Gallium-68 in infection imaging

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OUTLINE

Some words about infection .....

68-gallium & infection imaging

Preclinical application
(i.e. ^{68}Ga-labeled UBI)

Infection

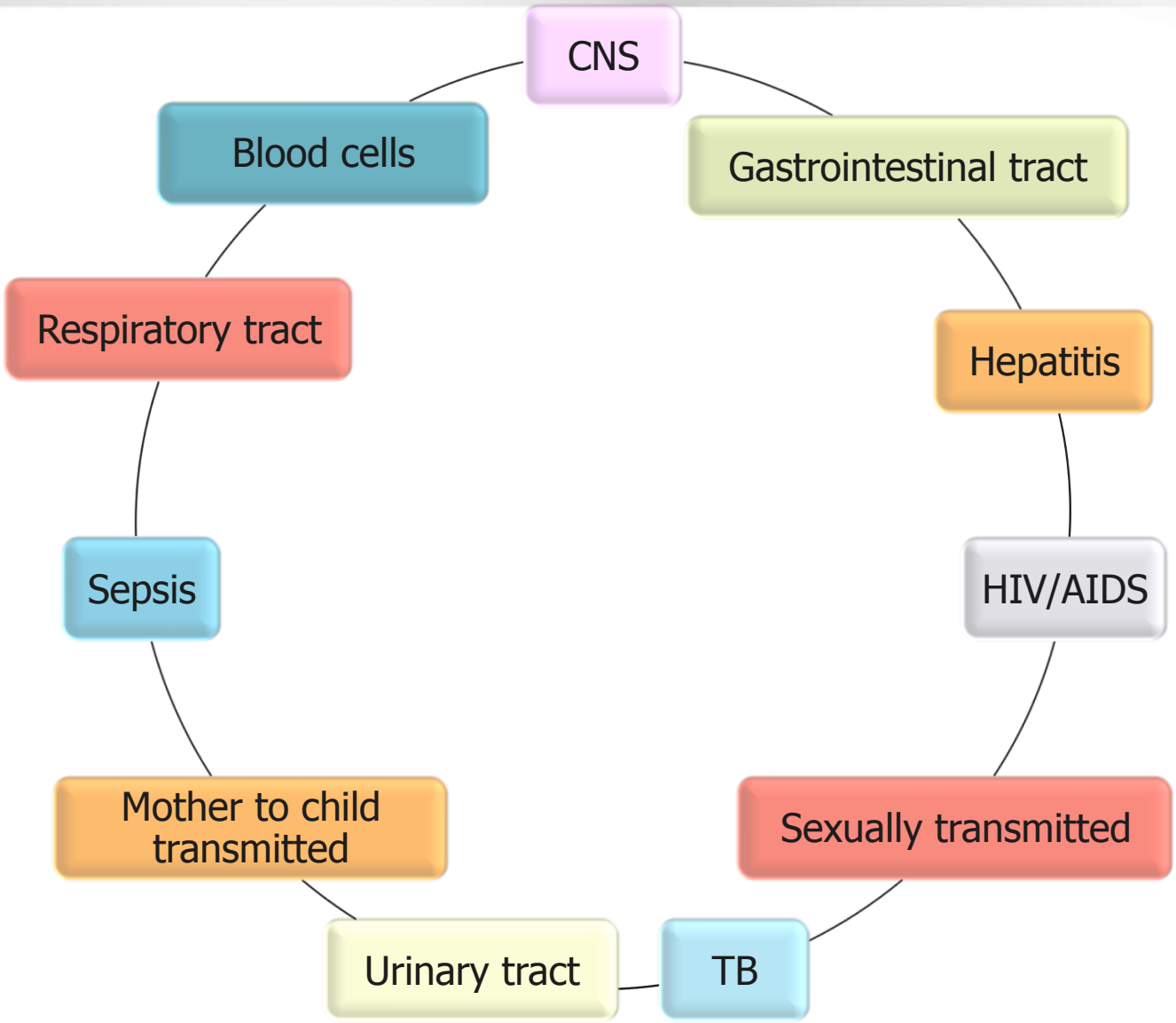
- **Infectious** diseases are caused by microorganisms (microbes): viruses, bacteria, and parasites etc.
  - the hosting organism provides the resources necessary for the infecting species to multiply

- **Inflammation**
  The complex biologic response of tissues to harmful stimuli.

Infection / inflammation are still a major cause of global morbidity and mortality despite advances in both diagnosis and treatment

Sathekge et al, Eur J Nucl Med Mol Imaging 2009, **36**: 1176-1184.

Main infection routes and targets



Public impact of infectious diseases

Pasteur Institute Annual Report 2007

17 million deaths each year due to infectious diseases

(***one third of annual mortality for the entire planet***)

- 43% of deaths in developing countries only 1% in industrialized countries.

ACUTE RESPIRATORY DISEASES	AIDS	DIARRHEAL DISEASES	TUBER CULOSIS	MALARIA	MEASLES
3.8 million	**3.5 million**	**2.5 million**	**2.0 million**	**1.0 million**	**0.9 million**
• du to bacteria (pneumococcus) or viruses (influenza)	• based on 2005	• shigellosis, rotavirus, E.coli pathogens , cholera, thyphoid fever	• MDR-TB	• up to 500 million cases	• even though a vaccine exists

Go figure....

235 million doses of antibiotics are consumed annually, 20% to 50% of that use is unnecessary.[1,2]

Over 60% of *Staphylococcus aureus* cases in hospital ICUs in some countries are now resistant to methicillin, oxacillin, penicillin and amoxicillin.[3]

Up to 75% of antibiotics are prescribed for respiratory tract infections (RTI),[4] even though around **80% of RTI are caused by viruses.**[5]

MDR-TB treatment is ca. **500 times more** expensive than traditional TB treatment.[3]

1. Centers for Disease Control and Prevention, 2000, New England Journal of Medicine, December 28, 2000
2. Christ-Crain M, Jaccard-Stolz D, Bingisser R, Genday MM, Huber PR, Tamm M, Müller B. Effect of PCT-guided treatment on antibiotic use and outcome in lower respiratory tract infections: cluster-randomised single-blinded intervention trial. Lancet 2004; 363:600-607
3. Laxminarayan, R., A. Malani. Extending the Cure: Policy responses to the growing threat of antibiotic resistance. Washington, DC, Resources for the Future 2007.
4. Christ-Crain M, Stolz D, Bingisser R, Müller C, Miedlinger D, Huber PR, Zimmerli W, Harbarth S, Tamm M, Müller B. Procalcitonin guidance of antibiotic therapy in community-acquired pneumonia. Am J Respir Crit Care Med. 2006; 174: 84-93.
5. World Health Report. 2003


Infection Imaging

^{68}Ga-candidates for infection imaging

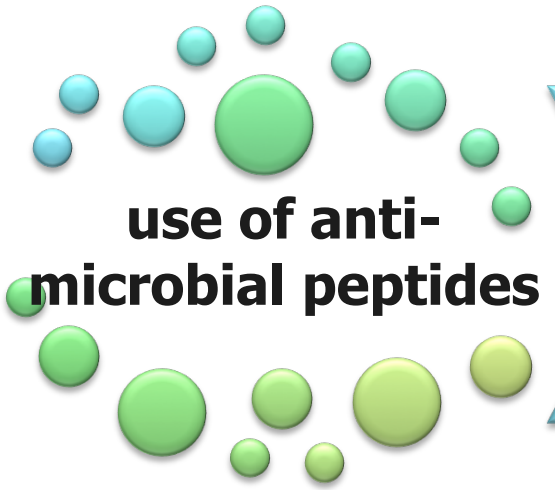
Since 1964 there are **over 750 publication** available involving ^{68}Ga (500 publication in the last decade)

SHEALY CN, ARONOW S, BROWNELL GL.
GALLIUM-68 AS A SCANNING AGENT FOR INTRACRANIAL LESIONS. J Nucl Med. **1964** Mar;5:161-7

Tracer	classification	published
^{68}Ga-CITRATE	Citrate (citric acid)	Hnatowich DJ, 1975 Kumar et al. 2009 Rizello et al. 2010 Nanni et al. 2009
^{68}Ga(3+)	Gallium(III)chloride	Maekinen et al. 2005
^{68}Ga-DOTA-VAP-P1	peptide	Ujula et al. 2009
^{68}Ga-TAFC, -FOXE	siderophores	Petrik et al. 2010
^{68}Ga-TF	apo-transferrin	Kumar et al. 2011
^{68}Ga-NOTA-UBI$_{29-41}$	Antimicrobial peptide	Ebenhan et al. 2012

Peptides as biomarkers for infection?

