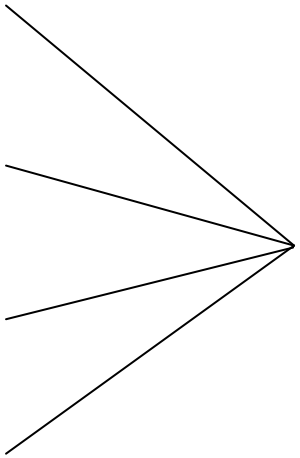
DETECTION OF FUNGAL INFECTIONS WITH RADIOLABELED ANTIFUNGAL AGENTS

Clinical history

Physical examination

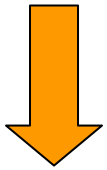
Laboratory tests

Imaging studies



signs and symptoms are suggestive of an infectious or a non-infectious cause

putative site of infection



microbiological cultures of body fluids and biopsies

identification of pathogens

> 85% of patients referred to hospital are febrile due to an infection

< 50% of febrile episodes can be attributed to infections

clinical manifestations of infection are subtle, non-typical, non-existent

identification of an infection at an early stage of the disease is critical for a favourable outcome

Laboratory tests

- **erythrocyte sedimentation rate**
- **white-blood-cell count**
- **acute-phase proteins**
- **cytokines**

Can nuclear medicine make an important contribution?

Current radiopharmaceuticals:

gallium-67-citrate (^{67}Ga)-labelled polyclonal human immunoglobulins

indium-111 (^{111}In)-labelled polyclonal human immunoglobulins

technetium-99m (^{99m}Tc)-labelled polyclonal human immunoglobulins

(^{111}In)-labelled autologous leukocytes

(^{99m}Tc)-labelled autologous leukocytes

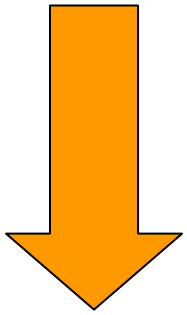
(^{99m}Tc)-labelled antigranulocyte monoclonal antibodies (or fragments thereof)

(^{99m}Tc)-labelled chemotactic peptides

(^{99m}Tc)-labelled interleukins

We need a radiopharmaceutical that binds to a variety of micro-organisms with little or no binding to host cells

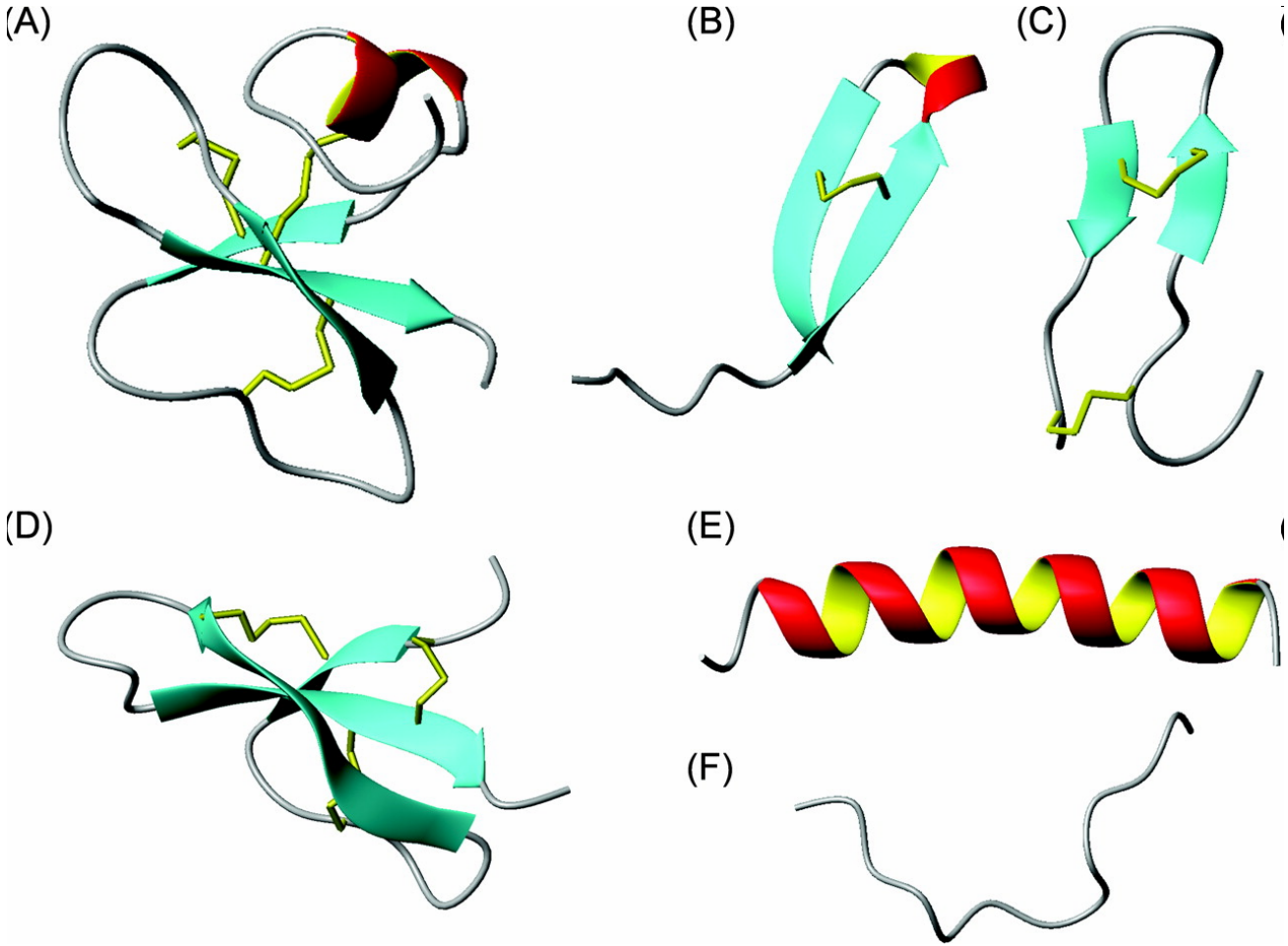
Antimicrobial peptides often display preferential binding to micro-organisms



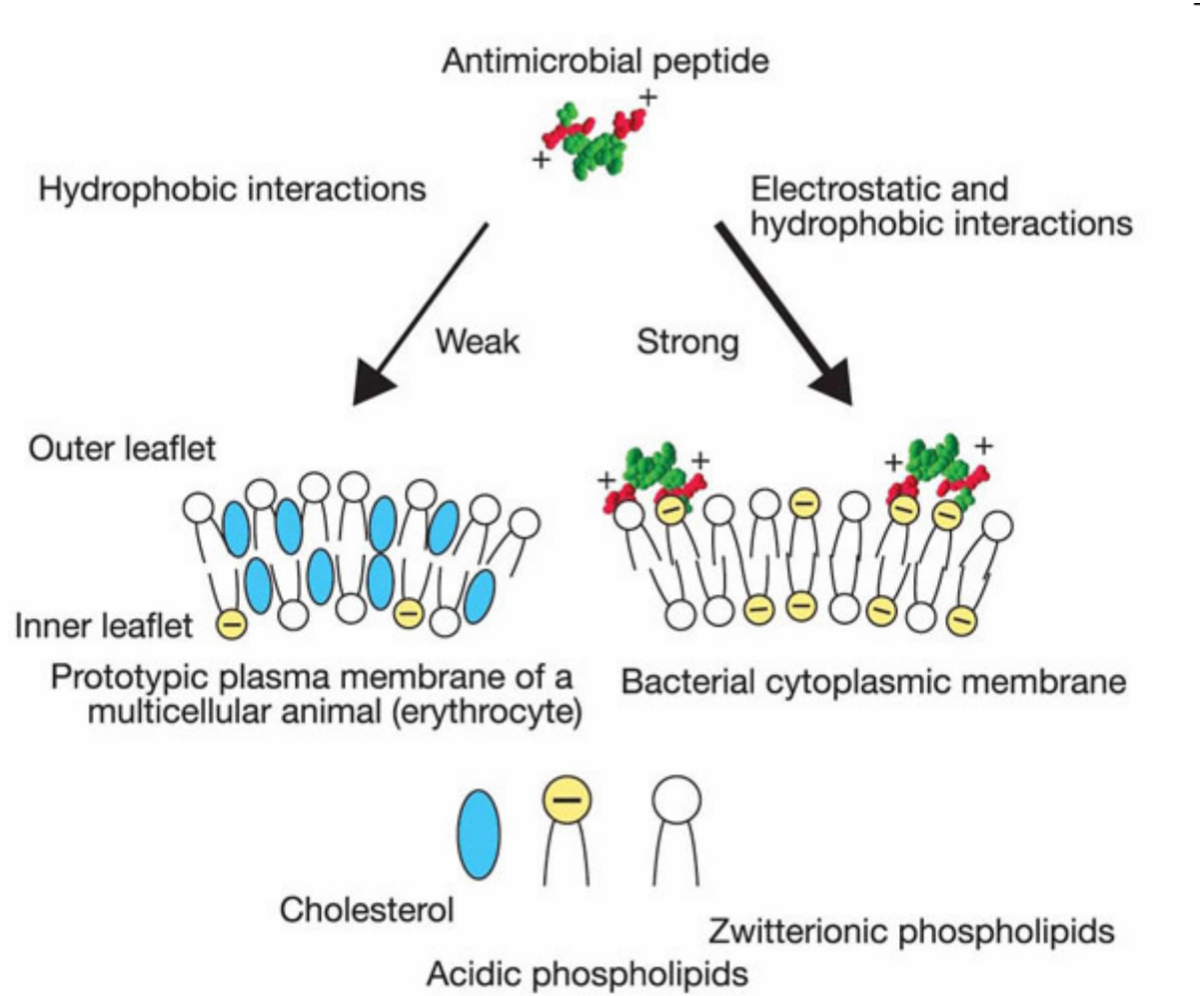
Radiopharmaceuticals recruited from human antimicrobial peptides can be promising candidates to discriminate infections from inflammations

