# Imaging Drug Delivery
## What can we learn from clinical experiences?

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NEXT-GENERATION DELIVERY INNOVATIONS

# Disclosures

I have **the following financial interest** or **relationship(s)** to disclose regarding the subject matter of this presentation:

Grant/research support/clinical trials: Crystal Therapeutics; CytomX; Boehringer Ingelheim; GSK; AstraZeneca

# Molecular Imaging with positron emission tomography (PET)

Quantitative, whole body, non-invasive technique to study biodistribution of radioactive labelled compounds

Highly informative during early drug development

- Target-mediated uptake of biologicals (simple mAb to modified/bispecifics etc)
- Biodistribution / delivery of non-targeted (nano) compounds

Considerations regarding interpretation of (quantitative) results!

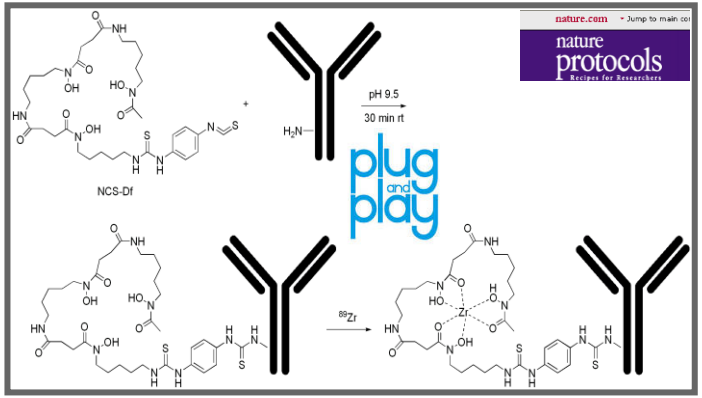
# Generic technology for radiolabeling of biologicals - Infrastructure from bench to bedside for clinical trials


Ronald Boellaard


Danielle Vugts


Guus van Dongen

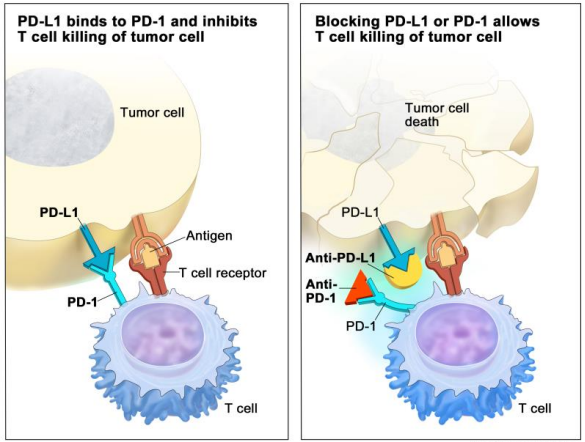
- Zirconium-89, half life 72h
- SOPs/GMP production
- Training/Automation
- IMAGING CENTRE Amsterdam/LAVOF PET
- Dedicated team for image / quantification analyses (Ronald Boelaard)
- Clinical trial unit with dedicated PIs

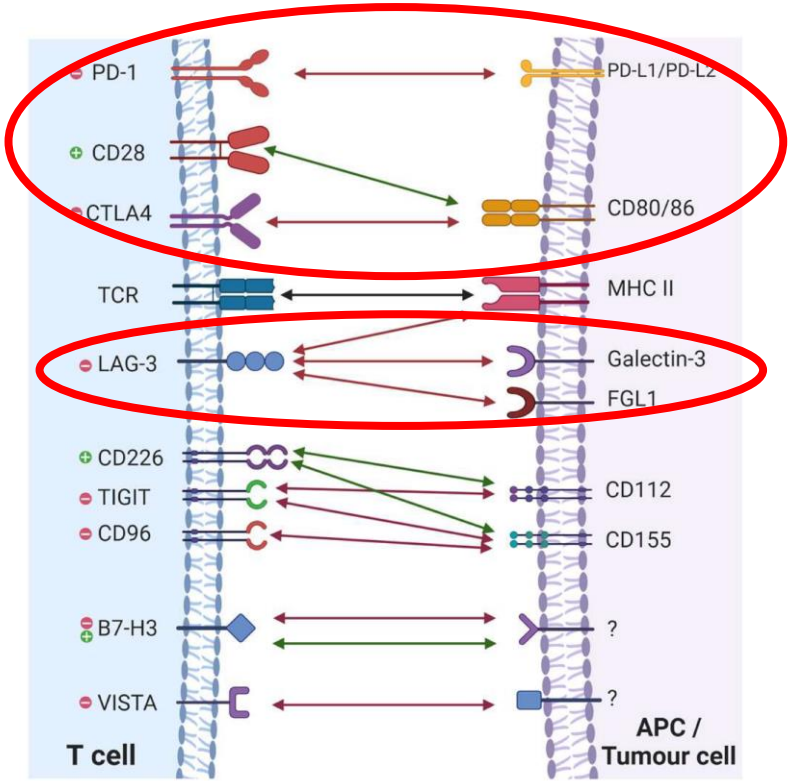


# Target-mediated drug delivery

# Blockbuster checkpoint inhibition …

….ever increasing number of discovered checkpoints and novel drugs developed to block or activate….

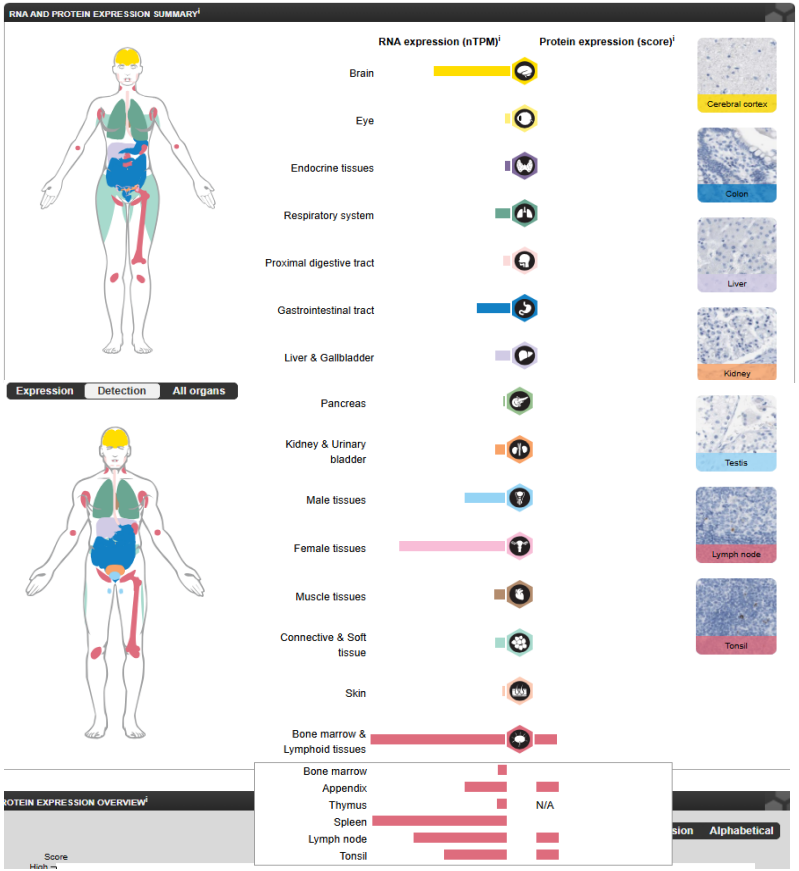



*Immuno* **2024**, *4*(3), 186-210; https://doi.org/10.3390/immuno4030013

# Checkpoint on tumor associated lymphocytes (TILs): LAG3

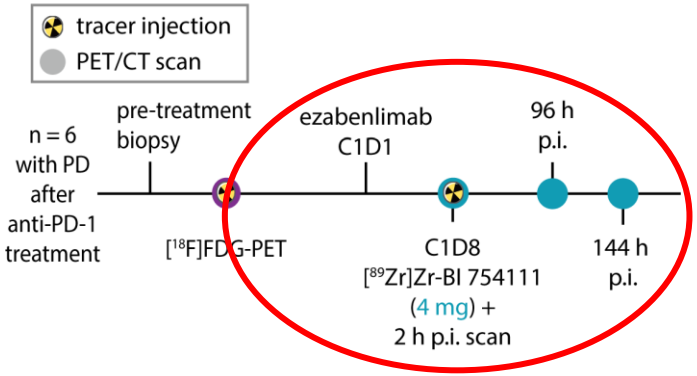
- Low expression, except lymphoid organs
- Increased expression upon T-cell activation
- Possible resistance mechanisme