Identify and develop radioactive compounds specific for infections?



use of anti-microbial peptides

occurence naturally in most multicellular organisms

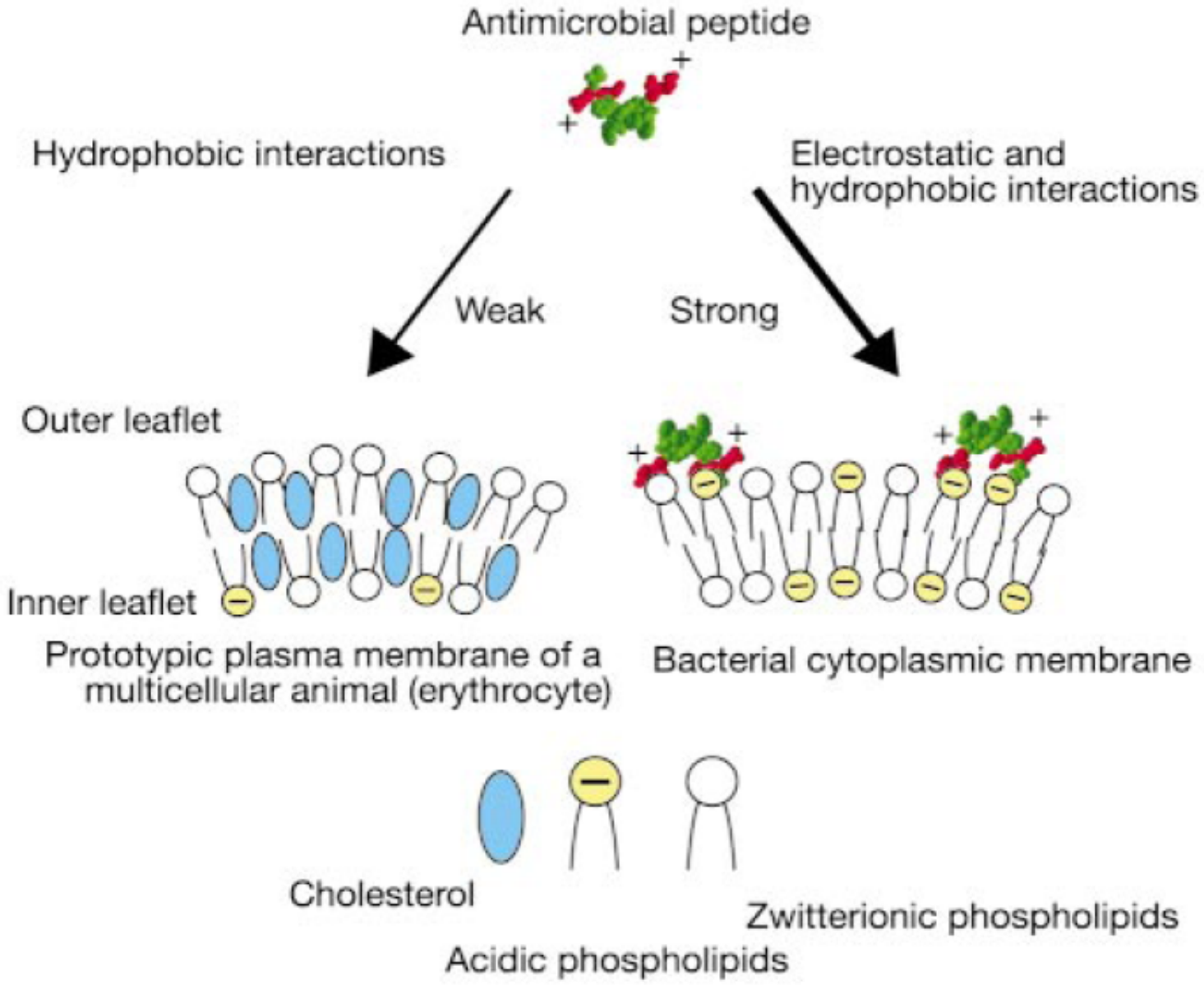
Peptide incorporates a *cation-rich domain*

Selective interaction with *anionic regions* of bacterial membrane expected

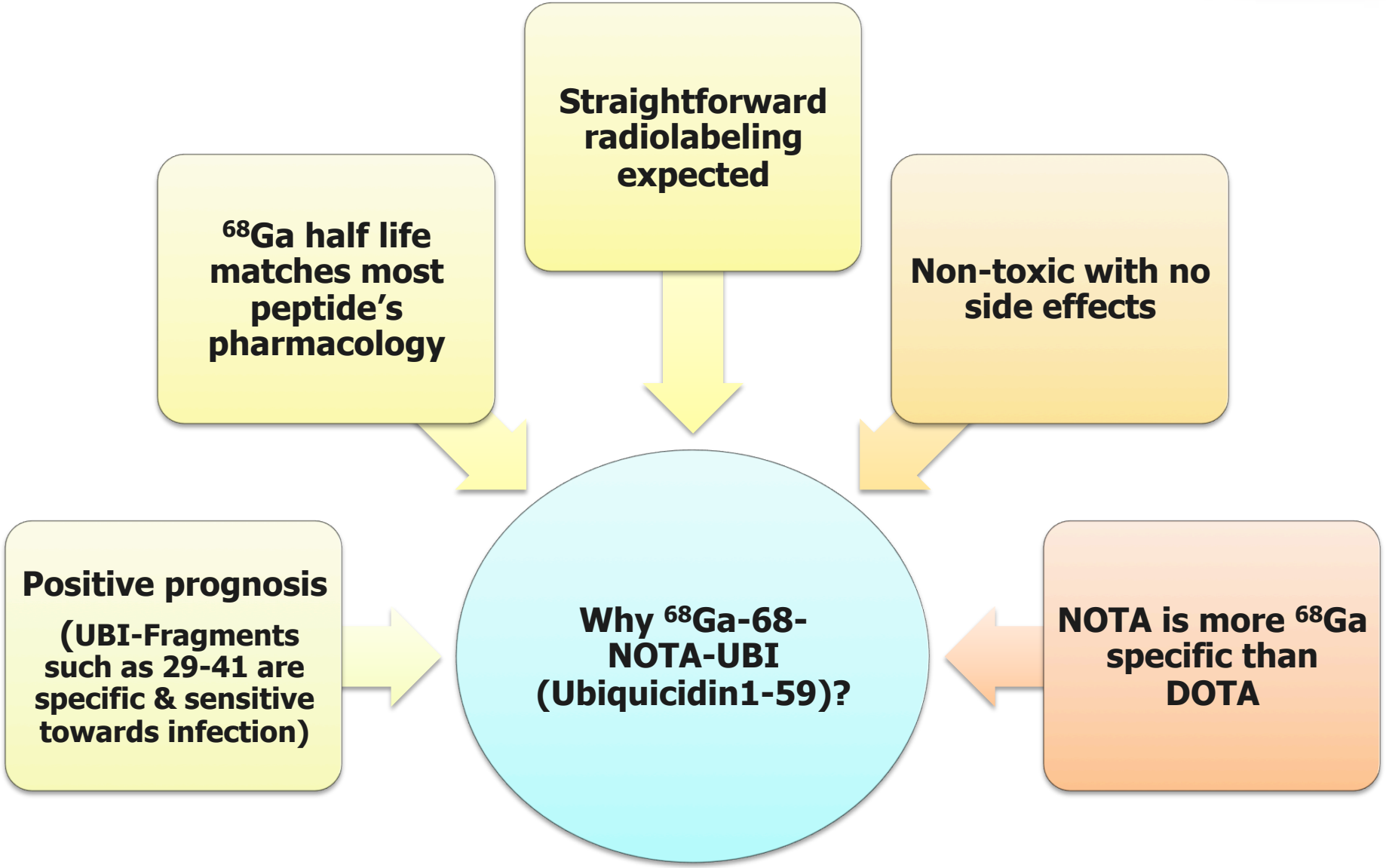
Differentiation of infection and inflammation?