Examples of antimicrobial proteins/peptides found in nature

Mammals	Humans	α-, β-Defensins
		Cathelicidins
		Histatins
		Lactoferrin
		Ubiquicidin
	Rhesus macaque	α-, β-, θ-defensins
		Cathelicidins
	Pigs	Protegrins
		PR39
		Prophenin
		Cecropin
		Cathelicidins
		β-Defensin-1
Frogs		Magainins
		Dermaseptins
		Esculentins
		Brevinins
		Bombinin
		Temporins
		Japonicins
		Tigerinins
		Ranatuerins
		Palustrins
		Ranalexins
		Gaegurins
		Nigrocins
Insects		Defensins
		Thanatin
		Drosomycin
		Cecropins
		Drosocin
		Attacin
		Diptericin
		Metchnikowin
Horseshoe crabs		Tachyplesins
		Polyphemusin
		Tachystatins
		Defensins
		Anti-LPS factor
Plants		Thionins
		Defensins
		Shepherins
Bacteria		Pediocin PA-1
		Leucocin A UAL-187
		Mesentericin Y105
		Sakacin P
		Curvacin A
		Nisin
		Cecropin-like peptide



Structural classes of antimicrobial peptides. (A) Mixed structure of human ß-defensin-2 (PDB code 1FQQ) (216); (B) looped thanatin (PDB code 8TFV) (156); (C) ß-sheeted polyphemusin (PDB code 1RKK) (202); (D) rabbit kidney defensin-1 (PDB code 1EWS) (165); (E) a-helical magainin-2 (PDB code 2MAG) (76); (F) extended indolicidin (PDB code 1G89) (212). The disulfide bonds are indicated in yellow, and the illustrations have been prepared with use of the graphic program MolMol 2K.1 (132).
From: Hancock *et al.* Clin. Microbiol. Rev. 19:491-511, 2006.



From: Zasloff M. Nature 415:389-395, 2002.

Production of sufficient amounts of cationic peptides under GLP conditions:

- recombinant cationic-peptide production by bacteria

- peptide synthesis:
  - chemical variants
  - introduction of chelators

Radiolabeling of peptides

the radionuclide should be firmely attached to the peptide

or incorporated into the peptide

labeling should not affect the binding activity of the peptide

Selection of ^{99m}Tc-labeled antimicrobial peptides for scintigraphic studies

- *in vitro* binding studies

- *in vivo* binding studies

- pharmacokinetics

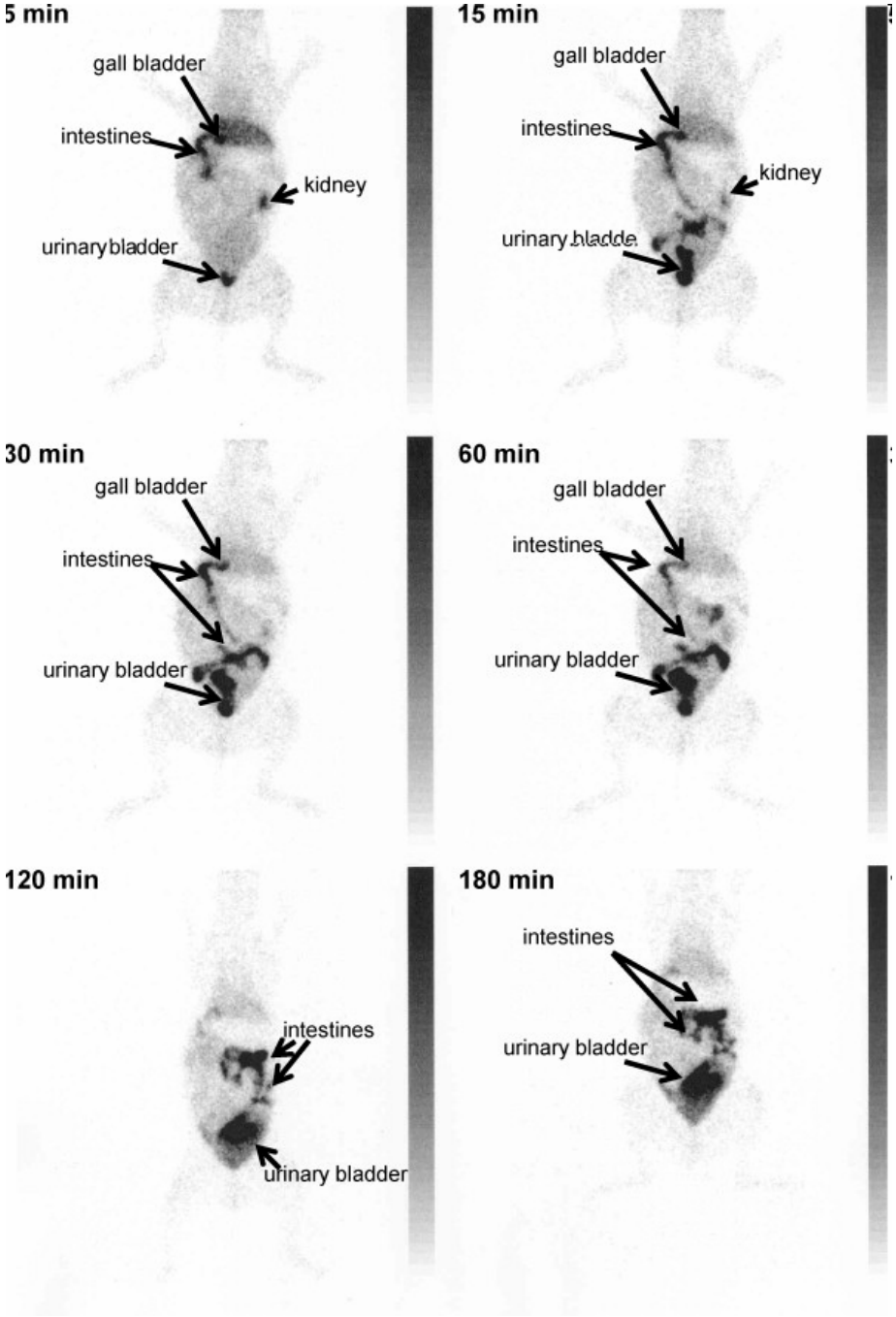
In vitro binding studies of ^{99m}Tc-peptides to micro-organisms and activated leukocytes

Peptide	Binding per 10^7 cells (% of added radioactivity)			
	Staphylococcus aureus	*Klebsiella pneumoniae*	*Candida albicans*	Activated leukocytes
^{99m}Tc-hLF 21-31	1±1	2±2	38±2	4±5
^{99m}Tc-hLF 1-11	24±1	25±8	31±4	11±15
^{99m}Tc-UBI 18-35	73±14	52±11	8±3	18±6
^{99m}Tc-UBI 31-38	63±16	34±13	2±1	5±3
^{99m}Tc-UBI 22-35	83±10	41±27	3±1	6±4
^{99m}Tc-UBI 29-41	41±6	15±6	11±1	4±2
^{99m}Tc-HNP 1-3	48±20	37±12	n. d.	13±4

Values are means±SEM of at least four observations.

