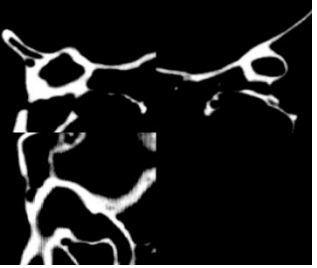
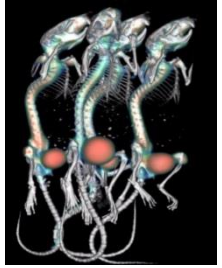
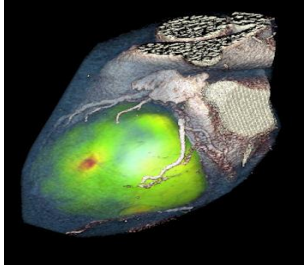
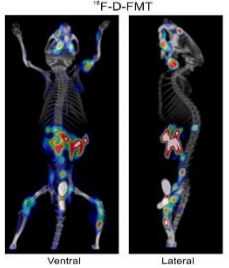

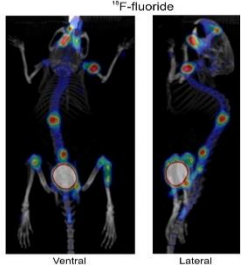

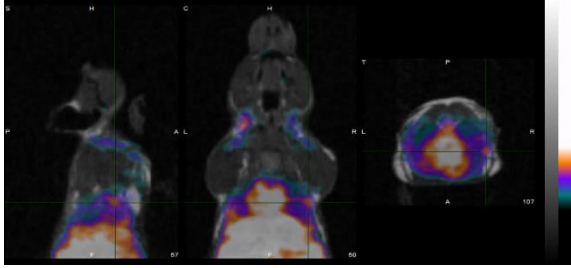

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Preclinical Imaging in Small Laboratory Animals

Instrumentation and Application

Infections & Inflammation

Molecular Imaging in Pharmaceutical Research

Sebastian Eigner, M.Sc.

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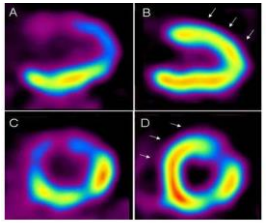
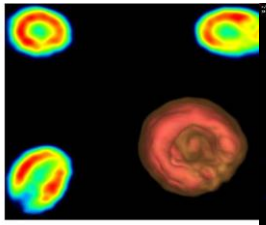
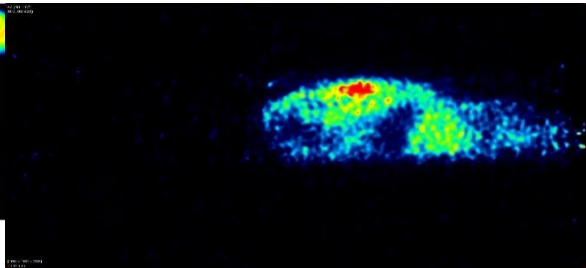
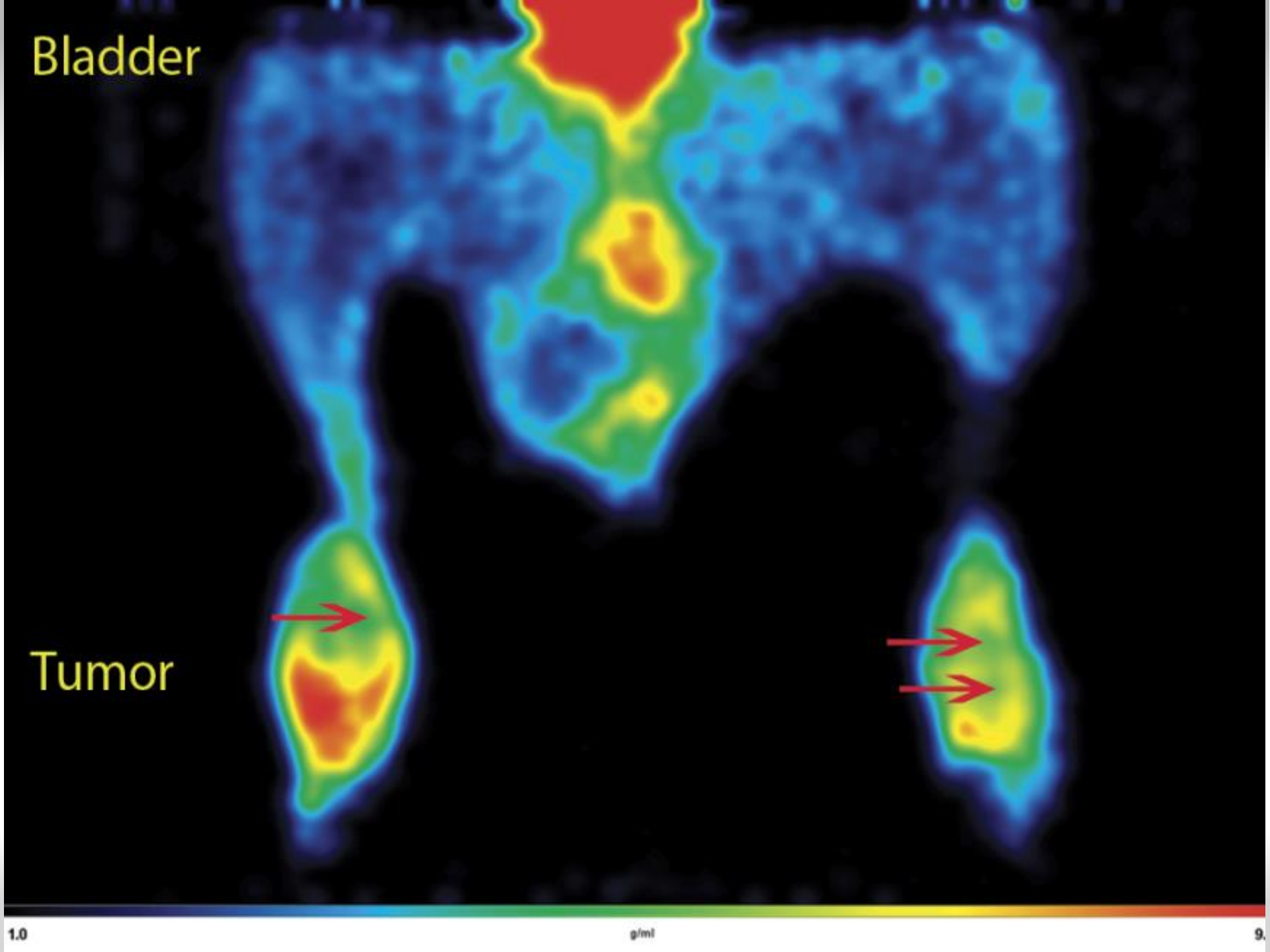
EVROPSKÝ FOND PRO REGIONÁLNÍ ROZVOJ
INVESTICE DO VAŠÍ BUDOUCNOSTI