Lupetti et al, Trends Biotechnol. 2003;**21**(2):70–3

Membrane peptide interaction

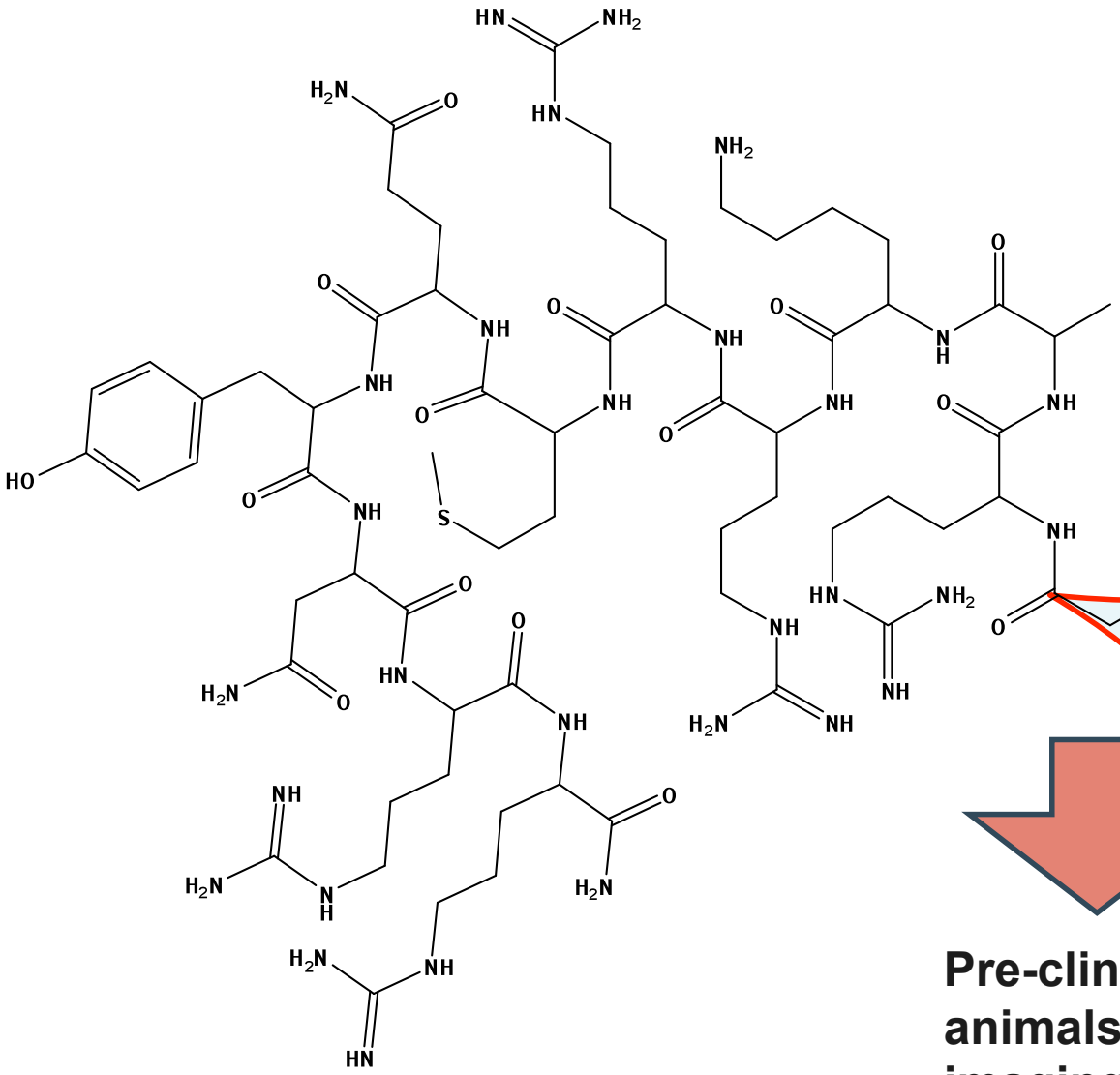


Tracer justification

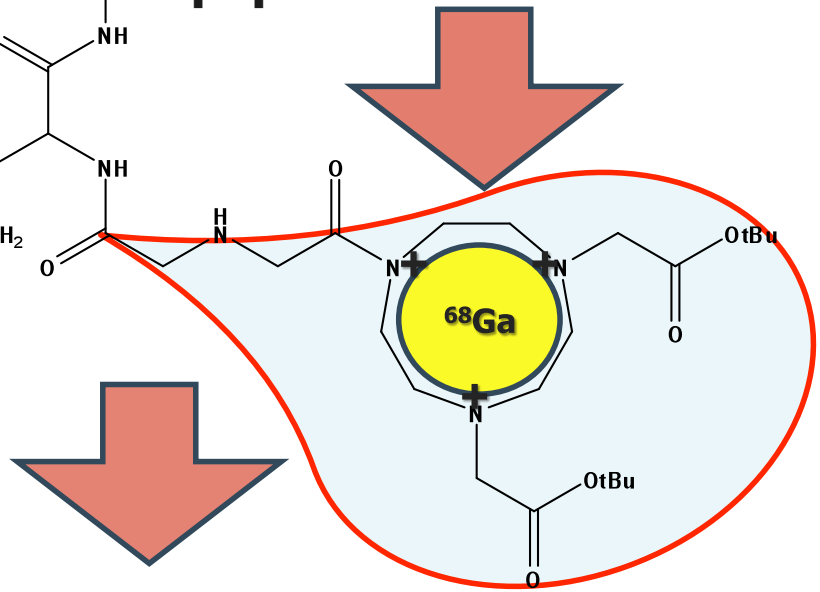


Sathekge, Nucl Med Commun 2008, **26**:663-65

^{68}Ga in biomolecules?