# Immuno-PET $^{89}$Zr-anti LAG3

Iris Miedema

## Target availability




[$^{89}$Zr]Zr-BI754111
37MBq=20mSv

- Patients with NSCLC, HNSCC, LAG3+
- Treatment with anti PD-1 and anti LAG3

**Aim**

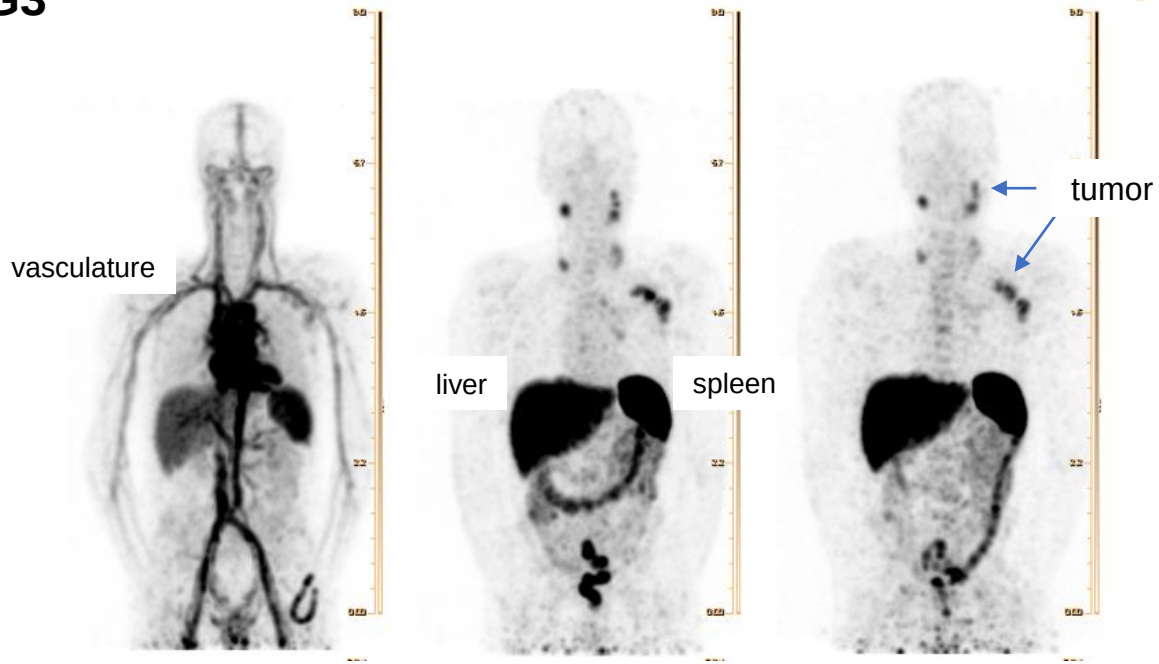
- Visualization/quantification of $^{89}$Zr anti-LAG3 tumor uptake

Boehringer Ingelheim

LAG-3, lymphocyte activation gene-3; PD-1, programmed cell death protein-1.

Miedema, IHC et al EJNMMI 2023

# Immuno-PET $^{89}$Zr-anti LAG3

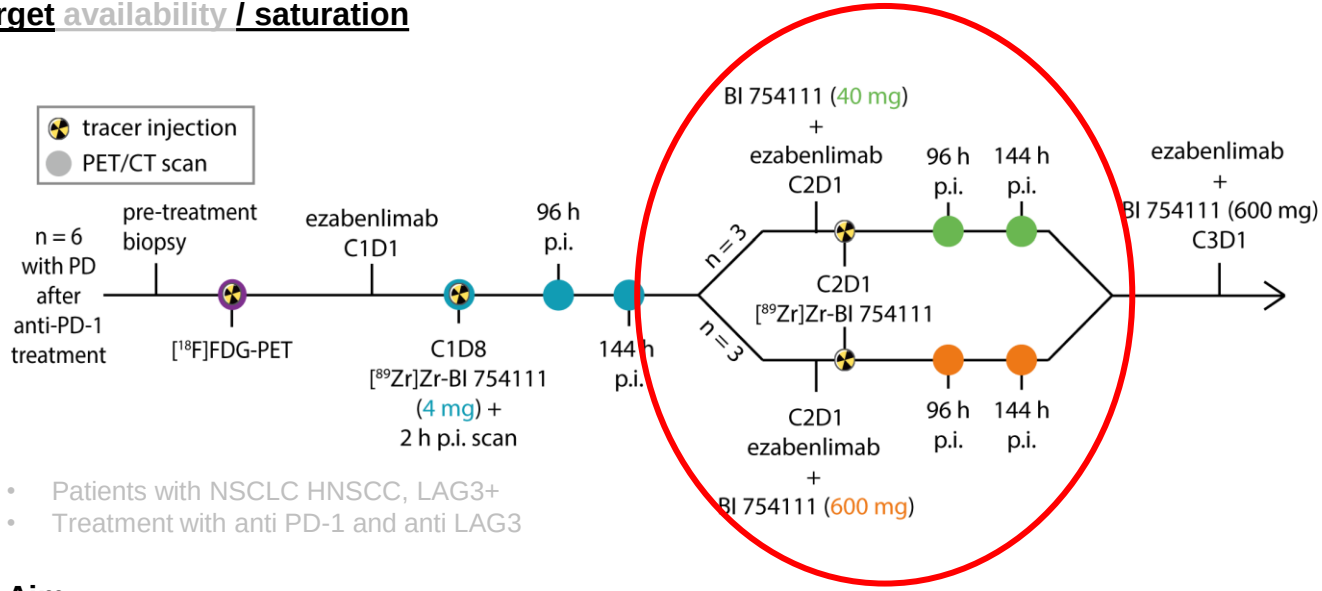



**Example whole body biodistribution of $^{89}$Zr-anti-BI 754 111 (anti-LAG-3) over time**

Adapted from Miedema et al., EJNMMI 2023, Supplementary Fig. 4

# Immuno-PET $^{89}$Zr-anti LAG3

**Target availability / saturation**




[$^{89}$Zr]Zr-BI754111

- Patients with NSCLC HNSCC, LAG3+
- Treatment with anti PD-1 and anti LAG3

**Aim**

- Visualization/quantification of $^{89}$Zr anti-LAG3 tumor uptake
- Effect different mass doses mAb on biodistribution/tumor uptake

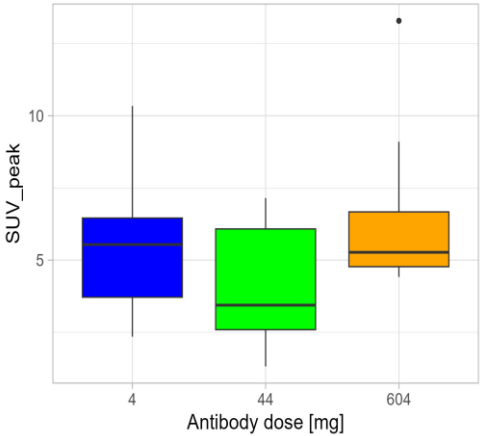
Boehringer Ingelheim

LAG-3, lymphocyte activation gene-3; PD-1, programmed cell death protein-1.

Miedema, IHC et al EJNMMI 2023

# Tumor uptake /saturation

- Tumour uptake was clearly visualised with 4 mg mass dose
- Decrease in visual uptake with both 44 and 604 mg

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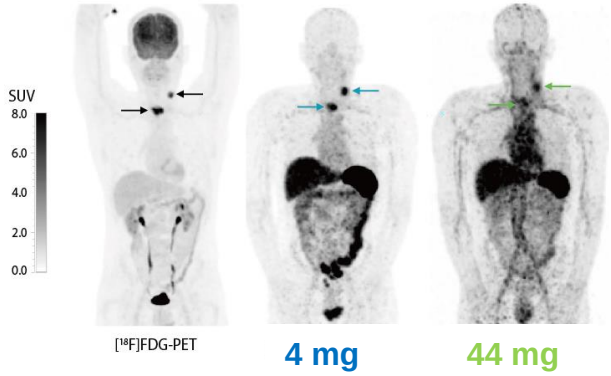
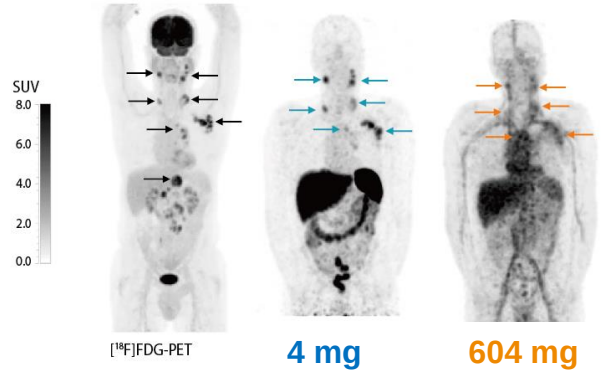
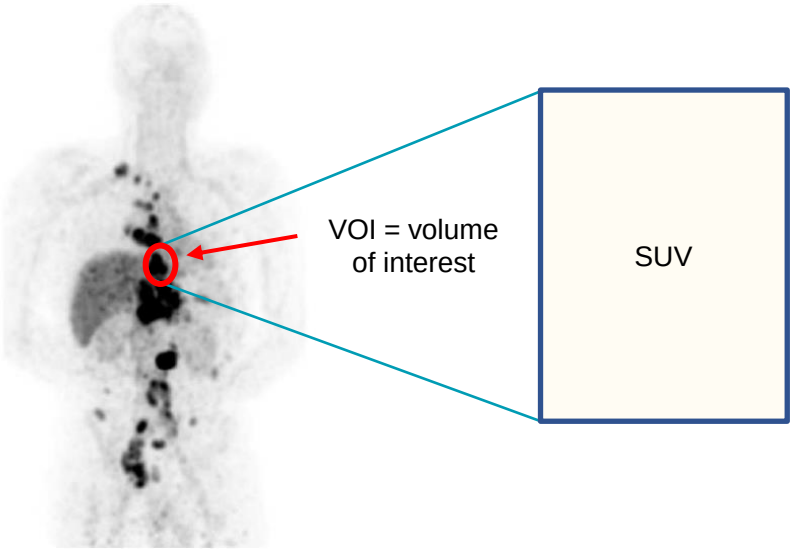


# Quantification using standard uptake value (SUV)




PET camera catches the radiactive signal (activity) emitted by the y-ray photons

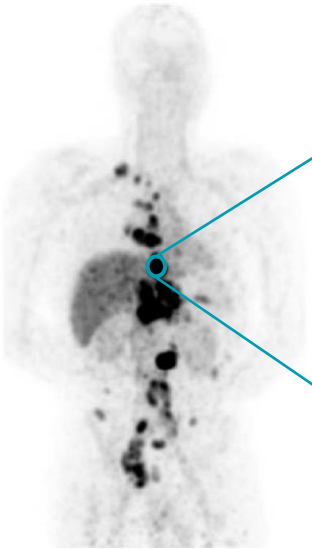
$$SUV = \frac{Act_{VOI}\,(kBq/mL)}{Act_{admin}\,(MBq)/BW\,(kg)}$$