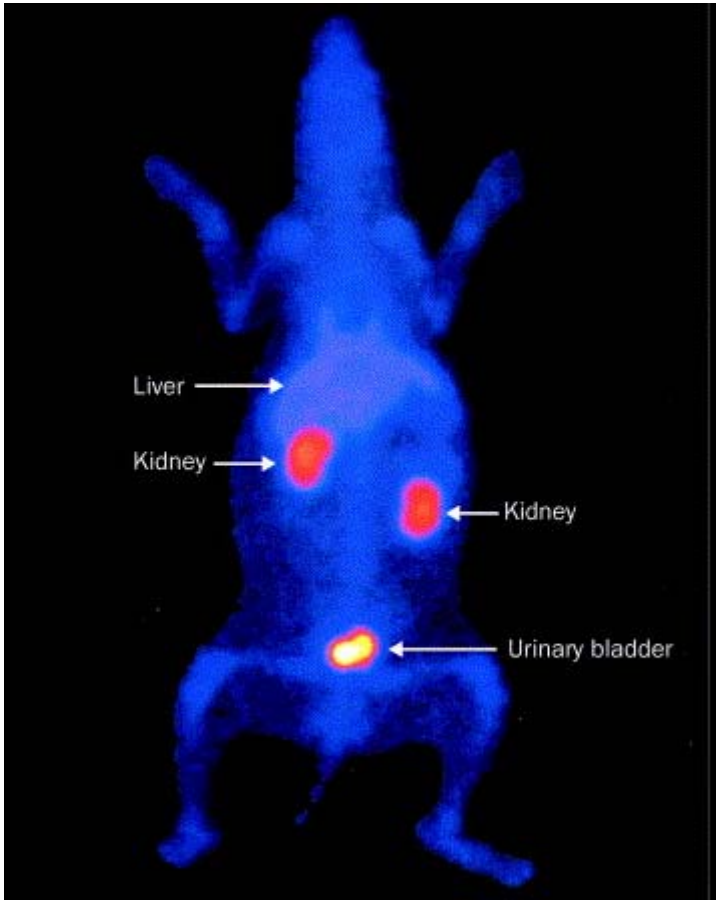
From: Lupetti *et al.* Quarterly J. Nucl. Med. 47:238-245, 2003

n.d. = not done



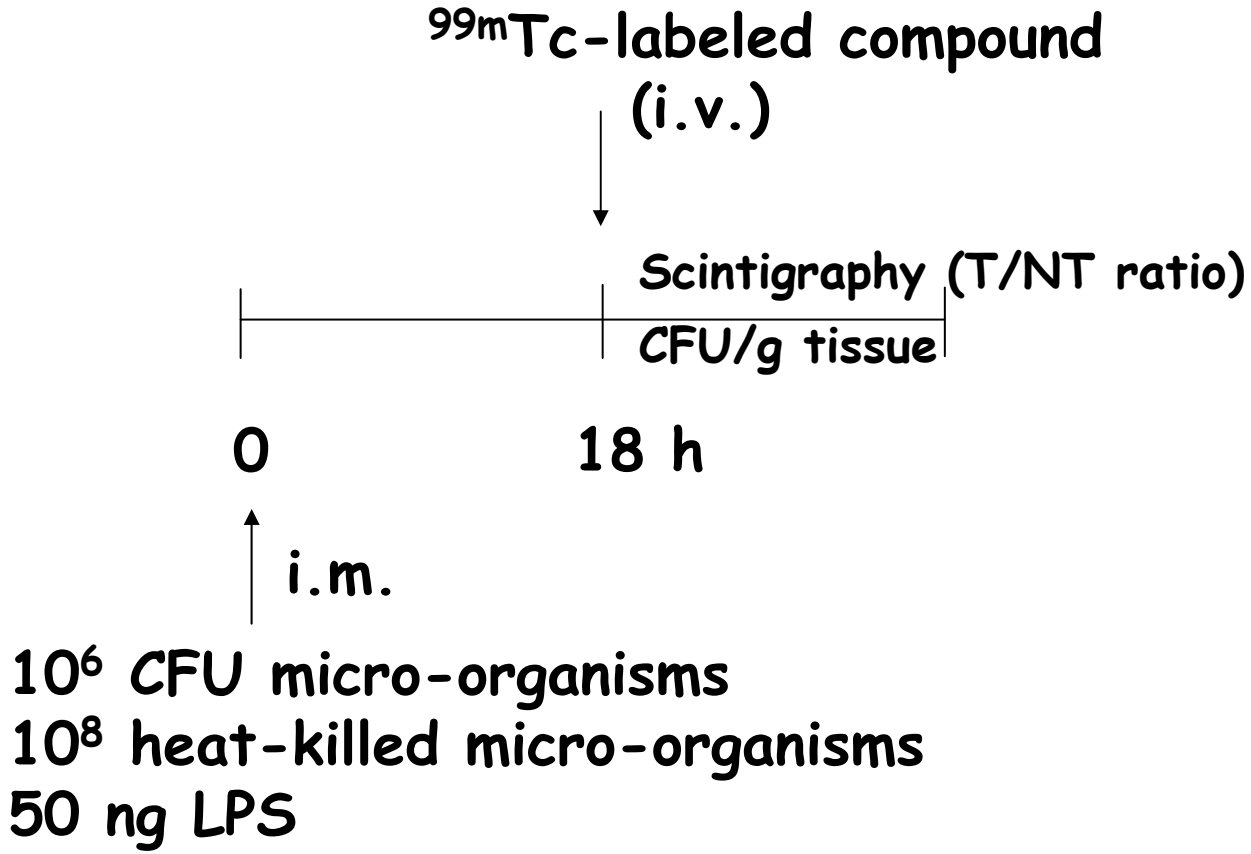
Biodistribution of ^{99m}Tc-labeled hLF 1-11 in a normal rabbit at various time intervals.

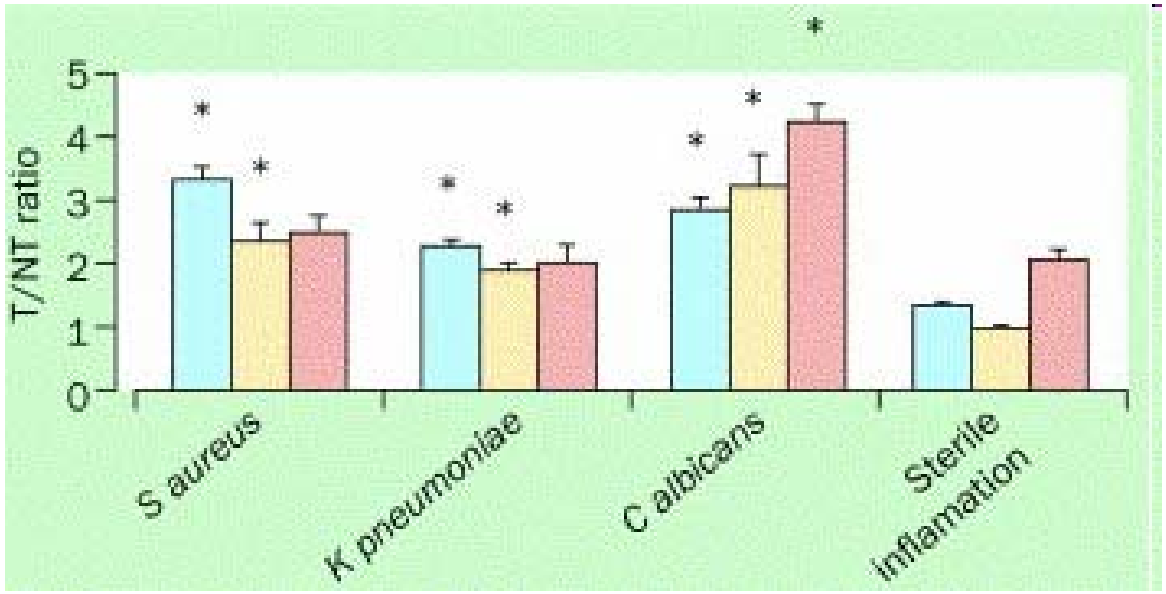
From: Brouwer *et al.* Peptides 29:1109-1117, 2008.