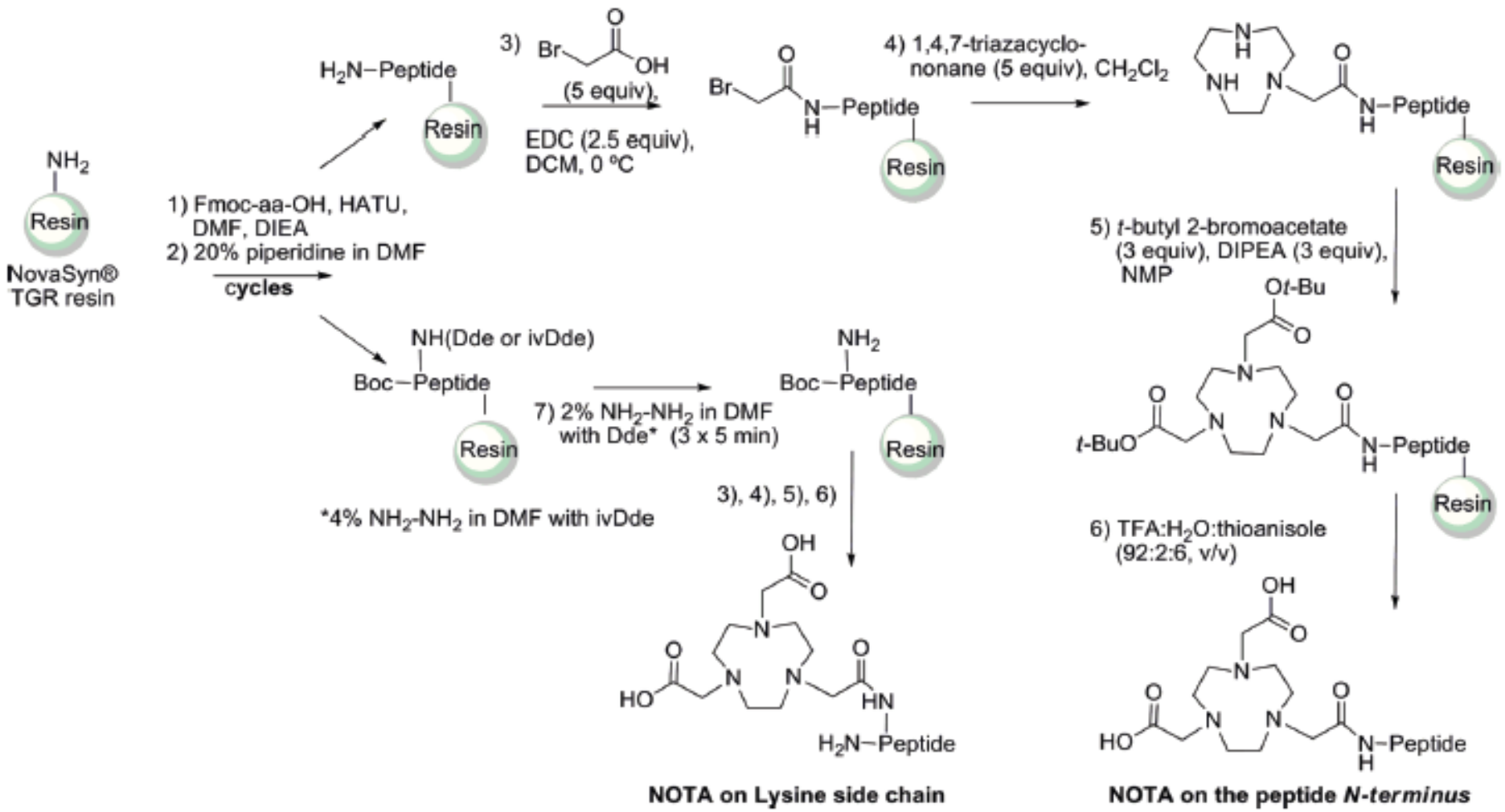
1,4,7-triazacyclononane-triacetic acid (NOTA) facilitates radiolabeling with Gallium-68 of known anti-TB active peptides.



Pre-clinical trials on laboratory animals for prospective PET/CT-imaging of infection (tuberculosis).

The challenge to conjugate NOTA to UBI

The NOTA group was conjugated with peptides using solid-phase



B. Guérin *et al. Org Lett* 2010; **12**:280-3

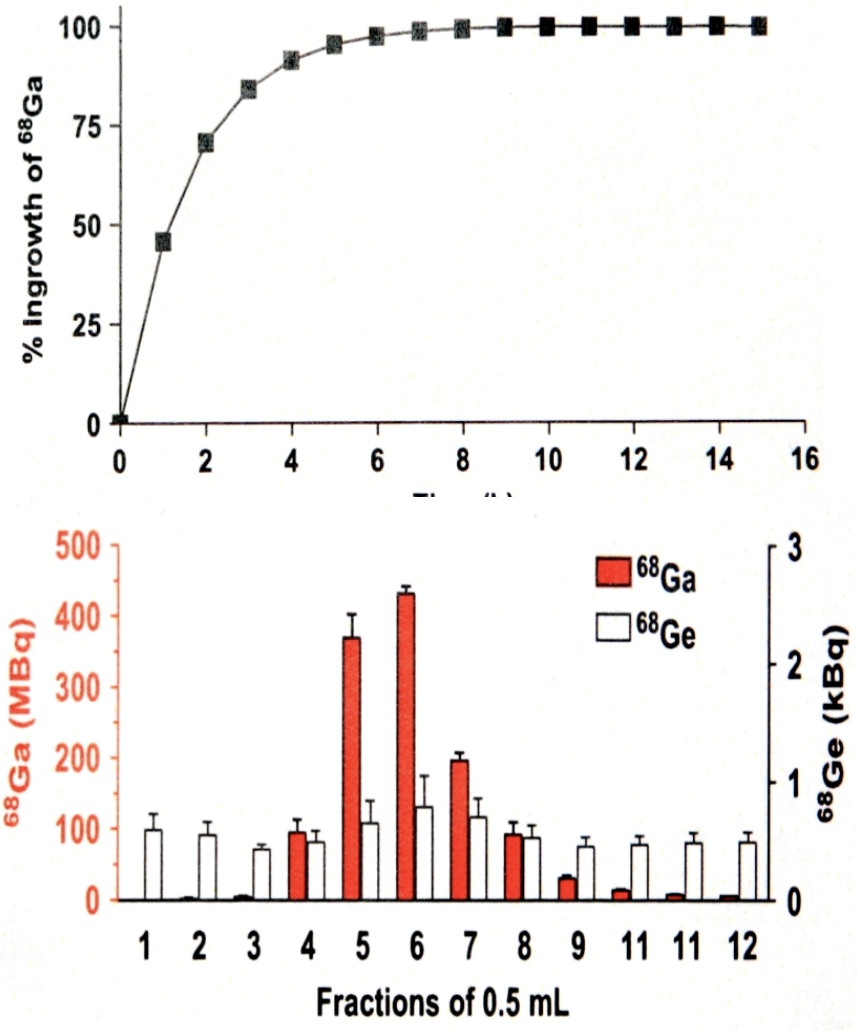
^{68}Gallium/ ^{68}Germanium generator

to **extract** the PET isotope **^{68}Ga** from a source of **decaying germanium-68**.

