impact on functionality after binding to T cells**

**Clinical translation**

Bailey et al. Mol Imaging Biol 2017; doi: 10.1007/s11307-017-1123-5

# Selection of suitable surface markers
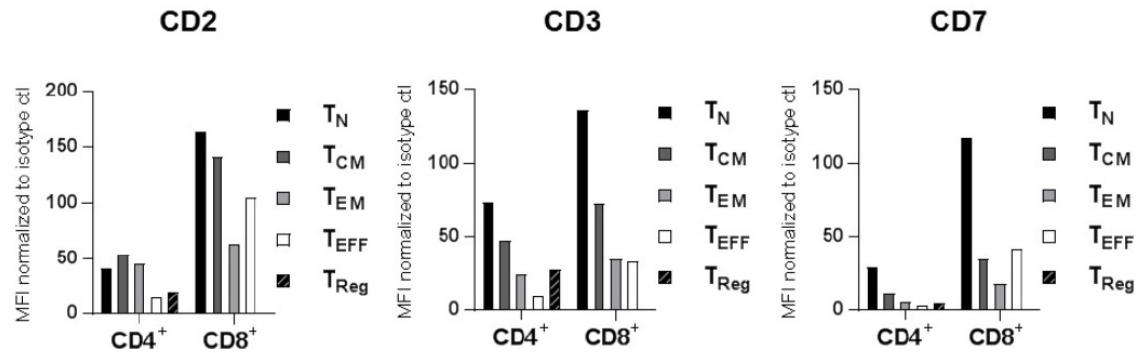


Expression on specifically activated T-cells



Mayer & Mall et al.  Theranostics 2018
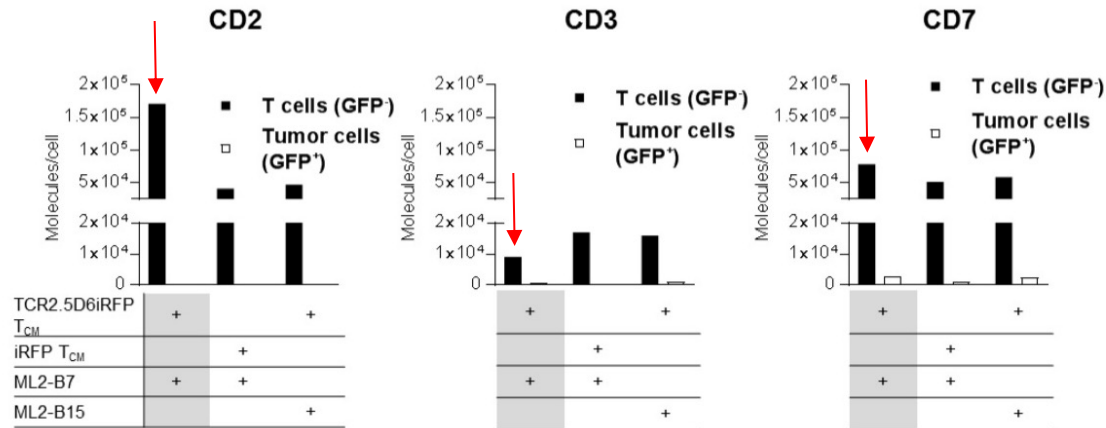
**Target selection**

# Impact of selected antibody-derived constructs on T cell function in vitro
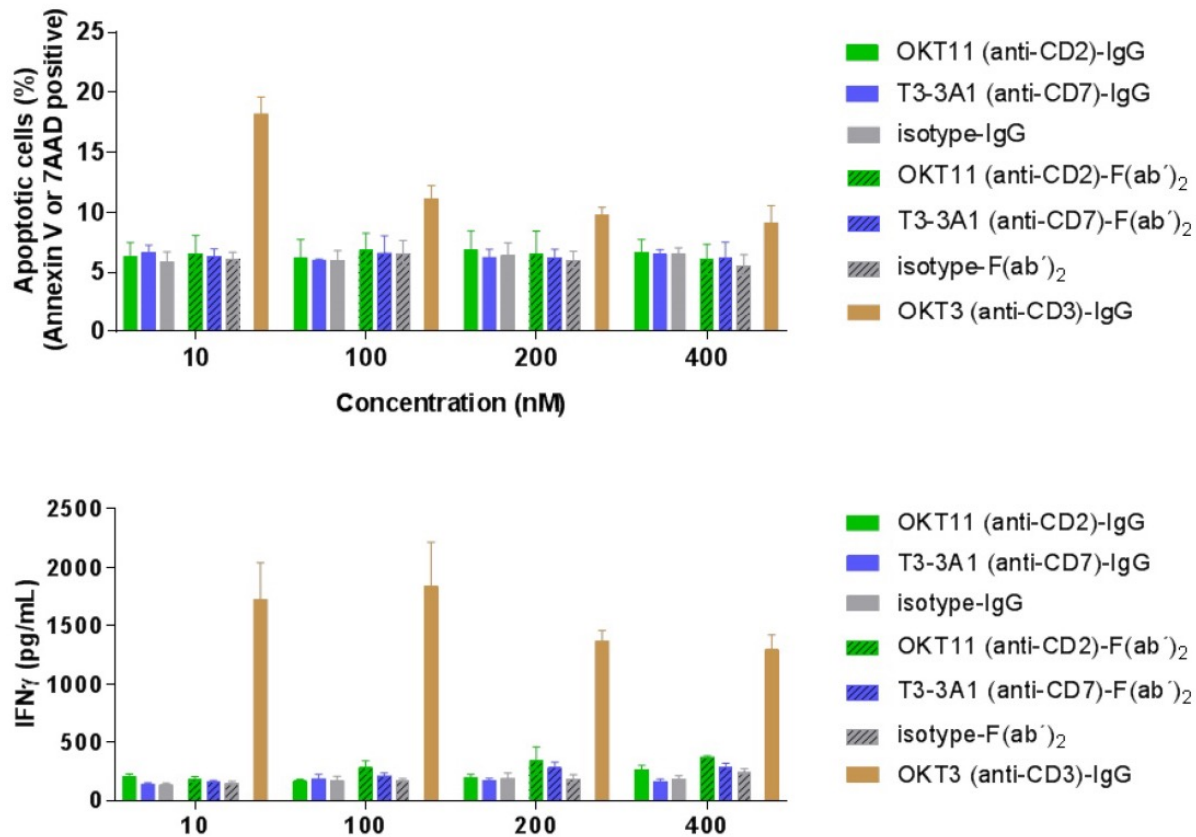
**Target selection**