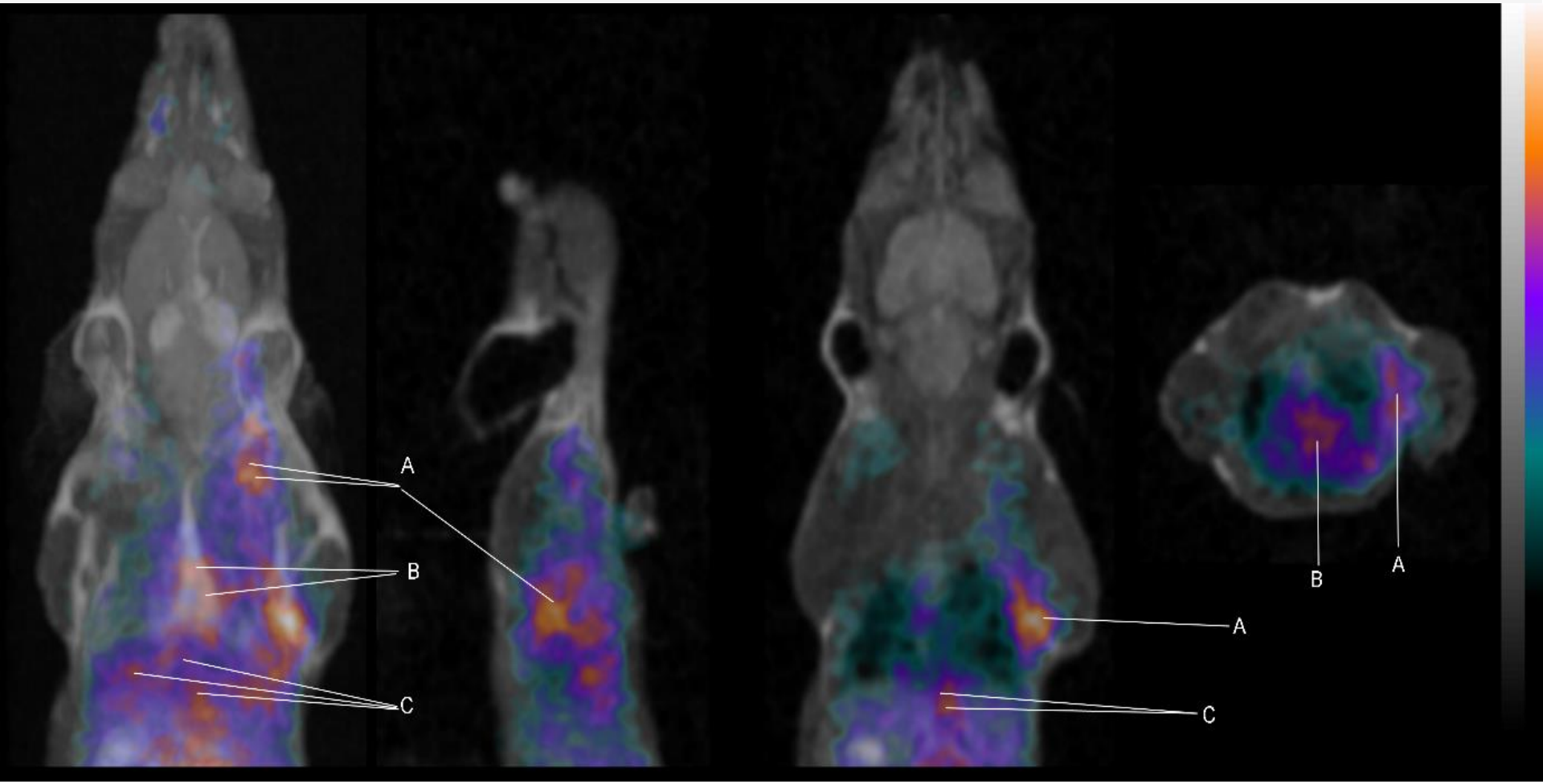
2007-13
OP Výzkum a vývoj pro inovace



^{18}F-FDG – human heart ^{A} ^{18}F-FDG –rat heart ^{B}


Bladder
Tumor
1.0
g/ml



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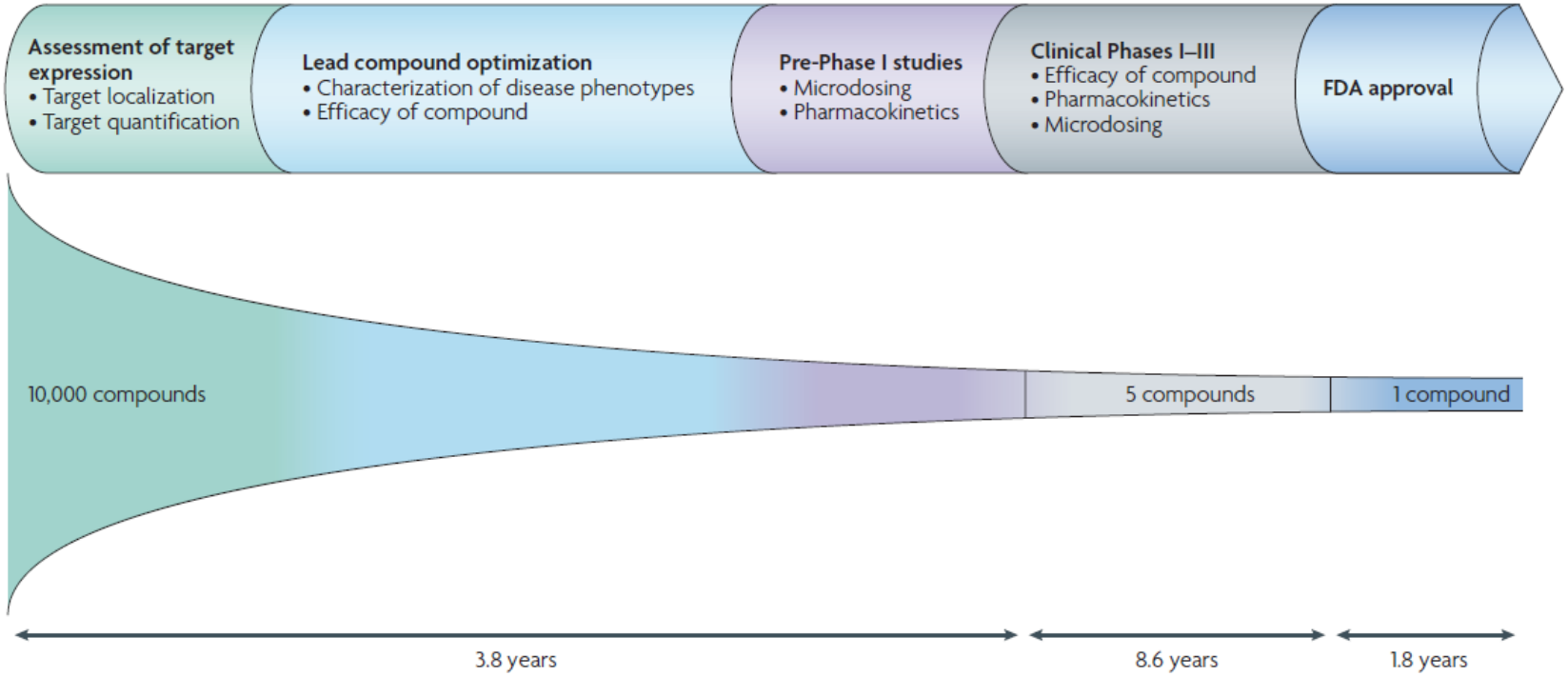
Drug Development Process



William JK et al., Nature Reviews 2008:7; 591- 607

Imaging Modalities

Dedicated for Small Laboratory Animals

Optical imaging

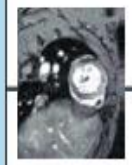
Advantages:	Disadvantages:
• High-throughput screening for target confirmation and compound optimization • High sensitivity	• Limited clinical translation • Low depth penetration

PET imaging

Advantages:	Disadvantages:
• Clinical translation • High sensitivity with unlimited depth penetration	• Cost

Magnetic resonance imaging

Advantages:	Disadvantages:
• Clinical translation • High resolution and soft-tissue contrast	• Costs • Imaging time

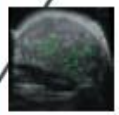


SPECT imaging

Advantages:	Disadvantages:
• Clinical translation • Unlimited depth penetration	• Limited spatial resolution

Ultrasound imaging

Advantages:	Disadvantages:
• Clinical translation • High spatial and temporal resolution • Low costs	• Operator dependency • Targeted imaging limited to vascular compartment



CT imaging

Advantages:	Disadvantages:
• High spatial resolution (bone/lung) • Clinical translation	• No target-specific imaging • Radiation • Poor soft-tissue contrast

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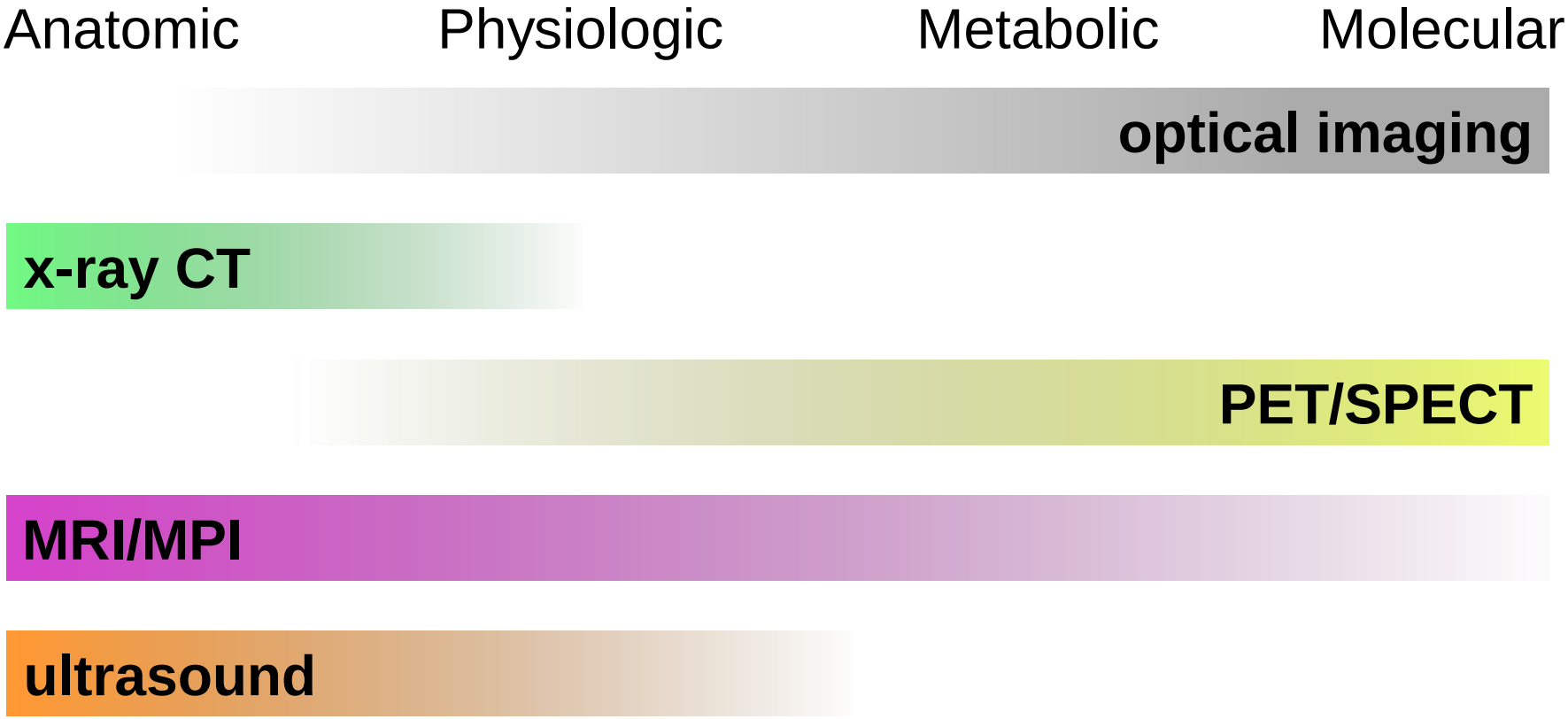
In vivo biomedical imaging technologies



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Spatial Resolution

Comparison of Clinical and Preclinical Imaging Modalities

Modality	Spatial resolution (mm)		Clinical-to-preclinical design refinement(s)
	Clinical	Preclinical	
MRI	~1	≤ 0.1	Higher field-strength magnets, improved gradient fields and coils
MRSI	~10	~2	Higher field-strength magnets, improved gradient fields and coils
PET	~5	1-2	Reduced detector element size, smaller-diameter detector rings
SPECT	~10	0.5-2	Pinhole collimation (and resulting magnification)
CT	1-2	≤ 0.2	Higher X-ray flux, smaller focal spot, and higher magnification
US	1-2	≤ 0.1	Higher-frequency scan heads

Fabian Kiessling and Bernd J. Pichler. “Small Animal imaging” Basics and Practical Guide. ISBN: 978-3-642-12944-5

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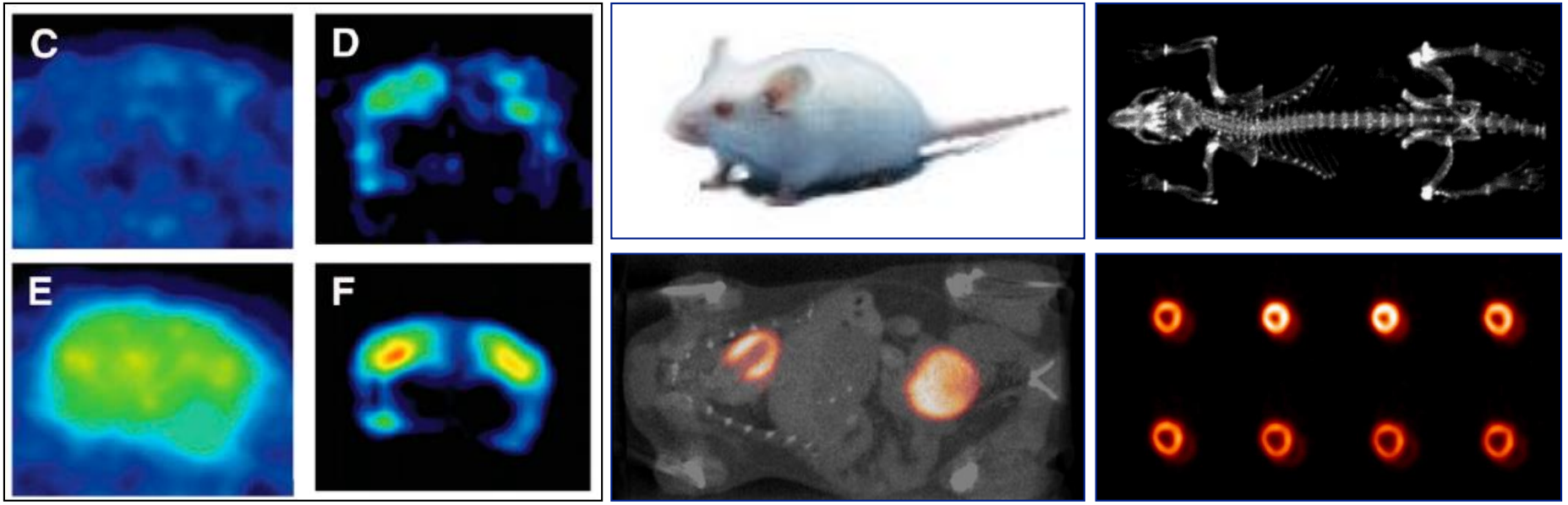
Small Animal Imaging