^{68}Ge half-life **271 days** vs. ^{68}Ga half-life of only **67,4 min**

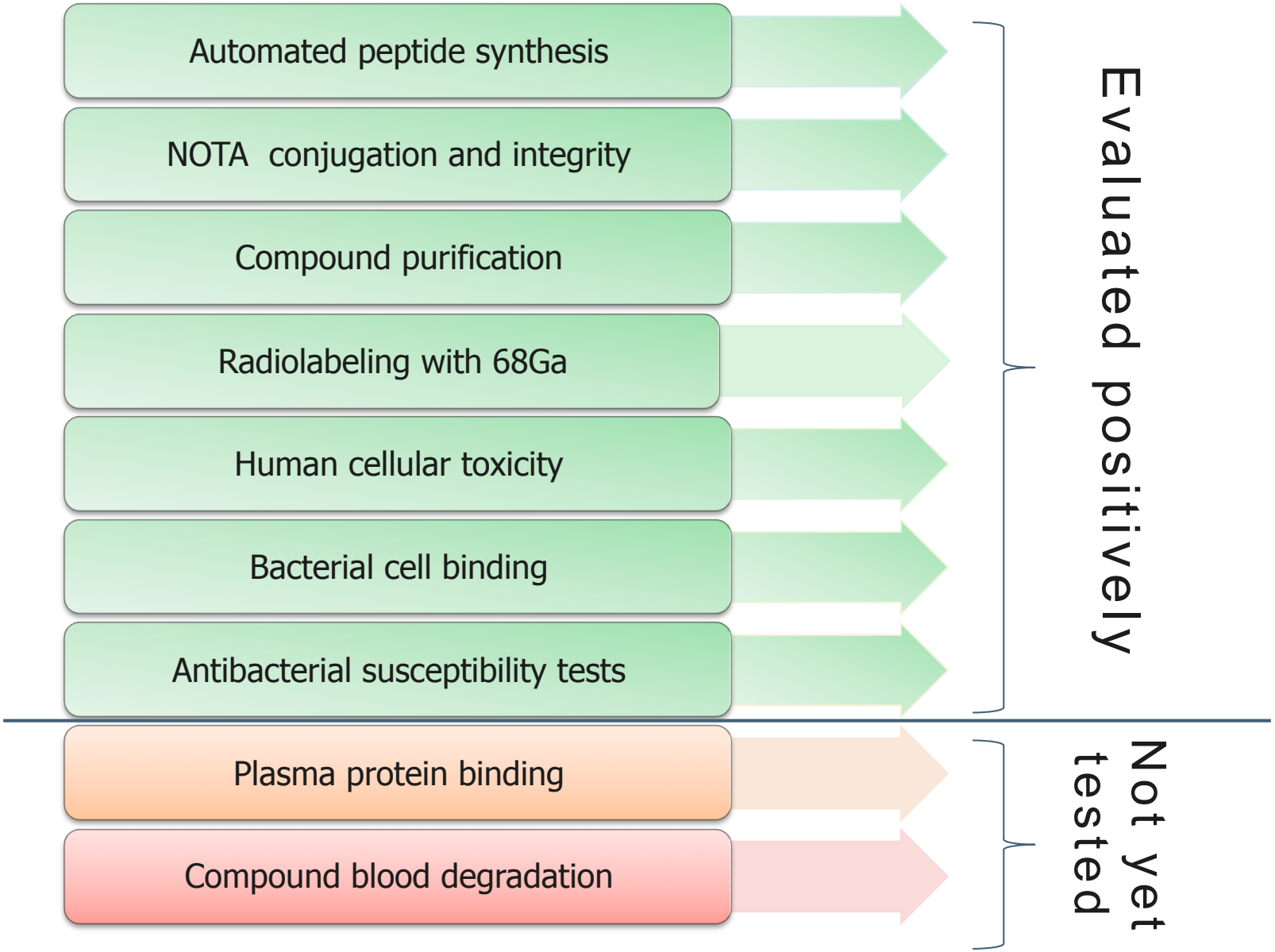
stationary phase alumina, TiO_2 or SnO_2, - germanium-68 is adsorbed.

mobile phase is a solvent (HCl) able to elute (wash out) gallium-68 (III) ($^{68}Ga^{3+}$)



W. A. Breeman *et al*. *Eur J Nucl Med Mol Imaging* 2005; **32**:478-85.; E. De Blois *et al*. *Appl Radiat Isot* 2010; **69**: 308-15

Synthesis & *in vitro* evaluation



Cellular Binding

Fluorescent Assay

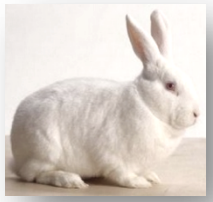
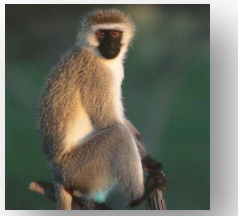
Compound binding affinity at 3µM pH 5 (N=3)

	CONTROL PEPTIDE *	(Ga)NOTA-UBI
Staphylococcus aureus (2x 10^7 CFU)	29,1 ± 4,8 %	97,9 ± 1,9%
Mt4 human leucocytes	9,4 ± 4,8 %	9,3 ± 3,4 %

* *NOTA-UBI-(Lys-Abz) (arginine (+) replaced by aspartic acid (-))*


Preclinical application

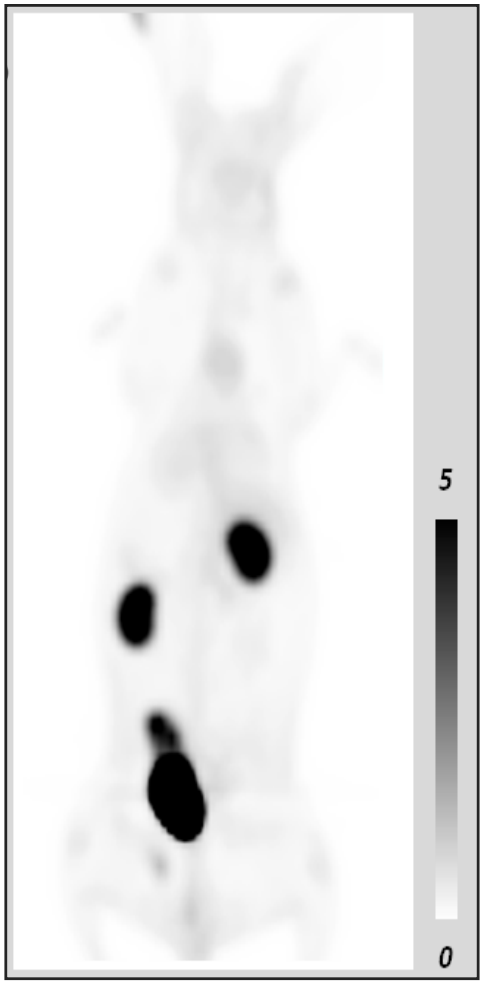
Preclinical application - ^{68}Ga-NOTA-UBI

Balb/c mouse (M)1	New Zealand white rabbit (M)2	Vervet monkey (M)2	
			
x	x	x	Imaging of healthy condition (Study of dosimetry and organ biodistribution)
	x	x	Fluids analysis for ^{68}Ga-activity (Blood & urine sampling for time-activity correlation)
x	x		Imaging muscular bacterial infection (Staphylococcus aureus)
	x		Imaging muscular inflammation (Turpentine oil induced)
	x		Imaging pulmonary inflammation (Ovalbumin induced asthma)
			

1) Preclinical μPET/CT/SPECT Scanner (GE Healthcare, TRIUMPH)
2) Clinical PET/CT camera (Siemens, Biograph True Point, 40 slice CT).

Imaging biodistribution- ^{68}Ga-NOTA-UBI

PET Imaging (Static scan 120 min p.i., ID 48.2 MBq/kg, BW 3.6 kg)