# Quantification of $^{89}$Zr-Immuno-PET


Marc Huisman


Jessica Wijngaarden


Iris Miedema



SUV

Blood and interstitium

Non-specific uptake

Target-specific uptake

higher mass dose mAb

Blood and interstitium

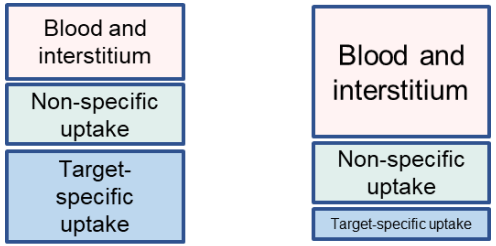
Non-specific uptake

Target-specific uptake

- Non specific uptake = catabolism (in endothelial cells ao)
- $^{89}$Zr residualizes in the cells once internalized

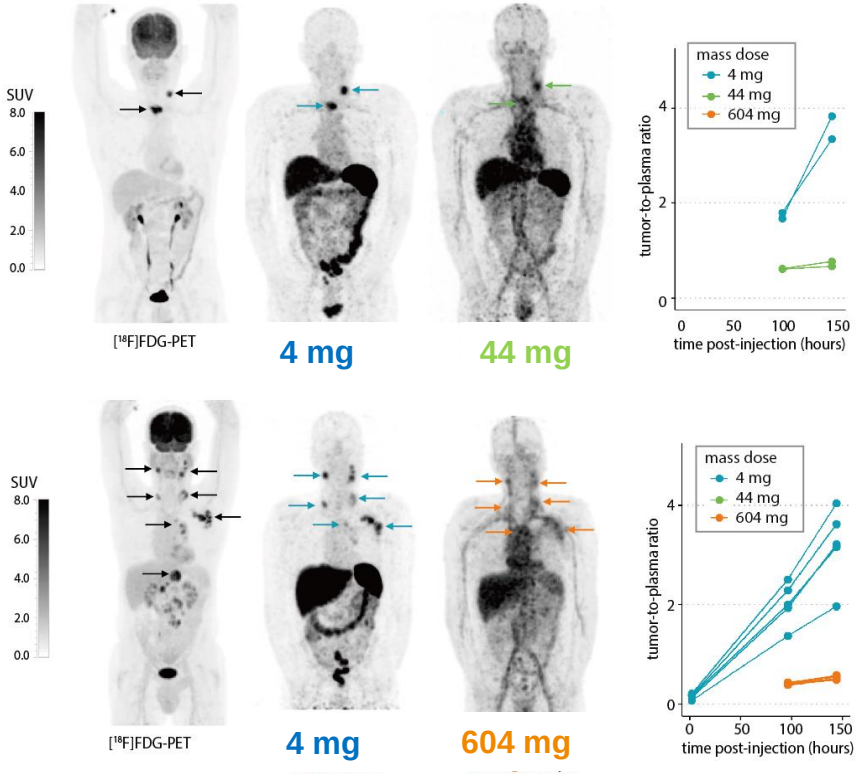
Miedema IHC et al. 2023 Cancers 15:5546

# How to discriminate target-mediated uptake / saturating effects?




*Correct the total signal with tracer availability in the blood* ➡

*Tissue to blood (or plasma) ratio*




Decrease in TPR was observed with both 44 and 604 mg mass doses

NB: spleen also showed a – even larger - decrease in TPR

Miedema, IHC et al EJNMMI 2023

# Modified mAbs

# Modified mAbs

Bispecific-trispecific

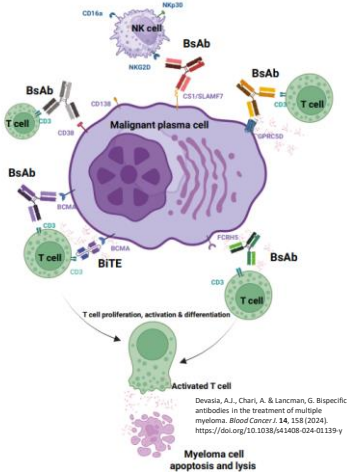
Glycosylated

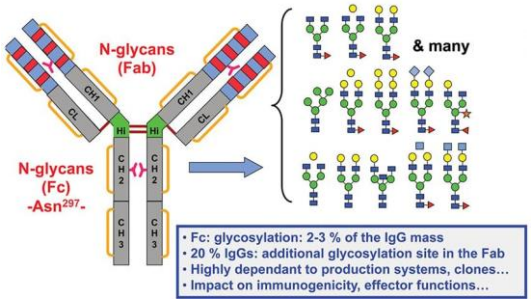
Capped

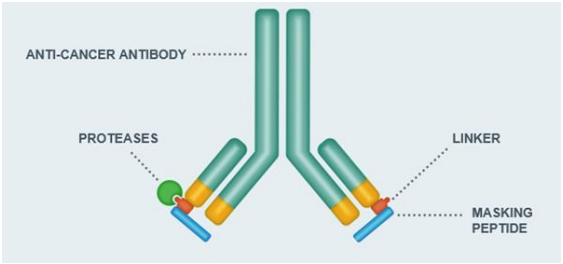
Antibody fragments

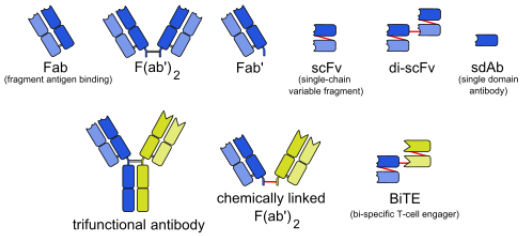
ADCs

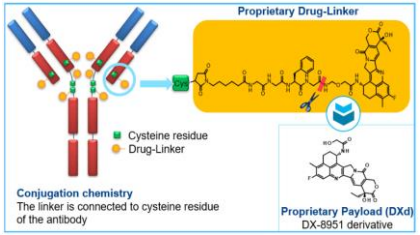
…..





Amsterdam UMC
University Medical Centers

Phase 1 trial: NCT04958239

# Target engagement of a bi-specific CD137 / FAP antibody as assessed with [$^{89}$Zr]Zr-BI 765179 PET-imaging

M. Stavenga[1,3], M.C. Huisman[2,3], G.J.C. Zwezerijnen[2,3], K. Ridler[4], B. Li[5], H. Maas[4], D. Radonjic[6], J. Pasek[7], D.J. Vugts[2,3], C.W. Menke-van der Houven van Oordt[1,3]

[1] *Amsterdam UMC location Vrije Universiteit Amsterdam, Department of Medical Oncology, De Boelelaan 1117, Amsterdam, The Netherlands*

[2] *Amsterdam UMC location Vrije Universiteit Amsterdam, Department of Radiology and Nuclear Medicine, De Boelelaan 1117, Amsterdam, The Netherlands*

[3] *Imaging and Biomarkers, Cancer Centre Amsterdam, De Boelelaan 1117, Amsterdam, The Netherlands*

[4] *Department of Translational Medicine & Clinical Pharmacology, Boehringer Ingelheim Pharma GmbH & Co. KG, Birkendorfer Strasse 65, 88400, Biberach.*

[5] *Department of Translational Medicine & Clinical Pharmacology, Boehringer Ingelheim Pharma GmbH & Co.KG, Binger Straße 173 D-55216 Ingelheim am Rhein, Germany.*

[6] *TA Oncology Medicine, Boehringer Ingelheim International GmbH, Binger Straße 173 D-55216 Ingelheim am Rhein, Germany.*

[7] *Early Clinical Operations, Boehringer Ingelheim Pharmaceuticals, Inc. 900 Ridgebury Road, Ridgefield, CT 06877*

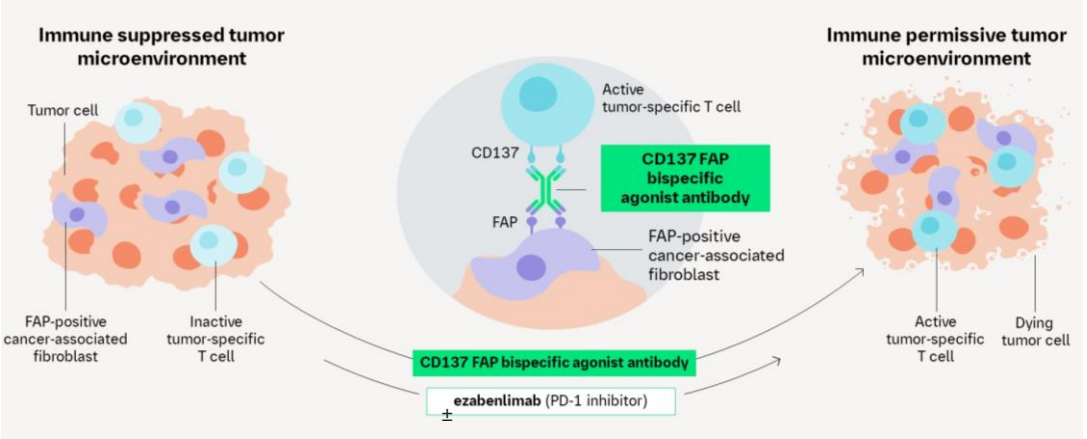
# Local CD137/4-1BB agonism – leads to stimulation immune response

**Bi-specific antibody** to ensure local effect: Designed so that binding to both is essential for activating CD137 pathway