# Impact of selected antibody-derived constructs on T cell function in vitro and in vivo



Mayer & Mall et al. Theranostics 2018

Probe selection

Nuclide selection
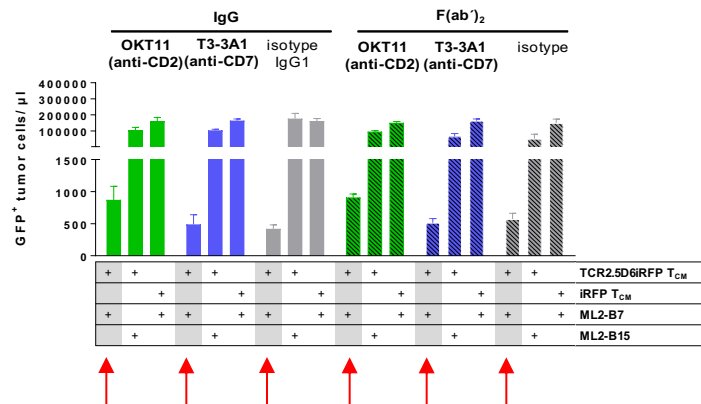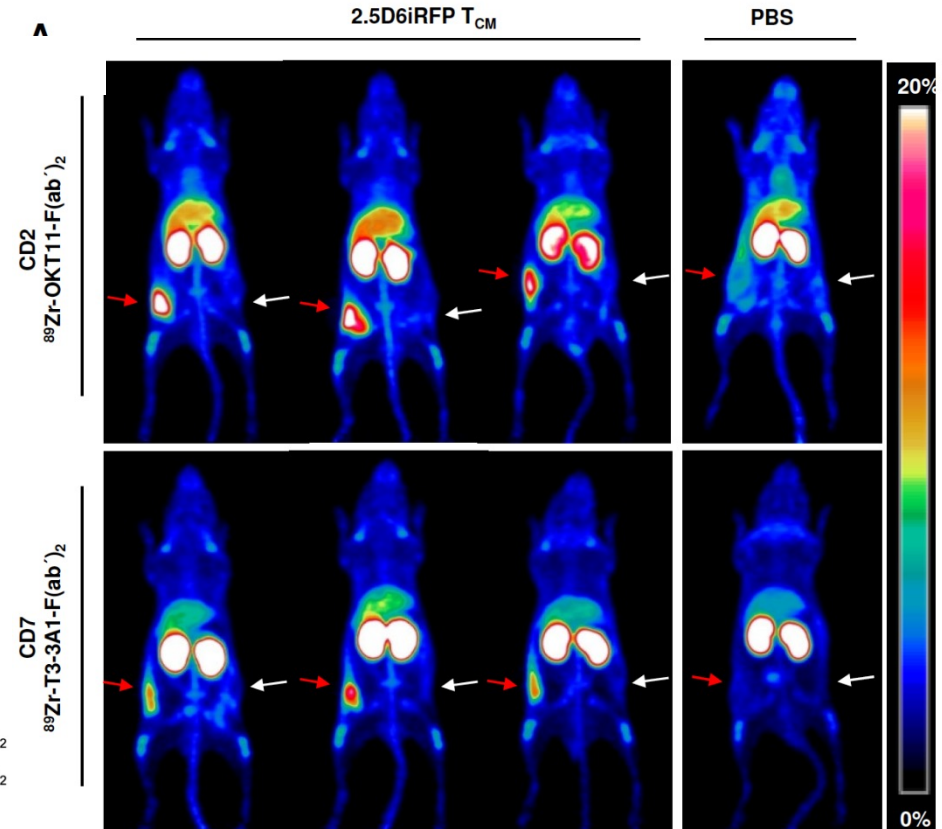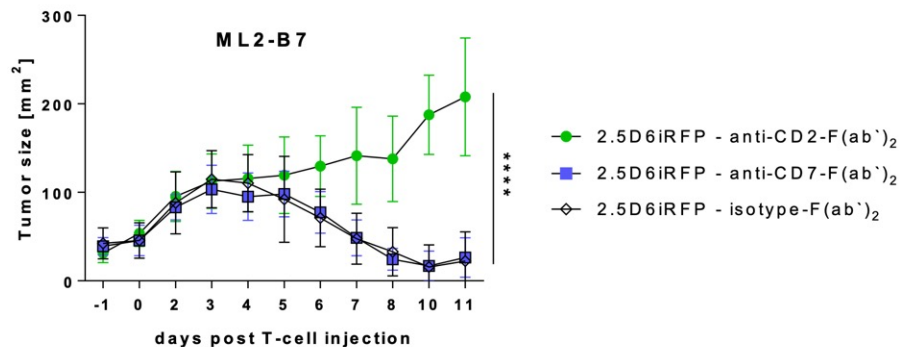
# Development of novel tracers: Al$^{18}$F method