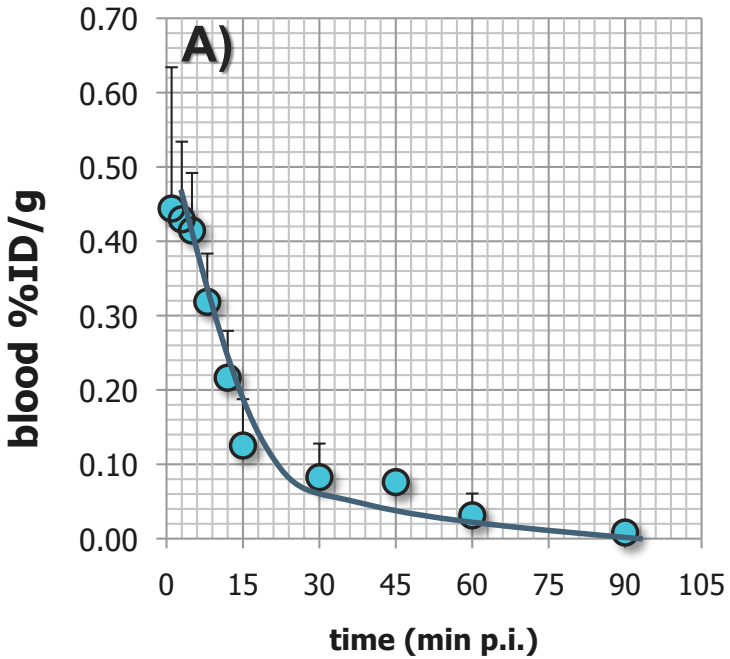
Coronal PET image of ^{68}Ga-NOTA-UBI

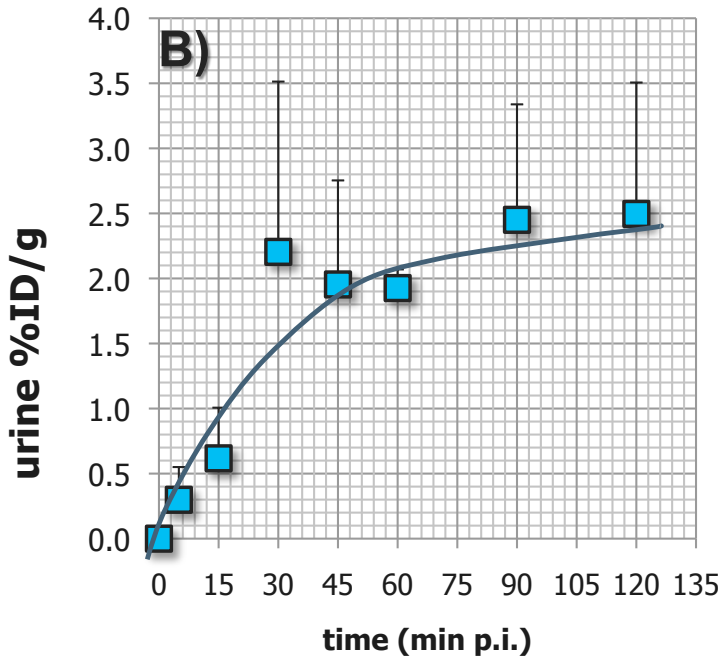
Quantitative 3D-VOI analysis (mean SUV) N=3-7

Scan time	30 min p.i.	60 min p.i.	120 min p.i.
Organ		**Mean SUV**	
heart	0.81 ± 0.23	0.65 ± 0.16	0.59 ± 0.36
liver	1.88 ± 1.16	1.80 ± 1.31	0.84 ± 0.47
spleen	1.69 ± 0.88	1.60 ± 0.94	0.57 ± 0.36
bladder	26.20 ± 15.63	12.55 ± 2.59	8.15 ± 7.83
kidney	3.21 ± 0.89	3.45 ± 0.71	2,78 ± 0.99
hind leg biceps	0,26 ± 0.10	0.20 ± 0.05	0.12 ± 0.03
front leg triceps	0.24 ± 0.05	0.21 ± 0.04	0.12 ± 0.05
lung	0.57 ± 0.22	0.51 ± 0.22	0.33 ± 0.07
brain	0.23 ±0.10	0.16 ± 0.07	0.09 ± 0.04

Fluid analysis - ^{68}Ga-NOTA-UBI activity

^{68}Ga-NOTA-UBI concentration per **A)** ml blood and **B)** ml urine in healthy rabbits (N=3-7)





c_{max} = 0.44±0.19%ID/g (max. blood concentration)

C_{Vd} = 23.4µg/L (distributed tracer concentration)

Δ Vol = 25µg/ml*min^{-1} (Bolus infusion)

$T_{1/2}$ = 19.8min (biological blood half-life)

Δct_{Blood}= -2.64%ID/h (blood elimination rate)

c_{max} = 2.56±1.09%ID/g (max. urine concentration)

TA_{50} = 22.6min (activity accumulation half time)

Δct_{Urine}= 4.40%ID/h (urinary accumulation rate)

$\Sigma_{urine120}$= 74±4%ID (urinary activity recovery 120 min)

Imaging biodistribution- ^{68}Ga-NOTA-UBI

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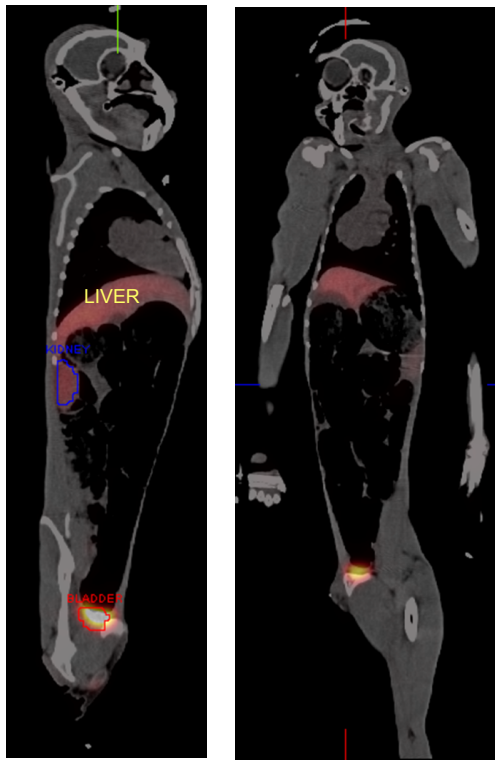
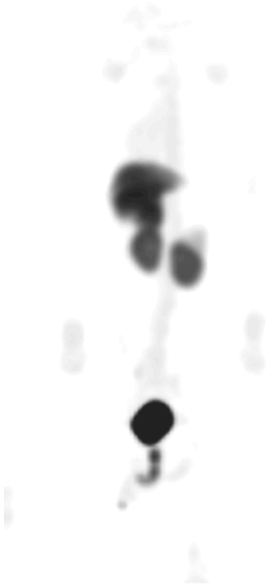
Imaging, 3D ROI calculation and fluid analysis of healthy vervet monkeys

- Static scan 120 min p.i.,
  ID 35.5 MBq/kg, BW 6.6kg



Sagittal

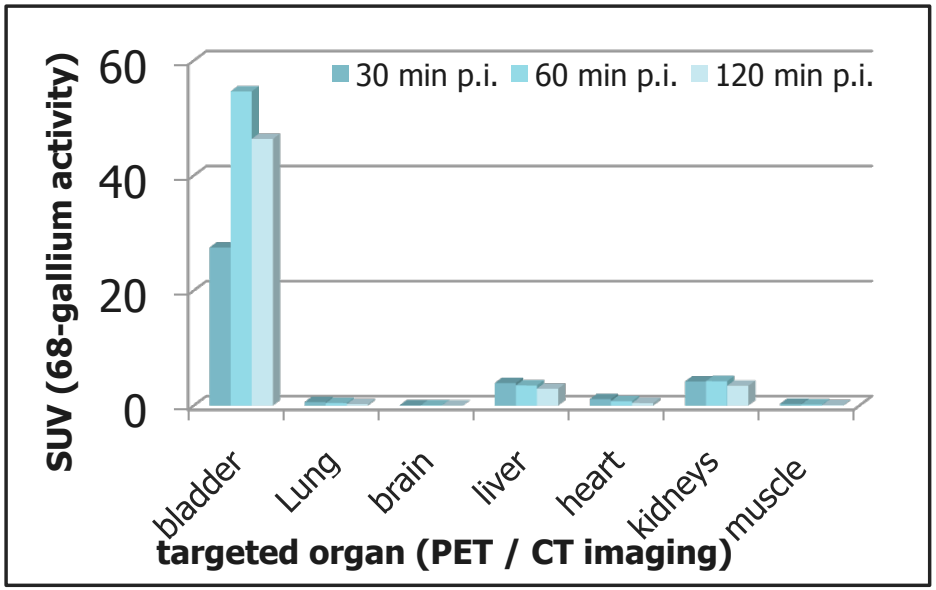
Coronal

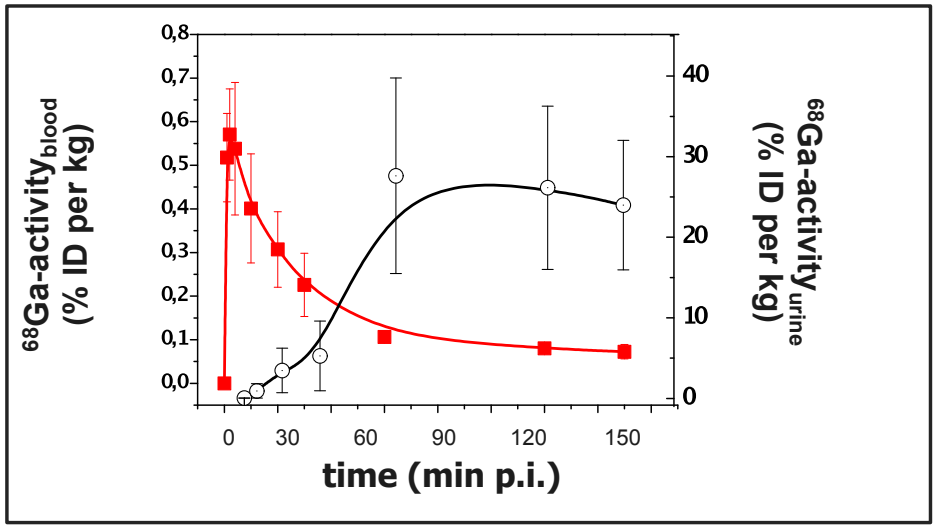


Coronal

Image fusion PET/ CT

PET(only)