Biodistribution of ^{99m}Tc-UBI 29–41 in a healthy rabbit at 2 h after i.v. injection of the radiolabeled peptide.

From: Lupetti *et al.* Lancet Infect. Dis. 3:223-229, 2003.


^{99m}Tc-labeled compound
(i.v.)
Scintigraphy (T/NT ratio)
CFU/g tissue
0
18 h
i.m.
10^6 CFU micro-organisms
10^8 heat-killed micro-organisms
50 ng LPS



^{99m}Tc-α defensins (HNP 1–3; blue bars),
^{99m}Tc-UBI 29–41 (yellow bars)
^{99m}Tc-IgG (red bars)

* $p < 0.05$ compared with the values for mice with an inflammatory process according to *Student* t test

From: Lupetti *et al.* Lancet Infect. Dis. 3:223-229, 2003.

the **ideal tracer** for infection imaging should fulfill the following criteria:

i) rapid uptake at sites of infection with little or no accumulation at sites of sterile inflammation;

ii) good stability of the labeled complex under physiological conditions;

iii) preservation of binding activity upon labeling;

iv) rapid clearance from the circulation with little or no accumulation in unaffected tissues,

v) little or no adverse effects, such as toxicity and immunological reactions

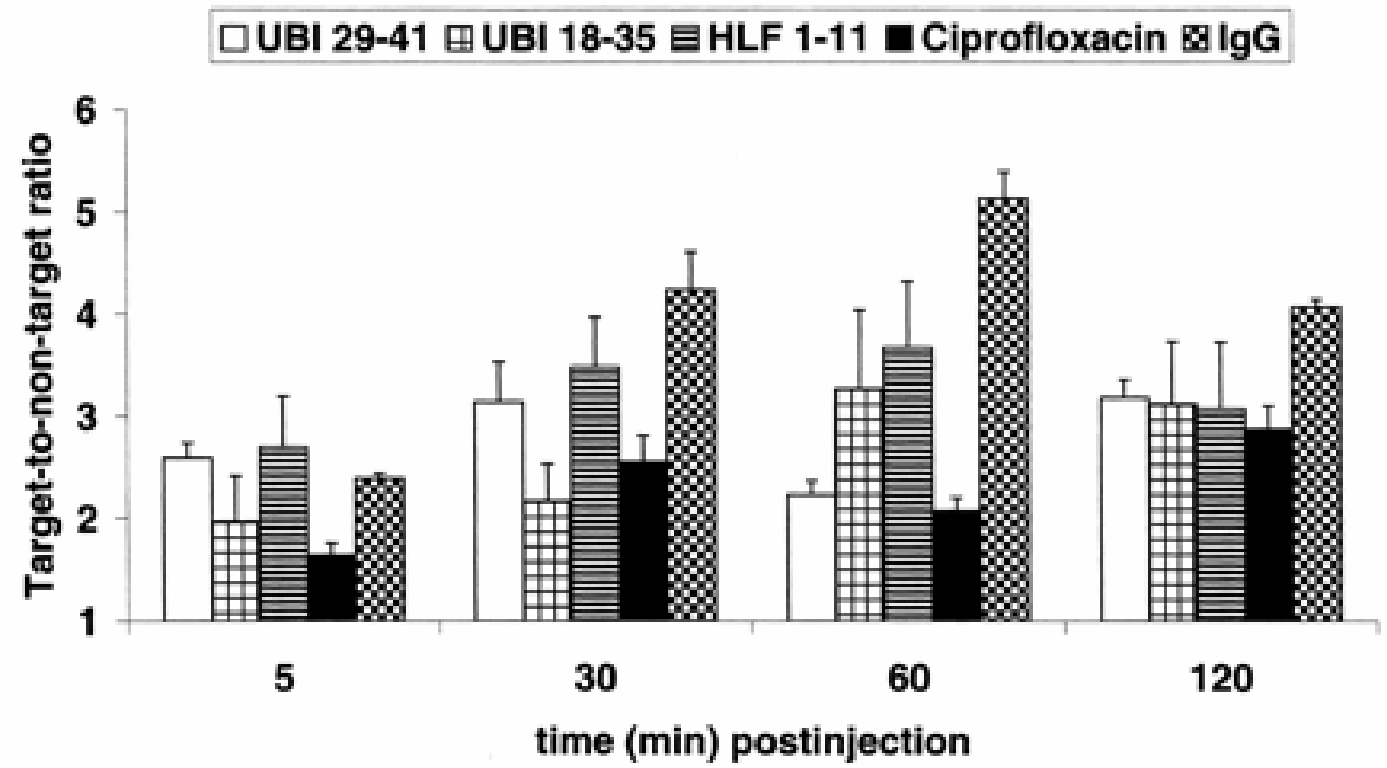
two new groups of tracers have been introduced

^{99m}Tc-labeled ciprofloxacin (^{99m}Tc-Infecton)

^{99m}Tc-labeled fluconazole

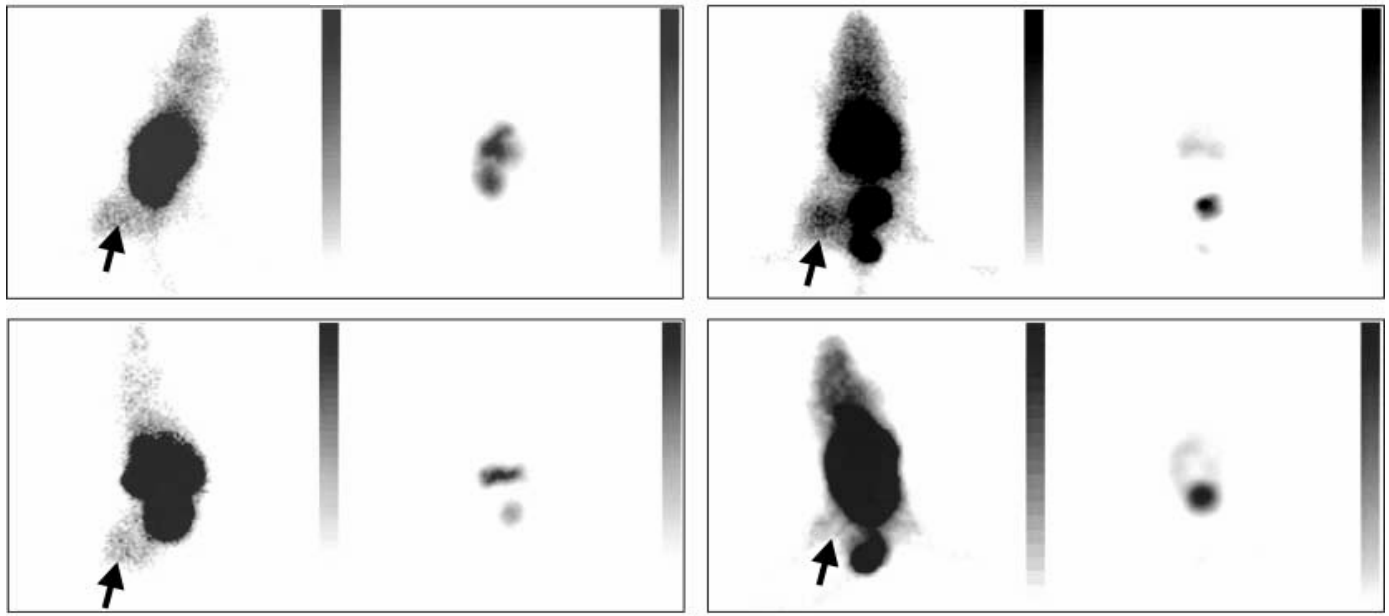
Can ^{99m}Tc-labeled antimicrobial peptides, ^{99m}Tc-fluconazole visualize *C. albicans* infections?

fluconazole-resistant *C. albicans*-infected mice



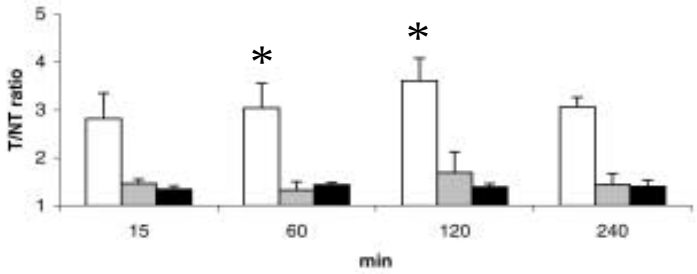
From: Welling *et al.* J. Nucl. Med. 42:788-794, 2001