## Radioisotope of choice → F-18

- Maximum positron energy of 0.635 MeV → *high resolution PET images*
- Half-life of 109.8 min → avoid prolonged irradiation of subjects but allow *in vivo* investigations
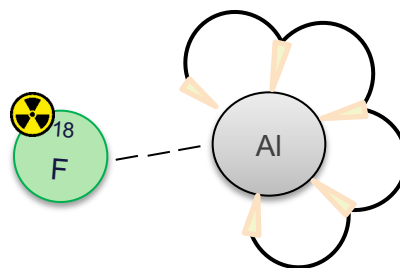
## Radiofluorination method → AlF-18

- **Al** forms octahedral complexes → *pentadentate* ligand is desired with *negatively charged oxygen donor groups*, leaving only one binding site open for the F-18 ion
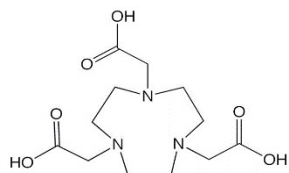
**NOTA**

- Radiolabeling T ∼ 100°C ⚠
- RCY ≥ 75%
- Serum stability (after 4h) → 87%
- High *in vivo* stability

AlCl$_3$ 2 mM in NaAcO

5 min, rt

12 min, 100/120°C

$^{18}$F

200-500MBq
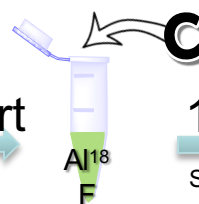
Al$^{18}$F

Sep-Pak Alumina N Plus Light cartridges

Al$^{18}$F

W. J. McBride *et al.*, *J. Nucl. Med.* **2009**, *50*, 991–998.

1
5

# AMP-based chelator Al$^{18}$Fmethod



3 step synthesis

4 step synthesis

2 step synthesis
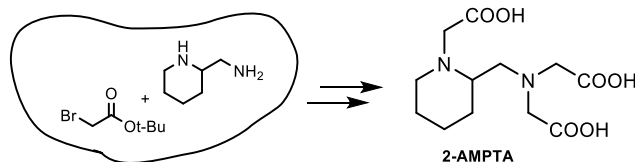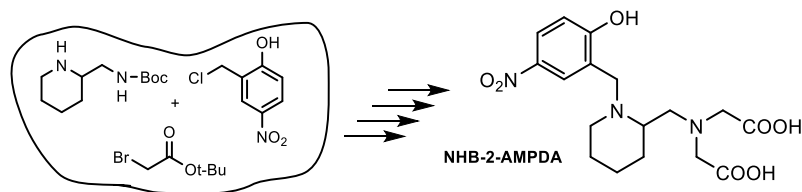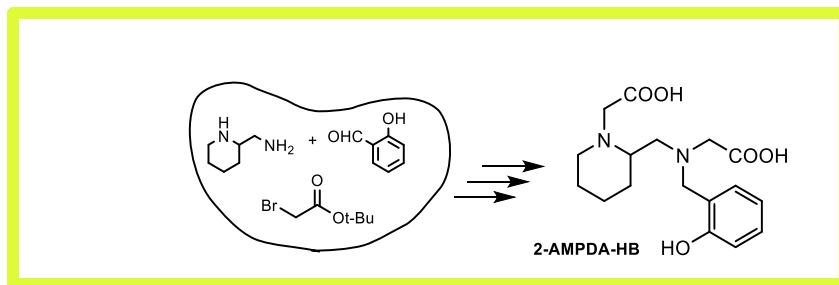
- Radiochemical yield of **55%** at pH 5 and **room temperature**.
- **High stability in Human Serum** was measured for [Al$^{18}$F(2-AMPDA-HB)]$^-$, with **90±4%** of F-18 complexed after 2h and **87±5%** after 4h.

Russelli L,….. D'Alessandria C. *ChemMedChem* 2020, 15, 1–10, https://doi.org/10.1002/cmdc.201900652

Probe selection

Nuclide selection

# In vivo PET/CT dynamic scan



ID: 2.3 MBq ± 0.2 in
0.9% NaCl

Biodistribution data of [Al$^{18}$F-(2-AMPDA-HB)]$^-$ on female healthy nude mice

Mice sacrificed 2h p.i.