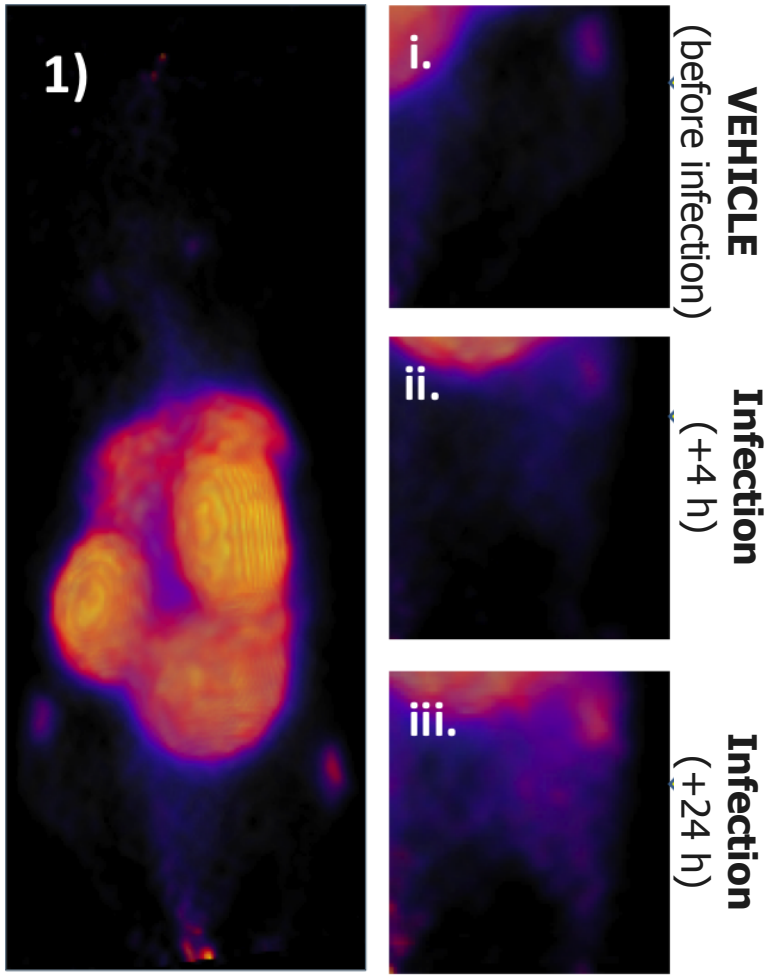




Infection Imaging - ^{68}Ga-NOTA-UBI

Balb /c mouse bearing infection

microPET imaging (Static scan 30 min p.i., ID 12 MBq, BW 35g)



Coronal microPET image of ^{68}Ga-NOTA-UBI

Method and results

5x10^6 cfu of viable Staphylococcus aureus bacilli inoculated into the hind thigh muscle (intramuscular / intradermal)

µPET/CT Scanner (GE Healthcare, Modell: TRIUMPH) at the Institute of Nuclear Medicine and Allied Sciences (INMAS, New Delhi).

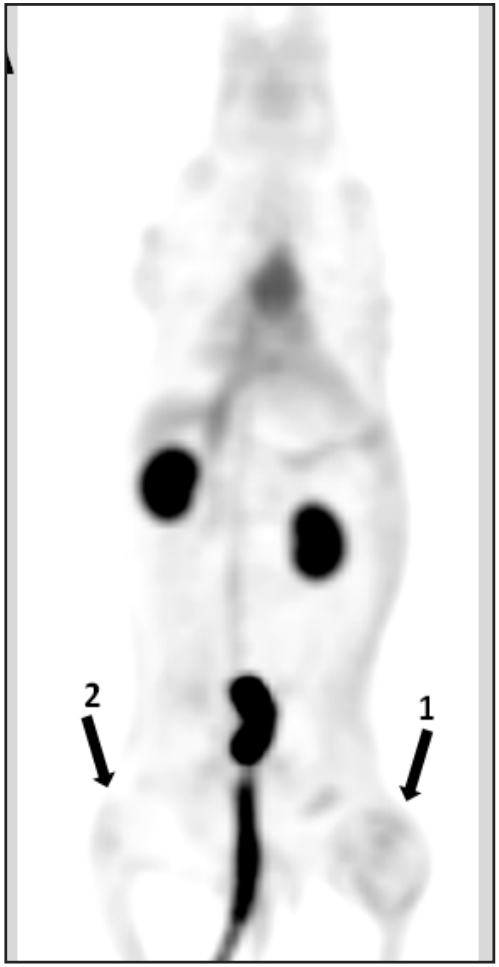
Standard inhalation anaesthesia: **Isoflurane**

Increasing, sensitive uptake of ^{68}Ga-NOTA-UBI, at infection site.

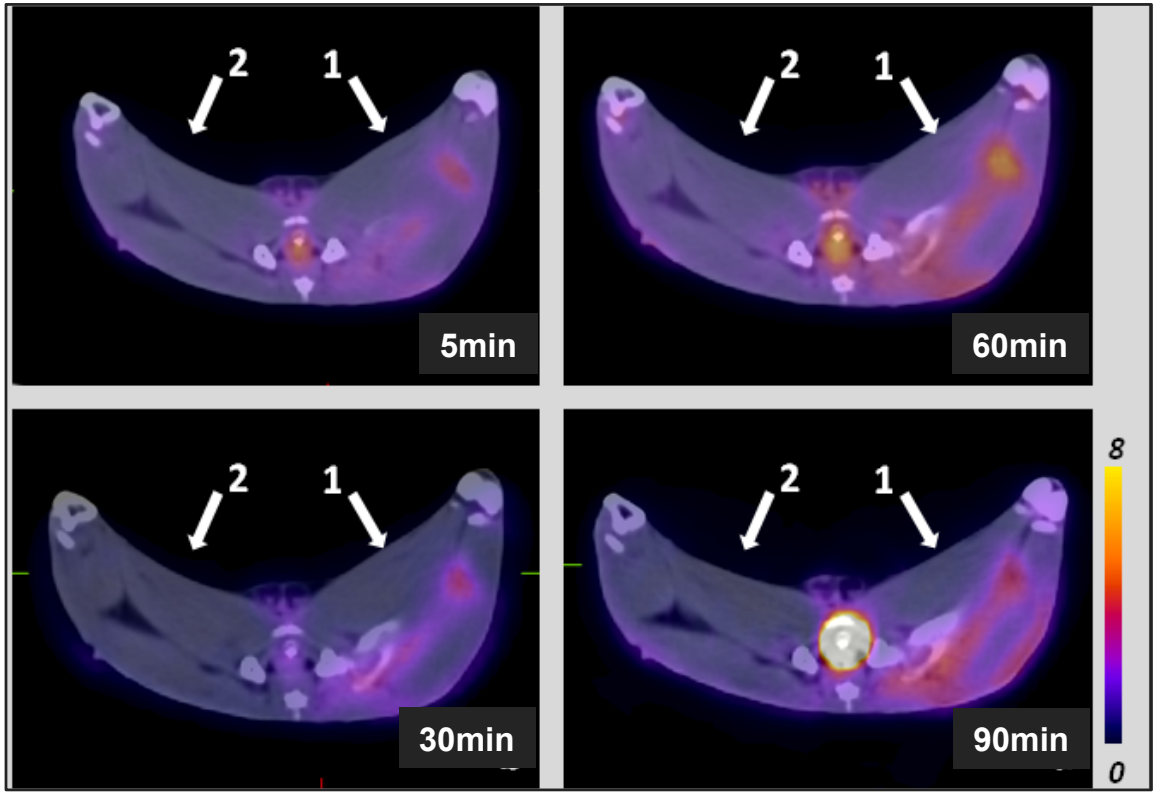
Specificity against sterile muscular inflammation?