C. albicans-infected mice

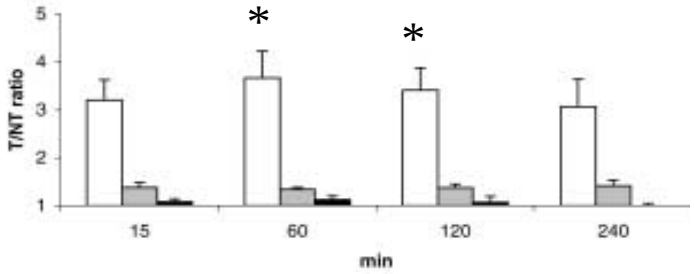


Right: scintigraphic imaging of the biodistribution of the tracers in the entire animal.
Left: scintigrams of the same animal with higher contrast visualising the thigh muscle infection/inflammation indicated by an arrow at 1 h after injection of the tracers.
From: Lupetti *et al.* Eur. J. Nucl. Med. 29:674-679, 2002

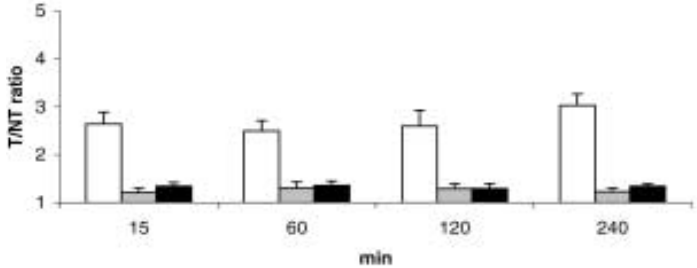
C. albicans-infected mice (open bars)
mice inflamed with heat-killed *C. albicans* (*hatched bars*)
or lipopolysaccharide (*LPS, closed bars*)



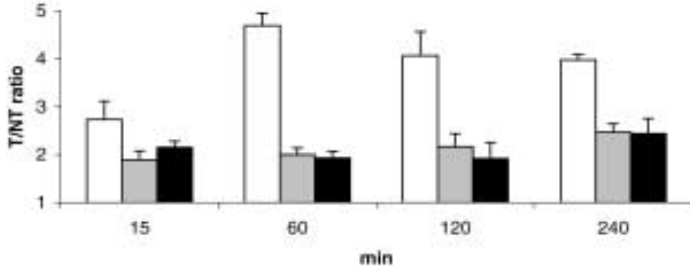
^{99m}Tc-fluconazole



^{99m}Tc-hLF 1-11



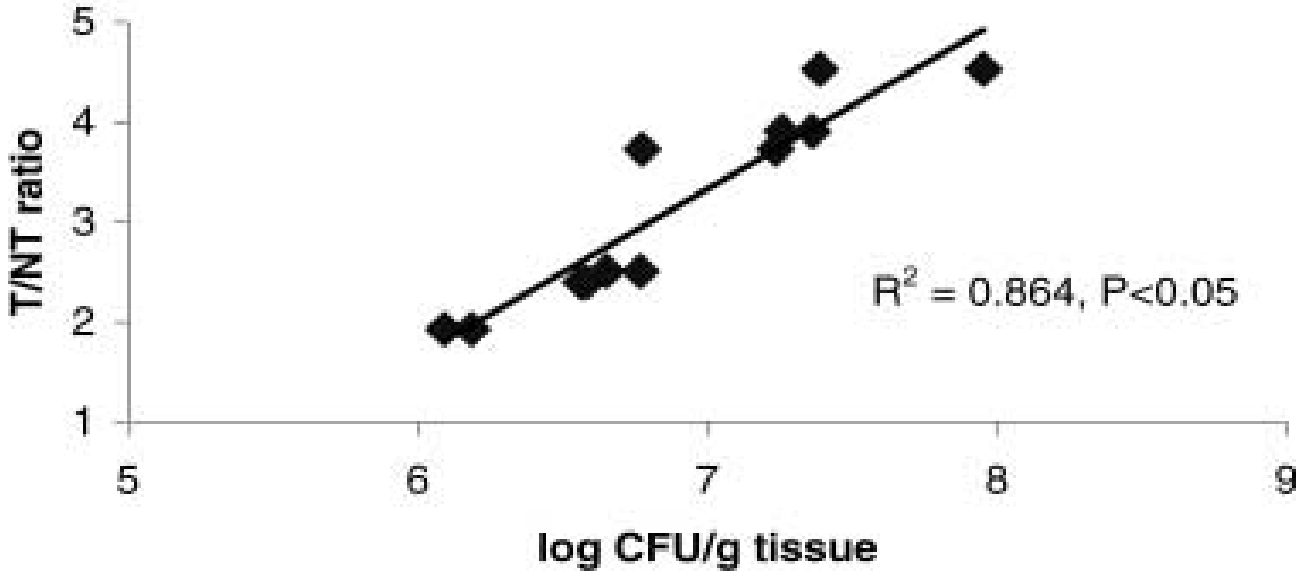
^{99m}Tc-UBI 29-41



^{99m}Tc-IgG

From: Lupetti *et al.* Eur. J. Nucl. Med. 29:674-679, 2002

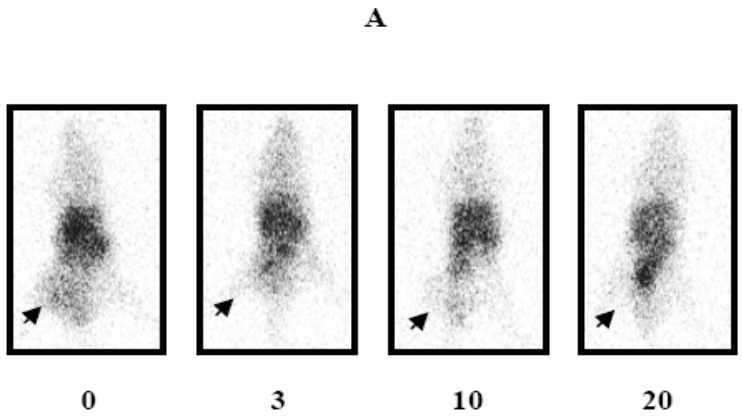
^{99m}Tc-labeled fluconazole



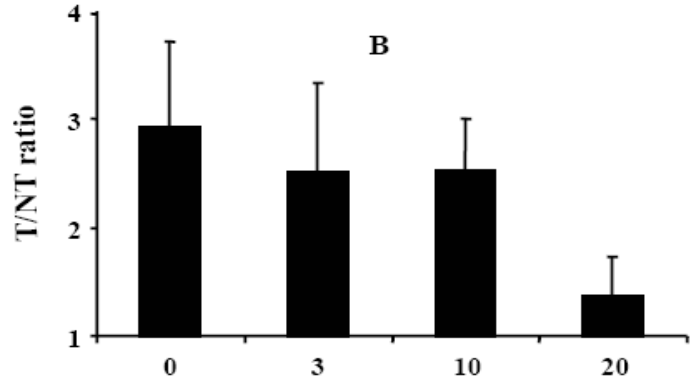
C. albicans

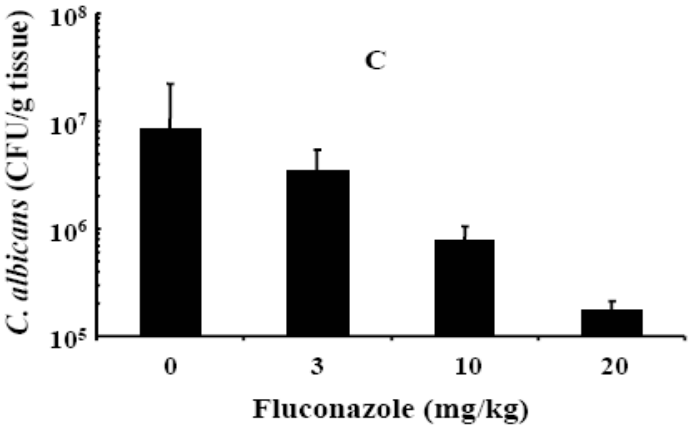
From: Lupetti *et al.* Eur. J. Nucl. Med. 29:674-679, 2002

Can nuclear medicine contribute in monitoring the efficacy of antifungal therapy?