Requirements



High spatial resolution

- mouse organs ~1000-fold smaller volume than human

High sensitivity

- number of targets also smaller, radiation dosimetry can be limiting

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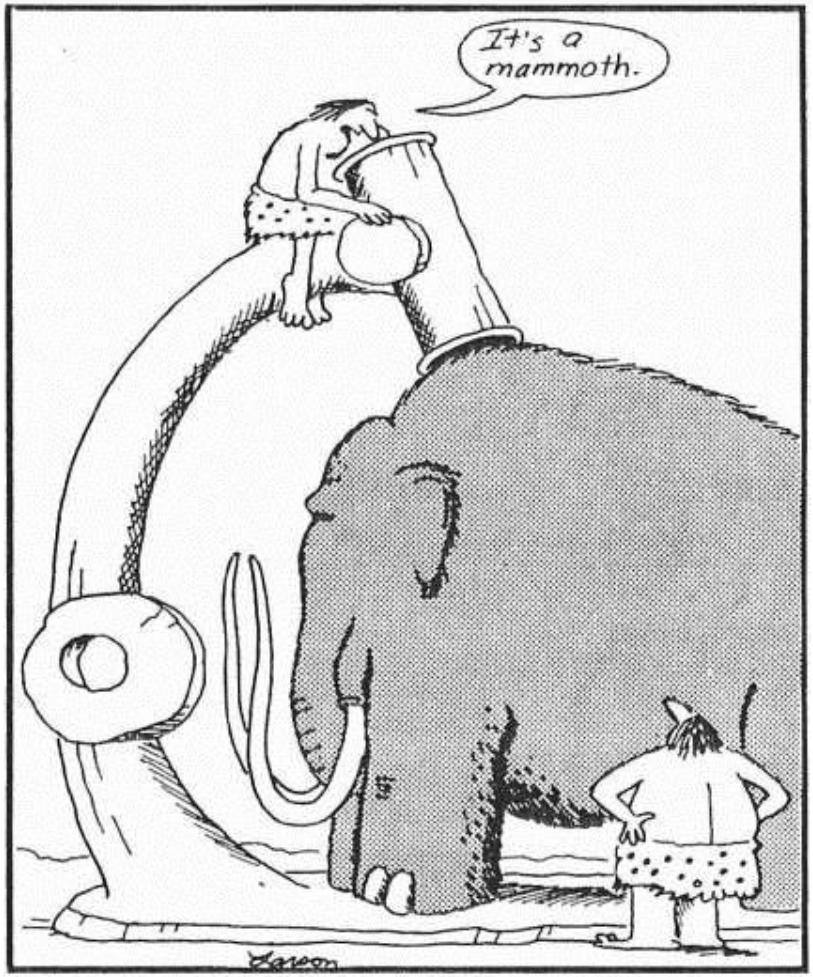
Molecular Imaging

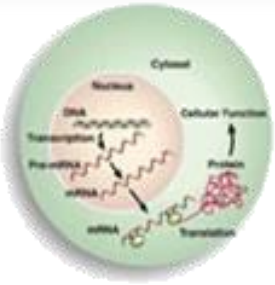
The Big Picture

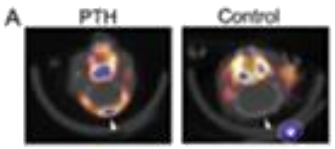


Early Microscope

Molecular Imaging

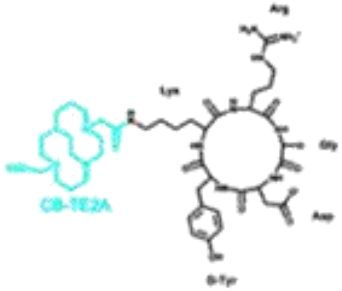
Pharmaceutical R&D



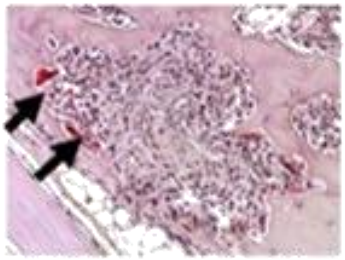


Pre-clinical imaging

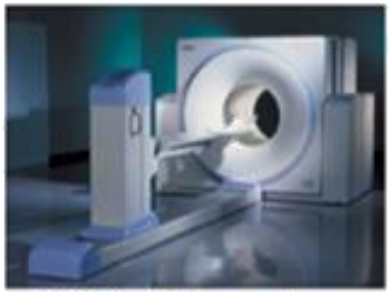
Targeting cell receptors and processes



Chemistry



Histological Validation



Clinical Imaging

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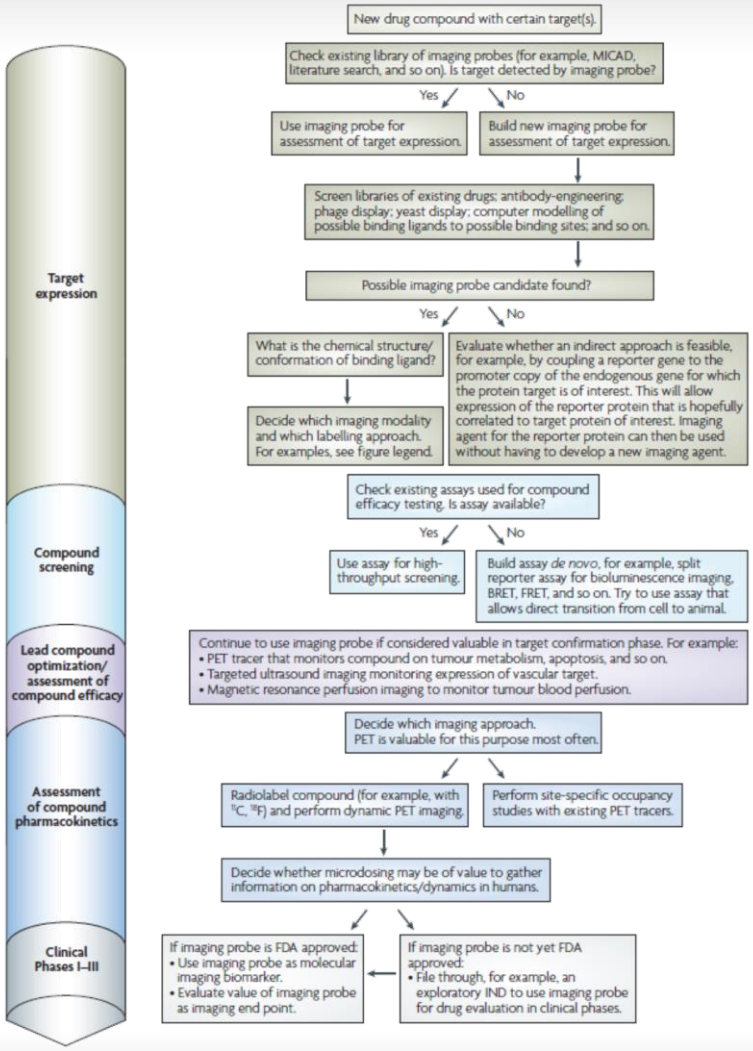
Molecular Imaging

Pharmaceutical R&D

- In vivo biological characterization
- Pharmacokinetics measurements
- Imaging biomarkers in clinical trials
- Prediction of treatment response
- Improve drug development successes
- Discovery of novel diagnostic imaging agents
- Improved diagnostics
- Improved patient outcomes
- Individualized treatment plans
- Identification of appropriate therapies
- Enhancement of resource utilization → saving money

Pharmaceutical R&D



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Ideal Tracer for Protein Synthesis?

- Pathway independent transport
- Uptake regulated by ribosomal activity (Metabolic Trapping)
- Ribosome entry independent from RNA-sequence and co-factors

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Biotin-Puromycin-Oligonucleotide

- Pathway independent transport
  - AMP transporter (permanently active in living cells)
- Uptake regulated by ribosomal activity
  - Bidirectional transport → steady state without incorporation
- Ribosome entry independent from RNA-sequence
  - No co-factors needed for coordination in ribosome A-site

Optical Imaging Systems

In-Vivo MS FX Pro

- front-illuminated 4 MP CCD
- ^{18}F, ^{90}Y, ^{99m}Tc
- QC settings: iTLC analysis
- Cell culture simulation on 12-well-plates
- In vivo imaging of ^{90}Y mAb in mouse

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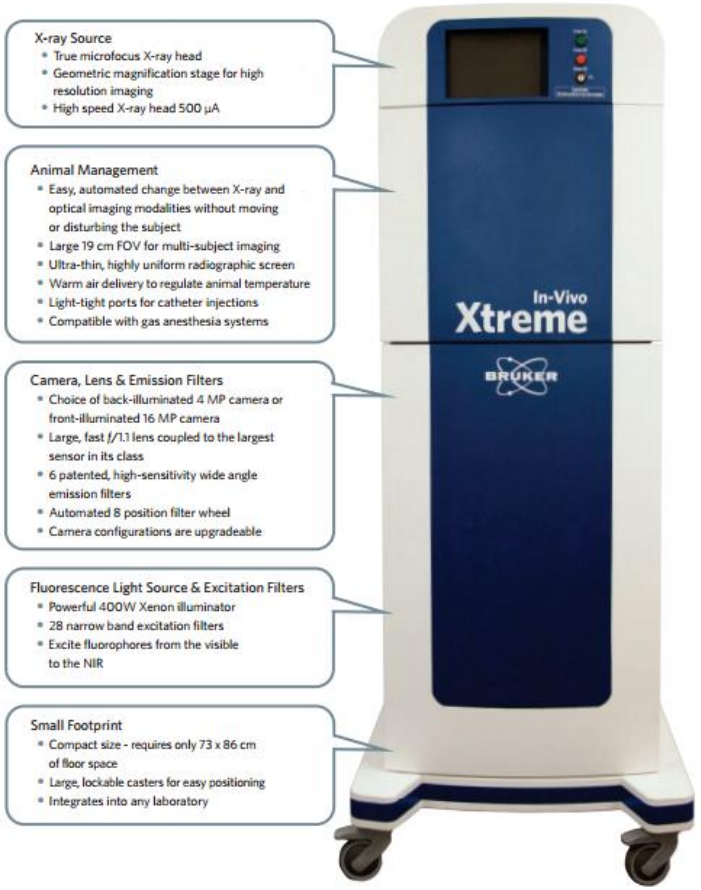
Optical Imaging Systems

In-Vivo EXTREME



- back-illuminated 4 MP CCD
- ^{18}F, ^{90}Y, ^{99m}Tc
- QC settings: iTLC analysis
- Cell culture simulation on 12-well-plates
- In vivo imaging of ^{90}Y mAb in mouse

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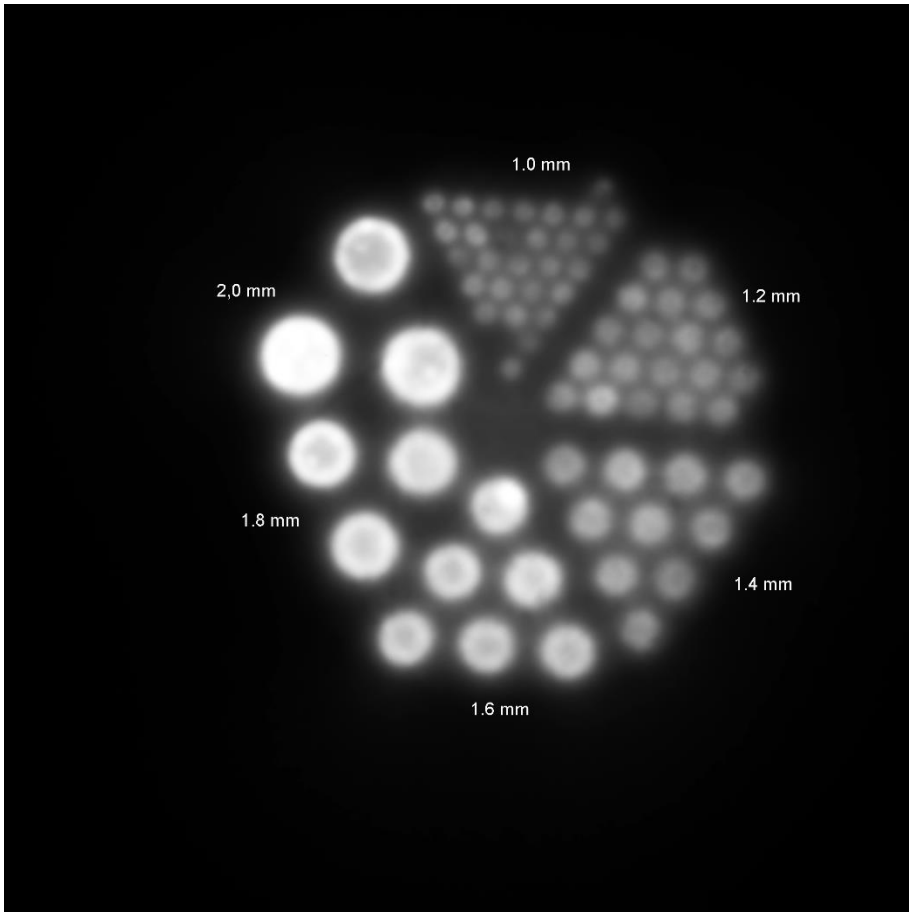
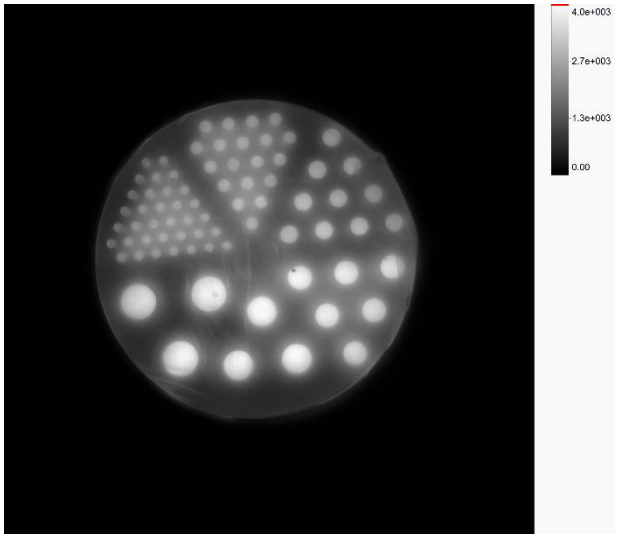
SPATIAL RESOLUTION