Infection Imaging - ^{68}Ga-NOTA-UBI

Rabbits bearing Staphylococcus aureus infection (72h post initiation)



Coronal PET image of ^{68}Ga-NOTA-UBI at 60 min p.i.



Axial PET/CT images show contra lateral hind legs at
(1) muscular infection site
(2) muscular inflammation site

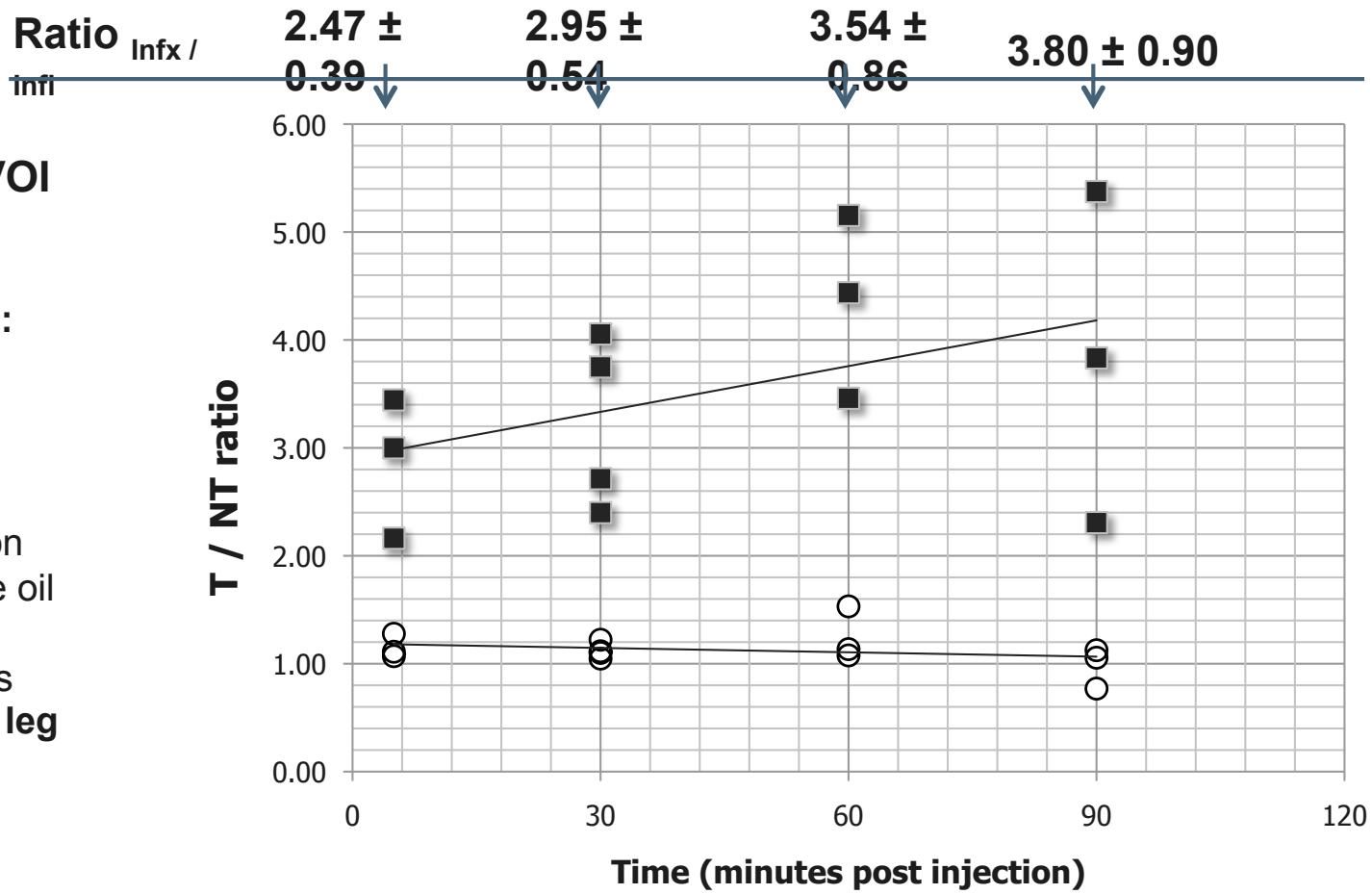
Infection Imaging - ^{68}Ga-NOTA-UBI

Quantitative 3D-VOI analysis (SUV)

Black solid squares: muscular bacterial infection site

Grey open circles: muscular inflammation induced by turpentine oil

Non-targeted tissue is represented by **front leg triceps**.



^{68}Ga-NOTA-UBI shows increasing, specific uptake in the muscle bearing bacterial infection compared to the contra lateral inflamed muscle

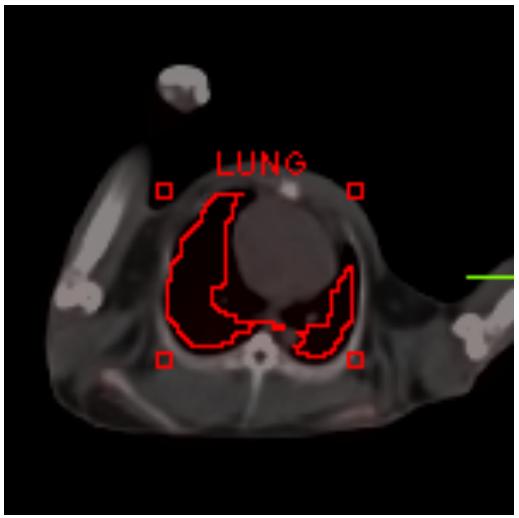
Imaging Inflammation? ^{68}Ga-NOTA-UBI

Pulmonary distribution of ^{68}Ga-NOTA-UBI in rabbits

target/ non-target ratio (Lung/front leg triceps)	PET/CT scan time post tracer injection	
	30 min	60 min
1) healthy	2.38 ± 0.63	2.48 ± 0.53
2) Infection/ Inflammation	2,51 ± 0.79	2.17 ± 0.45
3) Asthma*	2.42 ± 0.45	2.53 ± 0.44

* Initiation: - 5weeks, Induction: 5% ovalbumin aerosol inhalation

Representative axial image of PET/CT Scan (60min p.i.)



No significantly increased T/NT ratios (lung/muscle) were found in asthmatic inflamed lungs and rabbit lungs with extra pulmonary infection/inflammation compared to rabbits lungs in healthy condition.

Summary and Outlook

- The ^{68}Ga-NOTA-UBI fragments shows sufficient imaging and biodistribution in preclinical, healthy vervet monkeys and rabbits.

- The ^{68}Ga-NOTA-UBI fragment is capable to monitor infection (balb/c mice) & differentiate non pathogenic muscular infection from inflammation in rabbits.

- It also won't reflect asthmatic lung inflammation in rabbits.

Similar peptide candidates are currently investigated - *in vitro* and using *in vivo* imaging!

UP/NECSA will launch a multicenter clinical trial with NOTA-UBI

Project Network

NTeMBI

(Academic platform)

University Pretoria

SBAH (Nuclear Medicine Department)

UKZN Durban

Chemistry
Pharmacology
Biochemistry

University Pretoria

BRC,Onderstepoort

NECSA

Radiochemistry

INMAS

New Delhi, India

Medical Research Council, Pretoria


THANK YOU