**AIM**

1. To evaluate the distribution pattern of a bi-specific antibody against CD137/FAP

2. To investigate target engagement and saturating effects of the anti CD137/FAP antibody [$^{89}$Zr]Zr-BI 765179



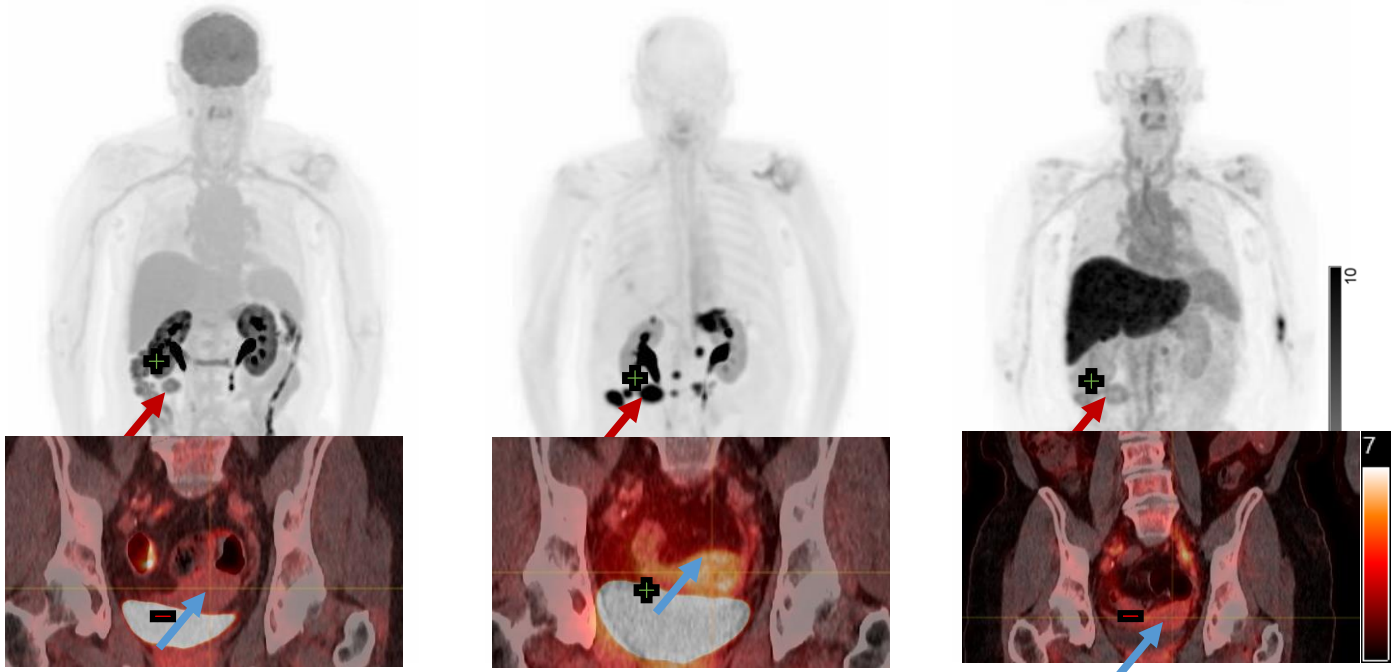


Bartkowiak T et al, Frontiers in Oncology, 2015
Claus, C et al, mAbs, 2023
Liu G et al, Frontiers in immunology, 2023
CD137 FAP Agonist | InOncology – Boehringer Ingelheim

# $^{89}$Zr-BI 765179: *accumulation presumably only in both CD137 and FAP expressing tissue*

Visually:
Tumor lesions:
FDG +
FAPI +
$^{89}$Zr +

FAP-expressing tissue (uterus):
FDG –
FAPI +
$^{89}$Zr not above background



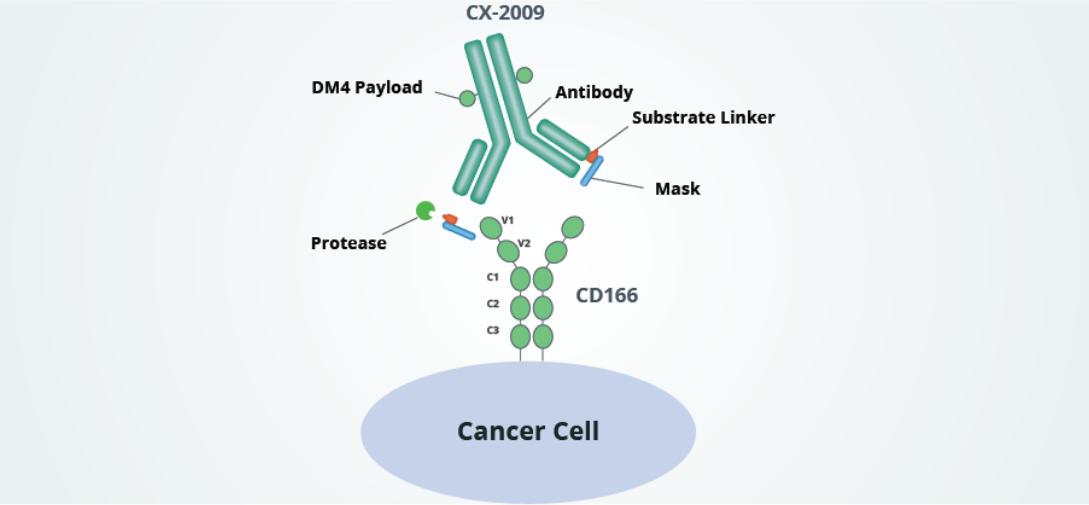

FDG | FAPI | $^{89}$Zr-BI 765179
Cycle 1, T = 144h

Dendl K. et al, EJNMMI, 2021
Taralli S. et al, Cancers, 2023
Zhang X. Clin. Nucl. Med., 2022
Stavenga M et al EMIM 2025