RADIOISOTOPIC PHOSPHOR SCREEN



Radio-Isotopic Phosphor Screen 15 sec exposure

- Derenzo Phantom
- 20 MBq 90Y
- Exposure: 15 sec



Cerenkov Imaging 5 sec exposure

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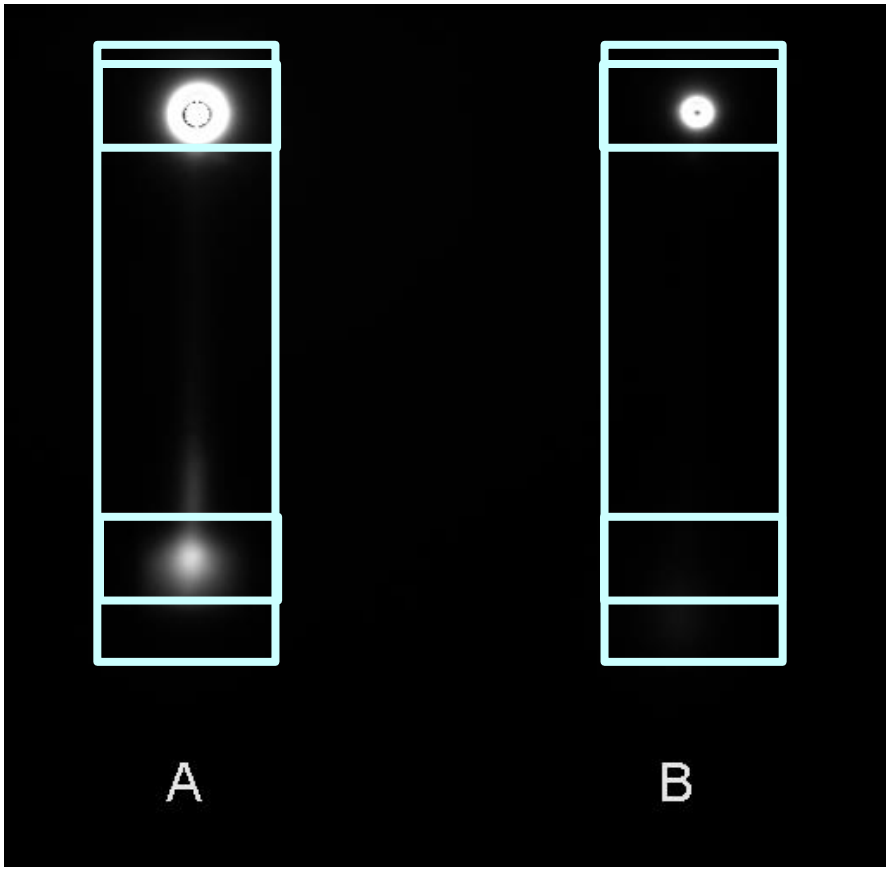
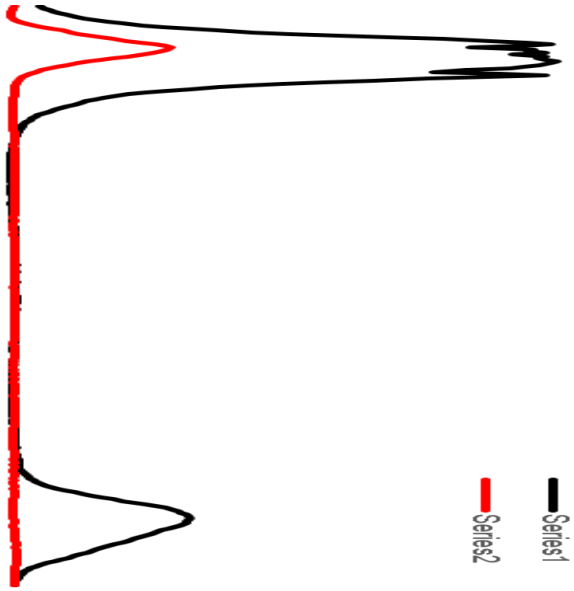
QUALITY CONTROL

iTLC





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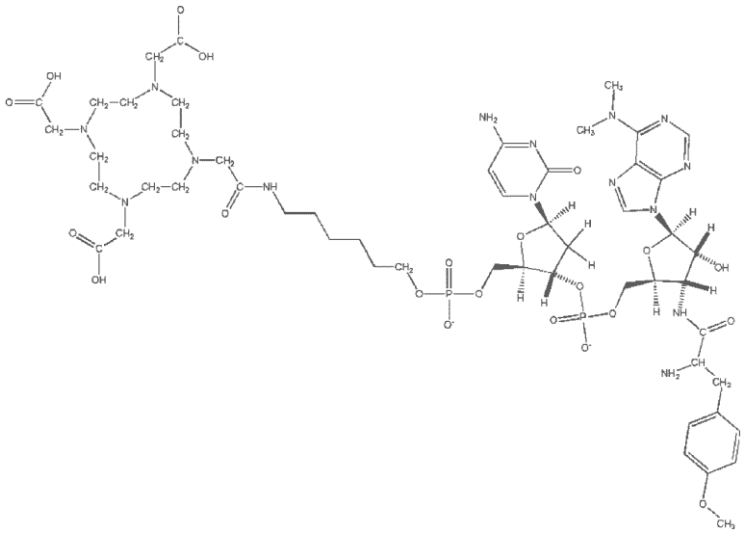
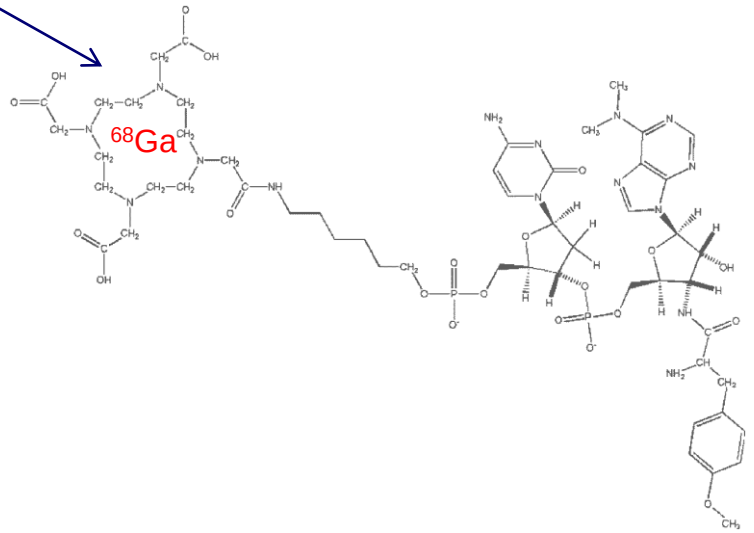
^{68}Ga-labeling of DOTA-Pur



^{68}Ga in 0.2 M HCl

HEPES 1M, pH = 3,0
110°C, 20 min
Purification on StrataX



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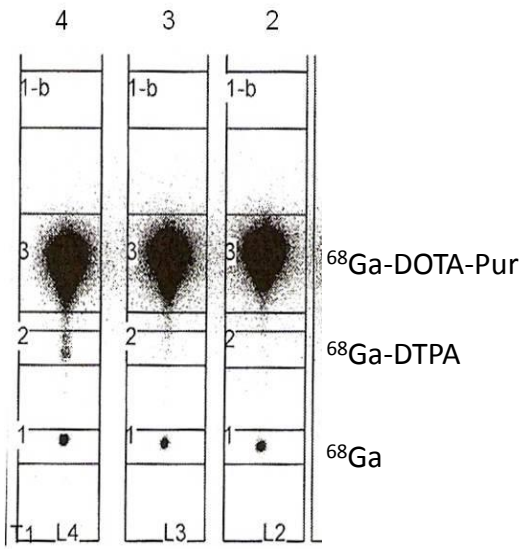
^{68}Ga-labeling of DOTA-Pur



- RM was purified on Strata X-columns following the protocol for purification of ^{68}Ga-DOTATOC (over all yield ≥ 93±2.8%)
- for injection EtOH was removed at 95°C in stream of air and the dried product resolved in 0,5M PBS (pH = 6,8)

Specific activity of [^{68}Ga]-DOTA-Pur : 1.5±0.1 GBq/μmol

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In vitro uptake and protein incorporation

Uptake in tumor cells (DU145) after 2 hours

2.0 ± 0.1% applied dose per 1×10^6 cells

Uptake in normal skin fibroblasts (BJ)

0.2 ± 0.1% applied dose per 1×10^6 cells

Tumor / Normal cells = 10:1

Protein incorporation in both cell lines was ≥ 93% of Uptake

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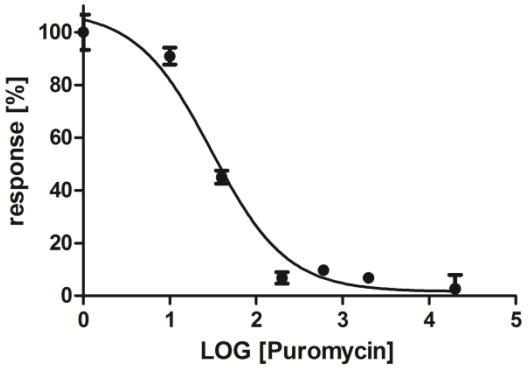
Inhibition of [^{68}Ga]-DOTA-Pur incorporation into proteins

Competitive Inhibition:

(Competition for ribosomal A-site)

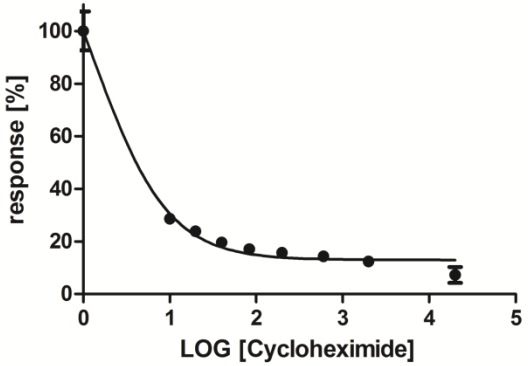
Puromycin dihydrochloride 10pmol/well and 20 nmol/well + 84 pmol/well of [^{68}Ga]-DOTA-Pur



Non-competitive Inhibition:

(blocking translational elomgation)

Cycloheximide 10pmol/well and 20 nmol/well + 84 pmol/well of [^{68}Ga]-DOTA-Pur



Cycloheximide: 3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl] glutarimide