Russelli L,…... D'Alessandria C. *ChemMedChem* 2020
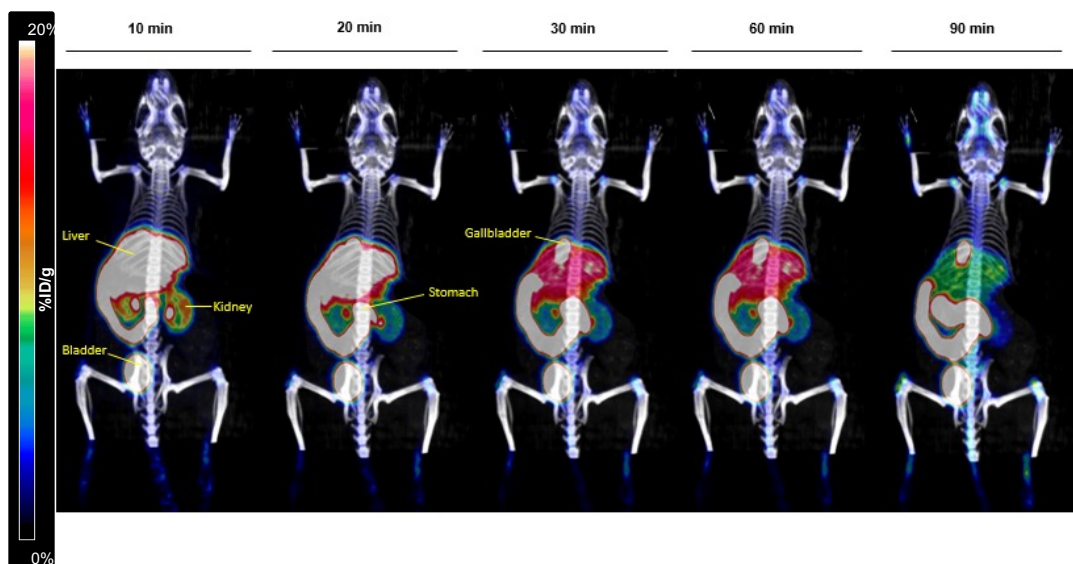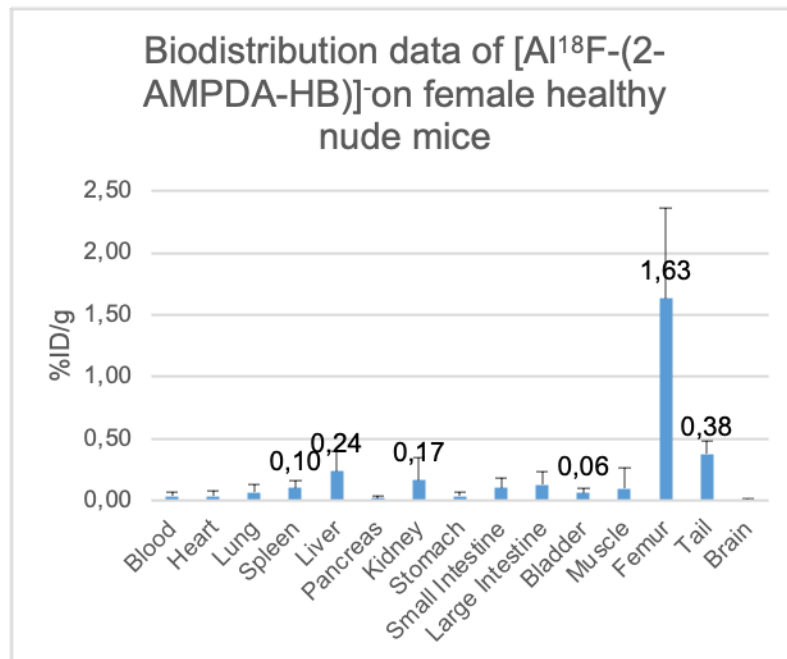
Probe
selection