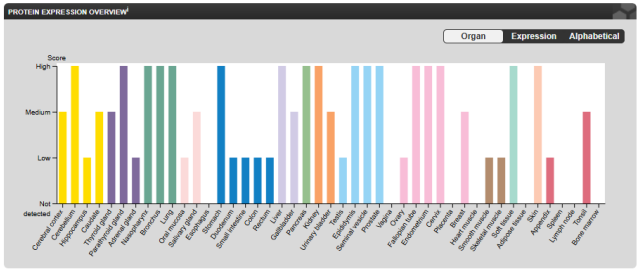
# Capped mAb - Probody concept

# Capped antibody drug conjugate (ADC) - Probody concept

Figure 1. CX-2009: A Probody Drug Conjugate Targeting CD166



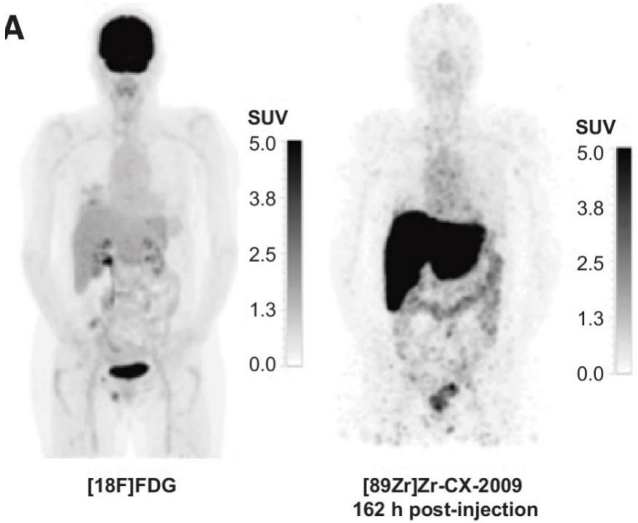

CD166 is expressed in nearly all normal tissues

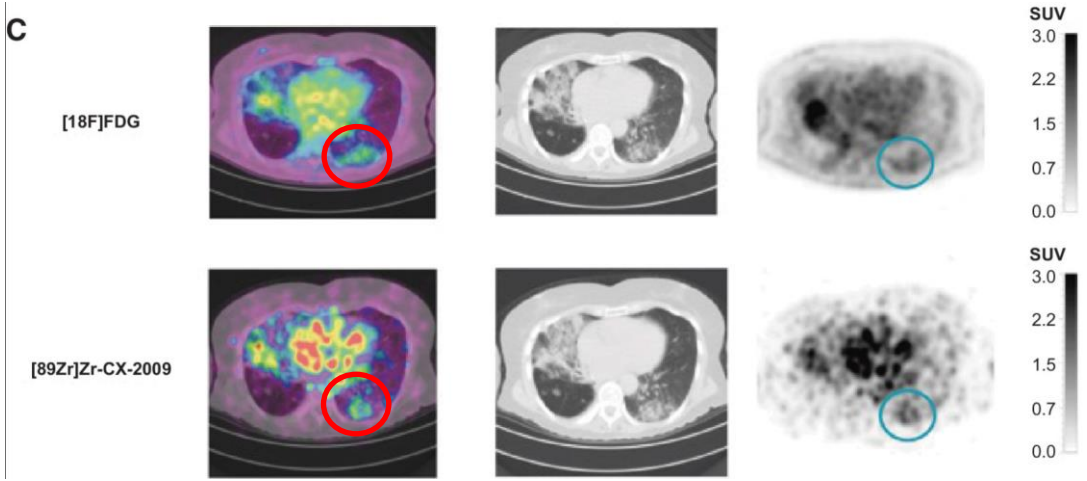



CytomX Therapeutics

# CD166 Probody – tumor accumulation

Iris Miedema

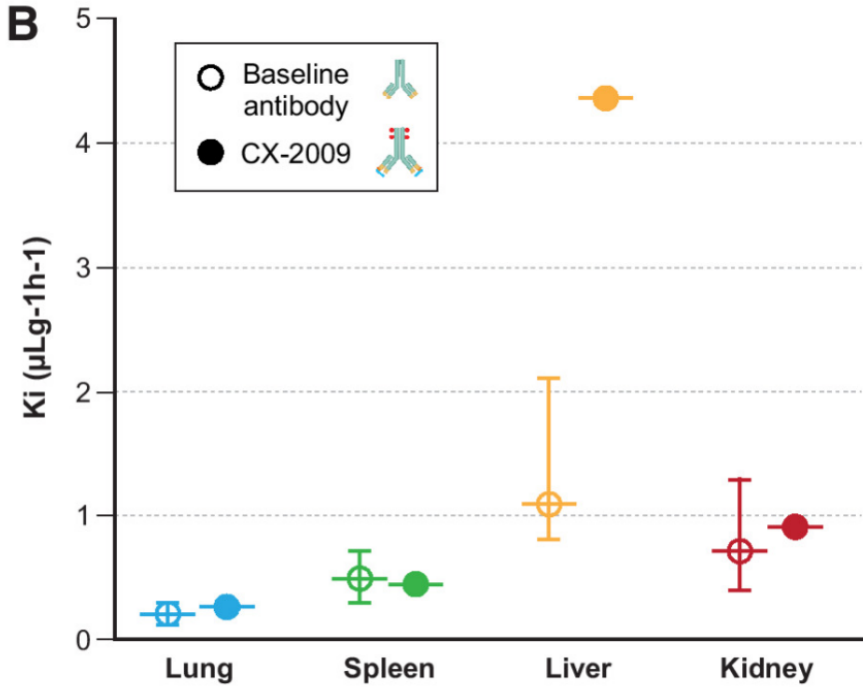



micropapillary adenocarcinoma of the lung

Boni et al CCR 2022

# CD166 Probody – no accumulation in (most) healthy tissues (Patlak)

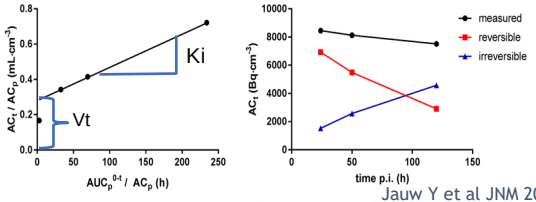



Boni et al CCR 2022

**PATLAK equation** – to describe irreversible uptake

Tissue signal (= SUV) consists of

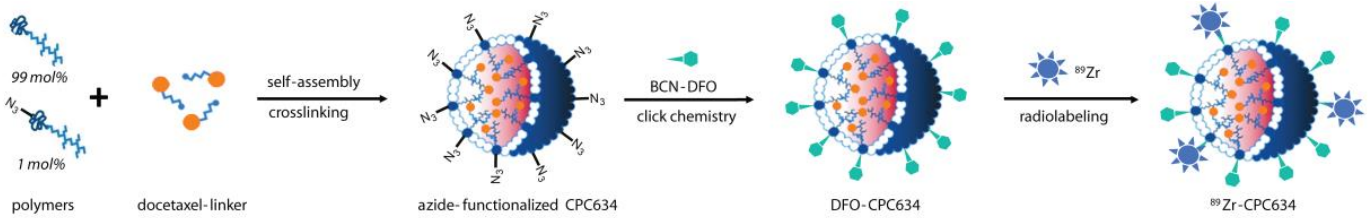
1. $^{89}$Zr-mAb in equilibrium with plasma (reversible) = $V_t$
2. internalization / residualization of $^{89}$Zr-mAb (irreversible) = $K_i$
   irreversible uptake = catabolism + target-mediated




Jauw Y et al JNM 2019

# Non-targeted compounds

# PET-CT imaging of non-targeted polymeric nanoparticles




*CriPec technology is based on amphiphilic methoxy-poly(ethylene glycol)-b-poly[N-(2-hydroxypropyl) methacrylamide lactate] (mPEG-b-pHPMAmLacn) block copolymers, which are designed to upon selfassembly covalently entrap active pharmaceutical ingredients (API) in core-crosslinked polymeric micelles (CCPM).*

Atrafi F *et al* J Contr Release 2020
Rijken CJF et al 2022 Adv Drug Delivery Rev 191: 14613

**$^{89}$Zr-CPC634 behaves identical to CPC634**

➡ **Prolonged exposure to docetaxel and reduced toxicity**

## Unmet clinical need

- To demonstrate tumor accumulation in a safe and reliable way
- To ultimately identify patients who are most likely to benefit from nanomedicine treatment

Cristal Therapeutics


Iris Miedema

# On-treatment imaging

**Study procedures**

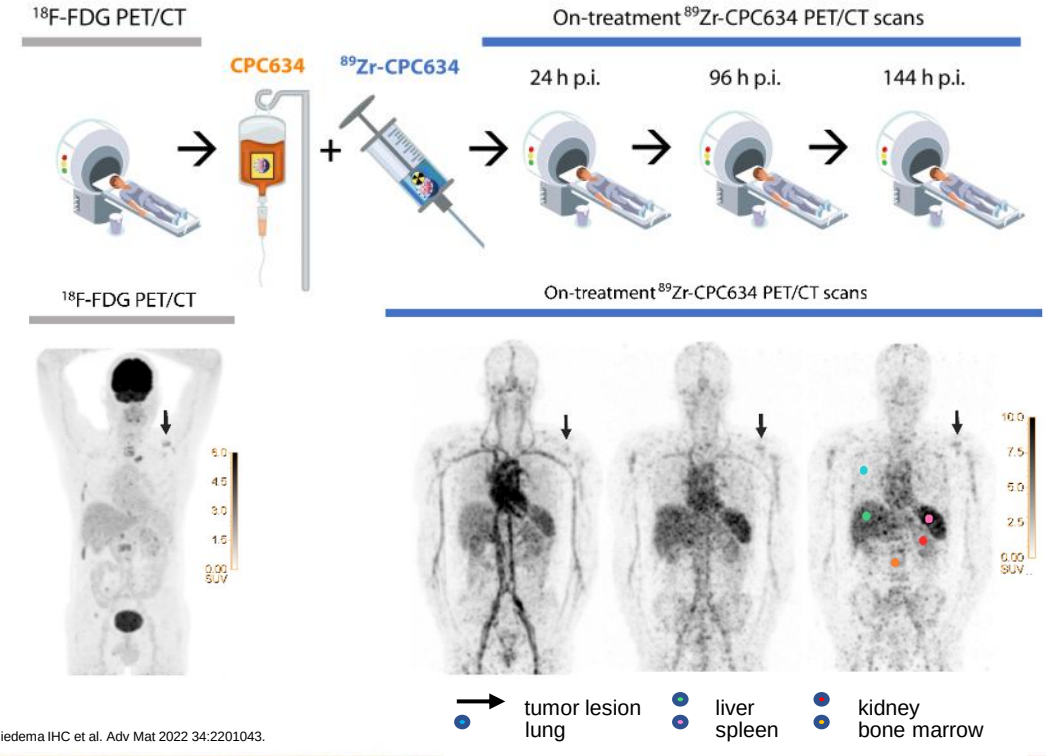
Patients with advanced solid tumors with no standard therapy option

Treatment dose CPC634 containing 60 mg/m2 docetaxel

Tracer 37MBq $^{89}$Zr-CPC634

**Biodistribution of $^{89}$Zr-CPC634**

- Circulation up to 144h p.i.
- Accumulation in spleen, liver and bone marrow, known sites of nanoparticle entrapment and elimination.
- Tumor accumulation was observed (black arrow).




Miedema IHC et al. Adv Mat 2022 34:2201043.

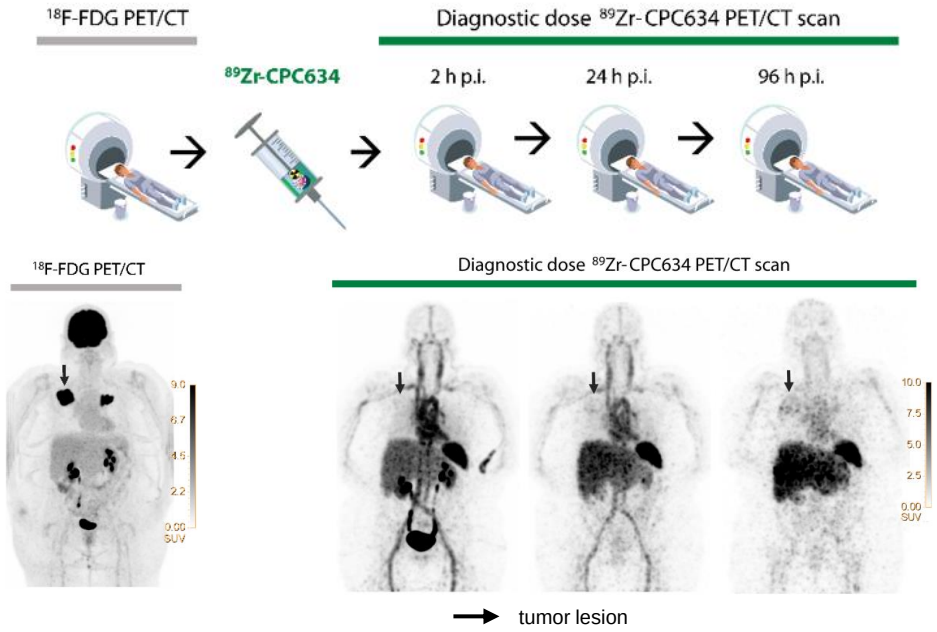
# Diagnostic-dose imaging

**Study procedures**

- Diagnostic dose = tracer only, 37MBq $^{89}$Zr-CPC634 with 1-2 mg docetaxel and 1.8 - 3.7*$10^{14}$ nanoparticles
- Treatment with CPC634 containing 60 mg/m$^2$ docetaxel after imaging

**Biodistribution/PK**

- Similar with diagnostic dose vs on-treatment (80x more nanoparticles)
- Similar tumor accumulation