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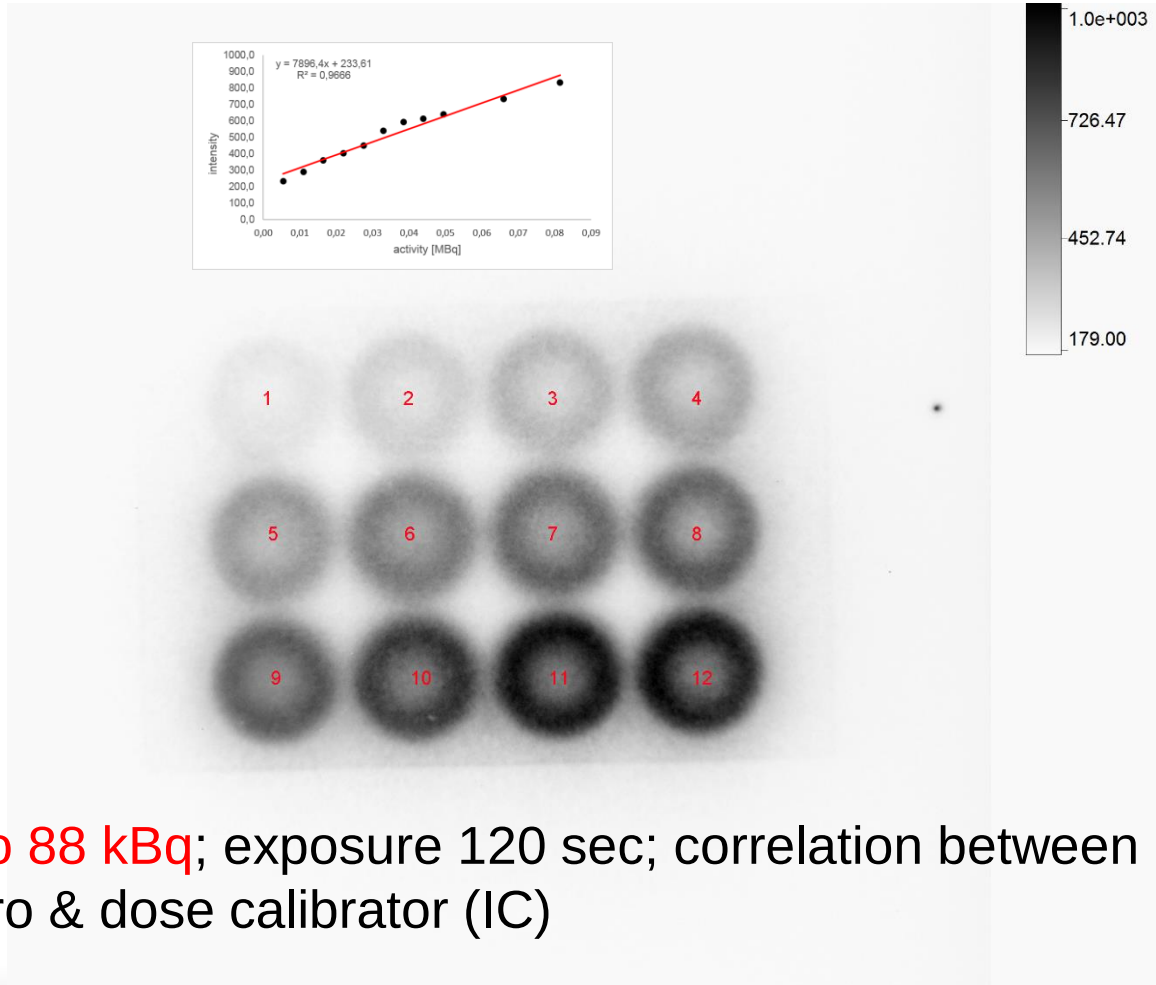
SENSITIVITY

LOW ACTIVITY – CELL CULTURE



5.5 kBq to 88 kBq; exposure 120 sec; correlation between in-vivo MS FX Pro & dose calibrator (IC)

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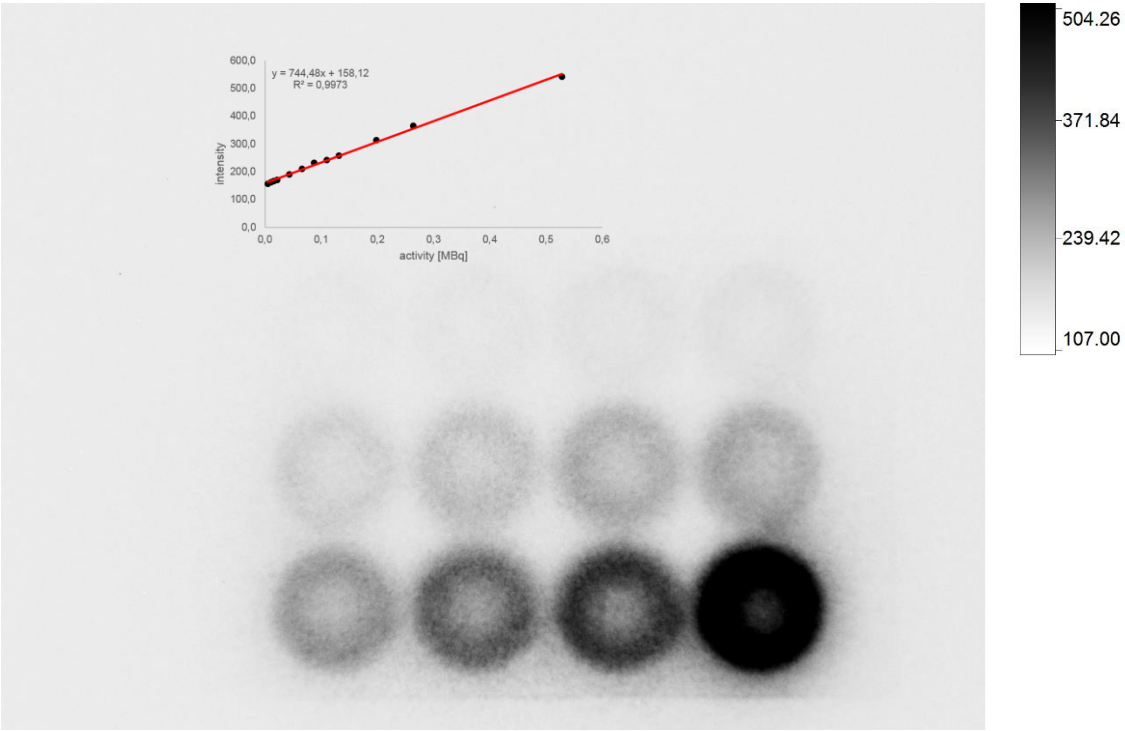
SENSITIVITY

INTERMEDIATE AKTIVITY – *EX VIVO* DIAGNOSTICS



5.5 kBq to 528 kBq; exposure 10 sec; correlation between in-vivo MS FX Pro & dose calibrator (IC)

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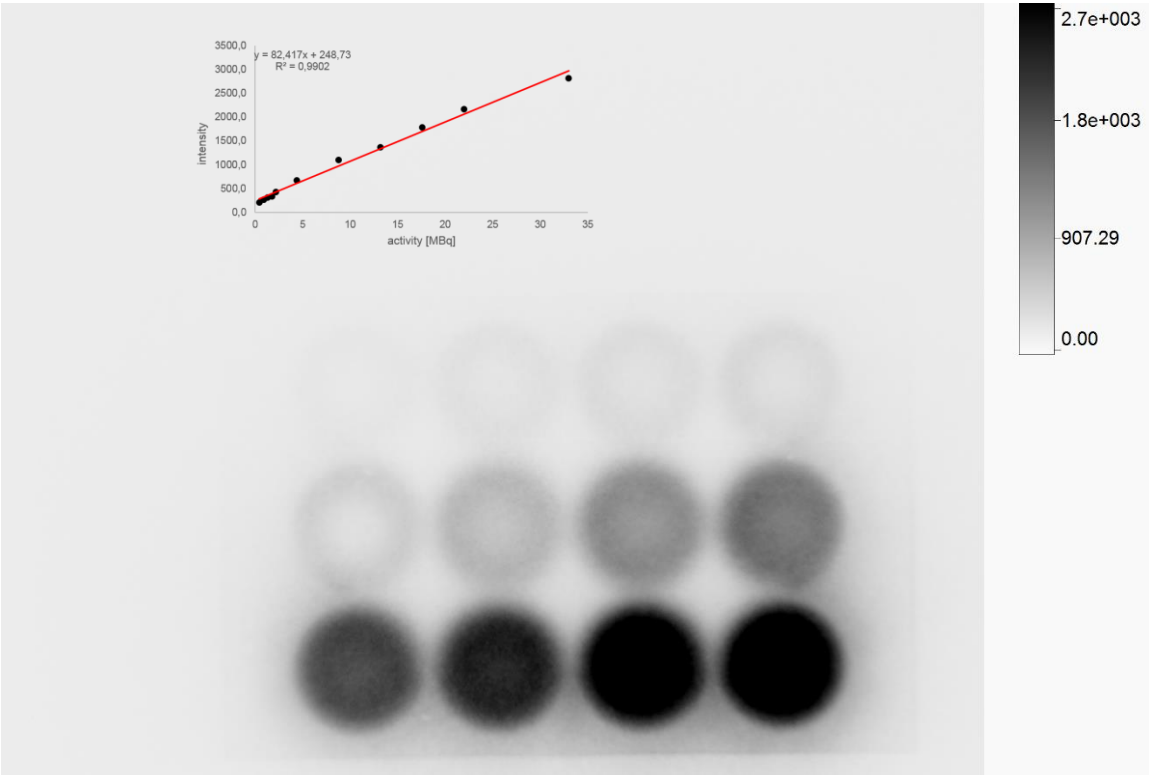
SENSITIVITY

HIGH AKTIVITY – *IN VIVO* DIAGNOSTICS



440 kBq to 33 MBq; exposure 5 sec; correlation between in-vivo MS FX Pro & dose calibrator (IC)

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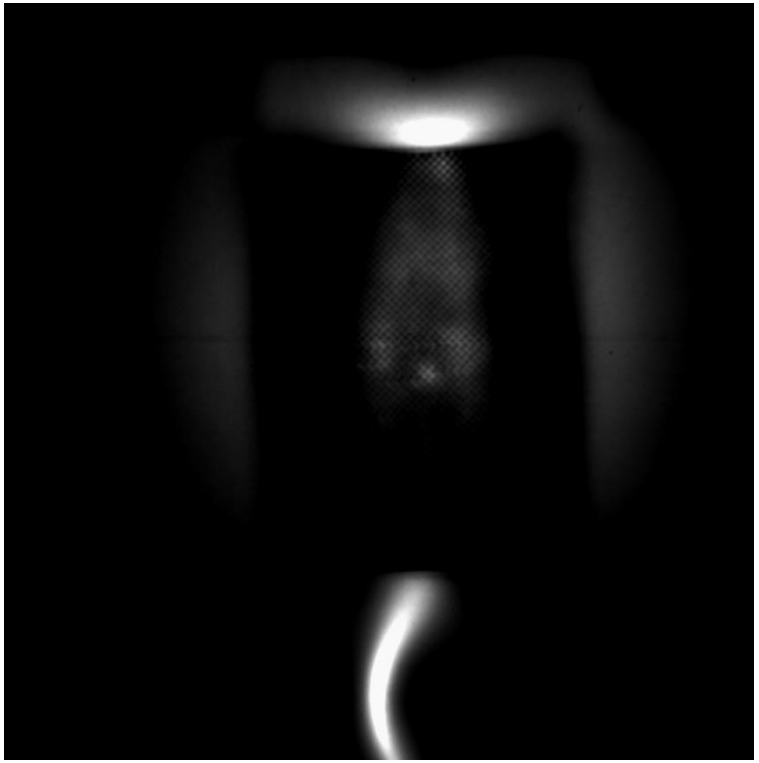
IN-VIVO SCREENING

^{90}Y-DOTA-hR3; 25MBq; 24h p.i.