Nuclide
selection

# Functionalised 2-AMPDA-HB



**1** Synthesis

**2** Conjugation
Lys
pH 8.7

**3** Labeling
Al$^{18}$F
rt, 12 min

Low RCY

**2-AMPDA-HB**

**2-AMPDA-HB-NCS**
MW=393.46

**2-AMPDA-HB-Lys**
MW: 539.65

*Hp:* Thiourea interfere with complexation

**2-AMPDA-HB-PEG4-mal**
MW: 749
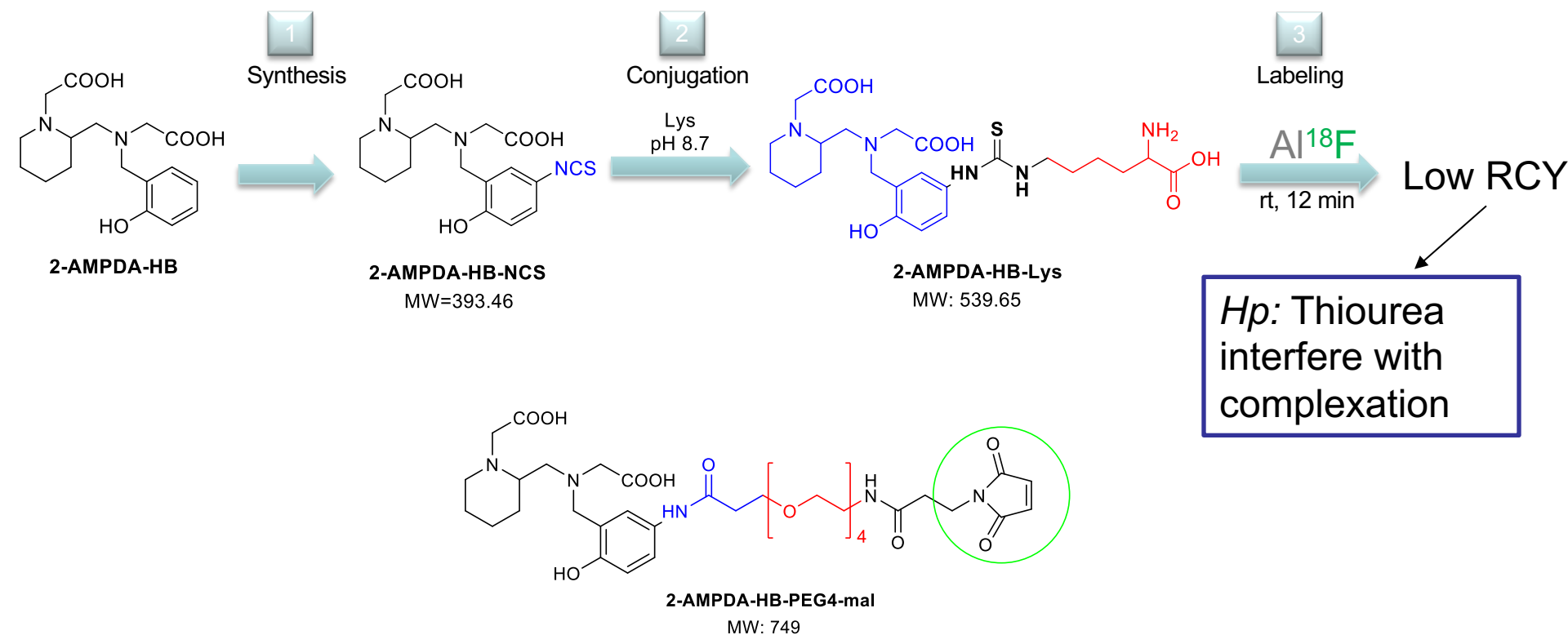
Probe
selection

Nuclide
selection

# Conjugation and labeling with $^{68}$Ga-NOTA



$^{68}$Ga

**C** =

**p-SCN-Bn-NOTA**

·3HCl

linker

HO

NCS

**1**
Conjugation

**2**
Labeling

**C** +

antiCD2-scAb
or
antiCD7-scAb
Buffer carbonate
pH 8.7

2h, rt

PD-10 purification
NaOAc 0.1M

$^{68}$Ga
in HCl

+

NOTA-antiCD2-scAb
or
NOTA-antiCD7-scAb

10 min, rt

PD-10 purification
NaCl 0.9% + ascorbic acid

$^{68}$Ga

NOTA-antiCD2-scAb
or
NOTA-antiCD2-scAb

Xavier et al., *Mol Imaging Biol* 2019, 21, 898-906, DOI: 10.1007/s11307-018-01302-5
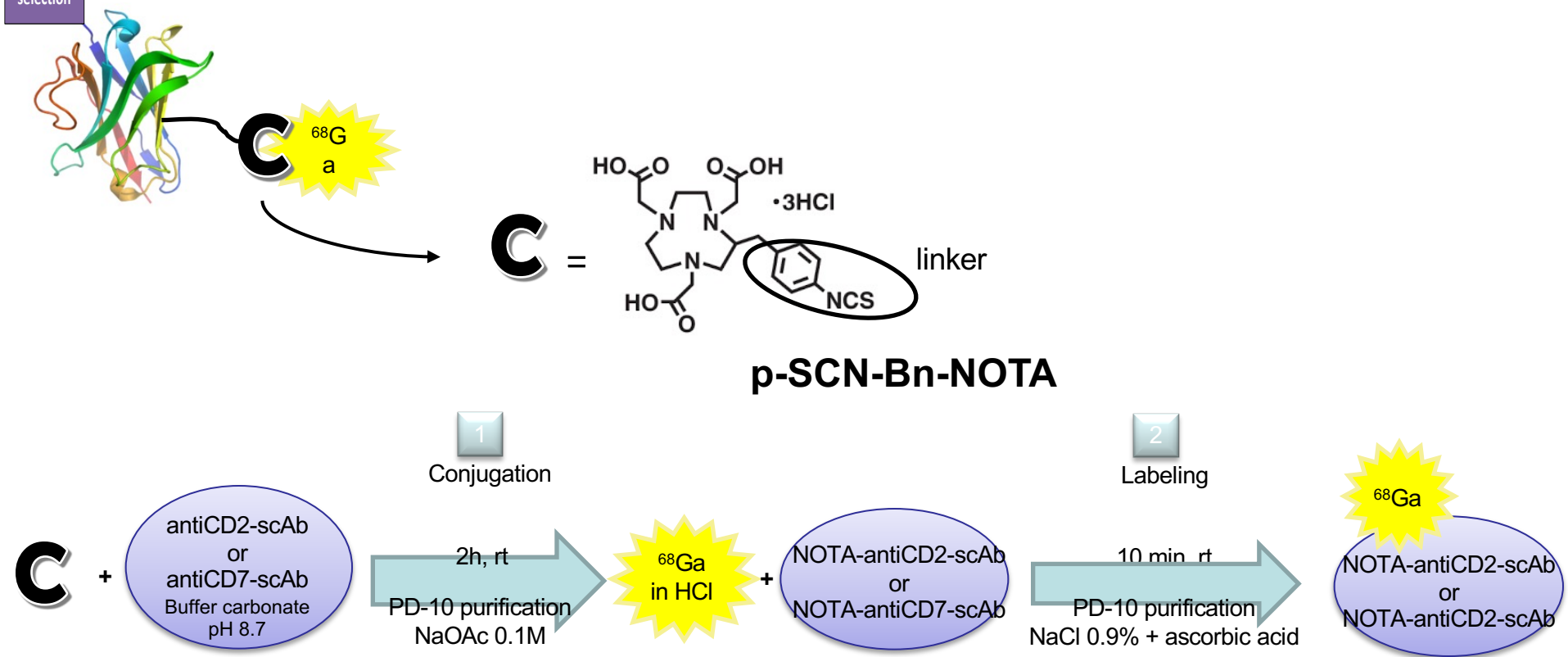
Probe selection