Monitoring the efficacy of antifungal therapy by accumulation of ^{99m}Tc-UBI 29-41



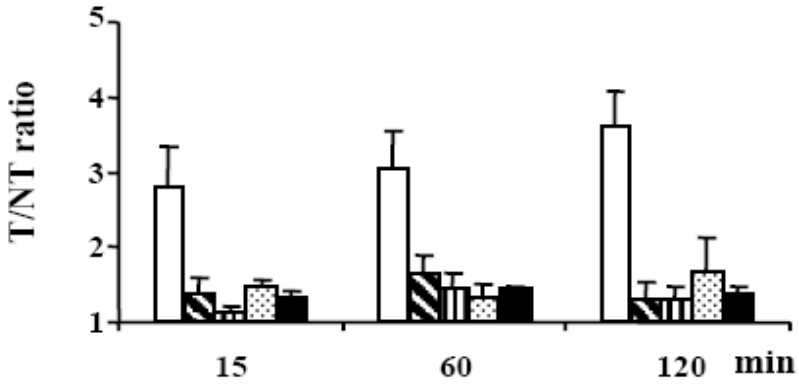


Is ^{99m}Tc-fluconazole able to discriminate between *C. albicans* and bacterial infections?

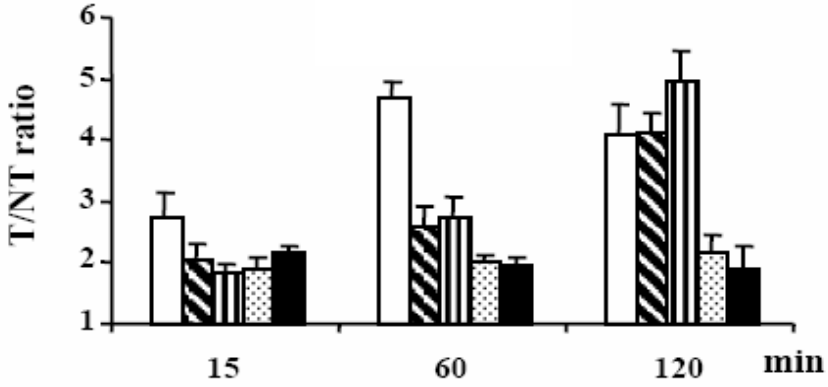
Mice infected/inflamed with:

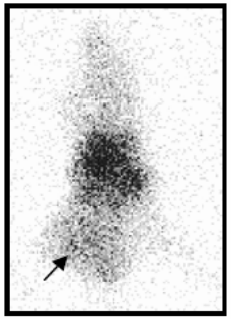
C. albicans (open bars)
MRSA (diagonally hatched bars)
K. pneumoniae (vertically hatched bars)
heat-killed *C. albicans* (dotted bars)
LPS (closed bars)

^{99m}Tc-fluconazole

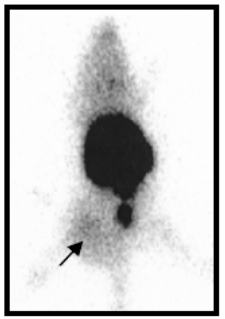


^{99m}Tc-IgG





C. albicans



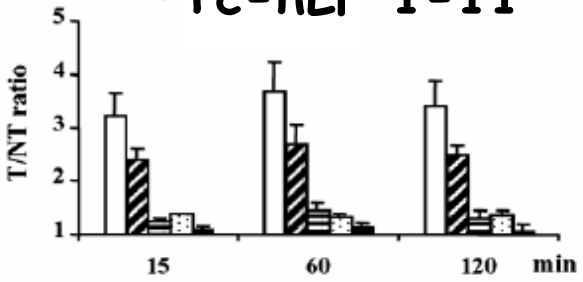
C. albicans

Are ^{99m}Tc-antimicrobial peptides able to discriminate between *C. albicans* and bacterial infections?

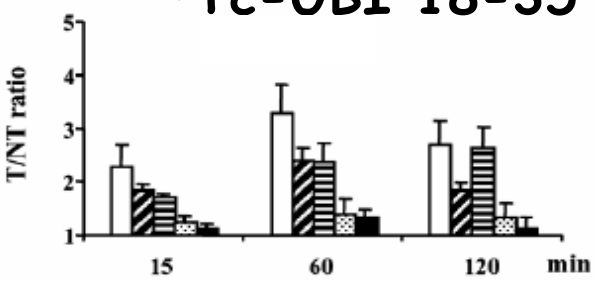
Mice infected/inflamed with:

C. albicans (open bars)
MRSA (diagonally hatched bars)
K. pneumoniae (horizontally hatched bars)
heat-killed *C. albicans* (dotted bars)
LPS (closed bars)