Suppine position; 5 min exposure; 1% isoflurane



prone position; 5 min exposure; 1% isoflurane

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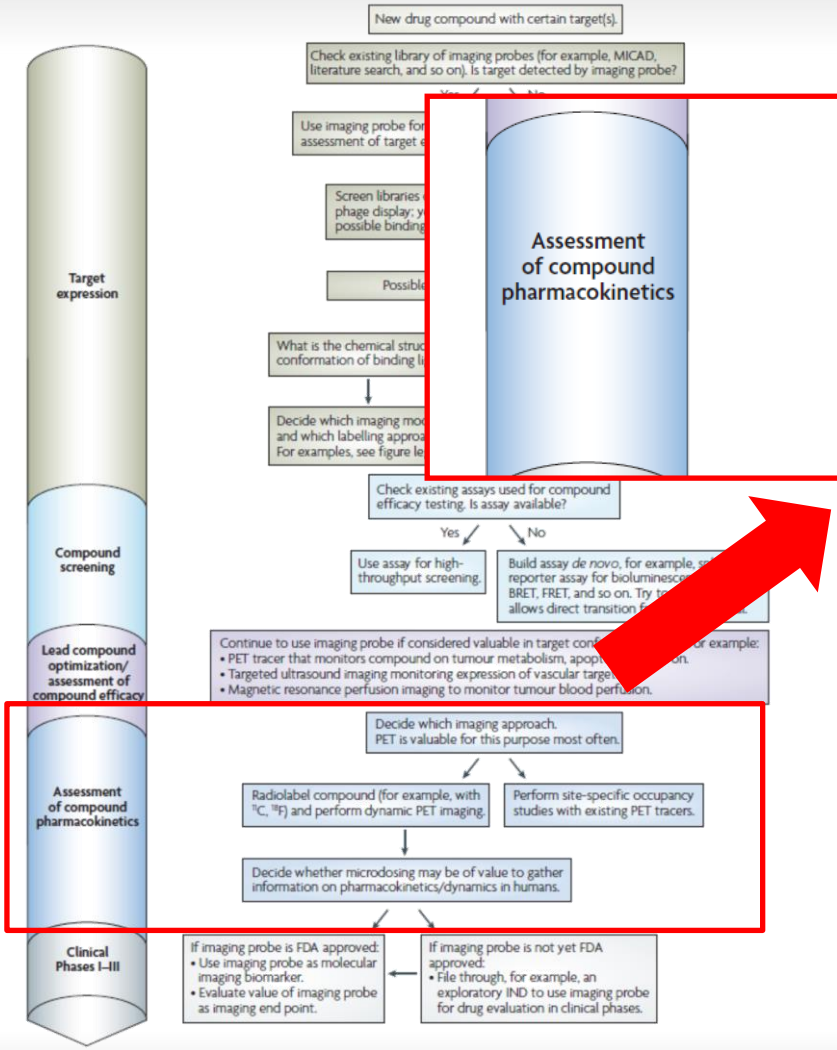
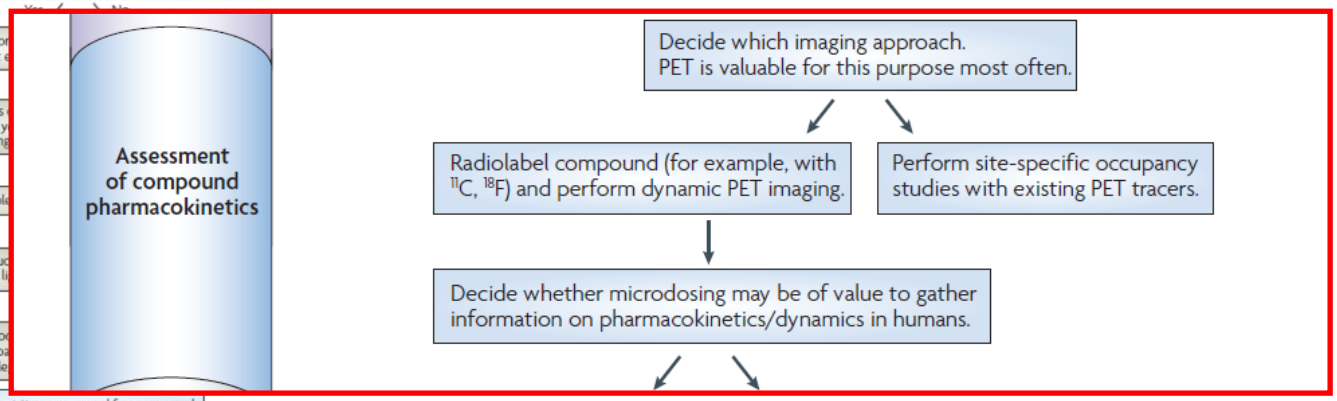
Pharmaceutical R&D





Most efficient and cost effective method in assessment of pharmacokinetic data for new compounds

→ dynamic PET accquisition

→ followed by quantitative image analysis and

→ Kinetic Modelling

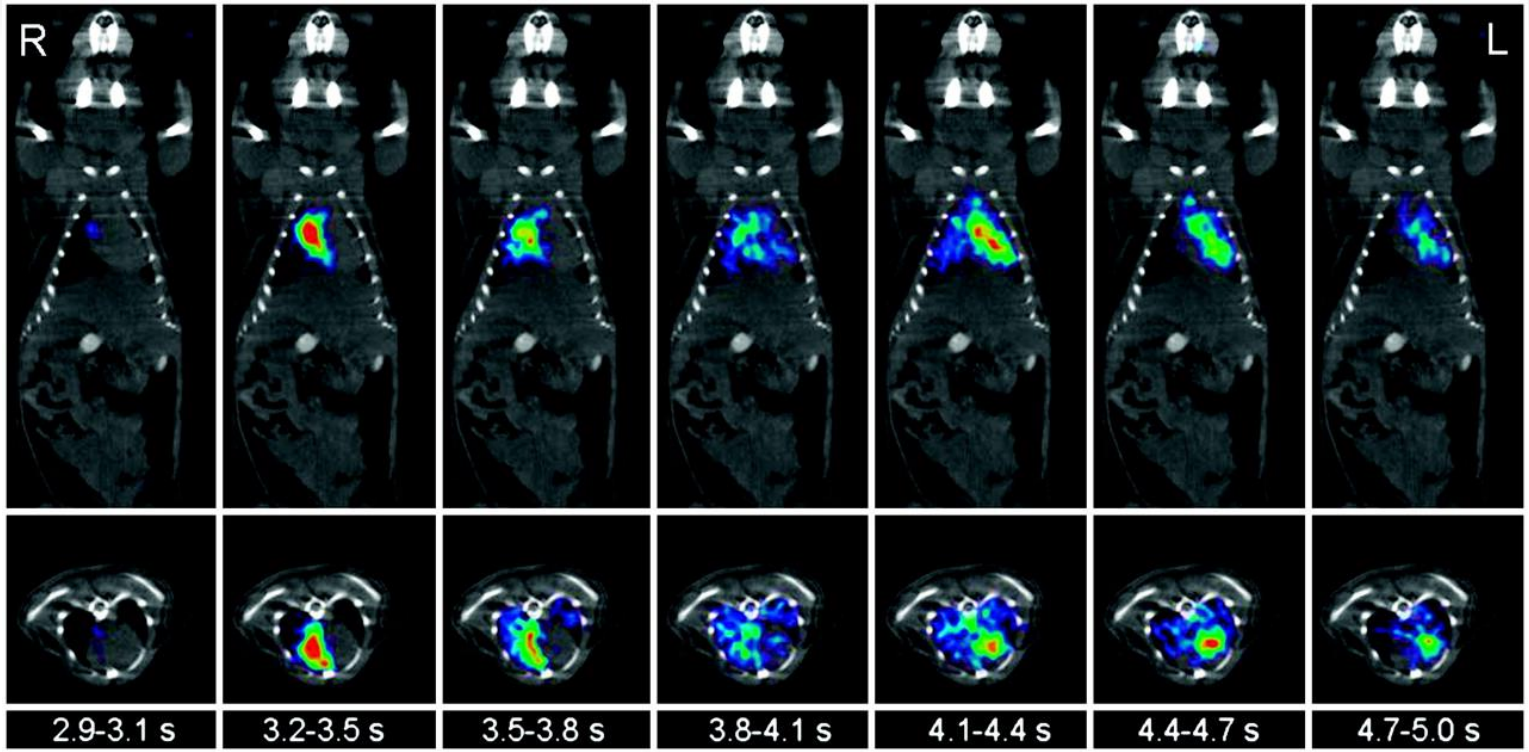
Positron Emission Tomography

in vivo PET imaging

- Tomographic imaging modality
- Functional information
- Non-invasive
- High sensitivity – pmol
- Short lived radioisotopes
- Large variety of labeled compounds
  - Energy metabolism (FDG)
  - Amino acid metabolism (^{18}F and ^{11}C labeled AA)
  - Protein biosynthesis (DOTA conjugated puromycin analogues)
  - Neurotransmitter
  - Receptor imaging (neuro, onco,…)
  - Hemodynamic parameters
  - Gene expression
  - Cell tracking (stem cells)
- 0.8 – 1.2 mm spacial resolution
- 6-10 % sensitivity
- temporal resolution < 0.5 sec
- QUANTIFIABLE

Positron Emission Tomography

Temporal resolution



Consecutive 0.3-s frames show passage of tracer bolus through RV cavity, lungs, and LV chamber of mouse on coronal and transverse slices. Times are those after start of image acquisition / injection. For better anatomic orientation, PET scan is overlaid with coregistered CT scan.

Michael C. Kreissl et al. J Nucl Med 2006;47:974-980

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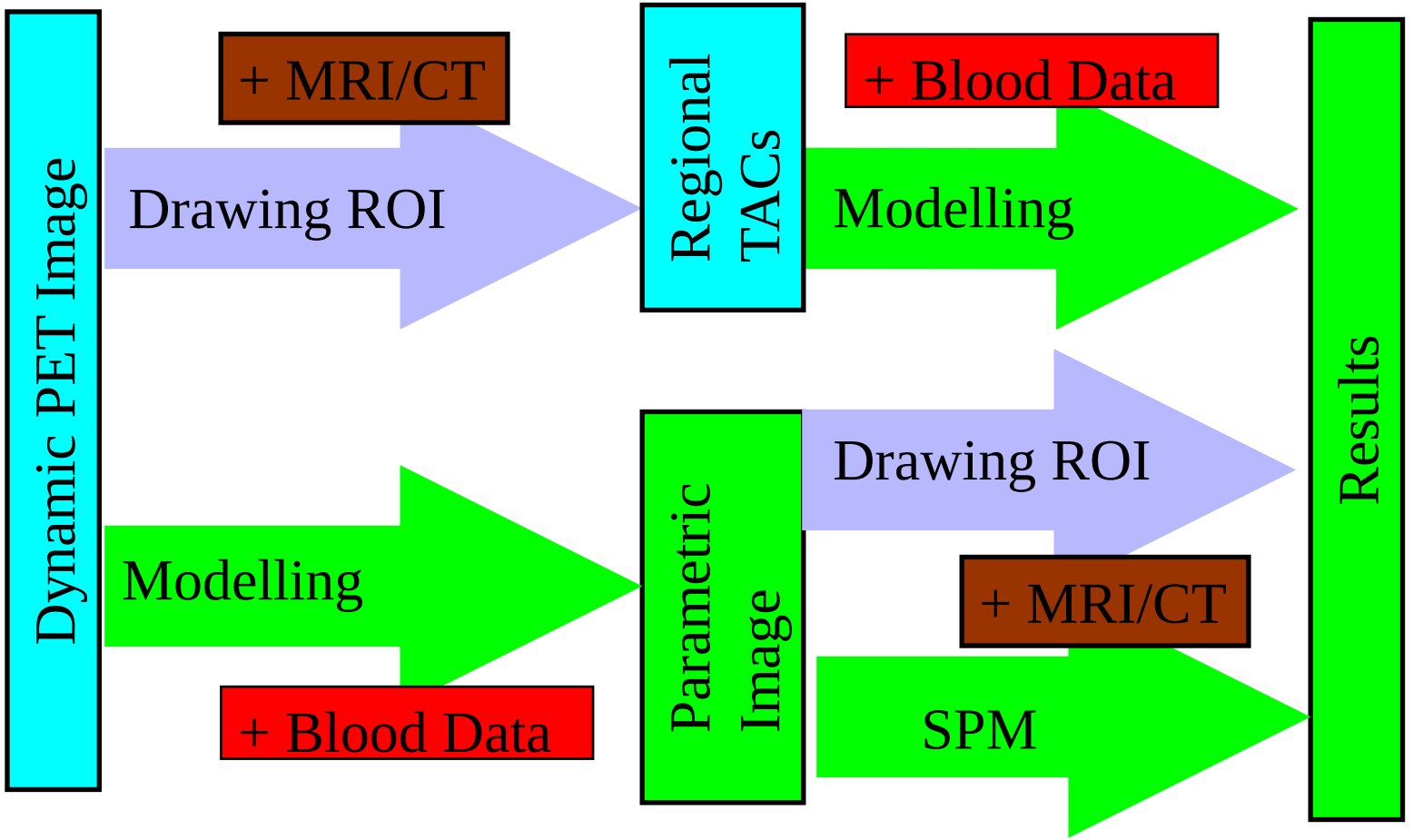
Dynamic PET Scan