Nuclide selection

# CyAAZTA publication Tei/D`Alessandria

## "*A rigidified AAZTA-like ligand as efficient chelator for $^{68}$Ga radiopharmaceuticals*"
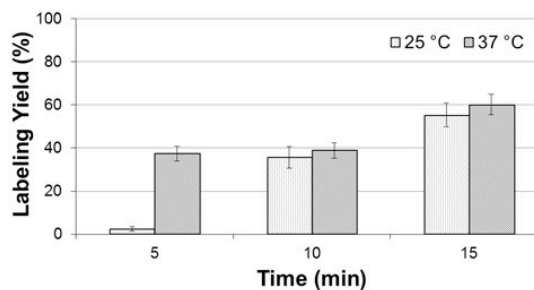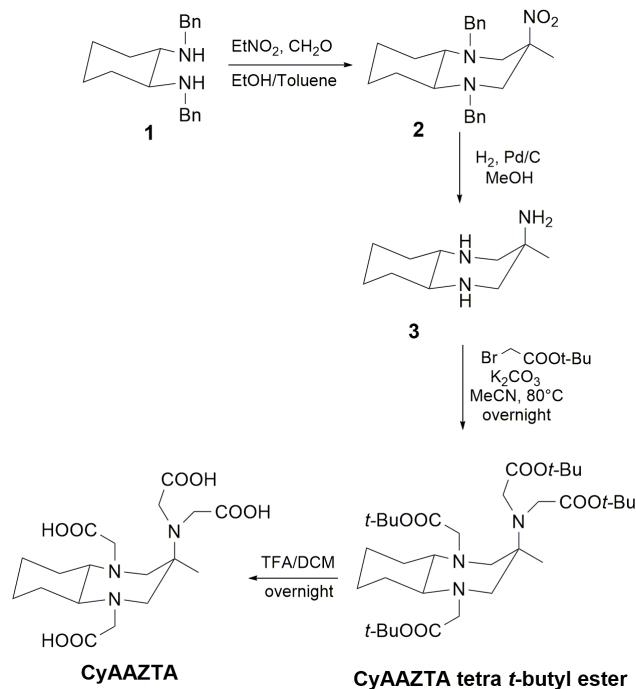


RCY at rt, 15min → 80.7%
RCY at 90°C, 5min → 94.7%

$^{68}$GaCyAAZTA is stable in HS and DTPA over 90 minutes (HPLC data)

Vágner A, D'Alessandria C et al., *ChemistrySelect* 2016

# CyAAZTA functionalized synthesis



CyAAZTA-mal
MW: 623.66
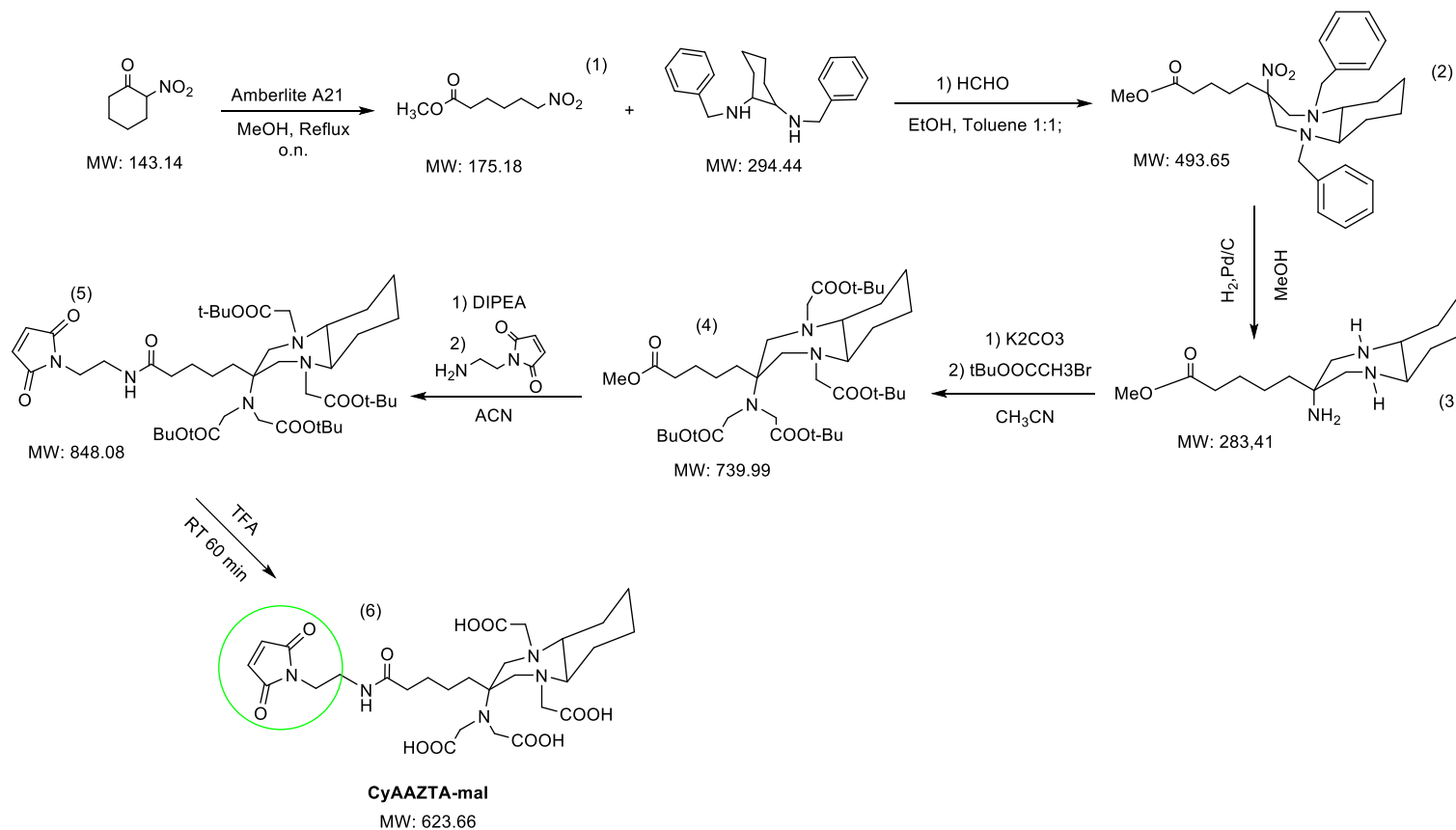
# Metabolic pH imaging