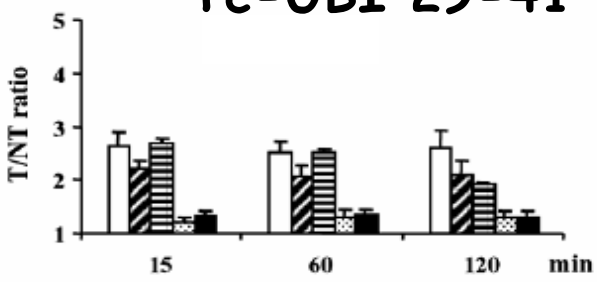
^{99m}Tc-hLF 1-11

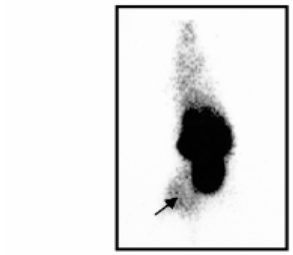


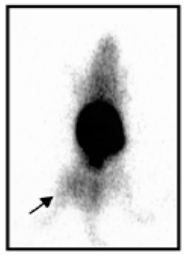
^{99m}Tc-UBI 18-35

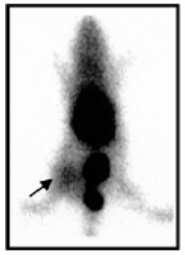


^{99m}Tc-UBI 29-41





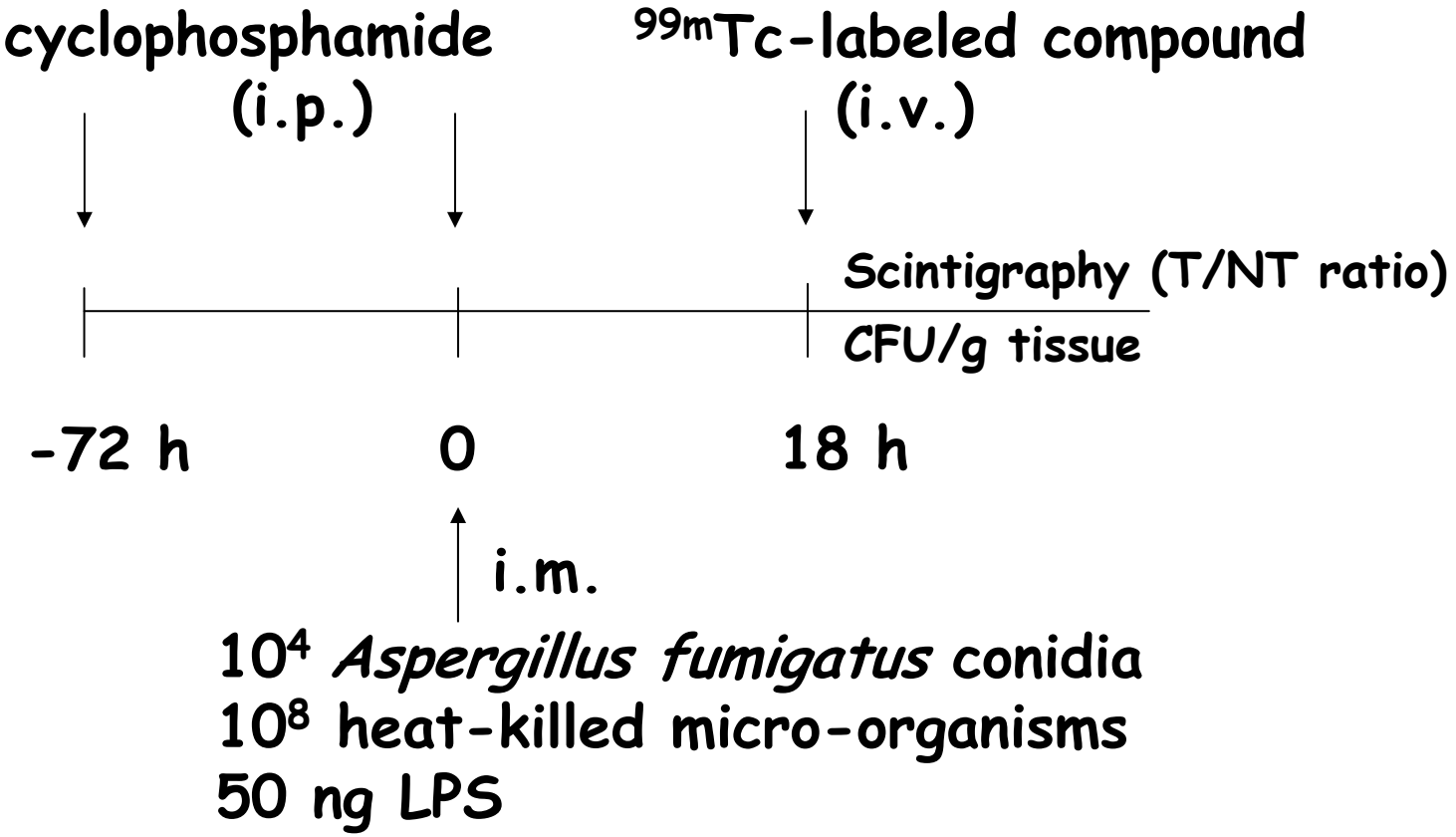




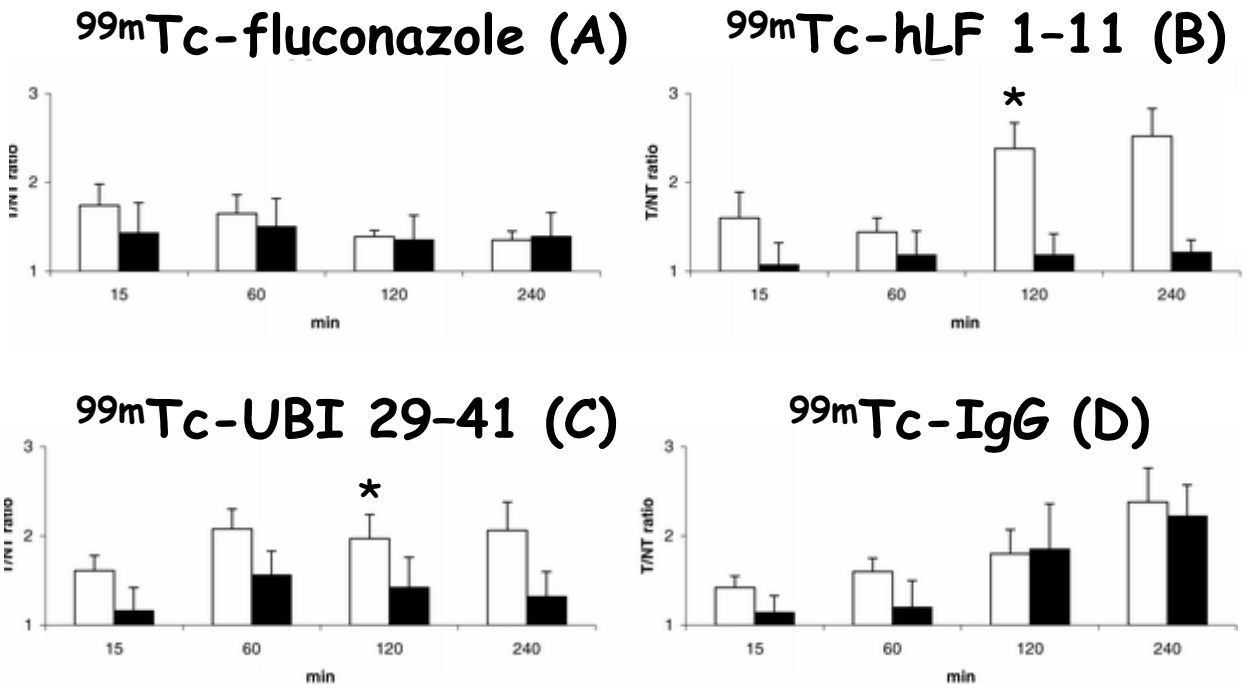
***C. albicans* infected mice**

Can ^{99m}Tc-labeled antimicrobial compounds

visualize *A. fumigatus* infections?


cyclophosphamide
(i.p.)
^{99m}Tc-labeled compound
(i.v.)
Scintigraphy (T/NT ratio)
CFU/g tissue
-72 h
0
18 h
i.m.
10^4 *Aspergillus fumigatus* conidia
10^8 heat-killed micro-organisms
50 ng LPS

A. fumigatus infected leukocytopenic mice

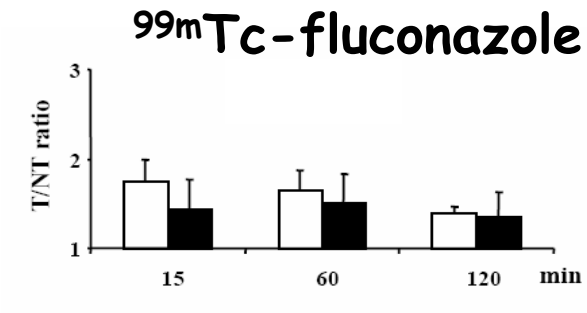


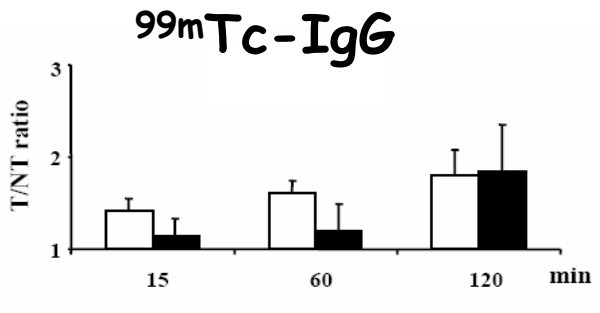
***A. fumigatus* (*open bars*)**

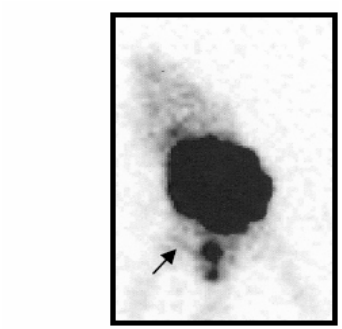
***LPS* (*closed bars*)**

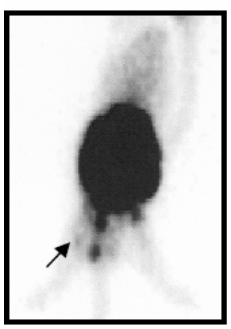
From: Lupetti *et al.* Eur. J. Nucl. Med. 29:674-679, 2002