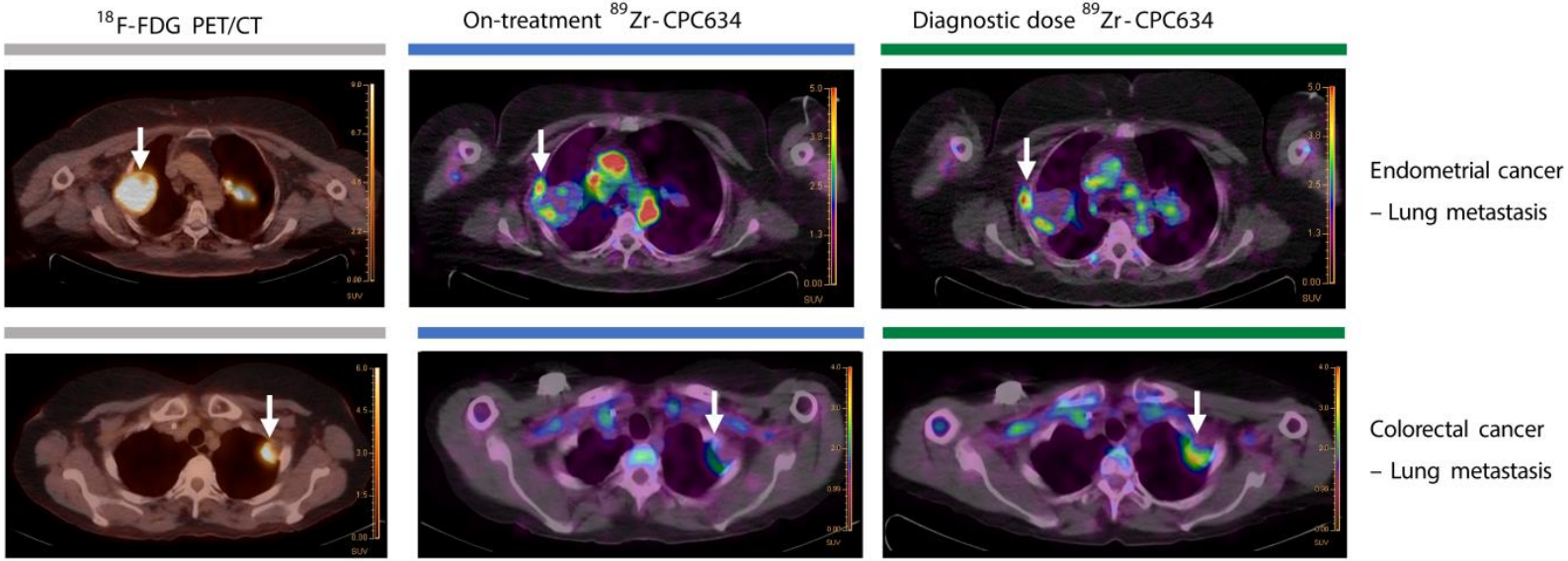



Miedema IHC et al. Adv Mat 2022 34:2201043.

# Tumor accumulation

**(***Heterogeneous) tumor accumulation of $^{89}$Zr-CPC634 was observed in 6 out of 7 patients*

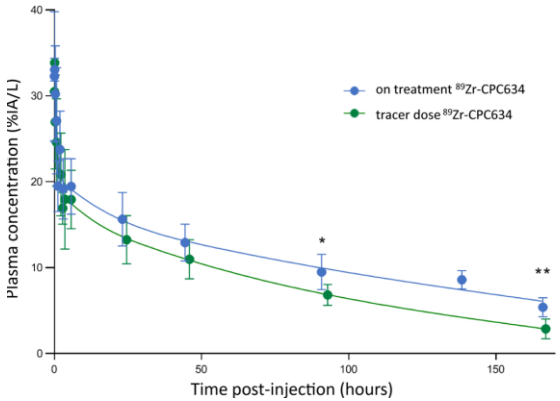
**Two patient examples**

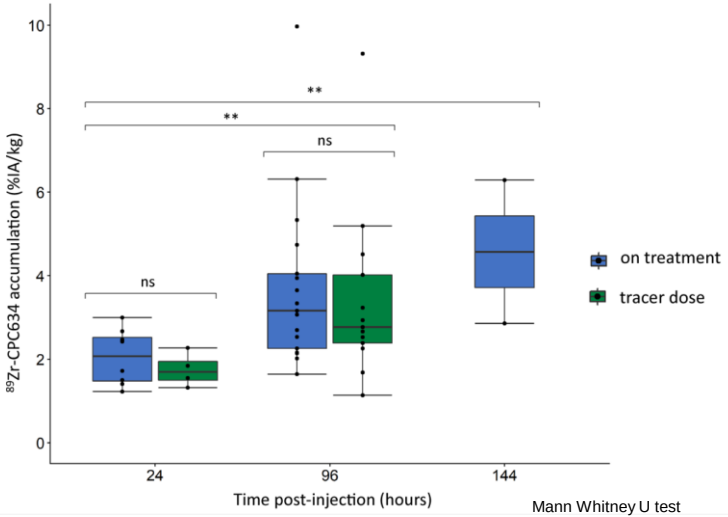



Miedema IHC et al. Adv Mat 2022 34:2201043.

# Tumor accumulation

*Tumor accumulation of $^{89}$Zr-CPC634 is not significantly different on-treatment vs diagnostic dose*



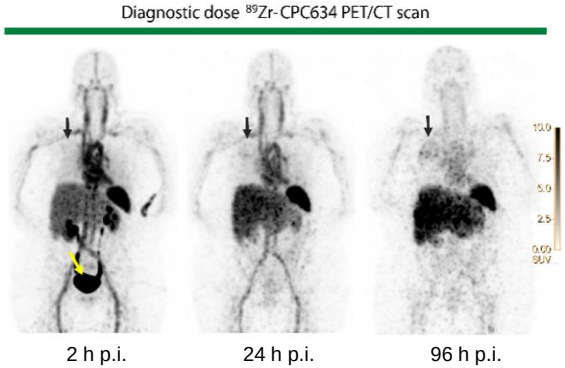



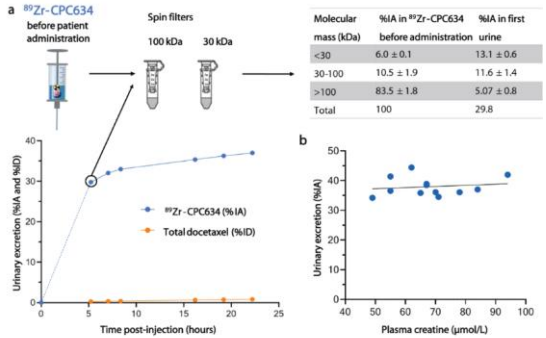

Mann Whitney U test

**Tumor accumulation**

- Heterogeneous between patients, between tumor lesions of each patient and within the tumor lesions itself
- In 21/46 tumor lesions with on-treatment imaging (46%; in 7 patients)
- in 13/32 tumor lesions with diagnostic dose imaging (41%; in 5 patients).

Miedema IHC et al. Adv Mat 2022 34:2201043.



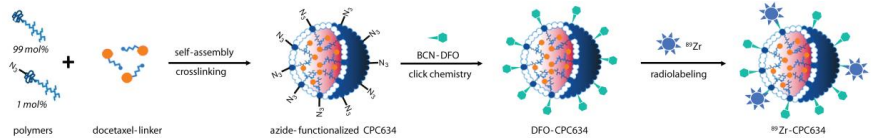




urinary excretion of $^{89}$Zr-labeled product

# Excretion $^{89}$Zr-labeled products in the urine

*most likely non-crosslinked block copolymeres labeled with $^{89}$Zr*

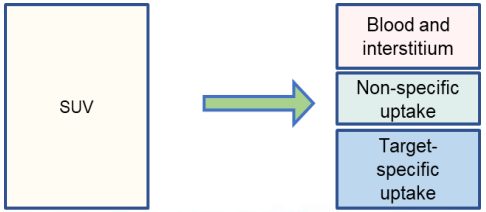
- Urine excretion $^{89}$Zr-labeled products is in all patients more or less the same, 38.2 ± 2.8%.
- Urine docetaxel is negligible (0.82 %ID in 24h).
- Free $^{89}$Zr would accumulate in bone
- Thus, we assume non-crosslinked block co-polymeres present in CPC634 were labeled with $^{89}$Zr.
- Imaging tool using $^{89}$Zr-CPC634 is not compromised




Miedema IHC et al. Adv Mat 2022 34:2201043.

# Conclusions

- Molecular imaging offers quantitative, whole-body information on (target-mediated) biodistribution and tumor uptake of biologicals ($^{89}$Zr-anti LAG3).
- It is an in vivo technique to inform on effectiveness of compound modifications (ao bispecific $^{89}$Zr-anti CD137/FAP mAb;  capped $^{89}$Zr-anti CD166 ADC).
- (Non-targeted) nanoparticle imaging appears feasible with low doses (number of nanoparticles 10x below estimated threshold to saturate rapid uptake by Kupffer cells; $^{89}$Zr-CPC634 PET).
- Smart, small clinical studies can support go/no-go decisions early in drug development.
- Important to realize that SUVs is not always suitable for quantification! Need to tailor quantification metrics to the pharmacokinetic profile of the tracer molecule / properties radionuclide.