## Imaging of pH in vivo using hyperpolarized $^{13}$C-labelled cymonic acid

Franz Schilling

Martin Grashei



24h post Fab-Inj.     48h post Fab-Inj.     72h post Fab-Inj.

pH-map (MRT)

$^{89}$Zr-F(ab')$_2$-PET

# Multispectral epi-illumination cryoslicing fluorescence imaging



Vasili
Ntziachristos

Dimitri
Gkorpas

3D reconstruction of ML2-B7 tumors

# Publications

Robu S, Richter A, Gosmann D, Seidl C, Leung D, Hayes W, Cohen D, Morin P, Donnelly DJ, Lipovšek D, Bonacorsi SJ, Smith A, Steiger K, Aulehner C, **Krackhardt AM**, Weber WA: Synthesis and Preclinical evaluation of 68Ga-labeled Adnectin, 68Ga-BMS-986192 as a PET agent for Imaging PD-L1 expression. J Nucl Med 2021, doi: 10.2967/jnumed.120.258384

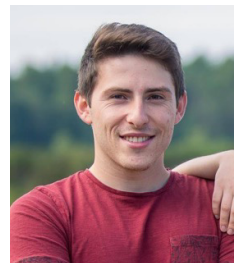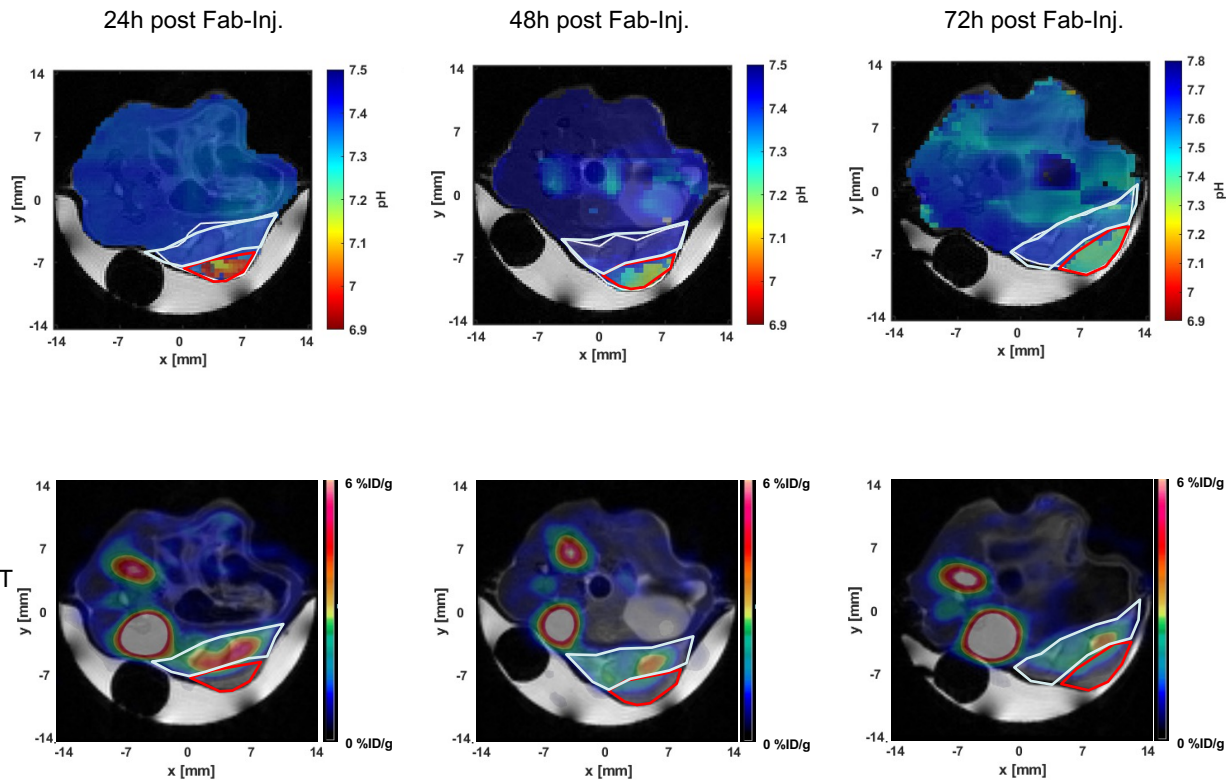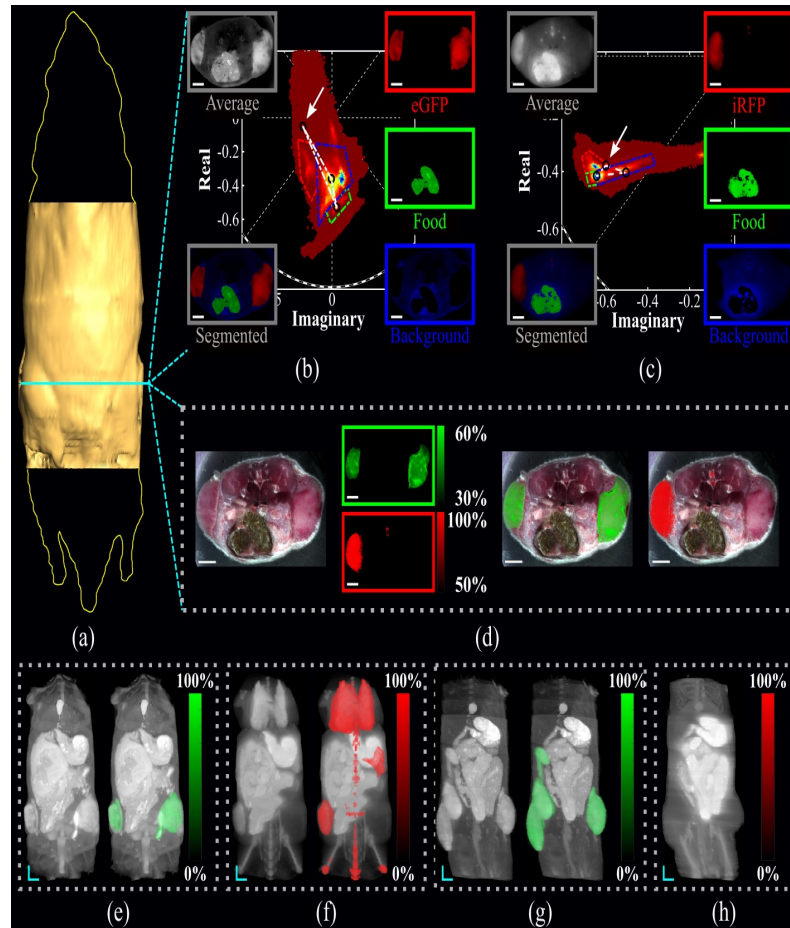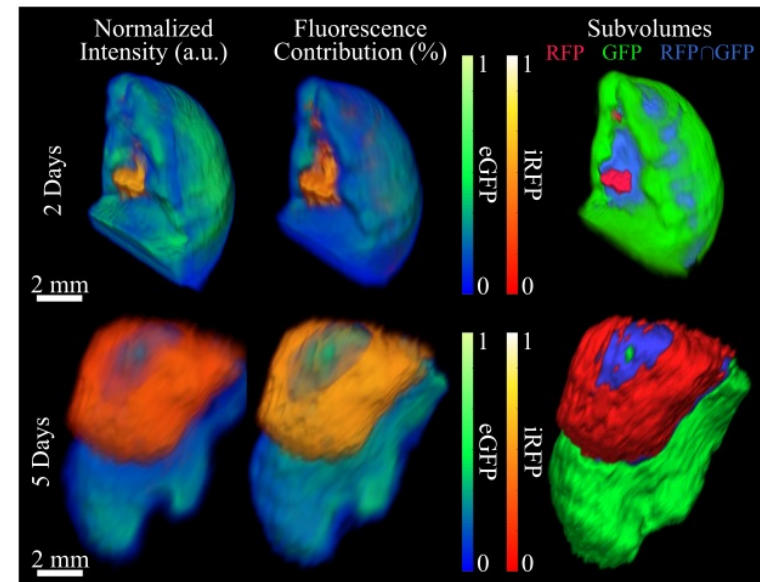Russelli L, Martinelli J, De Rose F, Reder S, Herz M, Schwaiger M, Weber W, Tei L, **D'Alessandria C**. Room Temperature Al18F Labeling of 2-Aminomethylpiperidine-Based Chelators for PET Imaging. ChemMedChem. 2020 Feb 5;15(3):284-292.