A. fumigatus infected leukocytopenic mice (open bars) or inflamed with LPS (closed bars)



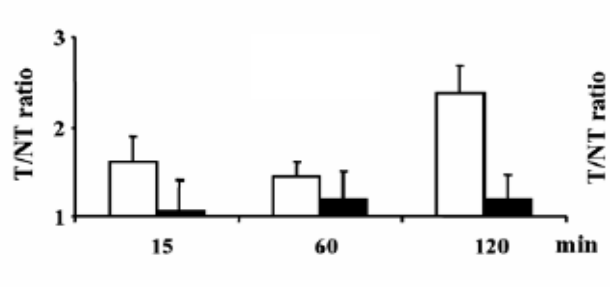




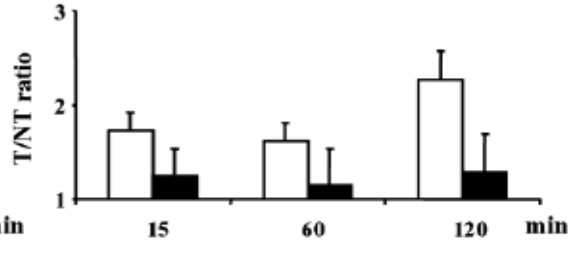


A. fumigatus infected leukocytopenic mice (open bars) or inflamed with LPS (closed bars)

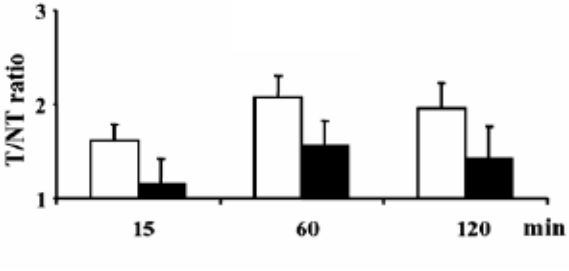
^{99m}Tc-hLF 1-11

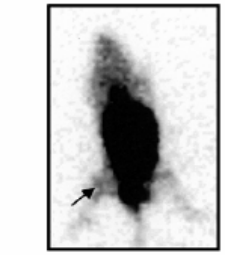


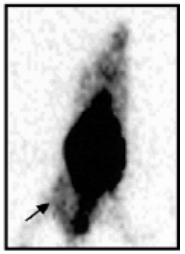
^{99m}Tc-UBI 18-35

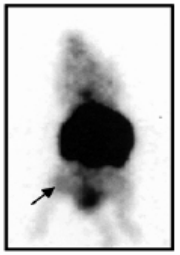


^{99m}Tc-UBI 29-41









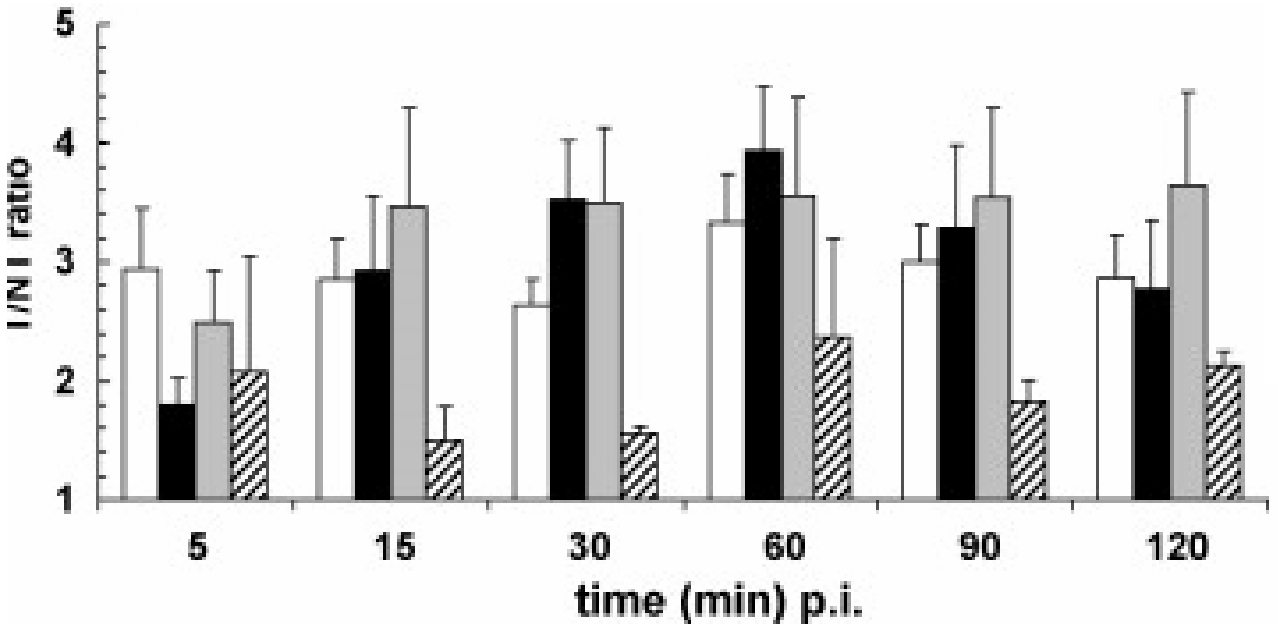
Biodistribution of ^{99m}Tc-labelled compounds in mice infected with *C. albicans*

^{99m}Tc compound	Injected radioactivity (% injected dose)								
	Bladder			Kidneys			Liver		
	15 min	60 min	240 min	15 min	60 min	240 min	15 min	60 min	240 min
Fluconazole	29±3	34±2	29±7	24±2	22±7	22±4	19±2	10±2	8±7
hLF 1-11	12±2	18±3	27±3	15±3	15±2	19±2	24±2	26±2	38±2
UBI 29-41	23±3	32±5	17±3	19±2	22±2	12±2	17±2	14±2	10±1
IgG	17±3	47±2	7±3	14±7	20±2	18±2	17±2	14±2	10±1

Values are the mean±SD of at least four observations

From: Lupetti *et al.* Eur. J. Nucl. Med. 29:674-679, 2002

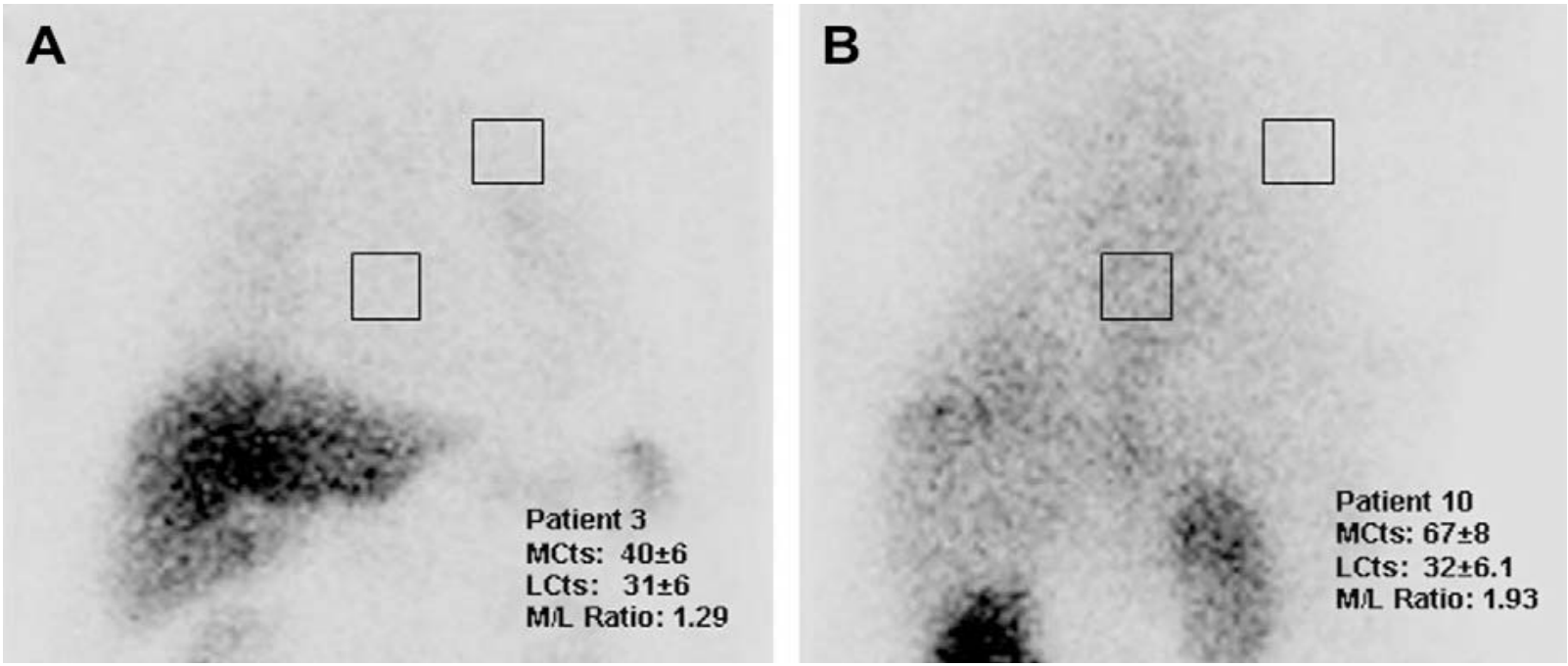
ROUTES OF ADMINISTRATION



Accumulation of ^{99m}Tc-labeled hLF 1–11 in MRSA-infected thigh muscles in mice at various intervals after different routes of administration:
iv. (open bars)
ip. (closed bars)
subcutaneous (grey bars)
oral (hatched bars)
Results are the means ± S.E.M. of at least eight animals.

From: Brouwer *et al.* Peptides 29:1109-1117, 2008.

… and in clinical studies?



(A) Patient with a negative ^{99m}Tc-UBI 29-41 scintigraphy. (B) Patient with a positive ^{99m}Tc-UBI 29-41 scintigraphy. Dose injected 740 MBq (20 mCi), 500 kilocounts (kcts) per scan. MCts, mediastinum counts; LCts, lung counts; M/L ratio, mediastinum/lung counts ratio.
From: Vallejo *et al.* Arch. Med. Research 39:768-774, 2008.

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