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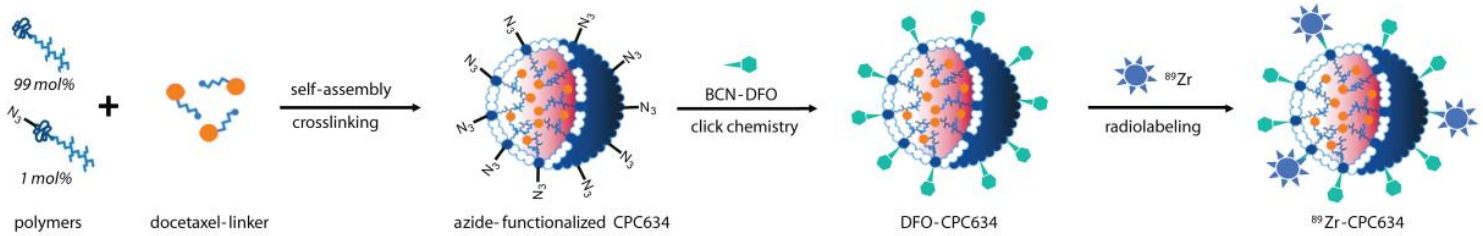
KWF

# Development of $^{89}$Zr-CPC634

**Manufacturing**




DFO = desferal , BCN = biclononyne

**Preclinical data in healthy/tumor-bearing mice**

Identical blood levels of total docetaxel (%ID/g) with CPC634 and $^{89}$Zr-CPC634

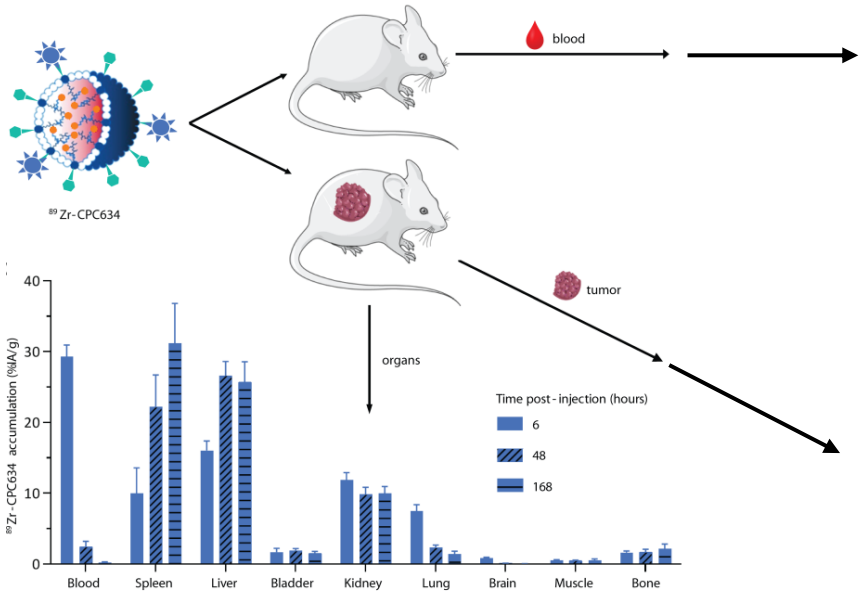
Expected biodistribution of $^{89}$Zr-CPC634 with predominant accumulation in spleen, liver and kidney

Similar intra-tumoral levels of total docetaxel (%ID/g) with CPC634 and $^{89}$Zr-CPC634

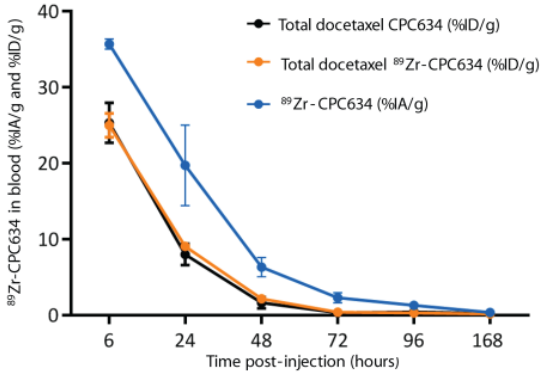
**$^{89}$Zr-CPC634 behaves identical to CPC634**

Miedema IHC et al. Adv Mat 2022 34:2201043.

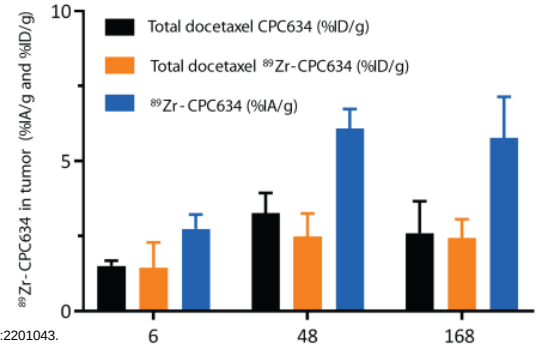
# Pre-clinical evaluation of $^{89}$Zr-CPC634




*Ex vivo biodistribution of $^{89}$Zr-CPC634 (%IA/g) with predominant accumulation in spleen, liver and kidney*




*Identical blood levels of total docetaxel (%ID/g) with CPC634 and $^{89}$Zr-CPC634*

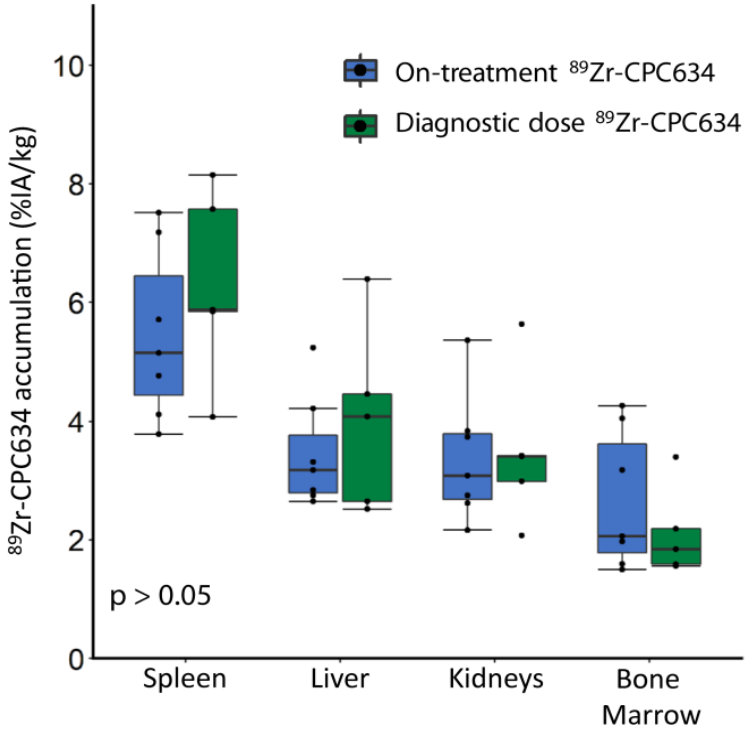



*Similar intra-tumoral levels of total docetaxel (%ID/g) with CPC634 and $^{89}$Zr-CPC634*

Miedema IHC et al. Adv Mat 2022 34:2201043.

# Organ accumulation

Quantification of $^{89}$Zr-CPC634 biodistribution shows no significant difference between on-treatment vs diagnostic dose imaging




96 h p.i.

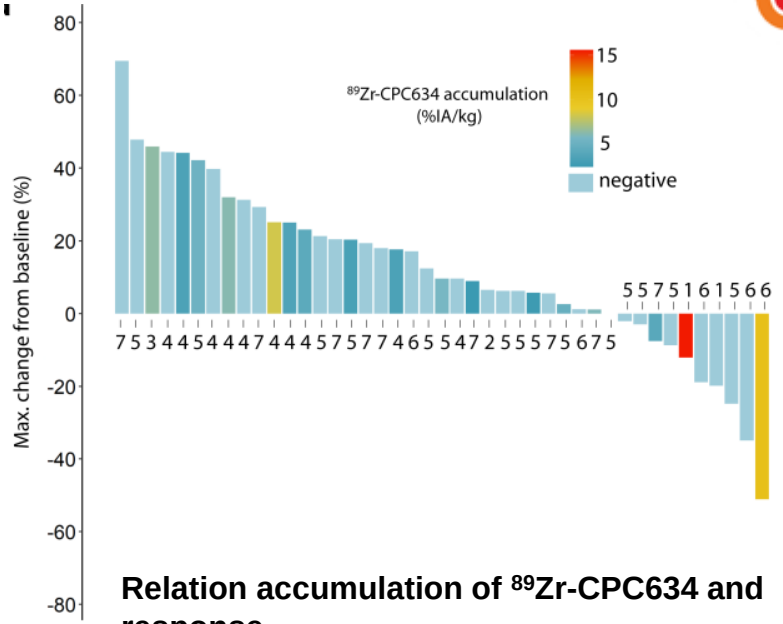
Kruskall Wallis test

Miedema IHC et al. Adv Mat 2022 34:2201043.

# Patient outcome

*3 out of 7 patients had stable disease as best response*

| Subject no. | Tumor type | Positive lesions - no. / total (%) | Best response |
|---|---|---|---|
| 1 | Ovarian carcinoma | 1 / 2 (50) | SD |
| 2 | Esophageal carcinoma | 0 / 1 | PD |
| 3 | Endometrial carcinoma | 2 / 2 (100)* | PD |
| 4 | Colorectal carcinoma | 6 / 10 (60) | PD |
| 5 | Myoepithelial carcinoma | 6 / 17 (35)† | SD |
| 6 | Breast cancer | 1 / 5 (20) | SD |
| 7 | ACUP | 3 / 9 (33) | PD |

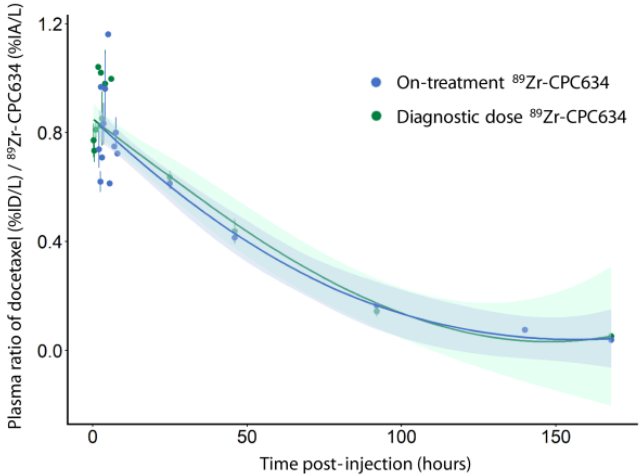



**Relation accumulation of $^{89}$Zr-CPC634 and response**

The highest accumulation of $^{89}$Zr-CPC634 was in two lesions with 12% and 51% decrease in diameter

Miedema IHC et al. Adv Mat 2022 34:2201043.