Steps of Analysis



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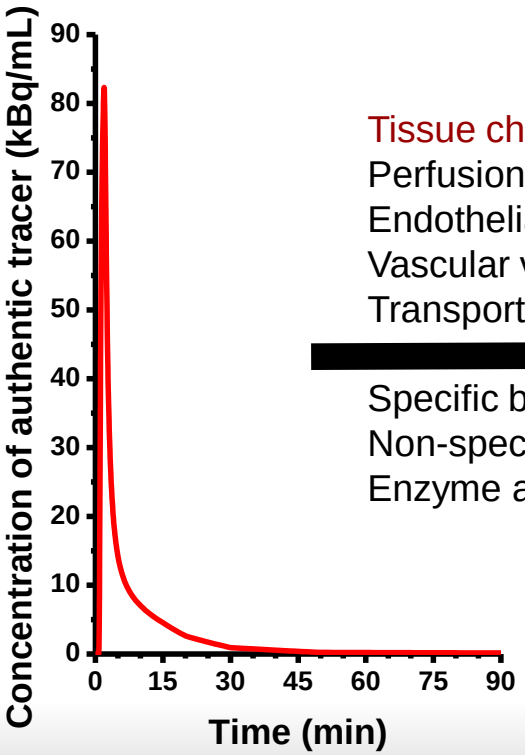
Dynamic PET Scan

Time Activity (concentration) Curves (TACs)

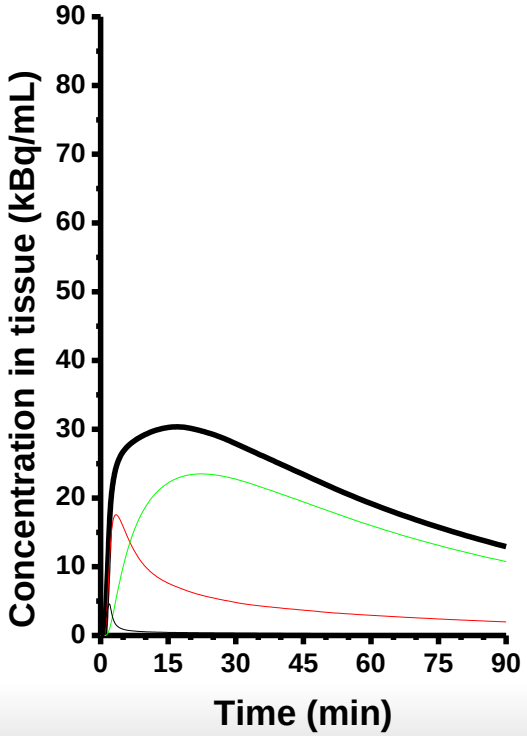
TAC of tracer concentration in arterial blood



Tissue characteristics:
Perfusion
Endothelial permeability
Vascular volume fraction
Transport across cell membranes

Specific binding to receptors
Non-specific binding
Enzyme activity

TAC of concentration in tissue measured by PET scanner



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μPET-imaging in tumor bearing rats

- Focus 120 small animal PET (Siemens/Concorde)
- Anaesthetized (3% Isoflurane in oxygen) animals (230-270 g body weight) were placed tail first supine in the field of view
- 20–25 MBq of [^{68}Ga]-DOTA-puromycin in 0.4-0.7 mL 0.9% NaCl-solution via tail vein
- TAC: varying time frames (1-5 min), measuring interval 45 minutes, PET list-mode, histogrammed in 12 frames for reconstruction

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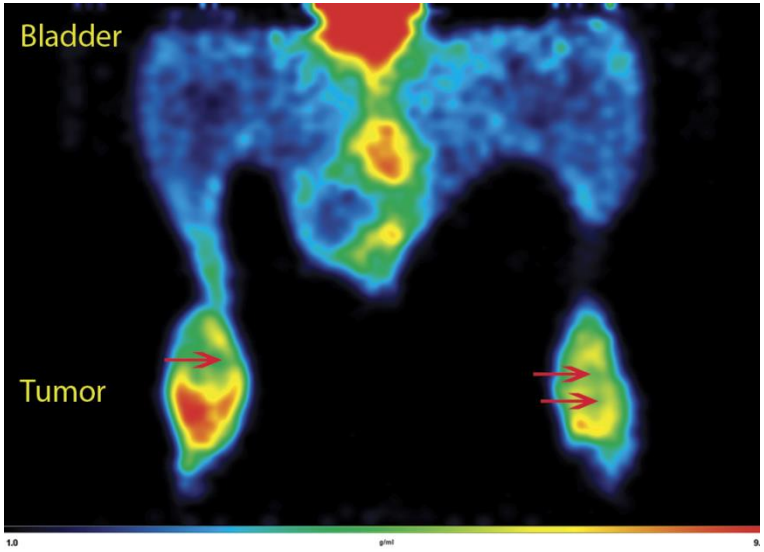
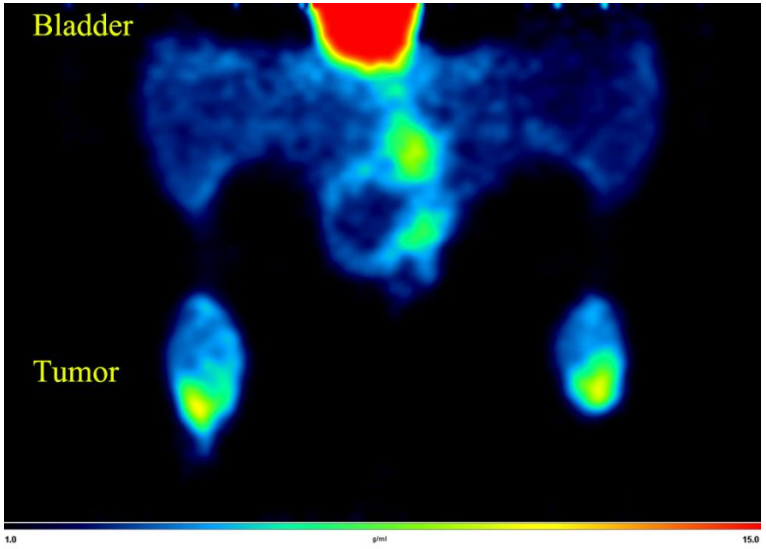
μPET-imaging in tumor bearing rats



Summarized μPET-image, coronal slice, colors expressed as SUV (0-9) (3-20 min, dynamic scan) of AT1 tumors on hind feet of Copenhagen



Summarized μPET-image, coronal slice, colors expressed as SUV (0-12) (3-20 min, dynamic scan) of Walker carcinomas on hind feet of CD rats and

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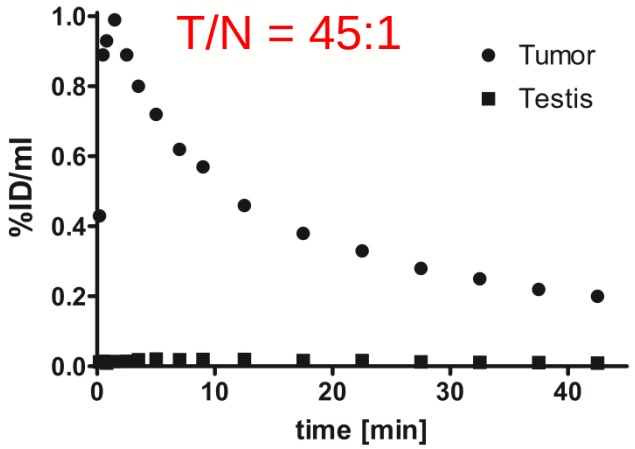
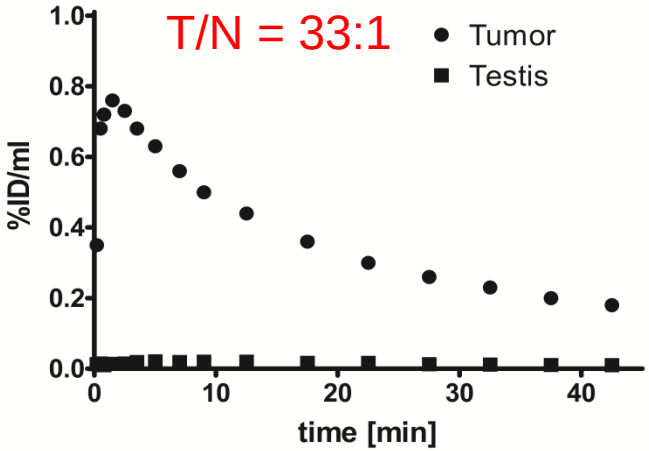
μPET-imaging in tumor bearing rats





0-120 min TAC´s of tumor and testis (reference) of AT1 tumor on hind feet of Copenhagen rats; steady state reached after approximately 65 minutes

Walker Carcinoma: Obtained TAC looks like cell uptake and slow wash out caused by retention of the [^{68}Ga]-DOTA-Pur as aa-tRNA-analogon within eukaryotic ribosomal A-site.

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Parametric Image

- **Dynamic information is converted to functional information with dedicated software**
  - **Not a series of scans (smaller file size)**
  - **image voxel value = the value of the studied physiological parameter (perfusion, glucose consumption, receptor density)**

- **More sophisticated analyses possible**
  - **requires careful evaluation of alternative models before choosing the right model**

PET quantification

- Radioactivity concentration (tissue or plasma) can be easily converted to drug concentration:

$$drug\ concentration = \frac{radioactivity\ concentration\ [kBq/cm^3]}{specific\ radioactivity\ [GBq/\mu mol]}$$

- Drug concentration is used to measure tissue function *in vivo:* e.g. perfusion, glucose consumption, receptor density, enzyme activity, etc.