Audehm S, Glaser M, Pecoraro M, Bräunlein E, Mall S, Klar R, Effenberger M, Albers J, Bianchi HO, Peper J, Yusufi N, Busch DH, Stevanovic S, Mann M, Antes I, **Krackhardt AM**: Key features relevant to select antigens and TCR from the MHC mismatched repertoire to treat cancer; Front Immunol 2019; 10: 1485; doi: 10.3389/fimmu.2019.01485

Albers J, Ammon T, Gosmann D, Audehm A, Thoene S, Winter C, Secci R, Wolf A, Stelzl A, Steiger K, Ruland J, Bassermann F, Kupatt C, Anton M, and **Krackhardt AM**: Gene editing enables T cell engineering to redirect antigen specificity for potent tumor rejection; Life Science Alliance 2019, doi: 10.26508/lsa.201900367.

Mayer KE, Mall S, Yusufi N, Gosmann D, Steiger K, Russelli L, Bianchi H, Audehm S, Wagner R, Bräunlein E, Stelzl A, Bassermann F, Weichert W, Weber W, Schwaiger M, D`Alessandria C, **Krackhardt AM**: T-cell functionality testing is highly relevant to developing novel immuno-tracers monitoring T cells in the context of immunotherapies and revealed CD7 as an attractive target; Theranostics 2018; 8: 6070-6087.

Varasteh Z, De Rose F, Mohanta S, Li Y, Zhang X, Miritsch B, Scafetta G, Yin C, Sager HB, Glasl S, Gorpas D, Habenicht AJR, Ntziachristos V, Weber WA, Bartolazzi A, Schwaiger M, **D'Alessandria C**. Imaging atherosclerotic plaques by targeting Galectin-3 and activated macrophages using (89Zr)-DFO-Galectin3-F(ab')2 mAb. Theranostics. 2021 Jan 1;11(4):1864-1876.

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Hannes Rolbieski
Michael Herz

## Biomedical Magnetresonance
Franz Schilling
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