# Estimation of intra-tumoral concentration of CPC634-entrapped docetaxel




Fraction of CPC634 still containing docetaxel is the same on treatment and with diagnostic dose.

Slow release of docetaxel from the nanoparticle.

Miedema IHC et al. Adv Mat 2022 34:2201043.

**Assumptions**

In tumor lesions there will be the same fraction of CPC634 still containing docetaxel as in plasma.

The %IA/L in the tumor lesion on PET represents the %ID/L of CPC634.

**Estimations** of nanoparticle-entrapped docetaxel in tumor lesions

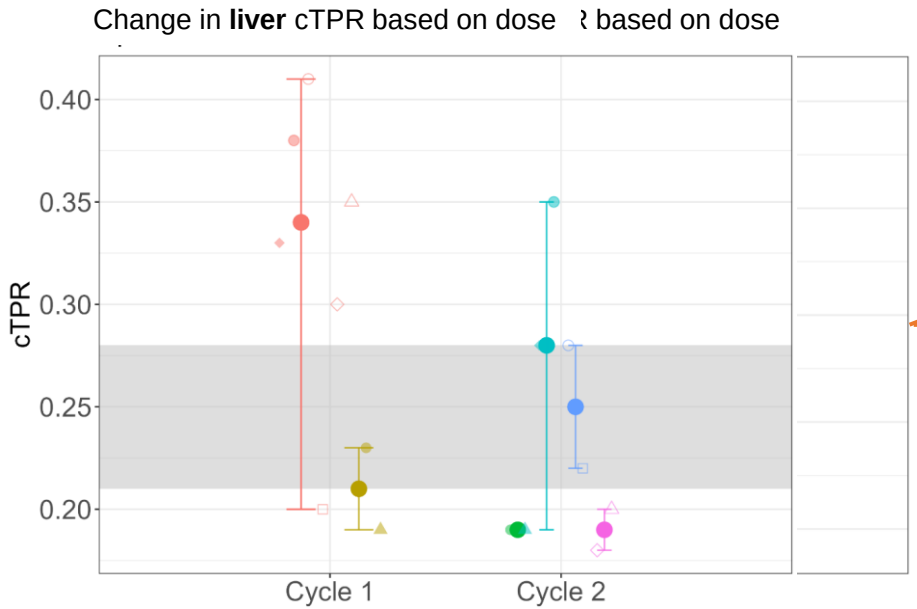
- 2.29 (IQR 2.00 – 3.23) ng/mg after 24 hours
- 0.83 (IQR 0.48 – 1.21) ng/mg after 96 hours

Total docetaxel with CPC634 in tumor biopsies 3.41 ng/mg; 95% CI, 2.56–4.55 ng/mg

Atrafi F *et al* CCR 2020

# $^{89}$Zr-BI 765179 accumulates and is saturable

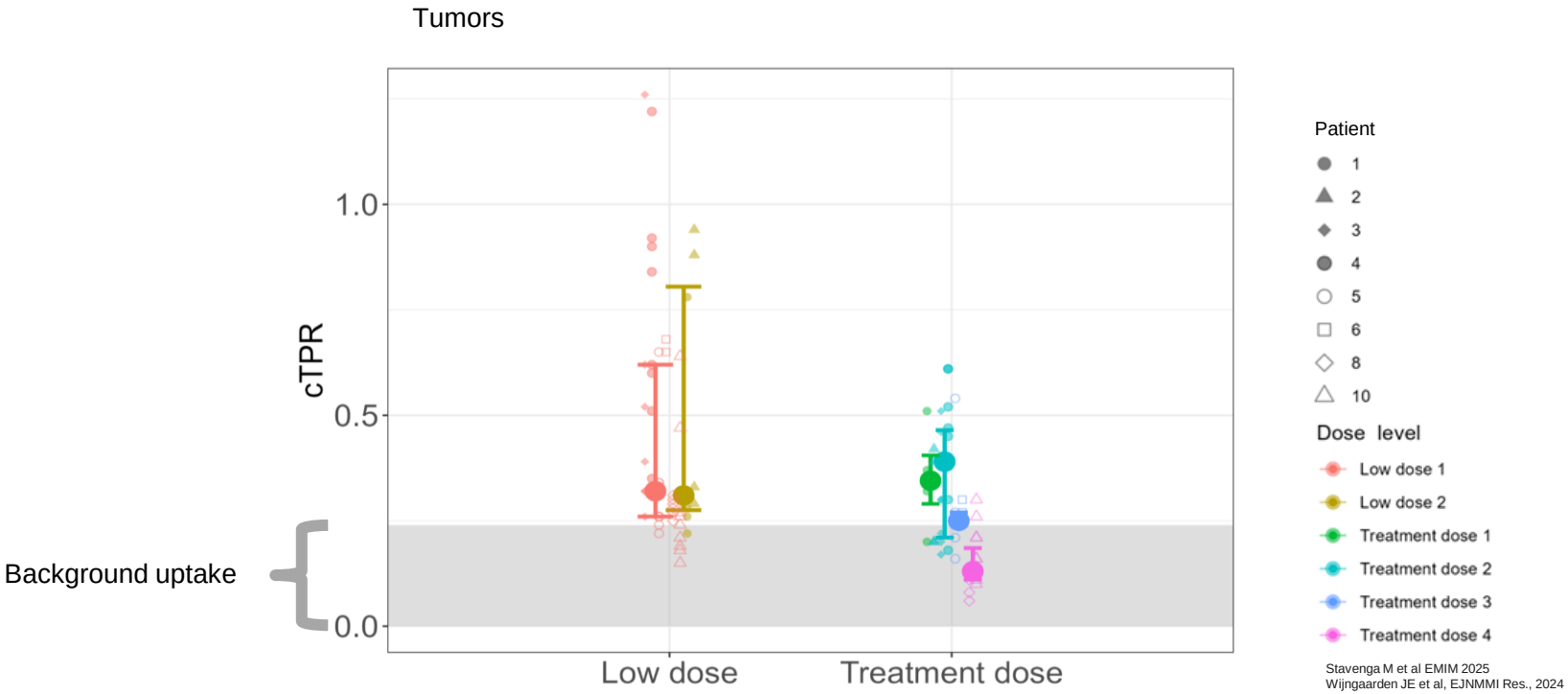
In spleen (minimal), bone marrow (minimal) and liver





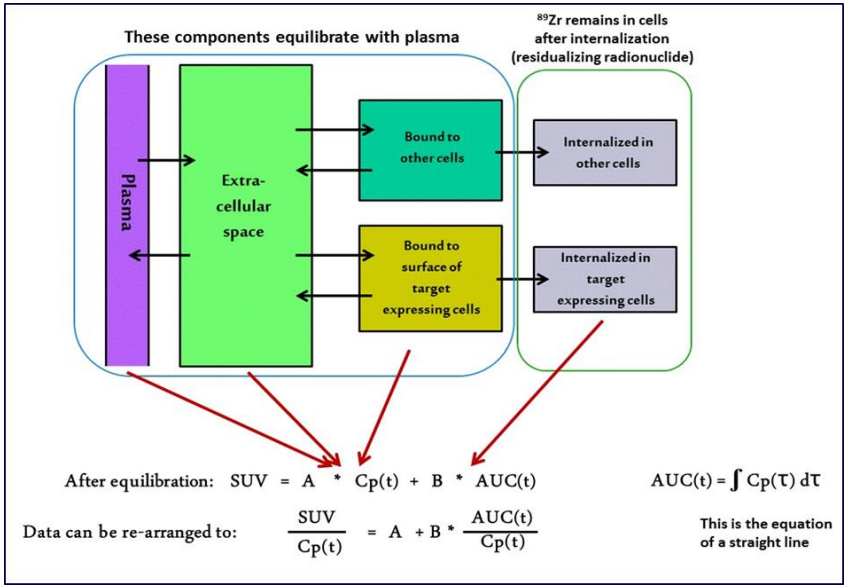
Stavenga M et al EMIM 2025
Jauw YWS et al, 2019 J Nucl Med

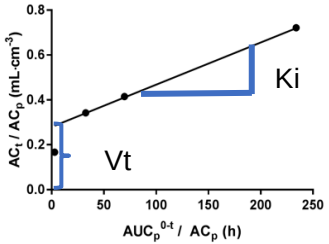
# Increasing treatment doses result in saturating effects

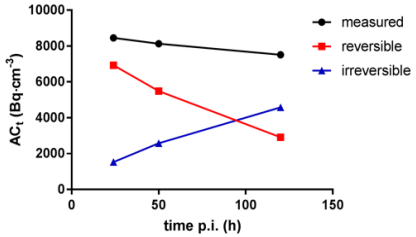



Stavenga M et al EMIM 2025
Wijngaarden JE et al, EJNMMI Res., 2024

# Patlak equation




$$\frac{SUV}{Cp(t)} = A + B * \frac{AUC(t)}{Cp(t)}$$

Offset = distribution volume Vt (**reversible** non-specific uptake);
Slope = net rate irreversible uptake Ki **(irreversible** (non)-specific uptake)

Jauw YWS et al, 2019 J Nucl Med