μPET/MRI & SPECT/CT

- ^{68}Ga-DOTA-Pur

  20 minutes static PET images of BCG infected (3 months prior to study) mice were acquired from 30 to 50 minutes after i.v. bolus injection of 5 to 8 MBq ^{68}Ga-DOTA-Pur followed by a spoiled GRE 3D MRI sequence
- ^{18}FDG

  20 minutes static PET images from 40 to 60 minutes after i.v. bolus injection of 5 to 8 MBq ^{18}FDG followed by a spoiled GRE 3D MRI sequence
- ^{67}Ga-Citrate

  24 h post injection multiple pinhole SPECT followed by a high resolution CT

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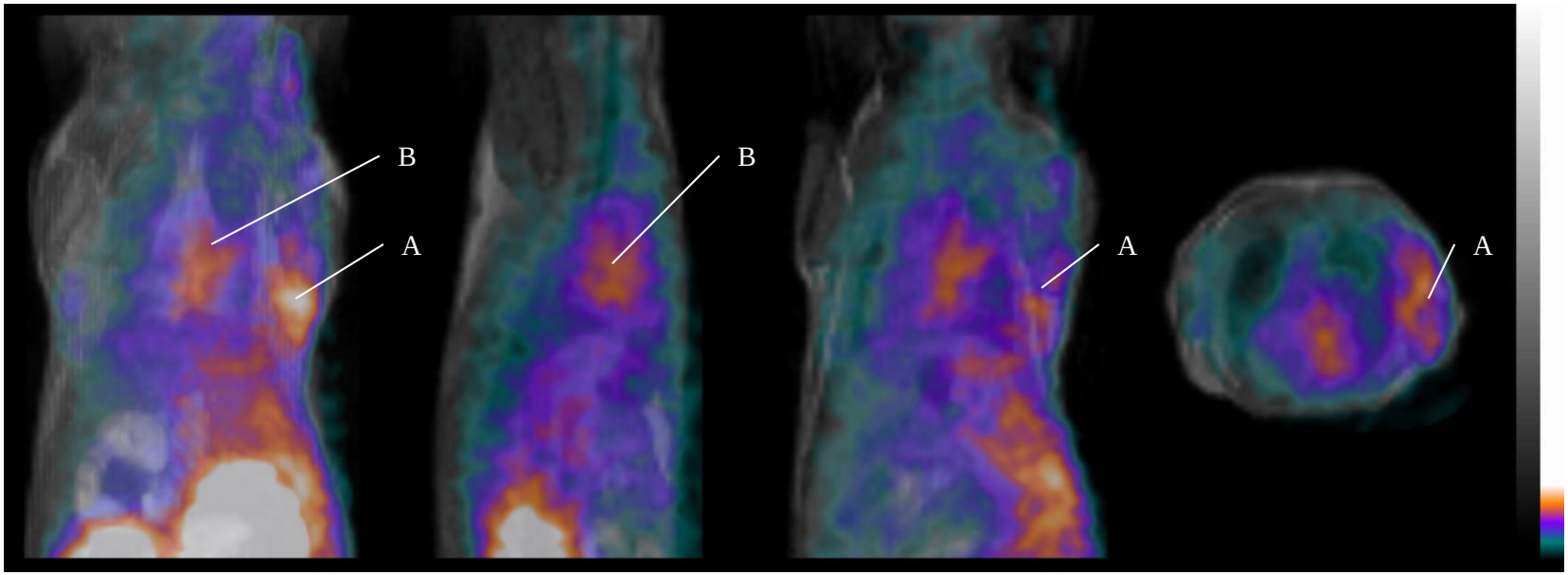
^{18}FDG PET/MRI



^{18}FDG µPET/MRI of BCG infected mouse; A) BCG infection in armpit; B) prefunded heart

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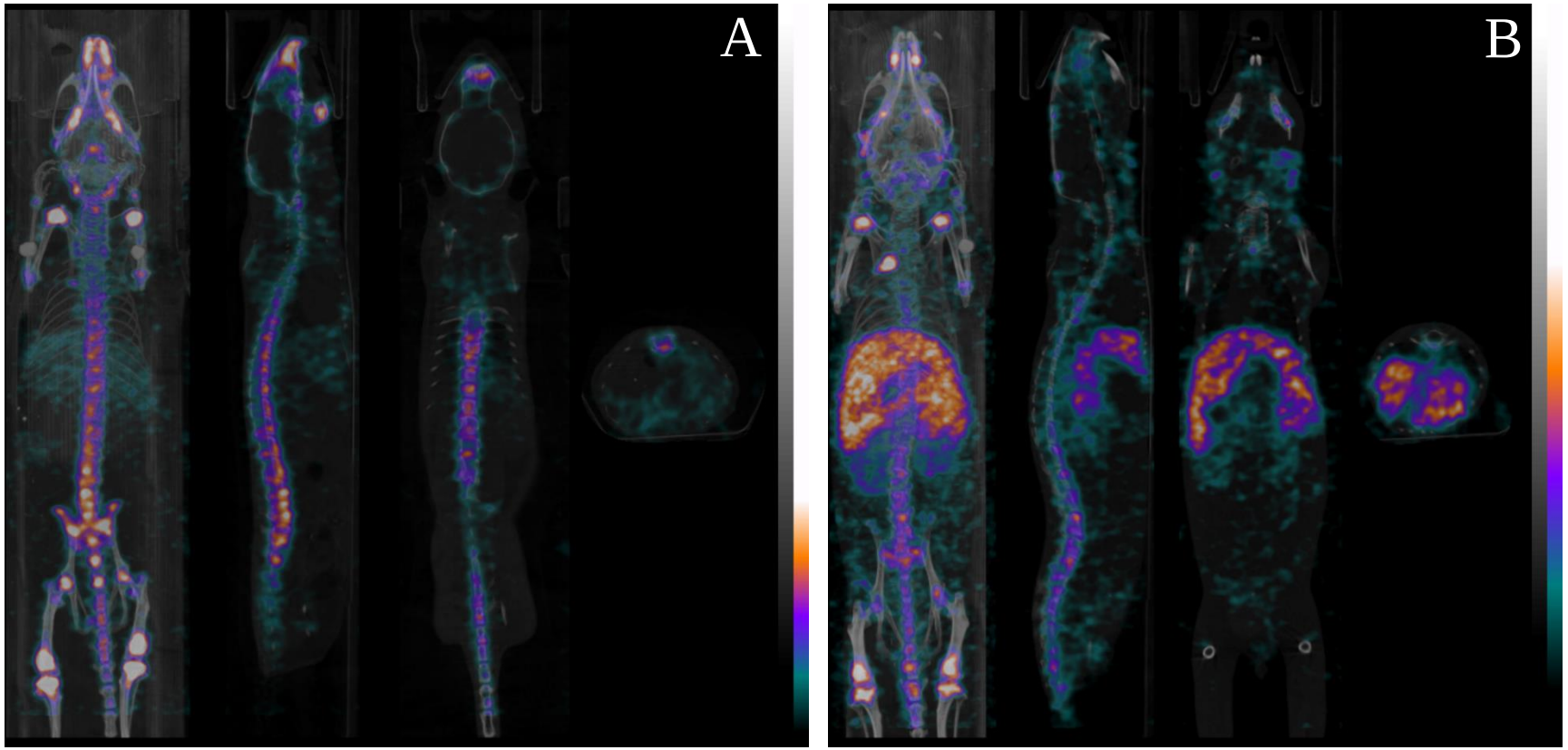
^{67}Ga Citrate SPECT/CT



^{67}Ga-citrate SPECT/CT of healthy and BCG infected mouse;
A) healthy; B) BCG infected

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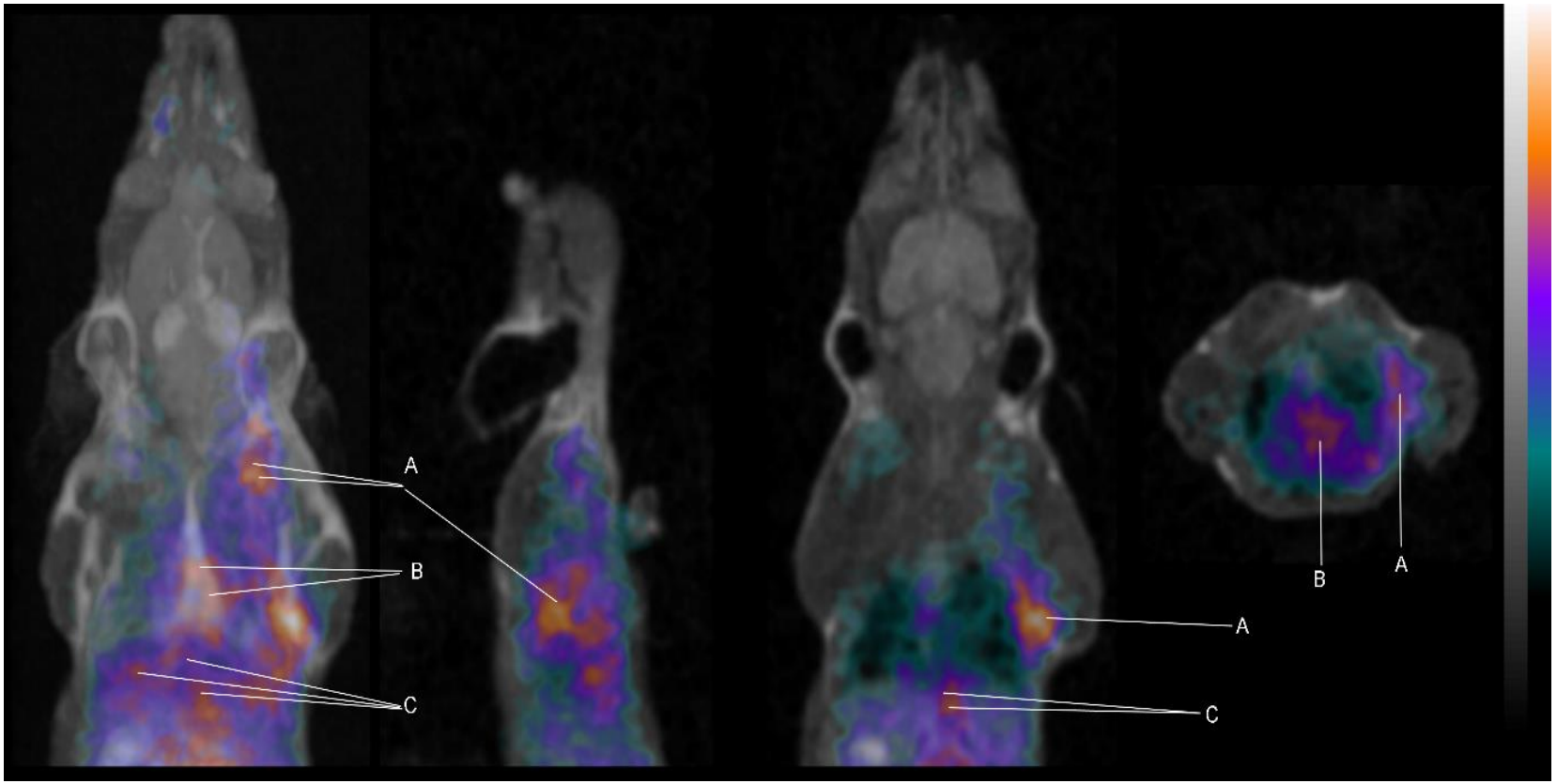
^{68}Ga DOTA-Pur PET/MRI



^{68}Ga-DOTA-Pur µPET/MRI of BCG infected mouse; A) BCG infection in armpit; B) prefunded heart; C) BCG foci in liver

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Histology

- Stainings: Ziehl-Neelsen for BCG; H&E for inflammation
- Granulomae were found near vessel walls in the armpit & inflammation at vessel walls in BCG infected area and lymph nodes correlating with ^{18}FDG uptake
- systemic mycobacteriosis was seen without inflammation in spleen and liver (single granulomae in liver) correlating with ^{68}Ga-DOTA-Pur uptake
- Spearman's correlation test
  $p<0.089$ for ^{68}Ga-DOTA-Pur and ZN level
  $p>0.2$ for ^{18}FDG and ZN level

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Summary

- Molecular imaging modalities can be utilized during all steps of radiopharmaceutical development
- Implementation of „alternative“ visualization techniques can save time and material
- Advanced analysis of dynamic PET scans enables absolute quantification of biochemical processes in various tissues
- Compared to *ex vivo* analysis advanced dynamic PET imaging saves up to 80% of animals and reduces lab-time to less than 15%

→ Implementation of molecular imaging in (radio)pharmaceutical R&D ultimately saves time and money and delivers translational data for planning and conducting of clinical trials

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